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Chancellor's Message

Prof. Dr. Khalil Ahmad Behsoodwal
Chancellor, Nangarhar University

To create new knowledge and find answers to complicated and unresolved problems need research. In the endeavors of research, the synthesis and analysis of information is scrutinized to the extent that it mostly leads to some innovative and creative outcomes. Currently, accessing approved domestic information and data within Afghanistan is quite a challenging task. For that reason, Nangarhar University has recently launched an online journal entitled "Nangarhar University International Journal of Biosciences (NUIJB)", which shares the results of the researches within and outside Afghanistan; introduces the achievements of the new researches on domestic and international level; facilitates the exchange of views and knowledge among the research scholars; and paves the way to resolve complex academic problems. It is, indeed, a time of rejoice and pride that NUIJB is publishing its second issue. We try our best to ensure publishing standardized and free of error research articles. Despite our best efforts, if the respected readers still face any academic or professional problems in the articles, they are requested to formally inform the administration of the journal for the intended corrections.

With the hope of a better, peaceful, and advanced Afghanistan!

Regards,

Prof. Dr. Khalil Ahmad Behsoodwal
Chancellor, Nangarhar University

Message of Editor-in-Chief

Assoc. Prof. Ihsanullah Nasih
Editor-In-Chief, NUIJB

I wish to extend my gratitude to the members of the boards and personnel involved in the successful launching of Nangarhar University International Journal of Biosciences (NUIJB). The journal invites articles in the areas of Medical, Agricultural, Veterinary and Animal sciences – a broad theme that should appeal to the largest possible audiences. It is also worth mentioning that NUIJB has already acquired ISSN and has been recognized by the Ministry of Higher Education (MoHE). The main aim and objective of NUIJB are to bridge the gap so that authors get a wider audience for their high quality scientific achievements. I believe we will be publishing a significant number of high quality original research article and scientific reviews from authors around the word. We also cordially welcome comments and suggestions that could improve the quality of the journal.

Regards,

Assoc. Prof. Ihsanullah Nasih
Editor-In-Chief, NUIJB

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NUIJB, 2023

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Blood Metabolites and Hormonal Profiles in Dairy Cows during Estrus Cycle in Jalalabad Afghanistan

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ABSTRACT

Background: The study was aimed to investigate the productivity and fertility status of the crossbred and Watani (local) cows in Jalalabad and role of blood metabolites in reproductive cyclicity.

Materials and Methods: A total of 100 dairy cows were selected in five zones comprising city, north, west, south and east of Jalalabad. Fifty crossbred cows (French Friesian x Watani) and 50 Watani cows were selected. Blood samples were collected (10 ml) for blood metabolites and hormonal profiles on day 1, 4 and 17th of the estrus cycle and analysed through UV/Vis spectrophotometer and hormonal profiles through ELISA using Humareader.

Findings: The concentrate intake in crossbred cows was 2.00 ± 0.20 ranging from 1.63 to 2.42 kg day^{-1}, while in Watani cows the values were 2.20 ± 0.21, 1.80 and 3.30 kg day^{-1}, respectively. The milk production for crossbred cows was 7.64 ± 0.14 ranging from 7.28 to 8.00 kg day^{-1}, while in Watani cows the values were 3.75 ± 0.49, 2.00 and 4.88 kg day^{-1}, respectively. Mucus discharge in crossbred cows was 2.00 ± 0.22 (1.50 to 2.50 scales) against 2.45 ± 0.19 in Watani cows. Standing for mounting in crossbred cows was higher (0.43 ± 0.86) than Watani cows (0.26 ± 0.09; scale 0 to 4), Triglycerides varied from 100.94±2.79 to 117.52±5.77 mg/dl (P=0.024) with significant effect of estrus days but no effect of region. Serum glucose in crossbred cows was 67.18±1.50 mg/dl while in Watani cows, it was 65.20±1.33. Protein was 7.62±0.11 in crossbred cows and 6.99±0.13 g/dl in Watani ones. The respective values for triglycerides were 110.69±3.43 and 104.03±3.40 mg/dl. Progesterone concentrations were 0.581±0.076 ng/ml and 0.569±0.09 ng/ml while LH was 28.41±3.31 and 24.20±2.64 ng/ml in the two breeds. All the blood metabolites and hormonal concentrations were not different among regions, however triglycerides showed a constant increase during the succeeding phases while glucose manifested an opposite pattern during the estrus cycle. The level of glucose in the city animals was higher (69.21±2.37 mg/dl, (P=0.127). Progesterone concentration increased on day 4th and declined on 17th day while LH showed a consistent increase during the period.

Conclusion: This study concluded that breeding of the local Watani cows with French Frisian semen resulted in production of crossbred cows. The crossbred cows showed higher concentrations of blood glucose, triglycerides, proteins, progesterone and LH hormones, reflecting higher metabolic activities than the local Watani cows.

Keyword: Blood; Metabolite; Hormone; Dairy Cow; Estrus Cycle

INTRODUCTION

The breeding efficiency of dairy cows gets lowered with a number of reproductive disorders like endometritis, anoestrus and repeat breeding. It adversely affects the productive and reproductive performance of cows, and results in great economic losses to dairy farmers. The basic causes of the reproductive problems in a herd are multiple. They include management, nutritional and pathological factors. The dairy farming has been supporting national economy through production of milk and beef for meeting the protein demand of the rapidly expanding human population (Qureshi, 2007., Dutta et al., 1988). Reproductive and productive disorders are associated with crossbreeding in cattle. Out of the existing cattle population of Pakistan, more than 25% are crossbred animals while the remaining 75% are local cows. According to the breeding policies, the indigenous cows in plain-irrigated areas are crossed with exotic Holstein-Friesian breed while the cows in hilly and arid areas are crossed with Jersey breed. However under the existing artificial insemination practices more than 50% of exotic blood level is used which leads to a decreased immunity and resistance to stress conditions. Increase in genetic purity coupled with poor nutritional and management conditions affect the reproductive and productive performance badly. The stress comprises nutritional, health and thermal factors and is revealed by the inability of an animal to cope with its environment, a phenomenon that is often reflected in a failure to achieve genetic potential (Qureshi, 2007).

Blood glucose, total proteins, urea and cholesterol levels in cyclic, non-cyclic, and endometritic crossbred cows in Seventy-five crossbred cows kept at the Livestock Experimental Station, Qadirabad, and District Sahiwal. The animals were divided into three equal groups i.e. cyclic, non-cyclic and endometritic. The results revealed significantly higher values of glucose and cholesterol in endometritic cows as compared to cyclic and non-cyclic cows. Protein level was highest in endometritic, followed by non-cyclic and lowest in cyclic cows. However, serum level of urea did not differ in cyclic, non-cyclic and endometritic animals (Ahmad et al., 2004).

Endocrines and milk yield of crossbred cows treated with recombinant bovine (rbST) somatotropin to investigate the effect of rBST on the blood metabolites, hormones and milk yield in lactating crossbred cows. Thirty lactating cows were divided into 2 groups as control (n=10) and treated (n=20). Treated animals were injected subcutaneously with 250 mg of rbST at 0, 14 and 28 days, whereas control animals were given place of 2 ml normal saline. Blood metabolites (glucose, blood urea nitrogen (BUN), triglycerides, total proteins, albumin, globulin, sodium and potassium) and hormones (thyroxine (T4) and insulin) were not altered by (rbST) injection. Serum growth hormone (GH) increased significantly ($P<0.001$) due to rbST injection but not milk GH. However, a significant ($P<0.001$) decrease in triiodothyronine (T3) level was observed in rbST-treated group compared to control group. The weekly average milk yield showed a significant increase of 33% in the rbST-treated group compared to the control group. It was concluded that 250 mg rbST could be used for short duration to increase the milk yield of crossbred cows (Qureshi et al., 2002).

This study was designed to monitor the changes of the blood metabolites and hormonal profiles in crossbred and Watani dairy cows during estrous cycle in Jalalabad, Afghanistan, with the following objectives:

1. To document the lactation and fertility status of crossbred dairy cattle in comparison to Watani cows under field conditions in Jalalabad, Afghanistan.

2. To investigate the relationship among the status of reproductive cyclicity, hormonal status and blood metabolites in crossbred and Watani (Local) dairy cows.

MATERIALS AND METHODS

Study Design

This study was conducted in Jalalabad and surrounding villages in three months in private farms; 5-10 cows from each farm. Fifty animals were selected from each; F1 cross of French Friesian with Watani cows and fifty pure Watani cows. Lactation status, fertility indicators and nutritional status were recorded for each animal. Lactation status comprised the level of daily milk yield (kg/day^{-1}) and lactation length. The fertility indicators comprised age at puberty, service period, calving interval, services per conception and number of calves born. Data on nutritional status was collected having concentrates which comprising the cotton seed cake and wheat bran 2-3 kg/day^{-1} and the quantity of 20-30 kg/day^{-1} of berseem were provided. T test was applied for determining difference between local and crossbred cows' concentrates intake, milk yield, mucus discharge and standing for mounting. Analysis of variance was applied for determining means for the five zones, in the result of the aforementioned parameters.

Selection of Animals

Total number of 30 cows including 15 each of F1 cross of French Friesian with Watani cows and pure Watani cows were selected for this study. The animals were divided into younger and older groups falling with an age range 4 to 8 years and 9 to 11 years respectively.

Data Collection

The data on milk yield and quantity of concentrates was collected on the pre –designed questionnaire. Each animal was studied for one estrus cycle. Animals were selected from five zones comprising city, north, west, south and east of Jalalabad.

Blood sampling and analysis

To determine the hormonal and metabolic profiles of the experimental cows (10 ml) samples of blood were collected on day 1, 4 and 17th of the estrus cycle. The samples for blood were collected during June, July and August where the temperature was in the range of 40^{0C} .The blood was collected from jugular vein of all selected animals with 16 gauge sterile needle at 8:00 AM to 9:00 AM after 2 hours of feeding time. Serum was separated from the blood after poured into centrifuge tubes, was kept for 40 minutes up to formation of clot and was finally centrifuged at 2000 rpm for about 10 minutes then it was stored in a freezer at -20^{0}C till further analysis. Blood metabolites including total blood protein, glucose and triglycerides were determined through the available kits.

Determination of Protein levels

The protein level was determined by Biuret reaction method using the commercial protein kit (AMEDA Labordiagnostik GmbH. Krenngasse 12, 8010 Graz, Austria). Twenty µL serum samples were dispersed into labeled test tubes and an amount of 1.0 ml of the available reagent was added. Then the prepared mixture was incubated for 10 minutes at 37^{0}C. Then absorbance was read for the standard (AbsStd) and the sample (AbsS) at wavelength of 540nm against the blank reagent using (UV/ Vis Spectrophotometer (model U2020 Geesthacht Germany). The result was expressed in (g/dl).

Determination of Glucose levels

The level of glucose was determined by enzymatic – colorimetric method using the commercial glucose kit (AMP Medizintechnik GmbH. Stattegger Strasse 31b, 8045 Graz, Austria). 10 µL serum samples were dispersed into labeled test tubes and an amount of 1.0 ml of the available reagent was added. The prepared mixture was incubated for 10 minutes at 37^0C. Then absorbance was read for the standard (Abs Std) and the sample (Abs S) at wavelength of 510 nm against the blank reagent using (UV/ Vis Spectrophotometer (model U2020 Geesthacht Germany). The result was shown in mg /dl.

Determination of Triglyceride levels

The level of serum triglycerides was determined by enzymatic – colorimetric method using the commercial triglyceride kit (AMEDA Labordiagnostik GmbH. Krenngasse 12, 8010 Graz, Austria). 10 µL serum samples were dispersed into labeled test tubes and an amount of 1.0 ml of the available reagent was added. Then prepared mixture was incubated for five minutes at 37^{0C}. Then absorbance was read for the standard (AbsStd) and absorbance for sample (AbsS) at wavelength of 500 nm against the blank reagent by (UV/ Vis Spectrophotometer model U2020 Geesthacht Germany). The result was shown in mg /dl.

Determination of luteinizing hormone (LH)

Luteinizing hormone (LH) was determined by using commercial ELISA kit (AmGEnix International, INC. 3444 Pinotin Ct. San Jose, CA 95148, USA). The principle for the Enzyme Linked Immune Sorbent Assay for LH is based up on the direct binding of LH to sheep polyclonal antibodies. The assay system consists of sheep polyclonal anti LH antibodies coated in the micro titration plate. The mouse monoclonal antibodies conjugate with enzyme that is horseradish peroxidase. The LH molecule is sandwiched between these antibodies and by adding substrate they read enzyme and produce color. The LH absorption is directly proportional to the color intensity of the last sample. In procedure of LH 50 µl, standards, specimens, and controls were pipette in micro titer plate. After that 100µl of enzyme conjugate reagent was added in every well and mixed for 39 seconds. Incubation was made in room temperature (18-25^0C) for 45 minutes. Then extra mixture was removed from the exist plate and wells were washed five times by distilled water. If residues were found, they were removed with paper towels. 100µl of TMB reagent was added in each well and they were gently mixed for five seconds. The micro titer plate was incubated in room temperature in the dark place for twenty minutes. The action was stopped adding up 100µl of stop solution to every well and slowly mixed for 5 more seconds. The color changed from blue to yellow. Absorbance was read at 450 nm with ELIZA plate reader (Humareader Plus.Cat-No.18500 P. Human GmbH Diagnostica Worldwide D65205 Wiesbaden Germany) within 15 minutes.

Progesterone Assay for Serum

The Progesterone level was determined by using the commercial progesterone kit (AmGEnix International .INC. 3444 Pinotin Ct. San Jose, CA 95148, USA).The progesterone ELISA is based on the principle of competitive binding between progesterone in the last sample and progesterone – HRP for a constant amount of rabbit, anti-progesterone antibodies. During incubation fixed amount of progesterone- HRP conjugate, compete with the progesterone of the standard and last sample for a fixed number of binding sites of the specific progesterone antibodies. Thus the amount of progesterone- HRP conjugate immunologically binds to the wells. It will be decreased as the concentration of progesterone in the standard and test sample. Thus the intensity of the color formed is directly proportional to the progesterone-HRP conjugate and as inversely proportional to the progesterone (unlabeled progesterone) of the standard in test sample.

In procedure of progesterone 25µl standards, specimens and controls were pipette into appropriate wells. Then 100µl of Working Progesterone – HRP and Conjugated Reagent were added in each well. Then 50µl of Rabbit Anti –Progesterone Reagent was added to each well and mixed for 30 seconds. Incubation was made at room temperature (18-25^{0}C) for 90 minutes. The micro wells were washed for 5 times with distilled or deionized water. 100 µl of TMB reagent was dispensed into each well. They were gently mixed for 10 seconds. The microtiter plate was again incubated at room temperature (18- 25^{0}C) for 20 minutes. The reaction was stopped by adding 100 µl stop solution to each well and mixed for 30 seconds. The reaction was completed when blue color changed to yellow. Absorbance read at 450 nm through ELIZA plate reader (Humareader Plus.Cat-No.18500 P. Human GmbH Diagnostica Worldwide .D65205 Wiesbaden Germany) within 15 minutes.

Rectal Palpation and Insemination

The experimental cows were clinically examined. Data was recorded and rectal examination was conducted for estrus detection and status of the reproductive tract. Reproductive parameters were recorded comprising mucus (scale 0-4) and standing for mounting (scale 0-1). The animals were artificially inseminated at appropriate time in consultation with the owner and it was palpated for pregnancy diagnosis per rectum on 60th day post-breeding.

Data analysis

The data was collected and maintained in computer based MS Excel files. SPSS-19 software was used for data analysis using guidelines provided by Steel and Torrie (1980). Means were compared through analysis of variance for zones. The dependent parameters were blood glucose, triglycerides, total protein, progesterone and luteinizing hormones.

RESULTS

Effect of feeding status and region on fertility and milk yield

Measure for the daily concentrates intake in two breeds (Watani and crossbred) cows and their association with fertility and milk yield are reported in **Table 1**. Statistical analysis of the data indicated that the average concentrates intake was higher in Watani than crossbred dairy cows. It was 2.20 kg versus 2.00 kg day^{-1}. The average milk yield of Watani cows was lower than the crossbred cows and was 3.75±0.49 versus 7.64±0.14 kg day^{-1}. The table shows that the local Watani cows consumed more concentrates while produced less milk than the crossbred cows.

Fig. 1 & 2 show the daily intake of concentrates and milk yield in different regions. Highest concentrates intake was found in west region in Watani dairy cows the two parameters showed opposite pattern, the west was followed by north, south, city and east reaching to the final values of 1.8 concentrates and 4.5 kg day^{-1} milk. In the crossbred cows the concentrates feeding and daily milk yield was almost parallel across the four regions. The north zone showed the best efficiency producing 8 kg day^{-1} milk with consuming 2.3 kg concentrates as compared to the east zone showing milk yield the same level 7.2 kg day^{-1} against concentrate intake. Higher concentrates intake (2.7 kg day^{-1}) was found in city region.

The average mucus discharge of Watani cows was higher (2.45±0.19) on estrus day, ranging from 2.00 to 2.90 than the crossbreed cows (2.00±0.22) ranging from 1.50 to 2.50. Standing for mounting in Watani cows was found as 0.26±0.09 on estrus days, ranging from 0.00 to 0.54 as compared to 0.43±0.86, ranging from 0.28 to 0.67 in crossbred cows.

Mucus discharge and standing for mounting in the city were the highest values of those parameters while the west zone showed the lowest values in Watani cows. For crossbred cows showed the most vigorous stress symptoms

in southern zones, mucus discharge was lowest in the north and standing for mounting was lowest in the city and east.

Table 1. Concentrates feed offered, Daily milk yield, Mucus discharge and Standing for mounting in Watani (local) and crossbred dairy cows.

	Breed	Min	Max	Mean±SE
Concentrates offered (kg day^{-1})	Watani	1.80	3.30	2.20±0.21
	crossbred	1.63	2.42	2.00±0.20
Milk yield (kg day^{-1})	Watani	2.00	4.88	3.75±0.49
	crossbred	7.28	8.00	7.64±0.14
Mucus discharge scale (0-4)	Watani	2.00	2.90	2.45±0.19
	crossbred	1.50	2.50	2.00±0.22
Standing for mounting scale (0-1)	Watani	0.00	0.54	0.26±0.09
	crossbred	0.28	0.67	0.43±0.86

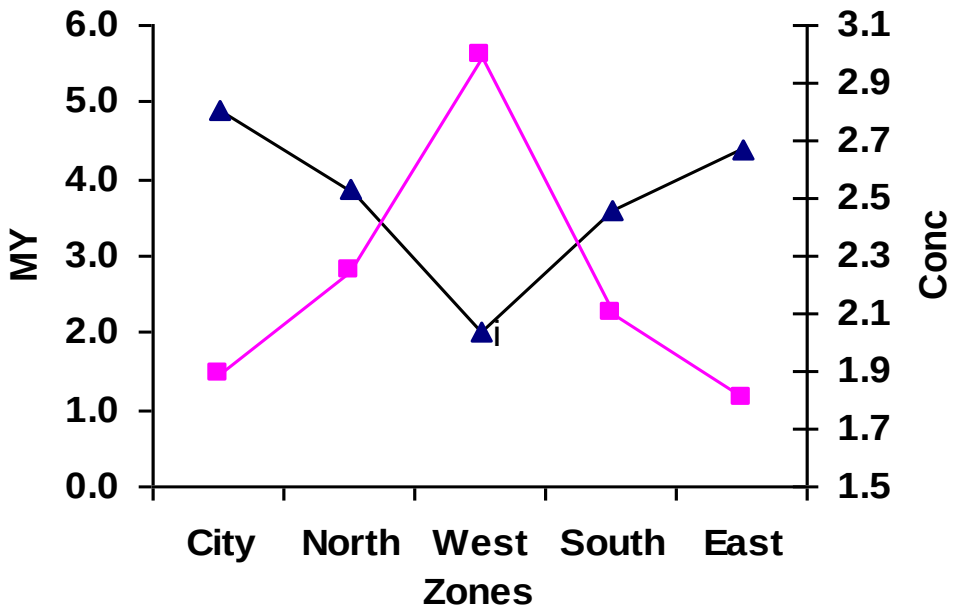

Fig.1. Milk Yield (kg day^{-1}, (▲) and concentrate intake (Conc. kg day^{-1}, (■) by Watani Dairy Cows in Jalalabad.

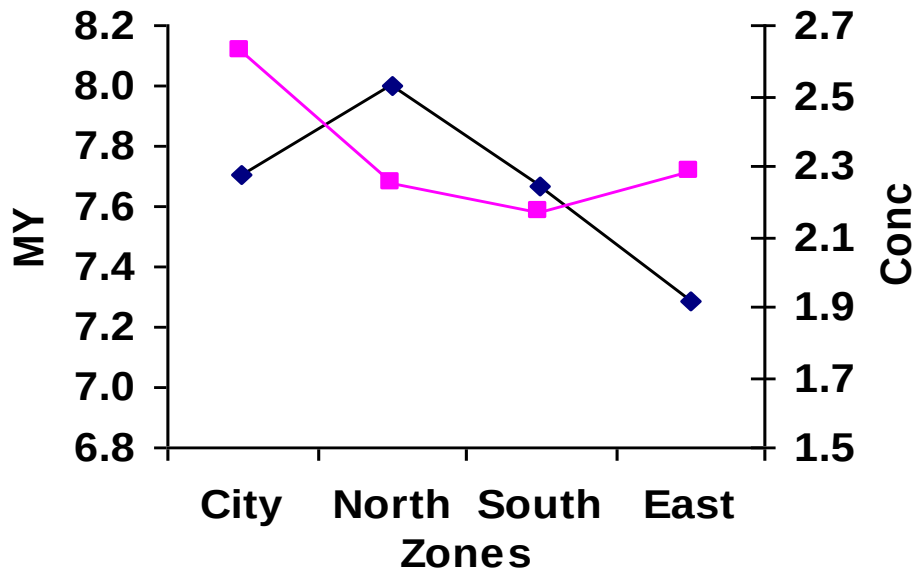

Fig. 2. Milk Yield (kg day^{-1}, (■) and concentrate intake (Conc, kg day^{-1}, (■) by Crossbred Dairy Cows in Jalalabad.

Effect of estrus days on blood metabolites and hormonal profiles

Table 2 shows the effect of estrus days on blood metabolites and hormonal profiles determined in dairy cows. Statistical analysis of data showed that the glucose remained higher on day 1 and 4 and decreased on day 17 (P= 0.650). Triglycerides showed an upward trend throughout the study period and is varied from 100.94±2.79 to 117.52±5.77 mg/dl (P=0.024) and the result was significant. Protein remained higher on day 1 and 4 and decreased on day 17 (P=0.385) which varied from 7.39 to 7.42 g/dl and the result was non-significantly different. Progesterone was lower on day 1 and increased gradually on day 4 with the developing of luteal tissue constituting the luteal phase up to day 16. Later on progesterone level went on decreasing on day 17 (P= 0.231). LH hormone was lower from day 1 to day 4 of estrus cycle increased from day 4 till it reached the maximum level on day 17. (P= 0.655).

LH hormone was lower from day 1 to day 4 of estrus cycle, but increased from day 4 till it reached the maximum level on day 17. The ovulation is expected on day 21 which needs the highest level of LH in the form of LH surge. Progesterone was lower on day 1 increased gradually on day 4 with the developing of luteal tissue constituting the luteal phase up to day 16. Later on progesterone level went on decreasing due to regressing of corpus leutum reaching its minimum on the day of ovulation.

Table 2. Blood Metabolites and Hormonal concentration as influenced by the days of estrus cycle in dairy cows (Mean ± SE, n=81)

Day	Glucose mg/dl	Triglyceride mg/dl	Protein g/dl	Progesterone ng/ml	LH ng/ml
1	67.18±1.67	100.94±2.79	7.39±0.15	0.47±0. 01	25.48±3.16
4	67.12±1.74	106.23±3.83	7.42± 0.15	0.71±0.16	25.70±3.68
17	64.23±1.85	117.52±5.77	7.11±0.17	0.54±0.03	28.48±4.56
P. value	0.650	0.024	0.385	0.231	0.655

Fig.3 shows that both glucose and protein were higher and parallel, on the day of estrus and remained so up to day 4. This may probably be supportive for estrus activities exhibited by the animals through enhanced metabolism, mucus secretion, locomotion and other signs of estrus. This was reflective of higher metabolic rates and the metabolites may have been derived from the nutrient pools in the body reserves.

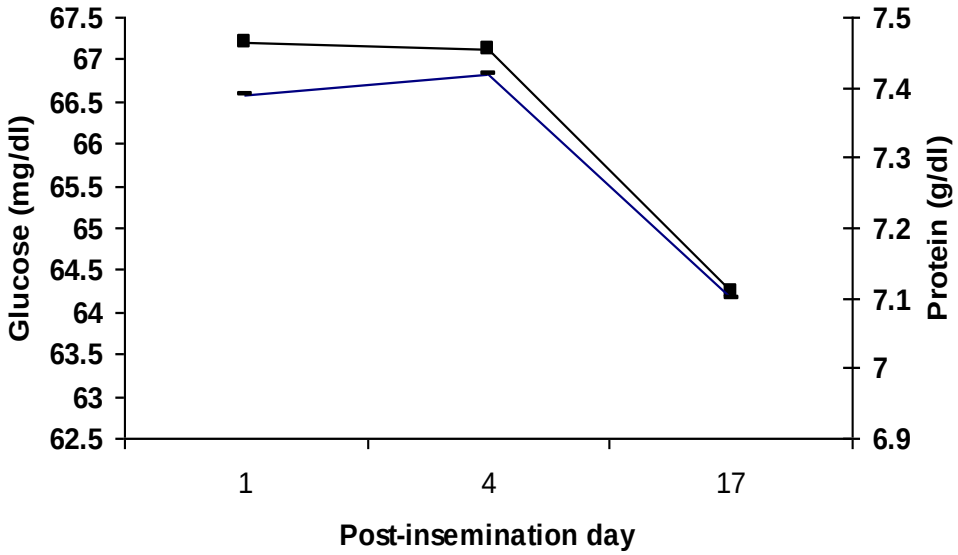

Fig. 3. Changes in blood glucose (solid squares) and protein (lines) levels in dairy cows with the post-insemination day

Effect of Regions on Blood metabolites and hormonal profiles

Data regarding effect of regions on blood metabolites and hormonal profiles in dairy cows in Jalalabad is reported in **Table 3** .Statistical analysis indicates that the average serum glucose concentration was similar in all regions, the differences being non-significant. Triglyceride and Protein concentration in serum were almost similar in all regions, triglycerides (P=0.624) and protein 7.33±0.09 (P=0.104) the result were non-significant. LH and Progesterone hormones concentration in serum was also non-significant difference among regions, the average of LH was 26.39±2.13 (P=0.450) and Progesterone was 0.57±0.059 (P=0.340).

Table 3. Blood Metabolites and Hormonal concentration as influenced by Region wise in dairy cows in Jalalabad. (Mean ± SE, n=81)

Region	Glucose mg/dl	Triglyceride mg/dl	Protein g/dl	Progesterone ng/ml	LH ng/ml
City	69.21±2.37	106.72±4.64	7.48±0.18	0.48±0.01	28.08±4.77
North	68.04±1.17	110.45±5.30	7.42±0.1	0.65±0.17	23.73±3.42
West	64.57±1.84	105.94±6.48	7.12±.26	0.69±0.23	29.14±5.94
South	63.49±2.68	105.91±5.79	6.96±.19	0.50±.02	23.02±4.44
East	63.51±2.77	108.3±5.80	7.54±0.31	0.59±0.06	27.85±5.22
P. value	0.127	0.624	0.104	0.340	0.450

Fig. 4 the level of glucose present in the city area is higher than those of north, west, south and eastern zones, 69, 68, 64, 63 and 63 mg/ dl. The protein also follows the same pattern as the glucose with the exception of the east, in which the protein level is highest (7.5 g/ dl) and lowest is in the south (6.9 g /dl). The region has a non-significant effect on serum glucose (P=0.127) and protein (P=0.104). **Fig. 5** shows that protein varied from 6.6 to 7.6g/dl shows the highest level in east followed by city, north, west and south; Triglycerides follows the same pattern except for the city.

Fig. 4. Effect of Region on Blood metabolites Glucose (Square) and Protein (Triangle)

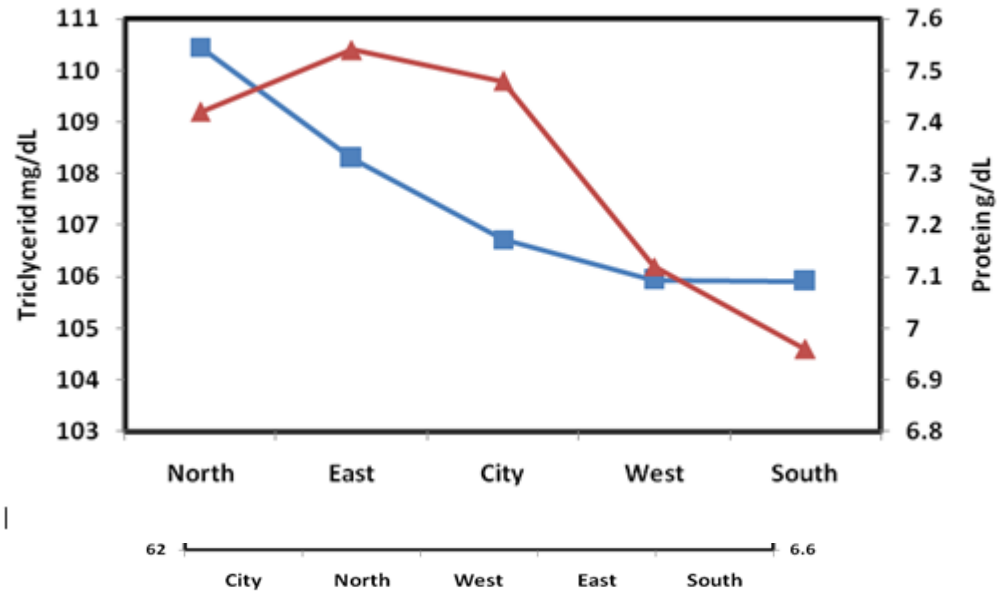

Fig. 5. Effect of Region on Blood metabolites (Triglycerides (Triangle) and Protein (Square)

Effect of breeds on Blood metabolites and hormonal profiles

Data regarding blood metabolites and hormonal concentration of crossbred and Watani dairy cows in Jalalabad is reported in **Table 4**. Statistical analysis indicated that the average serum glucose concentration in crossbred cows was higher 67.37±1.50 than Watani cows 65.20±1.33 mg/dl, the result was not significant.

Triglyceride concentration were also higher in crossbred than Watani cows 110.69±3.43 and in Watani was 104.03± 3.40 Protein concentration in crossbred was 7.62±0.12, and in Watani it was 6.99±0.13 mg/dl. Triglyceride in breeds was not significant while protein was significant in them (P=0.001).

LH and Progesterone hormones concentration in serum were also higher in crossbred and lower in Watani cows. The average of LH in crossbred was 28.41±3.31 and 24.20±2.64 ng/ml while Progesterone was 0.581±0.076 and 0.569±0.09 ng/ml in the two breeds respectively.

Table 4. Descriptive statistics for blood metabolites and hormones in crossbred and Watani dairy cows. (Mean ± SE, Minimum, Maximum)

	Glucose mg/dl	Triglyceride mg/dl	Protein g/dl	Progesterone ng/ml	LH ng/ml
Crossbred	67.371±1.50	110.69±3.43	7.62±0.11	0.581±0.076	28.41±3.31
	48.8	81.84	5.85	0.375	13.6
	87.2	167.60	9.68	3.630	109.6
	65.20±1.33	104.03±3.40	6.99±.13	0.569±.09	24.20±2.64
Local Watani	47.9	79.35	5.32	.375	13.6
	85.1	185.83	9.24	4.005	96.0
P. Value	0.288	0.173	0.001	0.919	0.328

DISCUSSION

The daily concentrates intake in Watani and crossbred cows and its association with fertility and milk yield was studied. The average concentrates intake was higher in Watani than crossbred dairy cows and (2.20 kg versus 2.00 kg day^{-1}) while the average milk yield of the former was lower (3.75±0.49 versus 7.64±0.14 kg day^{-1}). It shows an adverse effect of over feeding on milk yield in the low yielding breed. Watani cows have no genetic potential to utilize the extra amount of concentrates for milk synthesis. This phenomenon was extensively explored by our group (Qureshi, 2007). A same scale feeding was reported exposing the low yielding animals to adverse effects of over feeding. Intake of crude protein (CPI) varied between seasons and was positively correlated with serum urea levels (r=0.22, p<0, 01; Qureshi et al., 2002), In LMY buffaloes there seemed to be the stress of over feeding of degradable protein. In previous studies (Rahman et al., 1987; Husain and Mostafa, 1985; Khan and Khatun, 1998) it was observed that the daily milk yield was 8.10, 9.74 liters and 7.35 liters for SL x Pabna, Fx Pabna and Pabna x Pabna genetic groups respectively.

The present study shows that different regions have significant (P<0.05) effect on daily intake of concentrates and milk yield. In the crossbred cows the concentrates feeding and daily milk yield was almost parallel across the four regions. The north zone showed the best efficiency producing milk 8 kg day^{-1} while consuming 2.3kg concentrates as compared to the east zone showing milk yield is 7.2 kg day^{-1} consuming the same amount of concentrate intake. Higher concentrates intake (2.7 kg day^{-1}) was found in city region, while

higher milk yield (8 kg day^{-1}) was recorded in north zone. The best efficiency of the north zone may be attributed to the well-developed irrigation system supporting production of plenty of fodder which constitutes the best combination of practices for crossbred animals.

In the present study the Watani dairy cows show high mucus discharge than the crossbred dairy cows. Watani dairy cows are more adjusted to the local conditions as compared to the crossbred dairy cows, therefore they exhibit estrus symptoms with greater intensity as compare to the crossbred cows. The crossbred cows are not fully adapted to the local environment so fail to express full estrus signs.

The zones had a significant effect on mucus discharge and standing for mounting in Watani cows. The city showed the highest values of those parameters while the west zones showed the lowest values. The highest value is due to the special care, treatment facilities, feeding and attention of people of city, while the lowest value is due to low water availability, low land fertility, low treatment facility, feed scarcity and low attention of people of west zone.

In the current study, the mean concentration of glucose level in blood serum of local Watani and crossbred dairy cows during estrus cycle was (65.20±1.33 and 67.37±1.59 mg/dL) respectively. While Sarker et al. (2011) stated that blood glucose was 35.16±2.402 mg/ dl in a Red Chittagong (RC) cow and 35.37±2.403 mg/ dl in a local cow. Our result supported it. Anderson et al. (1930) stated that blood glucose level may alter because of the changing in its usage throughout the animal's life cycle. Ahmad et al. (2004) recorded blood glucose in cyclic cows as (50.72±1.12), and in non-cyclic as (50.56±1.13) mg/dl.

Glucose concentration varied with changing phases of estrus cycle on day 1 and 4 the concentration was higher while on day 17th it decreased by 3 mg/dl. The decreased concentration may be the result of glucose utilization for supporting estrus activities comprising restlessness, increase locomotion, mucus discharge and bellowing. Concentrations of glucose in plasma were affected by body condition score (Adams et al., 1987) and may influence reproductive performance in cattle.

Metabolism of glucose by bovine corpora luteal in vitro was influenced by the day of estrus cycle (Chase et al., 1992). The lack of estrus and failure of formation of functional corpora luteal in cattle was associated with inhibition of glycolysis (McClure et al., 1978). Glucose concentration increased and the GnRH- stimulated LH secretion with the infusion of propionate into heifers (Rutter et al., 1983). They reported cows that had luteal activity during the breeding season had 5.7 mg/dL more glucose than cows without luteal activity. Despite the significant difference, categorical data analysis indicated that individual glucose values were not determinants of luteal activity.

In our study total serum protein was 6.99±0.13 in Watani and 7.62±0.11 g/dl in crossbred cows. It observed from the present study that blood protein level increased from day 1^{s} to day 4th of estrus cycle and then 0. 28 g/dl blood protein level is reduced on day 17th of estrus activity, the decreased protein concentration maybe due to its utilization for supporting reproductive activities.

Sarker et al. (2011) studied blood metabolites of Red Chittagong (RC) and Local cattle at Chittagong, the total serum protein was recorded 8.98± 4.70 (g/dl) and 8.997±0.588 (g/dl) in RC cows and local cows, respectively. Kapale et al. (2008) reported that cows had low total protein than the adult Gaolao cows. Total protein and glucose of serum are the major blood components responsible for the maintaining homeostasis and metabolism. Protein deficiency resulted in delayed onset of puberty, increased days open, decreased dry matter

intake and lead to energy deficit (Gaikwad et al., 2007). They reported that adequate protein intake is necessary for normal fetal growth and development.

Ahmad et al. (2004) also reported that total protein level differed significantly ($P<0.05$) among cows of all the three groups, being highest in endometritic (19.16 ± 1.00), followed by non-cyclic (15.23 ± 0.89) and lowest in cyclic (9.19 ± 0.45) cows.

Triglycerides during estrus cycle show significant variation in the present study. We have observed that the blood serum triglycerides level consistently increased from day 1st to day 17th of estrus cycle. In Watani cows the mean was 104.03±3.40 and in crossbred cows it was 110.69±3.43 mg/dl. The increased concentrations were probably required for the milk fat synthesis, metabolic activity in lactating animal.

In previous study variation in blood cholesterol contents were reported during estrus and pregnancy, as precursor of the steroid hormones (Iriadam, 2007). Lipid profiles have been used to predict peripartum diseases; circulating blood triglycerides contribute significantly to milk fat synthesis (Nazifi et al., 2002). Relative to protein metabolism, a decrease in blood protein concentration during the late stages of gestation was observed in sheep, witnessing the utilization of amino acids for protein synthesis in the fetal muscles (Antunovic et al., 2002). It was also reported that plasma urea levels increased during week 10 of pregnancy, reaching a peak at parturition (El-Sherif and Assad, 2001), which in domestic ruminants was ascribed to the cortisol-stimulated catabolism of proteins in the body (Silanikove, 2000).

Nazifi et al. (2002) confirmed that a decrease occurring during lactation compared to dioestrus could be ascribed to the increased cholesterol uptake by tissue involved in milk synthesis because of the normal insulin responsiveness compared to late pregnancy. It was also observed that circulating blood triglycerides contribute significantly to milk fat synthesis. Karapehlivan et al. (2007) has also been reported that blood biochemical parameters including triglycerides, free fatty acids, total protein and urea are important indicators of the metabolic activity of lactating animals.

Schlumbom et al. (1997) reported that blood serum lipids profile is characterized by increase concentration of triglycerides, total cholesterol and lipoprotein due to the diminished responsiveness of target tissues toward insulin that together with an increased mobilization of fatty acids from adipose tissue make available new sources for fetal growth. While Leroy et al. (2004) found the triglycerides concentration in follicular liquid was improved with 43% as increased in the amount of follicle from (<4mm) to large (>10mm).

The present study showed that LH hormone in serum of Watani cow was 24.20±2.64 and in crossbred 28.41±3.31 ng/ml. Progesterone in Watani cows was 0.569±0.09 and in crossbred 0.581±0.076 ng/ml. LH was lower from day 1 to day 4 of estrus cycle but raised from day 4th till it reached the highest level on day 17th. The ovulation is expected on day 21 which needs the highest level of LH in the form of LH surge. Progesterone was lower on day 1 increased gradually on day 4 with the developing of luteal tissue constituting the luteal phase up to day 16. Later on progesterone level went on decreasing due to regressing of corpus leutum reaching its minimum on the day of ovulation.

Sprague et al. (1971) reported that in beef cows, LH peaked sharply from zero to between 2.5 and 61 ng/ml with occurrence of estrus cycle, while in second estrus the peak was less recognizable, after the detection of estrus cycle progesterone levels were lowest for 20hr while it peaked between days 2 to 3 and declined on day 4 to a level which was similar to that observed 20hr after the detection of estrus. Between days 6 and 12, the concentration was gradually rose which remained above 2.5 ng/ml with peaks on day 9 and 12 and declined

thereafter to the next estrus cycle. Haruna et al. (2009) studied five pairs of cows, on day 8 after insemination LH was higher in non-pregnant than in pregnant cows but they reverse on day 16.

CONCULSION

The average concentrates intake was higher in Watani than crossbred dairy cows while the milk yield was lower. It shows an adverse effect of over feeding on milk yield in the low yielding Watani cows having poor genetic potential to utilize the extra amount of concentrates for milk synthesis. The north zone showed the best efficiency producing 8 kg day^{-1} milk while consuming 2.3kg concentrates which may be attributed to the well-developed irrigation system supporting production of plenty of fodder. Watani dairy cows showed higher mucus discharge than the crossbred dairy cows which may be due to its adaptability to the local conditions. Blood glucose in local Watani and crossbred dairy cows was 65.20±1.33 and 67.37±1.50 mg/dl respectively and it decreased on 17^{th} day of estrus cycle probably due to its utilization for supporting estrus activities. Blood triglycerides consistently increased from day 1^{st} to day 17^{th} of estrus cycle. Serum LH was 24.20±2.64 ng/ml in Watani and 28.41±3.31 ng/ml in crossbred cows. The values for progesterone were 0.569±0.09 and 0.581±0.076 ng/ml respectively. LH was lower from day 1 to day 4 of estrus cycle but reached the highest level on day 17^{th} probably for supporting ovulation.

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In-vitro propagation of pre-pubertal bovine Spermatogonial Stem Cell in prepation for transplantation

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ABSTRACT

Backgroud: We investigated the expression of stem cell markers in *in -vitro* cultivated speramatogonial stem cells (SSCs) with the intention of developing a useful system to produce progeny of SSCs.

Materials and Mehtods: Testes from 3, 5 and 7 months old calves were utilized to examine isolation, purity and *in –vitro* propogation of SSCs. Cells were isolated and purified with two-stemp enzymatic digestion combined with centrifugal separation on discontinuous Percoll density gradient layers.

Findings: Cell propagation and SSCs marker expression were determined at 5, 10 and 15 days post-culture. Immunostaining in conjunction with transcrioption based marker expression of cultured cells showed that stem cell markers (DBA, UCHL1, Oct4 and Sox2) were expressed in SSCs.

Conclusion: The results demonstrated marker expression of bull spermatogonial stem cells and showed that prior to transplantation *in-vitro* culturing of bull SSCs is implementable.

Keywords: Bovine; *in-vitro* propogation; Pre-pubertal; SSCs; Testis

INTRODUCTION

Adult spermatozoa production is depended on single cell self- renewal and differentiation, located in the basment membrane of seminiferous tubules called spermatogonial stem cell (SSC) (He et al., 2015; Kaavya et al., 2016; Olive et al., 2015; Ryu et al., 2004; Schulz & Miura, 2002; R. Zhang et al., 2016; Zhao et al., 2016; Zheng et al., 2014). SSCs transplantation in rodents by Brinster & Zimmermannt (1994) opened a new insight for germ cells technology application (Schlatt, 2002). One, such as transgenic progeny production, has already been achieved through SSCs genetic modification (Shinohara et al., 2004; Seandel et al., 2007). For that reason, SSCs could be a great genetic resource. However, compared to other testicular cells in testes, the number of SSCs is remarkably lower; 2 in 1000 testicular cells are SSCs (Khaira et al., 2005), comprise only 0.3% of germ cells (Aponte et al., 2005). Nevertheless, transplantation requires large quantity of cells; therefore, prior to transplantation, *in-vitro* culture is an important practice. *In- vitro* progeny production techniques have mainly been developed for mouse SSSs (He et al., 2015). Recent studies have shown, *in-vitro* SSCs could propagate and maintain pluripotent (Dobrinski and Travis, 2007; He et al., 2015; McLean et al., 2001; Yamada et al., 2016; Zhao et al., 2016). Despite, the methods has been developed for bovine SSCs, none of them have been recommended it for long-term culture. (Fujihara et al., 2011). Cultured bovine germ cells, gonocyte could proliferate until 1.5 months. However, SSCs were able to make colonies for a week. In addition, Aponte (2008) proposed a culture technique based on growth

factors. The results presented somatic cells, required for germ cells proliferation. Furthermore, functional proliferation of *in-vitro* SSCs transplanted into infertile testes have been reported in rodents (Shinohara et al., 2003) and porcine (Kim et al., 2008). In addition, cultured SSCs from neonatal mouse (Shinohara et al. 2004), adult mouse (Seandel et al., 2007) and humans (Conrad et al., 2008) have given rise to embryonic stem cell-like cells. An important essay of *in - vitro* SSCs detection is expression SSCs specific markers. One such is DBA used to classify and identify spermatogonia from neonatal calves testes (Herrid et al., 2007; Izadyar et al., 2002) at differentiation point of development (Manku & Culty, 2015). Moreover, the binding of UCHL1 (Ubiquitin carboxyl- terminal hydrolase deubiquiting enzyme) (Vansandt, 2014), known as protein gene product 9.5 (Zheng et al., 2014) expresses in cytoplasm of SSC is also a general marker for type A spermatogonia in bull (Reding et al., 2010). Sox2 and Oct4, which are critical factors for maintaining the pluripotency of stem cells and their synergetic action controls another pluripotency factor Nanog (Shi & Jin, 2010). In any case, in spite of a few endeavors to culture male germ cells in animals species counting cattle (Izadyar et al., 2003; Oatley et al., 2004; Aponte et al., 2006) have not been successful and recommended for long term culture.

We aimed to build a practical system to generate progeny of SSCs by investigating expression of stem cell markers in- vitro cultured SSCs. To this end, we examined expression of different markers specific for stem cells, further we cultured SSCs from pre-pubertal testis. SSCs ability of proliferation after culture was evaluated by expression of markers.

MATERIALS AND METHODS

Testes from 3, 5 and 7 months old calves were collected in Agriculture and Forestry Research Center at University of Tsukuba and National Institute of Livestock and Grassland Science, all the animal work was performed under the approval of Institutional Animal Care and use Committee of the University of Tsukuba (approval no. 18-397). Immediately, after castration, the testicles transported to laboratory within 1 – 2 hour in phosphate buffer saline (PBS) containing 50 iu/ml penicillin – streptomycin (Gibco USA, 1864865), on ice. Approximately 20 g of the testicular tissue used for each cell isolation and culture process.

Isolation, purification and culture of SSCs

Single SSC was isolated and purified, using two-step isolation and discontinuous Percoll density layers purification as described by (Izadyar et al. 2002) with minor modification. In brief, after de capsulation, testicular tissues were minced in DMEMF12 containing 10 % FBS (Biowest, S15064s1870), 50 iu/ml penicillin – streptomycin (Gibco USA, 1864865), 40 iu/ml gentamycin (Sigma aldrich, 125174778V), and 1.5 mg/ml collagenase IV, 2 mg/ ml hyaluronidase type II, 1.5 mg/ml trypsin and 30 µlg/ml DNase I (Worthington, X6A16385) enzymes. Then the suspension containing testicular sample digested at 37 C^{o} in shaking water bath, 122 cycle/ min for 60 minutes. After removal of interstitial cells by centrifugation at 80xg for 5 minutes, the testicular fragments suspended in DMEMF12 as mentioned and enzymatic digestion was performed as above for 45 minutes. Germ cells separated from seminiferous tubules fragments with centrifugation at 30Xg for 2 minutes and filtration with 70-µlm nylon cell strainer. To pellet germ cells centrifugation at 1500xg for 5 minutes was performed. Further purification was performed with discontinuous Percoll density layers of 20%, 30%, 40%, 50% and 60%. Isolated cells purity was assessed by immunocytochemistry for SSCs markers. Cells were stained with DBA-biotin (1:200 µll) diluted in PBS for 15 minutes. Positive cells in 100 cells were counted. 1 x 10^{5} cells/ ml

were seeded in DMEMF12 supplemented with 10% FBS (v/v), 10 ng/ml EGF (Discovery Labware, Inc, 354001), 40 iu/ml gentamicin and 50 iu/ml penicillin – streptomycin and the medium was changed every two days.

Immunohistochemistry of cultured spermatogonial stem cells

After 5, 10 and 15 days in culture, cells were immunohistochemically analyzed for DBA, Oct4, UCHL1, and Sox2 described by Fujihara et al. (2011) with some modification. Briefly, after two washes with PBS, cells were fixed in 4 % parafarmaldehyde for 30 minutes at room temperature, then washed with PBS, non– specific binding block was performed using 10% goat serum (v/v made in PBS). Then, the cells were rinsed in PBS and stained with primary antibodies against DBA biotin (Abgent, 1:200 µl), UCHL1 (Abgent, 1:300 µl), Oct4 (Cell Signaling Technology 1:400 µl) and Sox2 (Cell Signaling Technology, 1:200 µl) (v/v in PBS) kept at 4 C° overnight. After washing twice with PBS, cells incubated with corresponding secondary antibodies Neutra Avidin Fluorescence FITC – conjugated (1:500 µl), goat anti mouse IgG (173667, 1:300 µl and texas red anti mouse IgG H+L (vector, made in horse 1:300 µl) for 90 minutes at room temperature. Analyzed under fluorescence microscope (Leica Microsystem, Germany).

Expression of spermagonia specific markers in cultured SSCs

To examine expression spermagonia specific marker UCHL1 and stemness factors Sox2, Oct4 in cultured SSCs, total RNA was extracted from the cells cultured using TRI Reagent Sigma (T9424). 800 µl of the reagent was added to cells, homogenized with disposable homogenizer (Nippi BioMasher Series Linup 891300). After being centrifuged at 2000 xg for 10 minutes at 4 C^O, DNA was dissociated by incubating the supernatant at room temperature for 5 minutes and biochemical phase separation was performed. To isolate RNA, Chloroform (0.1 x TRI reagent volume) was added, mixed with vigorous shaking, and then incubated for 5 minutes at room temperature. The mixture was centrifuged at 12000 x g for 15 minutes and the top part containing RNA was taken and mixed with iso – propanol (50% of TRI volume). After incubation for 5 minutes at room temperature, the cocktail centrifuged at 12000 x g for 10 minutes. Samples were washed twice with freshly prepared 75% ethanol (7500 x g centrifugation for 5 minutes at each wash), and pelleted RNA was placed under a fume hood until it dried completely. Then, the extracted RNA diluted with DNase and RNase free water (Millipore, F1AH74109) and RNAs amount was measured with Nanodrop lite spectrophotometer (Thermo Scientific). To amplify DNA, the 100 ng of extracted RNAs (14 µll) were mixed with 1 µll dNTP Mixture (TaKaRa) and 1 µll Oligo dt20 primer (Sigma). For RT-PCR, samples incubated for 5 minutes at 65 C^O and kept on ice. For reverse transcription, 4 µll 10X RT buffer (TaKaRa), 1 µll H2O (Millipore, F1AH74109) and 1 µll MMLV high performance reverse transcriptase (NIPPON GENE) were added and were incubated at 42 C^O for 50 minutes then 70 C^O for 15 min. RT - PCR amplification was carried out on 1 µll of the above cDNA solution per 19 µll PCR reaction mixture containing LA Taq HS, 10 × LA PCR Buffer II (Mg 2+ plus), dNTP Mixture (TaKaRa Hot Start version RR006A kit, TaKaRa BIOTECHNOLOGY) and H2O. They were mixed well and centrifuged briefly prior to PCR.

The following primers used for amplification of specific genes: Source: (http://bioinfo.ut.ee/ primer3-0.4.0/).

Table 1. Primers for amplification of germ cell marker genes

Genes	Forward	Reverse (5’ -3’)
UCHL1	GTCCCGGTCAAGAAACAAAA	TCTGGAACCAGGTCTTCACC
Oct4	GTTTTGAGGCTTTGCAGCTC	CTCCAGGTTGCCTCTCACTC
Sox2	GTTTGCAAAAGGGGGAAAGT	GAGGCAAACTGGAATCAGGA

The PCR product separated and visualized on 2% agarose gel electrophoresis containing 10 µll GelRed (Nucleic Acid Gel Stain, biotium.com). All PCR products were sequenced to confirm identity.

Statistical Analysis

The results are presented as mean ± SEM and statistical analysis was performed with single factor one-way ANOVA followed by Tukey HSD test for differences between means. Differences were considered significant when the P value was 0.05.

RESULTS

Cells isolation and purity

The antibody used in this study reported to identify spermatogonia at isolation time in many species including cattle. Isolated spermatogonial stem cell purity was assessed by comparing number of positive cells in total 100 isolated cells obtained from each discontinuous Percoll density gradient layer. Throughout different age of calves, spermatogonia enriched in fraction 40% Percoll density gradient was significantly higher than in others **(Fig. 1).** Interestingly, the average number of positive cells recovered in 40% Percoll gradient from 3 month was, 37.3 ± 3.7 from 5 months old 31.5 ± 0.8 similar to 7 months 32± 0.8. These positive cells manifested a typical morphology of SSCs: round shape, dense nucleus and with high nuclear cytoplasmic ratio.

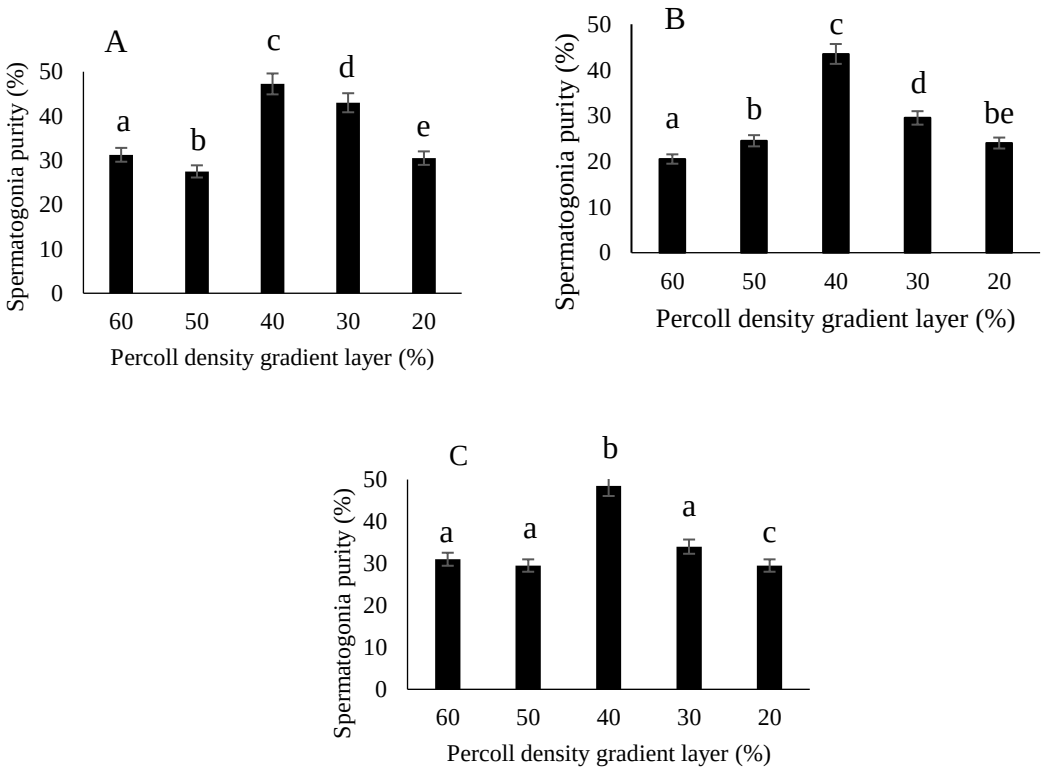

Fig. 1. Purity of isolated bovine spermatogonia at various stage of age. The result represent pooled data from 9 different experiments and the data is presented as mean ± SEM ($^{a-e}P<0.05$). (A-C) DBA positive cells/ 100 cells were counted under fluorescence microscope (Leica Microsystem, Germany). A, B, C, cells purified from 3, 5, and 7 months old calves respectively.

Colony formation and immunohistochemistry of cultured SSCs

All cultures begun with mixed population of spermatogonial stem cells and somatic cells from testicular tissue. On average 45% of cultivated cells were pure spermagonia. Colony like structures were formed in culture plates.

The colony forming cells approached each other and became packed as the colonies continued to grow. Between days 5 and 10 of culture, the colonies grew almost doubled in number; with further growth between days 10 and 15 of culture. As the development of colonies progress, the cells remained attached to the plate surface, constructing monolayer.

To characterize those colonies and cells, they were immunocytochemically analyzed after 5, 10 and 15 days of culture, showing that they were DBA, UCHL1, Sox2 and Oct4 were also positive **(Fig. 2A).** The number of colonies was almost doubled from 5 (110 ± 0.57) to 10 days (192 ± 1.43) and then further increased by 15 days (210.5 ± 1.21) **(Fig. 2B).** At 10 days of culture, the colonies had spherical shape, however, changed to long shape at 15 days of culture.

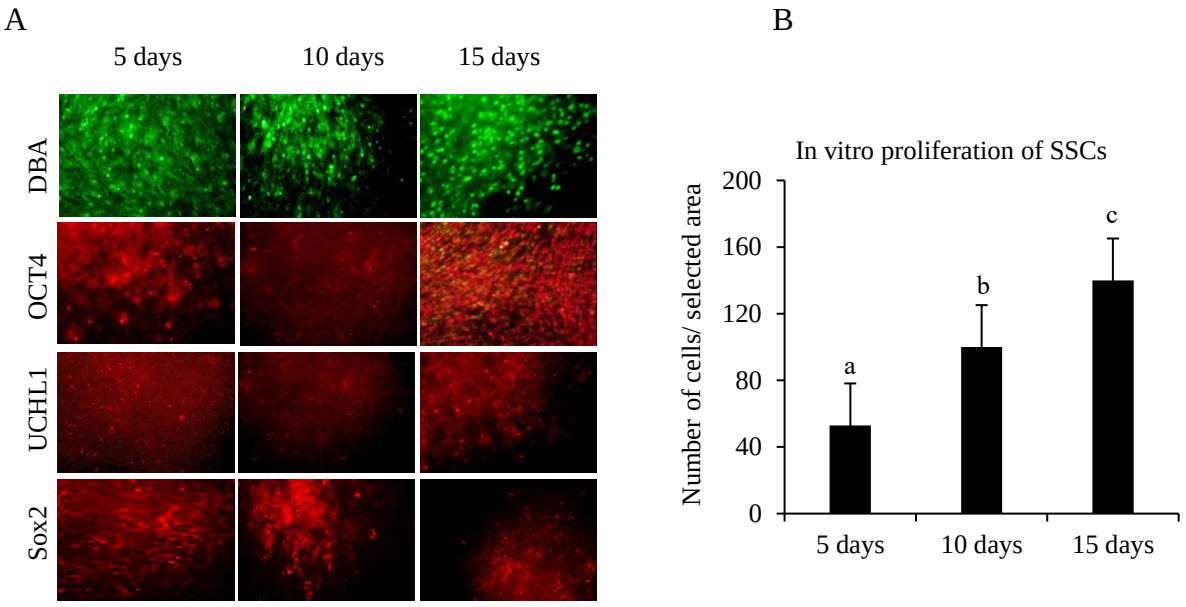

Fig. 2. *In vitro* culture of spermatogonial stem cells from pre- pubertal bovine testis. SSCs cultured, after 5, 10 and 15 day in culture the cells were stained with DBA, OCT4, UCHL1 and Sox2. Expression of SSCs markers maintained by 15 days post *in - vitro* culture. Images are representative of three independent experiments. (B) Number of DBA positive cells counted. 10 and 15 days post- in vitro showed significant increase of number of SSCs compared to 5 days in vitro. (A) Data are presented as mean ± SEM (n=9). (a-c $P<0.05$).

Expression of spermatogonia specific markers in cultured SSCs

In order to verify the stemness prosperities of the proliferating purified spermatogonial stem cells, the mRNA expression level for UCHL1, Oct4, and Sox2 quantified at day 15th of in- vitro culture, using RT – PCR. The analysis demonstrated that, spermatogonial stem cells specific marker UCHL1 and pluripotency factors Oct4 and Sox2 expressed around 300 bps **(Fig.3).**

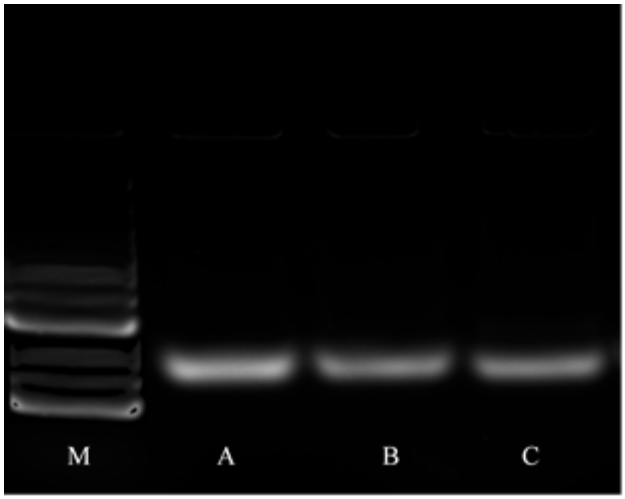

Fig. 3. Expression of transcription based markers in *in-vitro* spermatogonial stem cells from pre-pubertal calves. Total RNA quantified and amplified for UCHL1 (A), Oct4 (B) and Sox2 (C) expression. UCHL1 (A), Oct4 (B) and Sox2 (C) expressed at the predicted 300 base pairs. Images are representative of three independent experiments.

DISCUSSION

Isolation and purity of spermatogonial stem cells

The estimated number of SSCs is very low in testis, has limited efficient isolation of these cells for research activities. Based on cells characteristics, different isolation and purification methods have been used (R. Zhang et al., 2016). Despite the prevalence of isolation and purification techniques, viability of the cells during isolation is another concern that affects *in-vitro* function of SSCs. We used two -step digestion method for cell isolation followed by discontinuous Percoll density gradient layers enrichment. In our study, highly enriched population of spermatogonia to a final purity of 47 %, 43 % and 48 % could be isolated routinely from the pre- pubertal calves at the age of 3, 5 and 7 months old respectively (Fig. 1A, 1B, 1C). In terms of the most pure SSCs, 40 % of discontinuous Percoll density layer would be the most appropriate density to enrich SSCs. Combining two step enzymatic digestion, density separation and differential plating (Izadyar et al., 2002) achieved type A spermatogonia enrichment up to 67% relative to other cells. In addition to this, after first discontinuous Percoll density layers treatment (Fujihara et al., 2011) could enrich 2 – 11% of total cells, however, after second treatment the purity was as much higher as 40% with 75 % viability. Despite lack in differential plating and two times discontinuous Percoll density layers, we could obtain high number of spermatogonia. In that case, our results are comparable. Two step enzymatic digestion was established for the first time over 30 years ago (Bellvé et al., 1977), and is widely used for isolating SSCs of different species (Ahmad et al., 2013; Heidari et al., 2012; Izadyar et al., 2002; Kaavya et al., 2016; Liu et al., 2011; Rodriguez-Sosa et al., 2006; Shafiei et al., 2013; Zhao et al., 2016). Therefore, these techniques could be used to obtain undifferentiated spermatogonia from different species and will help further use of enriched cells.

SSCs culture techniques are not valid for large animal and yet to be successful for long- term. In present study, we successfully propagated pre-pubertal bovine SSCs in culture on stem cell specific medium and tested proliferation ability of SSCs, we found that, after 5 days in culture, SSCs were able to cover large portion of culture wells and make round shape colonies. The colonies grew further from 5 to 10 and 15 days in culture (Fig.2B). The number of colonies increased 100 folds from day 5 to day 10 with further increase until day 15.

However, the growth from day 10 to day 15 was 50 folds. For the first time, Izadyar et al. (2003) developed method for bovine SSCs long –term culture. Their results showed presence of SSCs in culture. However, at some point SSCs were not committed to differentiate. Additionally, SSCs cultured in medium containing different levels of growth factors (Aponte, 2008) and co-cultured with Sertoli cells in the presence of colony stimulating factor-1 (Shafiei et al., 2013), results in proliferation of SSCs. However, our results are not in arrangement. The fact, that the shape of colonies at 15 days of culture changed from spherical to elongated shape. Apparently, the growth factor and other components of the medium induce the growth and formation of organized structures *in- vitro.* Generally, bovine SSCs are in single form, however, it has been suggested that bull SSCs in and chain also have stem cells capability (Worbel, 2000). The round shape colonies do not seems to originate from aggregation of cultured cells but likely self- generate during early proliferation. As Sertoli cells are in spermatogonia suspension in range of 20 – 50 % (Izadyar et al., 2003), the contamination contribute in forming colonies. Immunohistochemistry of cultured SSCs shown the presence of somatic cells in colony (Aponte, 2008). Sertoli cells produce various elements required for maintaining proliferation capacity in SSCs (Aponte et al., 2008). Generally, the colonies happen to be round in shape. However, the use of growth factor EGF induced difference in morphology of the colonies. Due to the effect of EGF, colonies get together and make elongated form (Wahab et al., 2003).

Further, to investigate *in- vitro* stem cells characteristics of bovine SSCs, we examined expression of SSCs marker UCHL1 and self-renewal inducing factors Sox2 and Oct4 at both immunohistochemically and transcription levels. Interestingly, it was found that, the colonies were consistently positive for the spermatogonia specific markers until 15 day *in –vitro.* DBA, UCHL1, Sox2 and Oct4 (Fig. 2A) and expression of SSCs specific marker UCHL1 and self- renewal factors Oct4, Sox2 was maintained at 15th day post culture (Fig.3). DBA a germ cells specific marker have been used for spermatogonia classification and identification (Herrid et al., 2007; Izadyar et al., 2002). UCHL1 is a general marker for bull spermatogonia, bounding to the cytoplasm of SSCs (Fujihara et al., 2011; Reding et al., 2010) and other species (Goel et al., 2010; Vansandt, 2014; Zhao et al., 2016). Additionally, UCHL1 is present in SSCs that undergo asymmetrical cell division (Lou et al., 2009). Oct4 is transcription factor that is necessary for stemness (Pan et al., 2002) and its expression is observed in spermatogonia at different stages of bovine germ cells (Fujihara et al., 2011). However, in pig it is shown specific to differentiated type germ cells (Goel et al., 2008) and is present in mouse gonocyte (Shinohara et al., 2005). OCT4 and Sox2 members of pluripotency regulating protein network (Manku & Culty, 2015), promote stemness of stem cells and are crucial for maintaining proliferation capability (Deluz et al., 2016; H et al., 2014; Hagey et al., 2018; Oatley & Brinster, 2008; Zhang & Cui, 2014). Synergistically, Sox2 and Oct4 control Nanog activities (Yamaguchi et al., 2005) required for inducing self – renewal in stem cells. In mammals, these factors reported to be present in undifferentiated germ cells (Kim et al., 2014; Phillips et al., 2010). Since Sox2 and Oct4 are factors required for stemness and UCHL1 is spermatogonial stem cells specific marker, mRNA expression in cultured SSCs corroborated that SSCs retained stemness.

CONCLUSION

To conclude, spermatogonial stem cells with high survival rate could be enriched from pre- pubertal bovine testes, using two-step enzymatic digestion followed by Percoll ingredient layers, and isolated cells long term *in –vitro* cultured is possible until 15 days in culture. Further studies will investigate initiation of spermatogenesis in homologous recipient from cultured spermatogonial stem cells.

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Conflict of interest

All authors expresses no conflict of interest in any part of research, manuscript and submission to the journal.

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Comparison of Simple Ligation versus stump Invagination in open Appendectomy

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ABSTRACT

Background: Acute appendicitis (AA) is one of the most prevalent abdominal crises and faced by the surgeon in practice. The best treatment for the appendicular stump during open appendectomy is unknown. This is a randomized controlled trial comparing simple ligation with appendicular stump invagination.

Materials and Methods: The MASS (Modified Alvarado Score System) test was used to confirm the diagnosis of acute appendicitis. When necessary, an ECG & a chest X-ray were taken. All of the patients were put under spinal anesthesia for the procedure.

Findings: The appendicular stump of 305 patients were treated with simple ligation (161 patients) in group I, and ligation and invagination (144 patients) in group II. “Pyrexia, vomiting, serous discharge, wound infection, peritonitis, residual abscess, and post-operative pain in the right iliac fossa are all comparable after surgery. Paralytic ileus occurred in 2 (1.24%) of patients in groups I and 7(4, 36%) patients in group II, respectively, and was statistically significant.

Conclusion: Easy ligation of the appendicular stump after open appendectomy is advised since it is safe, simple, and takes less time.

Keyword: Acute appendicitis; Appendectomy; Appendicular stump; Simple ligation; Invagination

INTRODUCTION

In and around the Kathmandu valley, acute appendicitis (AA) is one of the most prevalent abdominal crises. Males have a 0.15 percent incidence of AA, while females have a 0.19 percent incidence, with a lifetime risk of 6-20 percent (Khan, 2010; SHAHID & IBRAHIM, 2004). Clinically, AA has been known as 'peri typhlitis' (associated with severe cecal inflammation) since the 16th century, but the first successful appendectomy was documented in 1736. Reginald Fitz initially documented the function of surgical excision of an inflamed appendix as a curative therapy in 1886 (Engström & Fenyö, 1985; Khan, 2010). In 1889, three years later, Mc Burney stressed the significance of early appendectomy. After five years, he developed his well-known muscle-splitting incision, which carries his name to this day. Appendicitis has been the most prevalent surgical emergency since

then. From skin incision through ligation and invagination of the appendectomy stump, the technique of appendectomy varies from surgeon to surgeon or from center to center. The best way to handle appendectomy stump has been debated for almost a century, and Ochsner and Lilly published a thorough historical assessment in 1937 (Engström & Fenyö, 1985). Following the ligation or Tran's fixation of the arteries, to help in diagnosis, several clinical and laboratory-based scoring systems have been developed. The Alvarado (MANTRELS) score is the most popular." 9 A score of 7 or above indicates a high likelihood of acute appendicitis. Abdominal ultrasonography or contrast-enhanced CT scan decreases the probability of negative appendectomy even more in individuals with an ambiguous score (Körner et al., 1997; Schein, 2010). The most frequent acute surgical disease of the abdomen is acute appendicitis." As a result, appendectomy is one of the most popular general surgical procedures (Cooperman, 1983). The popularity of conducting appendectomy through a laparoscopic method has grown due to the development of laparoscopic appendectomy (LA) and meta-analyses revealing lower post-operative discomfort and a shorter hospital stay following LA (Tate et al., 1993). However, open appendicitis is still a common surgery, with 34% of appendicitis patients in the UK undergoing it. In circumstances where laparoscopy is not an option and conversion is required due to technical challenges in removing the appendix safely, an open approach may be required. Total or sub-total appendectomy are two types of open appendectomy (Delaria, 1987). Appendicitis is the most common abdominal surgical emergency in developed countries, affecting approximately 6-10 percent of the general population and most commonly occurring in the second decade of life (Boswell, 1999), by maturity, one out of every six people will have had their appendix removed (Tate et al., 1993). It can strike anyone at any age, although it is most frequent in those between the ages of 20 and 40 years (Chhetri & Shrestha, 2005) Appendicitis affects about 8% of persons in Western nations at some point in their lives, with a peak occurrence between the ages of 10 and 30 (Schein, 2010). "Appendectomy has a 12-percent lifetime risk for males and a 25-percent lifetime risk for women, making it the most regularly done procedure in the world (Cooperman, 1983; Schein, 2010). Appendectomy for acute appendicitis is performed on about 7% of the population. Appendectomy, which can be done open or laparoscopically, is still the usual therapy for acute appendicitis (Scott-Conner et al., 1992). The appendix can be anywhere from 2 and 20 cm long, with an average length of 9 cm in adults (Delaria, 1987). The pathophysiology of acute appendicitis has long been thought to be the result of luminal obstruction caused by a fecality, hyperplastic lymphoid tissue, parasitic infestation, or tumor, with subsequent localized venous ischemia leading to mucosal disruption and invasive bacterial infection; viral ulceration may also be the cause of mucosal ulceration in some patients. Localized inflammation and uncomplicated, or supportive, appendicitis developed from infection restricted to the appendix. Appendectomy is one of the most often performed surgical procedures. The total prevalence of acute appendicitis is estimated to be approximately 14% (Khan, 2010). Although the introduction of laparoscopic surgery is a significant advancement in the area of surgery, open appendectomy is still a prevalent procedure (Neves et al., 2011). When the diagnosis of acute appendicitis is in dispute, most tertiary institutions in Pakistan and industrialized nations prescribe diagnostic laparoscopy. Surgical techniques may differ depending on the surgeon and the desire of the center (Neves et al., 2011). Simple wound infection to abscess development (superficial or deep), paralytic ileus, and other Colo-cutaneous fistula is a distinct postoperative complication, following appendectomy that causes intestinal blockage and is an uncommon consequence of stump appendicitis. As a result of these complications, the length of time spent in the hospital increased (IA & AA, 2005; Neves et al., 2011). Stump burial following appendectomy was once a common operation performed by surgeons, but it has recently been shown that this

surgery is linked to a rare complication known as stump appendicitis, which is difficult to diagnose and treat (Neves et al., 2011). Furthermore, invagination of the stump causes a bulk appearance during contrast investigations, causing diagnostic issues. (IA & AA, 2005). Another complication of stump closure is the creation of a fistula due to the passage of the needle into the gut lumen (Khan, 2010; Rafi et al., 2006). Many studies have found that appendicular stump invagination has a higher risk of problems than simple ligation. However, stump invagination is often essential when the base of the appendix is damaged (Asif Zaman & Muhammad, 2011).

MATERIALS AND METHODS

This was a prospective randomized study. It was conducted in the surgical ward of Nangarhar" University Hospital from December 29, 2019 till December 20, 2020. 303 patients with appendicitis were underwent surgery. The study was approved by the research committee; the research was conducted as a descriptive observation. Two groups of patients were formed. Operations performed on odd days of the week (Mondays, Wednesdays, Fridays, and Sundays) were assigned to Group I, which included simple appendicular stump closure, while operations were performed on even days of the week (Tuesdays, Thursdays, and Saturdays). They were placed in group II, which included a simple closure with a burial. The patient's criteria for admission were diagnosis of MASS, acute appendicitis, patient consent to participate in the trial, and the patient's suitability for spinal anesthesia. Appendicular perforation, appendicular abscess, appendicular mass, cecal edema, unwanted appendectomy with other abdominal diseases, and appendectomy with interval were all considered exclusion criteria. MASS test was used to confirm the diagnosis of acute appendicitis (modified Alvarado scoring system) **(Table. 1).** If necessary, ECG and chest radiograph were performed too. All patients were given spinal anesthesia during the operation. In group A, the appendicular stump was simply closed with 2-0 vicryl after opening the peritoneal cavity with a Mc Burney incision, while in group B, the appendicular stump was closed sero-muscular sutures with 2-0 vicryl on a 1 cm atraumatic needle. Around the base of the appendix," after simple ligation, it was invaginated into the cecum. Depending on the patient's weight, each patient received three doses of antibiotics, ceftriaxone IV and metronidazole IV. "The first dose was given before surgery after confirmation of diagnosis. The second dose was given during surgery and the third dose was given 12 hours after surgery. Ketorolac injection was provided if needed to manage postoperative pain. Oral fluids began when the patient passed bloating or intestinal sounds were heard. After 48 hours, it was checked whether the dressing was completed. On the seventh day after the operation, the stitches were removed. After discharge, patients were followed up for 3 to 7 days after surgery. The surgical site was evaluated for any wound complications such as wound infection, dilation and most severe discomfort, duration of ileus and length of hospital stay.

Table 1: An Alvarado scoring system

N0	Diagnostic Criteria	S/S	Point Value
1	Symptoms	Migration pain	1
		Anorexia	1
		Nausea/emesis	1
2	Sign	RUQ tenderness to palpation	2
		Rebound tenderness	1
		Pyrexia 37.3C	1
3	Laboratory values	Leukocytosis	2
		Left shift	1

RESULTS

In this research, 340 individuals with an appendicitis diagnosis (MASS score 5-9) were operated. The trial was terminated when 35 patients were found to be ineligible. 16 patients had cecal oedema, 4 suffered perforations, and 15 were lost to be follow-up. The research comprised a total of 105 patients. The ages of the participants varied from 15 to 65 years old. Patients in group I were 28.83 years old, whereas those in group II were 27.54 years old.

Table 2: Patients' statistics

N0	Total	Patients group I (161)	Patients group II(144)
1	Male	84(52.17%)	77(53.47%)
2	Female	77(47.82%)	67(46.52%)
3	Mean age (year)	38.83 year	37.54) year
4	average length of sickness	2.7 days	2.6 days
5	Time spent operating average	40.6 mint	43.5mint
6	Average stay in the hospital	8.5days	8.5days

Vomiting was reported by 46 (28.57%) of patients in group I and 36 (25.00%) of patients in group II. Fever was a symptom in 16 (9.93%) of group I patients and 11 (7.63%) of group II patients, respectively. All patients in both groups presented with pain in the right iliac fossa, followed by anorexia/ nausea in 98 (60.86%) of group I patients and 96(66.66%) of group II patients.

Table 3: shows the clinical signs and symptoms

No	Clinical features	Group -I (161) patients	Group – II(144)patients
1	Vomiting	46(28.57%)	36 (25.00%)
2	Anorexia/nausea	98(60.86%)	96(66.66%)
3	Fever	16(9.93%)	11(7.63%)
4	RIF pain	161(100%)	144(100%)
5	Blumberg sign	30(18.63%)	26(18.05%)

Table 4: Patient's presentation in the hospital after occurrence of symptoms.

N0	Time	Group -I(161)	Group -I (144)
1	1 -12 hours	6(3.72%)	7(4.16%)
2	13 – 24 hours	11(6.83%)	11(7.63%)
3	25 -36 hours	30(18.63%)	28(19.44%)
4	37 – 48 hours	40(24.84%)	34(23.61%)
5	>74 hours	71(44.09%)	61(42.36%)

Table 5: Duration of surgery

N0	Time	Group I	Group II	p. value
1	Minimum	23mints	25 mints	
2	Maximum	75 mints	72 mints	0.225
3	Mean operating time	38.40 mints	42.90mints	

Although group I mean operating time (38.40 minutes) was smaller than group II (42.90 minutes), the difference was not statistically significant.

Table 5: Complications following surgery.

No	Complication	Group -I	Group -II	p - value
1	Pyrexia	6(3.72%)	7(4.86%)	0.5968
2	Vomiting	8(4.96%)	10(6.94%)	0.4443
3	Paralytic ileus (24-48hor)	2(1.24%)	7(4.86%)	
	(48-72hour)	1(0.62%)	2(1.38%)	0.2
	>72 hour	0(0.00%)	0(0.00%)	
4	Peritonitis	0(0.00%)	0(0.00%)	-
5	Wound infection	5(3.10%)	5(3.47%)	0.4038
6	Serous discharge	6(3.72%)	7(4.86%)	0.9281
7	Residual abscess	0(0.00%)	0(0.00%)	-
8	OB due to adhesion	0(0.00%)	0(0.00%)	-
9	R I F pain Intestinal	3(1.86%)	3(2.08%)	0.4132

Patients in groups A and B experienced post-operative pyrexia in 6 (3.72%) and 7 (4.86%) cases, wound infection in 5 (3.10%) and 5 (4.86%) cases, and serous discharge in 6(3.86%) and 7(4.86%) cases, respectively. Which were not significantly difference between the two groups.

DISCUSSION

"Invagination of appendicular stump during appendectomy has traditionally been practiced by many surgeons in many centers despite lack of evidence from randomized clinical trials to justify its benefit (Lavonius et al., 1996). In this study we can compare the results of our study with the fact that there was a slight difference between the group I and the group II., which was as follows: vomiting was reported by 46 (28.57%) of patients in group I and 36 (25.00%) of patients in group II. Fever was a symptom in 16 (9.93%) of group I patients and 11 (7.63%) of group II patients, respectively. All patients in both groups presented pain in the right iliac fossa, followed by anorexia and nausea in 98 (60.86%) of group I patients and 96 (66.66%) of group II. Patients in groups I and group II, experienced post-operative pyrexia in 6 (3.72%) and 7 (4.86%) cases; wound infection in 5 (3.10%) and 5 (4.86%) cases; and serous discharge in 6 (3.86%) and 7 (4.86%) cases, respectively. We compared the results of our study with the result of the prospective randomized study which was conducted by Suvera et al. (2013).

The study showed mean operating time in minute was less in Group-II compare to Group-I. Mean length of Hospital stay also less in Group II patients. Postoperative wound infection was noticed in 3 (2.7%) patients in Group-I and 2 (1.8%) in Group-II. The difference between the two groups was not statistically significant. ($P > 0.05$). The rate of postoperative ileus was more in Group-I, 6 and 1 during first 48 hours and 72 hours respectively as compared to Group-II, which is significantly higher in Group-I. None of the patients had paralytic ileus for more than 72 hours in both the groups. No case of postoperative peritonitis, residual abdominal abscess and intestinal obstruction due to adhesions was noticed in both groups during the postoperative period According to Neves LJ et al. (2011). ligation and injection operations took 5.5 minutes longer on average than basic ligation operations. The group without intussusception had a much shorter mean operating time. Furthermore, Neves LJ et al. (2011) found that wound infection, which occurred in 9.7% of patients and there was no statistically significant difference in wound infection rates between the two groups which is consistent with our finding. In agreement with other randomized clinical studies. "Our study showed no advantages of invagination of the appendix stump over simple ligation (Dass et al., 1989; Lavonius et al., 1996). The present study showed no statistically significant differences in the rate of postoperative complications and postoperative hospital stay between the two groups which is in consistent with other trials (Engström & Fenyö, 1985; Lavonius et al., 1996).

In addition, "although, the mean operating time was not significantly shorter in the group without invagination, a finding consistent with that reported by others (Engström & Fenyö, 1985; MS & RU, 2006). Like in other studies no case of postoperative peritonitis, residual abscess and intestinal obstruction due to adhesions was noticed in both groups during the postoperative period and follow up."

CONCLUSION

The findings of this study suggest that simple appendicular stump closure is a safe and straightforward surgery with little downtime and postoperative complications. It does not create any intestinal wall distortion, which might subsequently be misinterpreted as an abdominal tumor. As a consequence, simple closure as a natural therapy for appendectomy is advised

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Conflict of interest

The authors declare no conflict of interest

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Norfloxacin-Repeated Dose Toxicity on Liver of Broiler Chickens

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ABSTRACT

Background: Norfloxacin is a second generation fluoroquinolone, primarily used in the treatment of urinary tract infections. However, later clinical studies have documented the efficacy of norfloxacin for a variety of Gram-negative infections including pneumonia, CNS/CSF infections, prostatitis and septicemia. The present study was conducted to investigate the toxic effect of norfloxacin during 28 day repeated dose toxicity on the liver tissue of broiler chickens.

Materials and Methods: The experimental birds acclimatized to laboratory housing condition were assigned as vehicle, control, treatment, and satellite groups (each group contained 6 birds). Stellate group, without administering Norfloxacin solution with partial control group was maintained for extra two weeks after the 28-day period.

Findings: Gross lesions in the liver of group IV and V observed were fatty change, enlargement, and increase of size and petechial hemorrhagic. Hepatocytes were swollen with mild vacuolar changes with granular cytoplasm. Focal areas of hepatocytes with degeneration infiltration of mononuclear cells, dilation of sinusoid, perivascular infiltration of inflammatory cells, bile duct epithelial hyperplasia and perivascular infiltration were noticed in the present study. The organ/body weight ratio percentages of liver for groups I, II, III, IV and V on 29th day were 2.81, 3.53, 3.54, 3.98 and 5.03 respectively. There was significant ($P<0.001$) increase in organ/body weight percentage ratio of group V as compared to the control group. On day 21 of treatment the serum ALT concentration was significantly increase in group V ($P < 0.001$) when compared to group I birds. On day 28 of treatment the mean serum ALT concentration was significantly ($P < 0.01$) higher in group V birds compared to group I birds. On 21st day of treatment the serum AST level of group V was significantly increased ($P < 0.001$) when compared to group I birds. On 28th day of treatment the mean serum AST was significantly high in-group V ($P < 0.001$) birds when compared to group I birds. These findings were accordance with finding of other authors.

Conclusion: According to gross pathology, histopathology and biochemical findings in the present study it was concluded that the norfloxacin has toxic effect on the liver tissue of chickens at the dose of 333 and 1100 mg/kg orally.

Keywords: Norfloxacin, Sub-acute toxicity; liver degeneration; Broiler chicken; liver; AST, ALT.

INTRODUCTION

Norfloxacin is a second generation fluoroquinolone, primarily used in the treatment of urinary tract infections. However, later clinical studies have documented the efficacy of norfloxacin for a variety of Gram-negative infections including pneumonia, CNS/CSF infections, prostatitis and septicemia (Mascellino et al., 1986). Norfloxacin usage in the treatment of gastrointestinal infections caused by Esherichia coli, Salmonella spp., Shigella spp. and Campylobacter spp. are also reported (Braunwald et al., 1987), which are the common disease causes of poultry.

Fluoroquinolones, including trovafloxacin, ciprofloxacin, ofloxacin, enoxacin, norfloxacin, and, gatifloxacin, have been associated with hepatotoxicity causes hepatocellular necrosis, which results in elevated ALT and AST concentrations (Coleman et al., 2002). Elevation in levels of hepatic enzymes occurred in 1.8%-2.5% of the patients florquinolones in clinical doses and these effects were reversible after the withdrawal of drug (Halkin, 1988). According to Hess (2016) SGPT being freely soluble in cytoplasm, was released from the hepatic cells even with very slight damage, which may not be detectable morphologically. Unlike SGPT, SGOT did not readily leak out from liver cells unless there was extensive injury to the liver. Gonzalez and Henwood (1989) observed elevated SGOT and SGPT concentration in patients undergoing treatment with pefloxacin. The most commonly reported abnormal laboratory finding in human patients treated with ciprofloxacin were increased glutamic pyruvic transaminase and glutamic oxaloacetic transaminase (Matsuno et al., 1995). Cornelius (1980) was the first to examine activity of glutamic oxaloacetic transaminase (SGOT) in fowls. The concentration of SGOT expressed was on the basis of the amount of pyruvate liberation per ml of plasma. On 28th day the amount of SGOT was 226.7 units in poultry. The fluoroquinolone antimicrobials norfloxacin and enrofloxacin were found to inhibit hepatic microsomal cytochrome P-450 monooxygenases in the livers of broiler chickens using dosages as given in commercial flocks (Shlosberg A, et al., 1997) impaired metabolism of a number of drugs has been associated with fatty liver. These findings suggest an association between increased lipid deposition and impaired CYP enzymes. (Gomez et al., 2009) Fluoroquinolones may accumulate, when they are repeatedly administered (Regmi et al., 2005). The present study was conducted to investigate the toxic effect of norfloxacin on the liver tissue of broiler chickens.

MATERIALS AND METHODS

Experimental birds acclimatized to laboratory housing condition were assigned as vehicle, control, and treatment groups, consisting of six groups of 6 birds in each group. Satellite group was given with high dose 1.1g /kg as high dose group and maintained along with the control group. Stellate group, without administering Norfloxacin solution with partial control group was maintained for extra two weeks after the 28-day period.

The norfloxacin powder was obtained from Trichem laboratories Bangalore. The norfloxacin, a yellowish white powder was not soluble in water. To make it soluble in water, an acetate buffer (Acetic acid 50 mmol/L and 50 mmol / L of sodium acetate with pH 4.5) was prepared. One g norfloxacin was first added to 0.25 ml of acetic acid and 2 ml of 50mmol/L acetate buffer maintained at pH 4.5 was added and mixed until the drug was completely dissolved. Thus prepared stock solution was used for further dilution.

The experiment was carried out under hygienic condition and standard management one-week-old broiler chicken procured from a reputed hatchery and divided into five groups each containing sex chickens.

The norfloxacin was administered at the doses of 111 mg/kg, 333 mg/kg and 1100 mg/kg orally for a period of 28 days based on LD_{50}= 549mg/kg.

Table 1. Details of group and doses administered in sub-acute liver toxicity.

Group	Dose
Group I (Control)	Distilled water
Group II (Low dose)	0.11 g/kg
Group III (Medium dose)	0.333 g/kg
Group IV (High dose)	1.1 g/kg
Group V (Vehicle)	Vehicle
Group VI (Satellite)	1.1 g/kg

The histopathological findings were based on histopathologic lesion in liver, sections in birds taken high doses and med doses of the drug. The satellite group shown recovery according to hematological, biochemical and histopathologic finding.

The liver for organ/body weight ratio percentage and tissues from liver of treated chickens were collected on day 29 for Histopathology. The blood samples were collected from jegulare vein on day 7, 14th, 21st and 28 for biochemical analysis (ALT, AST).

RESULTS

Gross lesions in the liver of group IV and V observed were fatty change, enlargement, and increase of size and petechial hemorrhagic.

Fig. 1. Sub-Acute toxicity (High dose) Gross pathology liver showing fatty degeneration (compared with low dose)

The organ/body weight ratio percentages of liver for groups I, II, III, IV and V on 29th day were 2.81, 3.53, 3.54, 3.98 and 5.03 respectively. There was significant ($P<0.001$) increase in organ/body weight percentage ratio of group V as compared to the control group. The other groups didn't show any significant increase in liver weight.

Table. 2. Table shows effects of norfloxacin (IP) on organ/body (%) weight in experimental broiler chickens in sub-acute toxicity study.

	Group I (control)	Group II (Vehicle)	Group III (0.11g/kg)	Group IV (0.333g/kg)	Group V (1.1g/kg)	Group VI (1. 1g/kg)
Liver/BW	0.028100	0.035300	0.035400	0.039800	0.050300	0.05470

In histopathological finding birds received high dose hepatocytes were swollen with mild vascular changes and granular cytoplasm. Focal area of hepatocytes degeneration was also seen with infiltration of leukocytes.

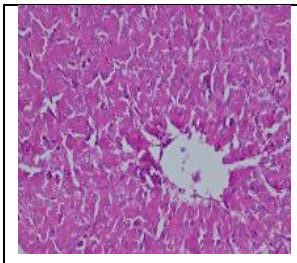

Plate 2. Hepatocytes were swollen with mild vascular changes in granular cytoplasm. Degeneration of focal area of hepatocytes -H&Ex500.

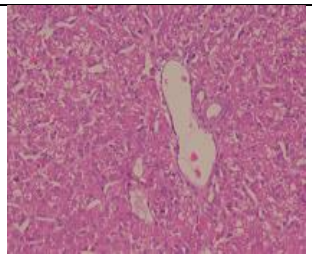

Plate 3. Infiltration of leukocytes, dilation of sinusoid, perivascular infiltration of inflammatory mononuclear cells -H&Ex500

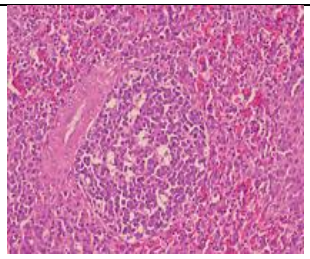

Plate 4. Bile duct epithelial hyperplasia and perivascular infiltration -H&Ex500

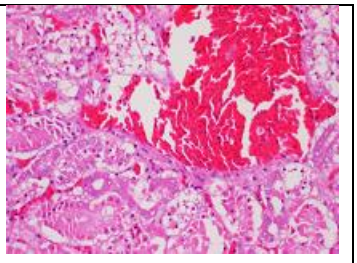

Plate 5. Congestion of vessels and dilatation of sinusoidal space. Perivascular hepatocytes degeneration with infiltration of few inflammatory cells.

In addition, dilation of sinusoids, perivascular infiltration of inflammatory cells and bile duct epithelial hyperplasia was noticed. (Plate. 1-3)

The same lesions in mild form were observed in liver tissue of broiler chicken received 333 mg/kg dose of norfloxacin but no lesions were observed in chickens received 111-mg/kg norfloxacin. Satellite group showed lesions: Congestion of vessels and dilatation of sinusoidal space. Perivascular hepatocytes degeneration with infiltration of few inflammatory cells (Plate 5). The histopathological lesions were simultaneously supported by biochemical findings. On day 21 of treatment, the serum ALT concentration of group I, II, III, IV and V were 9.23±1.78, 8.98±1.41, 11.81±1.44, 15.16±2.09 and 21.02±2.03 U/dl respectively. Significant increase in ALT concentration was observed in group V ($P < 0.001$) when compared to group I birds. On day 28 of treatment the mean serum ALT concentration of group I, II, III, IV and V were 9.66±3.08, 14.95±5.46, 22.91±5.84, 40.06±10.7 and 43.89±4.05 U/dl respectively. The serum ALT concentration was significantly ($P < 0.01$) higher in group V birds compared to group I birds.

Table 3. Effects of norfloxacin on ALT level of experimental broiler chickens

Groups	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I (control)	7.23±1.05	9.54±2.36	9.23±1.78	9.66±3.08	43.89±4.05	43.89±4.05
Group II (Vehicle)	8.40±1.84	8.98±3.35	8.98±1.41	14.95±5.46		
Group III (1.1g/kg)	8.41±1.44	14.76±2.55	11.81±1.44	22.91±5.84		
Group IV (0.333g/kg)	8.45±1.43	21.20±3.23	15.16±2.09	40.06±10.7		
Group v (1.1g/kg)	9.60±1.04	20.78±3.63	21.02±2.03***	43.89±4.05***	31.97±5.94**	29.86±3.88***

Values are Means ±SE

***P<0,001, **P<0, 01, *P<0, 05, the values on 35 and 42 pertain to satellite group.

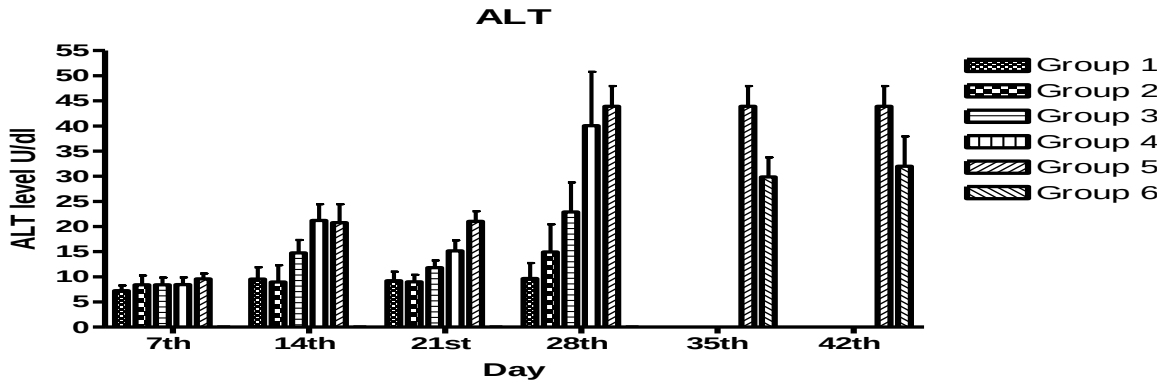

Fig. 1. Effects of norfloxacin on ALT level (U/dl) of experimental broiler chickens

On 21st day of treatment I, II, III, IV and V were 227.16±2.07, 234.83±8.20, 231.16±13.28, 281.50±19.04, 324.5±24.34 U/dl respectively. The serum AST level of group V was significantly increased ($P < 0.001$) when compared to group I birds. On 28th day of treatment the mean serum AST level of group I, II, III, IV and V were 224.83±10.08, 249.50±11.18, 246.33±7.20, 269.83±21.81, 330.83±14.70 U/dl respectively. It was significantly high in-group V ($P < 0.001$) birds when compared to group I birds.

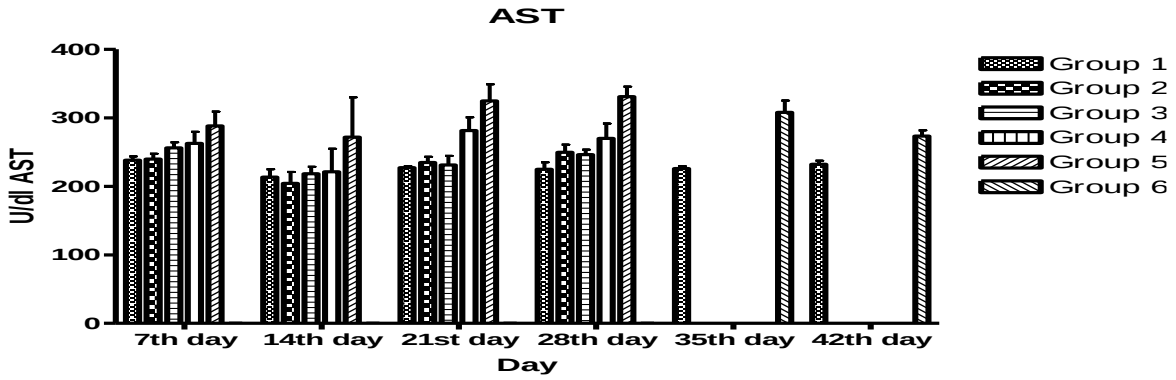

Fig. 2. Effects of norfloxacin on AST level (U/dl) of experimental broiler chickens in repeated dose sub-acute toxicity study

Table 4. Effects of norfloxacin on AST level of experimental broiler chickens in repeated dose sub-acute toxicity study

Groups	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Group I (control)	238.16±5.44	213.33±11.59	227.16±2.07	224.83±10.08	330.83±14.7	330.83±14.7
Group II (Vehicle)	239.66±7.89	204.33±16.54	234.83±8.20	249.5±11.18		
Group III (0.11g/kg)	256.16±8.142	218.33±10.29	231.16±13.28	246.33±7.2		
Group IV (0.333g/kg)	262.66±16.83	221.16±33.65	281.5±19.04	269.83±21.81		
Group v (1.1g/kg)	288.16±20.90	271.66±58.21	324.5±24.34**	330.83±14.7**	307.83±17.3**	273.33±8.43***

Values are Means ±SE, ***P<0,001, **P<0, 01, *P<0, 05. The values on 35 and 42 pertain to satellite group

DISCUSSION

The fatty change in liver, according to (Gomez et al. 2009), is impaired metabolism of a number of drugs, associated with fatty liver, which suggests an association between increased lipid deposition and impaired CYP enzymes. In the meanwhile, (Shlosberg et al. 1997) reported that fluoroquinolone antimicrobials norfloxacin and enrofloxacin were found to inhibit hepatic microsomal cytochrome P-450 monooxygenases in the livers of broiler chickens using dosages as given in commercial flocks or may be correlated with finding of (Regmi et al,. 2005) who observed accumulation of Fluoroquinolones when they were repeatedly administered. These are accordance with finding of (Coleman. C et al. 2002) who reported that Norfloxacin have been associated with hepatotoxicity causes hepatocellular necrosis and results in elevated ALT and AST concentrations.

The increase in ALT and AST values in the present study correlate with the finding of Hess (2016), who reported that SGPT being freely soluble in cytoplasm, was released from the hepatic cells even with very slight damage, which may not be detectable morphologically. Unlike SGPT, SGOT did not readily leak out from liver cells unless there was extensive injury to the liver. In the same time these findings are in accordance with observations of Amerson, which denotes that fluoroquinolones increased alanine transaminase, alkaline phosphatase, aspartate

transaminase activities the norfloxacin potential hepatotoxicity can be supported by an increase in serum ALT and AST concentration coupled with histopathological changes in the liver of broiler chicken (Amerson, 1982).

Conclusion

According to gross pathology, histopathology and biochemical findings in the present study, it was concluded that the norfloxacin has toxic effect on the liver tissue of chickens at the dose of 333 and 1100 mg/kg orally

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Effects of Probiotics on Oral Health

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ABSTRACT

Studies examining the function of the microbes living inside the human body in various contexts over the past few decades have shown how easily they can be controlled to treat and prevent diseases. For more than a century, probiotics have been used to treat infections and inflammation, one of their most notable uses. Despite the advantages of other probiotics, gastrointestinal infections, urogenital infections, tooth decay, and periodontal disease can all be treated or prevented with the help of *Bifidobacterium* and *Lactobacillus* species, the ones that are most often used. Influence of the gut microbiota, a probiotic therapeutic target, since a variety of bacteria species reside in the gastrointestinal tract that influences host metabolism and immune response either directly or indirectly, and can also be seen in the biology of the defense system. Therefore, it is not unexpected that probiotics have shown promise in reducing the symptoms of inflammatory disorders that affect humans, including inflammatory bowel illness; type 1 diabetic, multiple sclerosis, and rheumatoid arthritis, among others. This review's goal is to evaluate the possibility of probiotic-based treatment techniques to limit infection and the onset of inflammation in human patients.

Keywords: Probiotics; Halitosis; Periodontal diseases; Dental caries

INTRODUCTION

The name "probiotics" comes from the Greek language and sense "for life." They contain a diverse range of microbial species and strains (Isolauri and colleagues, 2001). When administered in sufficient concentrations, live bacteria are referred to as probiotics and are said to have health rewards (Reid et al., 2003). A few varieties of *bifidobacteria* and *lactobacilli*, as well as *Saccharomyces spp*., have been used as probiotics. However, some commensal *Escherichia coli*, *streptococci*, and *enterococci* have also been said to have favorable effects under specific conditions (Caglar et al, 2005; Reid et al, 2003; Picard et al, 2005; Moreno et al, 2006).

Live bacteria known as probiotics can help the host's health when provided in adequate amounts (Ai-Qun, 2016). Most probiotic products currently on the market contain lactic acid bacteria (LAB) from the genera *Lactobacillus* and *Bifidobacterium*. The preparation or composition of Probiotic products may have an impact on variety of results. Some probiotics were said to require viability and stability to be effective (Seal et al., 2007), whereas recent research revealed the therapeutic effects of living and sluggish LAB were comparable (Lopez, 2008; Fang, 2014). Synbiotics (combining prebiotics and probiotics) Moreover, mixtures of two or more probiotics can sometimes outperform a single probiotic strain (Le Leu, 2010). Probiotics have been demonstrated in numerous

studies to directly improve the host's gut flora in addition to this indirect benefit and have the potential to reduce tumor formation and metastasis due to increased gut barrier function, reduced bacterial translocation, immunological modulation, microbiota modulation, anti-inflammatory, anti-pathogenic activity, anti-inflammatory, and anti-pathogenic activity (Le Leu, 2010).

Probiotics have also been suggested by several researchers for dental health. Probiotics have been shown to extend the life of vocal prostheses by preventing undesirable microorganisms from adhering to them (Busscher et al., 1997; Schwandt et al., 2005). *Bifidobacterium* and *Lactobacillus* are two of the most regularly utilized probiotic species of bacteria (Saxelin et al., 2005). These bacterial genera are considered to be a natural feature of the human microbiome. Less than 1% of the oral cavity's total cultivable microbiota is made up of *lactobacilli;* however, there are no known species that are only found there. Certain species, on the other hand, are present in feces as well as oral samples (Ahrne et al., 1998; Maukonen et al., 2008). Species like *L. paracasei*, *L. plantarum*, *L. rhamnosus*, and *L. salivarius* are frequently isolated from saliva samples (Ahrne et al., 1998; Simark-Mattsson et al., 2007). According to culture-based research, among the *bifidobacteria* are the earliest anaerobes to colonize the oral cavity. Breast milk contains lactobacilli and *bifidobacteria*, suggesting early exposure to these bacteria in the oral cavity (Gueimonde et al., 2007; Abrahamsson et al., 2009). Oral samples have yielded *Bifidobacterium bifidum*, *Bifidobacterium dentium*, and *Bifidobacterium* long for isolation (Maukonen et al., 2008; Crociani et al., 1996; Beighton et al., 2008).

A chronic inflammatory disease called periodontitis is characterized by the resorption of alveolar bone and the loss of connective tissue (Allaker, 2017). The majority of treatments are aimed at slowing disease progression, restoring periodontal tissue, and harmonizing the host's reaction to pathogenic organisms' virulence factors. In 1998, Socransky described many bacterial combinations associated with periodontal health and disease. The most frequent microorganisms linked to periodontal disease are red and orange complex bacteria (Agarwal, 2015). Although there has been a drop in the prevalence of dental caries in western countries (Campus, 2009), it remains one of the most frequent diseases worldwide. The syndrome develops over time as a result of the interaction of cariogenic bacteria (mostly *mutant streptococci* and *lactobacilli*), a high-fermentable carbohydrate diet, and host factors including saliva production rate and buffering ability (Selwitz, 2007). For a very long time, *Mutants streptococci* (MS) were believed to be the most important pathogens in caries formation. However, it has recently been reported that caries cause changes in the microorganisms on the surface of teeth, to a majority of MS and other non-mutant bacteria, such as *lactobacilli* and *Bifidobacterium spp*, from a prevalence of *non-mutants streptococci* and *Actinomyces spp* (Takahashi, 2011).

Probiotic use in the oral cavity is a new idea. The purpose of this review is to summarize the effects of probiotics on oral health as well as potential mechanisms of probiotic bacteria in the oral cavity. They are crucial in the clinical therapy of periodontal, dental, and halitosis problems. As it is well known, probiotics are helpful in preserving the body's microbial balance.

Probiotic effects in the oral cavity and their method of action

The broad processes of probiotics are grouped into three categories: normalization of the gut flora, immune response modulation, and metabolic impacts (Parvez et al., 2006). The mechanisms of probiotic effect in the oral cavity may be similar to those previously discovered in the intestine. Possible ways that probiotics might affect oral health are summarized in **Fig 1**.

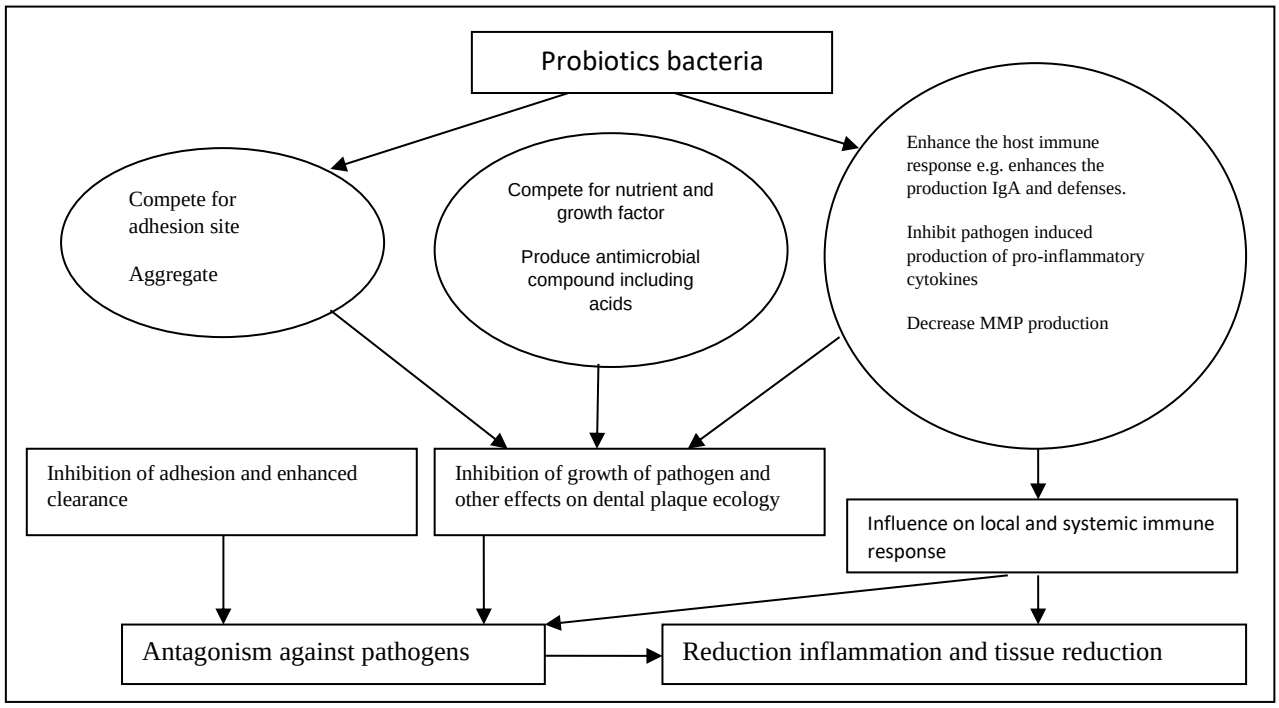

Fig 1. Potential ways that probiotic microorganisms may have an impact on dental health (Revised from 76; includes supplementary references) (Hatakka et al., 2008; Hojo K et al., 2007; Haukioja A et al., 2006).

Although total IgA levels in saliva appear unaltered by probiotic usage, oral colonization by probiotic bacteria has long been believed to be necessary for them to generate oral benefits. Despite this, systemic effects cannot be ruled out (Kekkonen et al., 2008; Paineau et al., 2008). Surprisingly, the content of breast milk appears to be influenced by maternal use of particular probiotic strains (Rautava, Kalliomaki, and Isolauri 2002). Several factors support the assumption that bacteria could be beneficial for the treatment or prevention of oral illnesses can be found in commensal oral microorganisms. Indeed, several probiotic benefits have been suggested to be shared by multiple species, rather than being unique to a few well-studied strains (Haukioja et al., 2006; Haukioja et al., 2008). According to the ecological plaque theory, selective force in the environment is able to alter the equilibrium between dental fitness and illness (Marsh, 2003). Because bacteria may impact their environment, and because bacteria in dental plaque have both synergistic and antagonistic interactions, Bacteria may contribute to the ecological plaque hypothesis's description of environmental pressure. Second, it is well known that a balanced oral microbiota guards against infections in the mouth. And finally, there exist bacterial species exactly as there link to oral disorders. Additionally, several species might be related to dental health (Becker et al., 2002; Stingu et al., 2008; Riep et al., 2009). However, it's unclear if bacteria consumed in food might affect the mouth's rather stable microbiota, especially in adults. It's also worth noting that the clinical evidence is mostly based on short clinical pilot trials, whereas all of the mechanisms discussed in this section are based on in vitro observations. Recent research has revealed that probiotics actively reduce the symptoms of gingivitis, dental caries, periodontitis, and halitosis by utilizing a variety of oral microbial species that are beneficial to oral health in place of the harmful ones (Agarwal et al., 2015 & Bartel, D. P. 2015). Additionally, probiotic strains' effectiveness in

enhancing oral health benefits has showed promise when combined with nitrate-reducing bacteria. (Haukioja A., 2010).

Periodontal ligaments and probiotics

Initial research on the benefits of probiotics for oral health centered on reducing periodontal inflammation (Kragen, 1954). Patients were treated locally with an *L. acidophilus* culture supernatant for periodontal conditions like gingivitis, periodontitis, and pregnant gingivitis. Almost every patient experienced a significant improvement. Recently, a lot of people are interested in employing probiotics to treat periodontal disease. *Bacillus subtilis*, *L. brevis* (CD2), *L. casei Shirota*, *L. reuteri strains*, and *L. salivarius* WB21 were among the probiotic strains employed in these investigations. Gingival health has improved with *L. reuteri* and *L. brevis*, as evidenced by reduced gum bleeding. (Krasse et al., 2006; Della et al., 2007; Twetman et al., 2009). *L. reuteri* ATCC 55730 and ATCC PTA 5289, two probiotic strains of chewing gum, also decreased GCF (Greatest common factors) levels of pro-inflammatory cytokines. According to (Twetman et al. 2009). MMP (Matrix metalloproteinase) (collagenase) activity besides other inflammatory markers in saliva was reduced when *L. brevis* was used (Della et al. 2007). There are insufficient experimental studies on the use of probiotics in periodontal problems, mostly because of a lack of knowledge regarding the precise etiology of the condition and the environments that encourage wellness. In contrast to a placebo group, patients who received one of two *L. reuteri* formulations for moderate to severe gingivitis showed lower plaque and gingivitis scores (Krasse et al, 2006). When compared to a placebo control group, regular (three times per day for eight weeks) administration of tablets containing *Lactobacillus* salivarius in smokers at high risk for periodontal disease led to advantages in terms of pocket probing depth and plaque index (Hamou, 2019). Further researches are sought to uncover creatures with the prospective to act as probiotics in the prevention of periodontal diseases. Several oral *streptococci* and *lactobacilli* (Koll et al.,2006; Sookkhee et al., 2001; Koll-Klais et al.,2005) and *bifidobacteria* (Hojo et al. 2007) strains have been shown to exhibit inhibitory effect against periodontal pathogens in vitro, some are more effective against *mutant streptococci* (Simark-Mattsson et al., 2007; Koll et al., 2008).

Probiotics and dental caries

An infectious microbial disease of the oral cavity known as caries is brought on by bacteria that ferment biological compounds, causing how much mineral is in the tooth construction to dissolve. *S. mutants* are the principal organism to be blamed. Dental caries is becoming more common, especially among children, as a result of increased refined sugar consumption and poor oral hygiene. (Tinanoff et al., 2019). When *L. reuteri*, *Bifidobacterium lactis* BB-12, and *L. rhamnosus* GG strain were consumed as probiotics, the amount of *S. mutants* was significantly reduced. They may limit the adherence of the surface of the tooth with germs and so minimize the incidence of dental caries by deterring the microbiota of dental plaque. There is a significant reduction in cariogenic bacteria when multiple species or strains of probiotic organisms are utilized (Meurman et al., 2007). Similarly, when the *L. rhamnosus* 1b21 strain is combined with fluoride in milk, the result is the same (Galdeano et al., 2007).

Probiotics and Halitosis

Halitosis is a broad term that refers to any unpleasant stench of expired air, regardless of its source (Aydin et al, 2014). *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Porphyromonas intermedia*, and *Trichophyton denticola* are the bacteria that generate the odor. The microbiota triggers putrefactive amino acid metabolism, which results in VSCs (hydrogen sulfide, methyl mercaptan, dimethyl sulfide) (Niles et al, 1996). The dorsal

surface of the tongue has many papillae surface acts as a habitat for the accumulation of bacteria. Probiotics aid in the reduction of microbial dysbiosis on the tongue, particularly the difficult-to-clean posterior portion (Gurpinar et al., 2019). *W. cibaria*, when given as a probiotic, inhibited *F.nucleatum*, resulting in a decrease in Hydrogen peroxide production (Kang et al, 2006). One of the pioneer probiotic strains, *Streptococcus salivarius* (K12 strain), secretes Bacteriocin, when taken in the form of lozenges, aids in the reduction of Solobacterium moorei, hence lowering oral bad breath (Wescombe et al, 2010). *L. reuterin* has also been shown to aid in the promotion of oral health and the reduction of bad breath. *F. nucleatum* and *P. gingivalis* manufacture methionine lyase by down-regulating the enzyme (Fujiwara et al, 2017).

CONCLUSION

This study's objective is to look at the available research on probiotics and their impact on oral health. It is well-recognized that probiotics, aid in the maintenance of the human body's microbial equilibrium. Probiotics are a relatively new notion within the mouth. They are important in the treatment of halitosis, dental caries, and periodontal illnesses in the clinic. These findings imply that probiotics can help to lower pathogen loads while also restoring the microbiota of the host. To fully comprehend the potential of probiotics, the probiotic organism's capacity for survival, reproduction and therapeutic effect needs further study. To identify dosages and delivery schedules, interactions with other substrates, and other factors, we also need well-designed, lengthy follow-up studies, and analysis of oral health hazards associated with long-term probiotic usage, so that we can highlight and practice the same appropriately.

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Prevalence of Systolic and Diastolic Ventricular Dysfunction in Nangarhar University Teaching Hospital

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ABSTRACT

Background: Heart failure occurs when the heart cannot efficiently circulate blood throughout the body. Due to the increasing use of echocardiography, a brand-new condition called heart failure has developed. CAD is the most common cause of heart failure. Patients with left heart failure may have dyspnea, even after rest, fatigue, weakness, cough, inability to sleep lying flat, reduced cardiac output, and increased pulmonary venous pressure. Heart failure affects around 6.2 million people in the US, and by 2030, it is expected to affect at least 8 million people. Ten percent of people over the age of 80 have heart failure. Our aim was to determine systolic and diastolic heart failure in Nangarhar University teaching hospital in the medical ward.

Methods and Materials: It is a cross-sectional hospital-based study carried out in the Nangarhar University Teaching Hospital of Internal Medicine ward in 2000 on heart failure from 2021 to 2022, which represents 323 patients.

Findings: We studied 2000 heart failure patients in Nangarhar University teaching hospital from 2021 to 2022, which represents 323 patients. In terms of gender, 153 (47.36%) patients were female and 170(52.63%) patients were male. In terms of age, 190(58.82%) patients were over 50 years old and 133(41.17%) patients were under 50 years old. The STDEV age was (57±11). 190(58.82%) patients had diastolic dysfunction, 133(41.17%) had systolic dysfunction, and 64(19.81%) had combined systolic and diastolic dysfunction. 198(61.10%) had a preserved ejection fraction and 125(38.69%) had a reduced ejection fraction. 160(49.53%) patients had shortness of breath, 163 (50.46%) patients had chest pain, and 38(11.74%) patients had combined shortness of breath and chest pain.

Conclusion: It is a cross-sectional study fulfilled in the internal medicine ward of Nangarhar University Teaching Hospital. The prevalence of heart failure is lower in females (153) (47.36%) than in men (170(52. 63%).According to the age group, heart failure is more common in over-50-year old patients 190 (58.82%).

The overall prevalence of diastolic heart failure is 9.5% and systolic heart failure overall prevalence is 6.65%. Diastolic dysfunction 190(58.82%) is more common than systolic dysfunction 133(41. 17%).Chest pain 163(50.46%) is more than shortness of breath of 160(49. 53%).The ejection fraction is preserved in 198(61.30%) patients and reduced in 125(38. 69%). Heart failure is diagnosed with clinic and echocardiography.

Keyword: Heart failure; Ejection Fraction; Systolic Dysfunction; Diastolic Dysfunction; Age

INTRODUCTION

The words "congestive heart failure" and "heart failure" are frequently used interchangeably. Congestive symptoms including dyspnea, orthopnea, neck vein enlargement or pulmonary or peripheral edema nearly always signify high right or left ventricular filling pressures. These elevated pressures can be the result of diastolic dysfunction or primary systolic failure (Grossman, 1991). Congestive heart failure (CHF) is a clinical condition with recognized signs and symptoms. Patients with CHF usually receive echocardiography in order to quantify the ejection fraction (EF) and establish whether their systolic function is reduced (systolic CHF) or preserved (diastolic CHF). In addition to determining the EF, comprehensive Doppler echocardiography may now characterize diastolic function directly (Redfield and Jacobsen, 2003). Because there are many patients with heart failure in the university teaching hospital and the incidence of systolic and diastolic heart failure is not known, therefore, we chose this research to find out the incidence of this disease. Because each of this problem has different treatment plan and outcome.

Heart failure (HF), a prevalent condition with multiple underlying causes, can cause the ejection fraction to drop (EF). Numerous studies have been conducted on the pathophysiology of HF with lower EF, and effective management techniques are widely accepted (Bursi, and Weston, 2006). Although clinical series, observational data, and clinical investigations have increased our understanding of HF and preserved EF 2, 3, there is still disagreement over a number of critical elements of this condition, including its incidence, clinical characteristics, and prognosis (Bursi, and Weston, 2006). No studies that we are aware of have looked at the prevalence and distribution of diastolic dysfunction in individuals with HF and reduced or retained EF have been published. Congestive heart failure (CHF) is growing to be a significant global public health concern. Bursi, and Weston, 2006).

Diastolic heart failure has become a recognized medical condition as a result of the extensive use of echocardiography in the diagnosis of CHF. The authors studied all Olmsted County residents receiving medical care for HF at Mayo Clinic inpatient and outpatient facilities. Many people who have a clinical diagnosis of heart failure fit within this category. Heart failure is a condition that affects many people and has a wide range of etiologies. Its ejection fraction could be increased or decreased (EF). Numerous studies have been conducted on the pathophysiology of HF with lower EF Bursi, and Weston, 2006). A frequent syndrome with increasing prevalence and incidence is heart failure. Heart failure currently affects 6.2 million people in the US, and by 2030, it's predicted that figure will at least increase to 8 million. In the US, hospitals release 809,000 individuals with a diagnosis of heart failure each year. More than 75% of new and continuing cases involve people over the age of 65, indicating that it is mainly an aging disease. Patients with heart failure make up 75% of those with the illness. Less than 1% of adults between the ages of 60 and 80 have heart failure. The majority of individuals exhibit both right-and left-sided failing symptoms or indicators (Bursi and Weston, 2006).

Systolic heart failure is most frequently caused by CAD in the UK, and it can lead to MI. Systemic hypertension is more common in the US and worsens the condition as well as LV or biventricular dilatation and wide systolic failure are characteristics of dilated or congestive cardiomyopathy (Bursi and Weston, 2006). Viral myocarditis, including HIV infections, is the most frequent cause. Infiltrative illnesses, metabolic disorders, cardiotoxins, and drug toxicity are examples of rare causes. LV or biventricular dilatation and wide systolic failure are hallmarks of dilated or congestive cardiomyopathy. Viral myocarditis, including HIV infections, is the most frequent cause. Infiltrative infections, metabolic conditions, cardiotoxins, and drug toxicity are examples of rare causes (Bursi

and Weston, 2006). Diastolic or systolic myocardial failure is the most common type of heart failure. Pressure-volume correlations in the left ventricle are the best indicator of a possible problem. Diastolic dysfunction could be the only physiologic disturbance or the dominant one (Warren & Grossman, 1991). Reduced cardiac output and increased diastolic filling pressure at rest and/or during activity are hemodynamic symptoms of cardiac insufficiency. Increased initial tension of muscle fibers (Frank-Law) Starling's or a sign of decreased myocardial compliance because the increase in diastolic filling pressure, that compensates for cardiac output (https://pubmed.ncbi.nlm.nih.gov/8351672/). Hemodynamic signs of heart failure include decreased cardiac output and increased diastolic filling pressure at rest and/or during exercise. An increase in the initial tension of the muscle fibers indicates systolic insufficiency. Diastolic congestive heart failure is frequently accompanied by decreased ventricular compliance and normal stroke function. (Federmann & Hess, 1994). Our aim was to determine systolic and diastolic heart failure in Nangarhar University teaching hospital in the medical ward.

MATERIALS AND METHODS

It is a cross-sectional hospital-based study carried out in the Nangarhar University Teaching Hospital of Internal Medicine ward from 2021 to 2022 in 2000 on heart failure, which represents 323 patients. First, we took the history and physical examination of the patients of our selected sample. Then, according to the format of the prepared questionnaire, we did the history and physical examination. Then we performed the echocardiography on the patients. Data collection was from patient files, echocardiographic registration and medical records of the hospital. Data analysis was done with excel and IBM SPSS statistic 26. Examinations of Echocardiography is free in Hospital. In this research, all the patients who had heart failure in terms of history and physical examination of the clinical record were included.

RESULTS

We considered 2000 heart failure patients in Nangarhar University teaching hospital from 2021 to 2022, which represents 323 patients. In terms of gender, 153 (47.36%) patients were female and 170(52.63%) patients were male. In terms of age, 190(58.82%) patients were over 50 years old and 133(41.17%) patients were under 50 years old. The STDEV age was (57±11). 190(58.82%) patients had diastolic dysfunction, 133(41.17%) had systolic dysfunction, and 64(19.81%) had combined systolic and diastolic dysfunction. 198(61.10%) had a preserved ejection fraction and 125(38.69%) had a reduced ejection fraction. 160(49.53%) patients had shortness of breath, 163 (50.46%) patients had chest pain, and 38(11.74%) patients had combined shortness of breath and chest pain. The overall prevalence of diastolic heart failure is 9.5% and systolic heart failure overall prevalence is 6.65%.

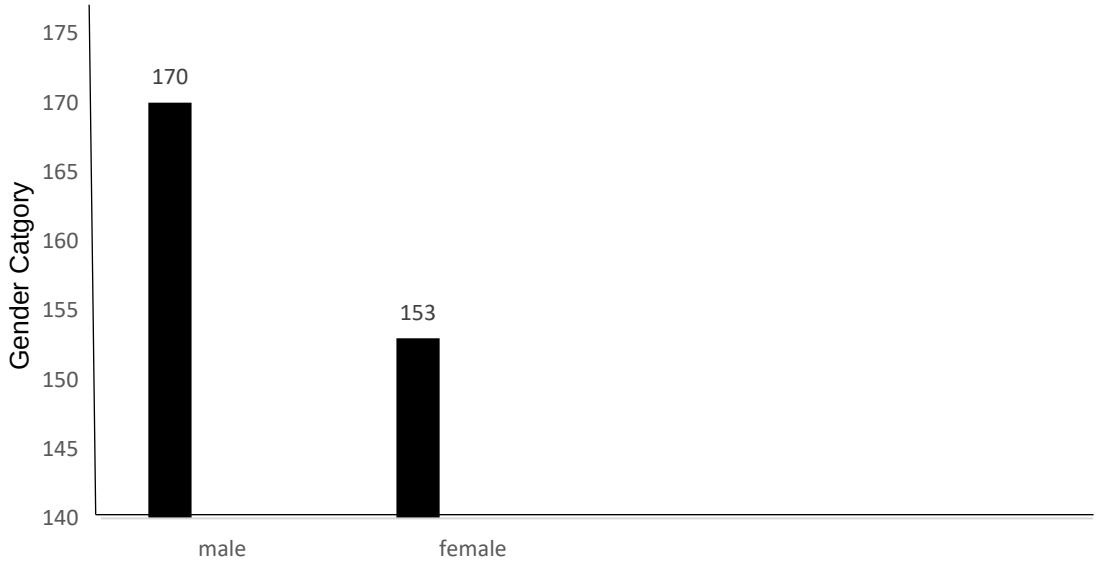

Fig. 1. Prevalence of Systolic and Diastolic Ventricular Dysfunction in Nangarhar University Teaching Hospital in Internal Medical Ward. Male prevalence was higher as compared to Female.

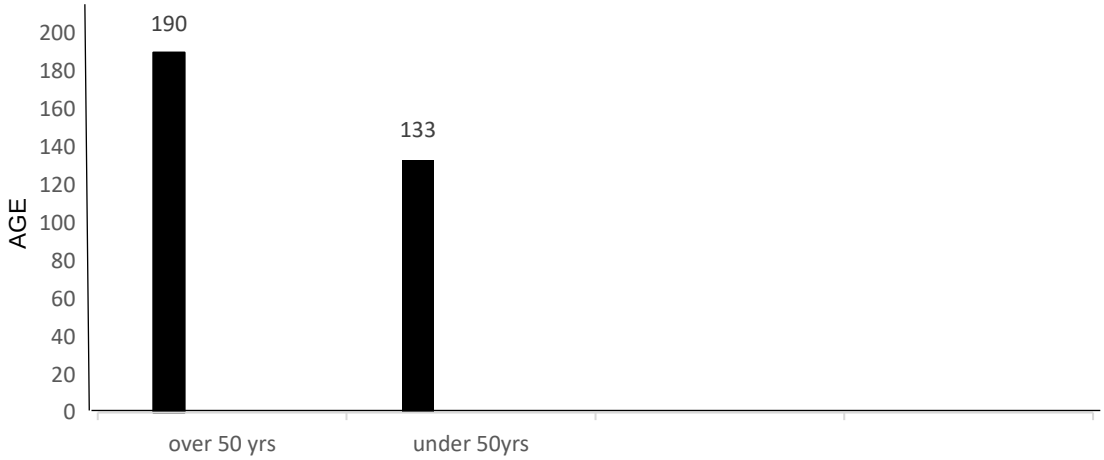

Fig. 2. Prevalence of Systolic and Diastolic Ventricular Dysfunction in Nangarhar University Teaching Hospital in Internal Medical Ward. Over 50 Years prevalence was higher as compared to under 50 Years old.

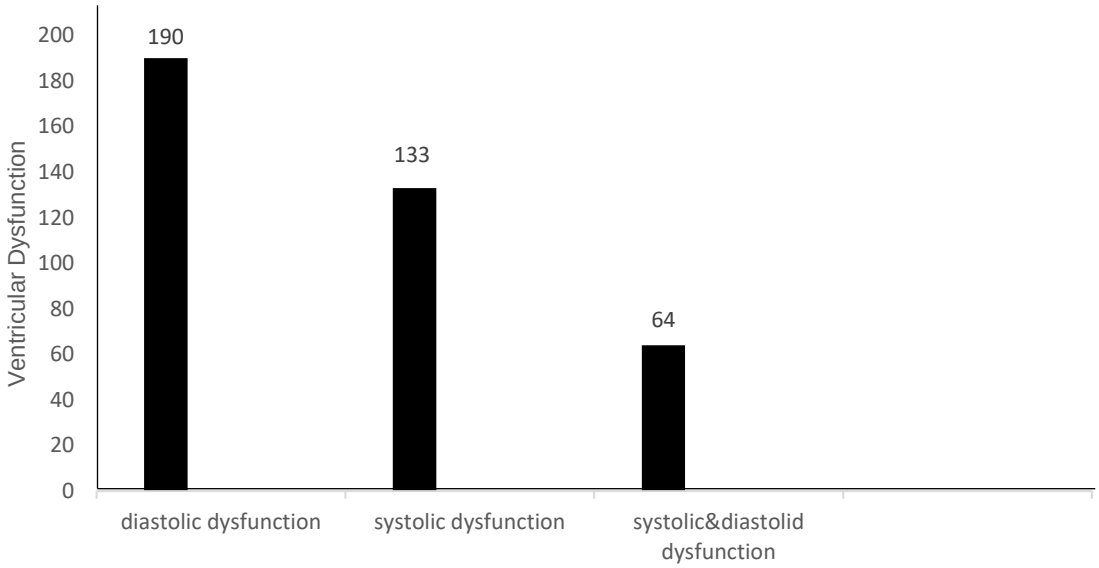

Fig. 3. Prevalence of Systolic and Diastolic and Systolic and diastolic Ventricular Dysfunction in Nangarhar University Teaching Hospital in Internal Medical Ward. The Prevalence of diastolic was higher as compared to systolic dysfunction.

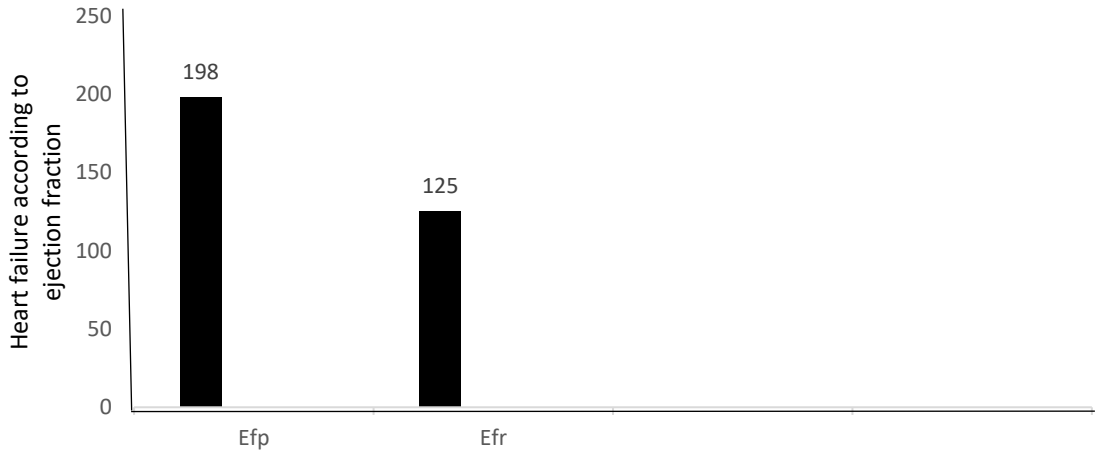

Fig. 4. Prevalence of preserved ejection fraction (HFpEF) and reduced ejection fraction (HFrEF) heart failure

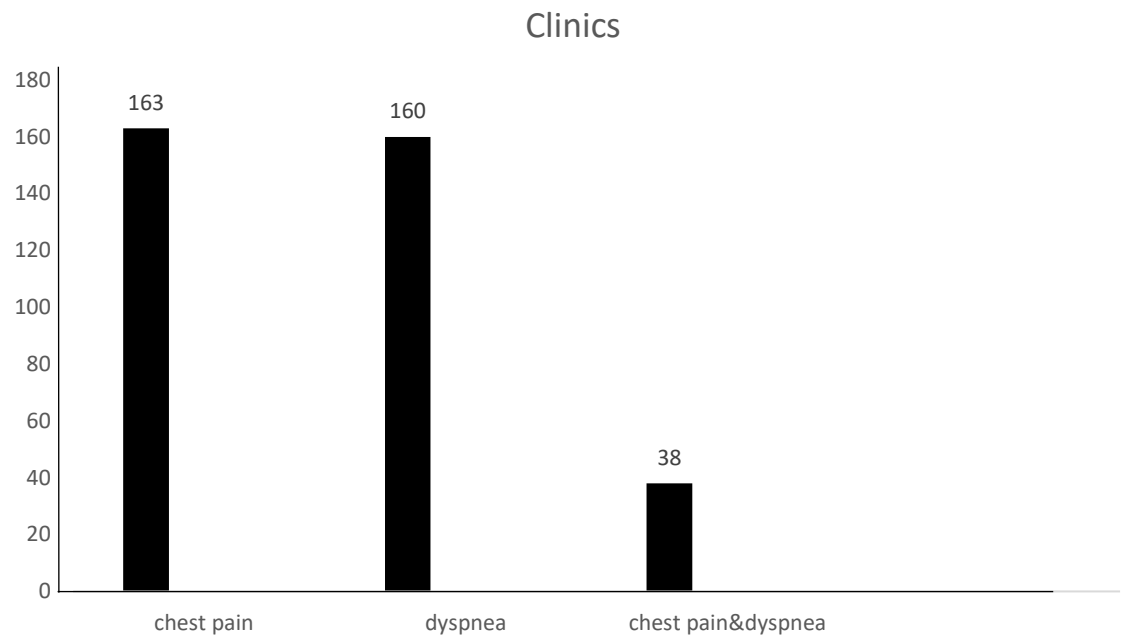

Fig. 5. Symptoms and signs of heart failure according to Clinics report

DISCUSSION

Our goal is to assess the heart's insufficiency by measuring systolic and diastolic ventricular dysfunction in the medical ward of the Nangarhar University Teaching Hospital. From 2000 heart failure patients we simply selected 323 people, the Diastolic dysfunction was present in 190 patients (58.82%) and systolic dysfunction in 133 patients (41.17%). 160 patients reported shortness of breath and 163 patients had chest discomfort. In terms of age, 190(58.82%) patients were over 50 years old and 133(41.17%) patients were under 50 years' old. From this, it was found that the incidence of diastolic heart dysfunction is more than that of systolic heart dysfunction, and the incidence of heart failure is more in the elderly. Preserved ejection fraction were more than reduced ejection fraction. We compared the research of our relatives with others and it was seen that diastolic heart dysfunction is more common than systolic dysfunction and the incidence of heart failure is high in elderly people because the incidence of hypertension, cardiac ischemia, blood lipid disorders, and heart arrhythmia is high in elderly people. There were no limitations in data collection, sampling selection, and data analysis. The Preserved EF was associated with older age, female sex and no history of myocardial infarction. Diastolic dysfunction with preserved EF was present in 242 (44%) patients. For patients with reduced EF, moderate or severe diastolic dysfunctions were more common (Bursi, and Weston, 2006). In the Switzerland, 44% of the population had a diastolic dysfunction (EF) above 50%, with 44% having an EF higher than 50%. The incidence of confirmed CHF was 2.2% (95% confidence interval [CI], 1.6%-2.8%) (Redfield and Jacobsen, 2003). The Diastolic dysfunction was more common in patients with reduced EF than in those with intact EF, according to a study by Cardiologists at the University of Bristol (Bursi, and Weston, 2006). The prevalence of normal ventricular systolic function among patients with congestive heart failure ranges widely from 13% to 74%. Diastolic heart failure has identical clinical signs and symptoms. The stated annual mortality rate does too, from 1.3% to 17.5% (Vasan and, Benjamin, 1995). The Echocardiographic Study of Latinos (ECHO-SOL) selected a total of 1818 patients from across the United States between the ages of 45 and 74. Participants underwent a rigorous echocardiography examination to find left ventricular systolic dysfunction (LVSD), prevalence was 3.6%, whereas LVDD was detected in 50.3%.

(Hardik and Anderson, 2016). The Compared to people with normal heart function, CHF was substantially more frequent in people with systolic or diastolic dysfunction. Overall, 6.6% of patients had any type of dysfunction, and 2.0% had moderate or severe dysfunctions (Margaret and Redfield, 2003). The Overt congestive heart disease (CHF) patients who have diastolic dysfunction without a reduced ejection fraction compensate approximately half of the population. (EF) Nevertheless, it is still unknown how prevalent diastolic dysfunction is in the general public or how it is linked to systolic dysfunction and CHF *(*Redfiel, and Jacobsen, 2003*).* The LV dysfunction was assessed using this double and Doppler echocardiography. Overall, 1.3% (or 13.7 million) of the adult Chinese double and Doppler echocardiography. Overall, 1.3% (or 13.7 million) of the adult Chinese population aged 35 or older had HF, 1.4% of individuals had LV systolic dysfunction (ejection fraction 50%), and 2.7% had LV diastolic dysfunction classified as "moderate" or "severe. (Hao, and Wang, 2019). The isolated left ventricular (LV) diastolic dysfunction has been estimated to be responsible for up to one-third of the occurrences of heart failure (HF), with an increasing prevalence in the senior population. Overall, 102 patients (78%) showed prevalent LV systolic dysfunction (LV ejection fraction 45%), while 29 patients (22%) had isolated LV diastolic dysfunction (Badano, and Albanese,2004). Higher altitude is associated with an increased risk of LVDD in those who live above 1500 meters, particularly in men (Zheng and Wang, 2021). The Prevalence of heart failure and systolic ventricular dysfunction in older Australians: the Canberra Heart Study (Abhayaratna et al., 2006).

CONCLUSION

The prevalence of heart failure is lower in females (153) (47.36%) than in males (170) (52.63%). The ejection fraction is preserved in 198(61.30%) patients and reduced in 125(38.69%) patients. Heart failure is diagnosed by clinic and echocardiography. The overall prevalence of diastolic heart failure is 9.5% and systolic heart failure overall prevalence is 6.65%. Diastolic dysfunction is more than systolic dysfunction, and the incidence of heart failure is higher in elderly patients. Heart disease is a leading cause of death, but it's not inevitable. While you can't change some risk factors — such as family history, sex, or age — there are plenty of ways we can reduce the risk of heart disease. We can prevent heart disease by living a heart-healthy lifestyle. Here are strategies to help us protect our heart. We'll stop smoking or using smokeless tobacco. Aim for at least 30 to 60 minutes of activity daily. Eat a heart-healthy diet. Maintain a healthy weight. Get good quality sleep. Manage stress well. Get regular health screenings. Support in daily cardiology patient management. Primary prevention is usually aimed at people who already have developed cardiovascular risk factors, such as high blood pressure or high cholesterol. As with secondary prevention, primary prevention focuses on controlling these risk factors with making healthy lifestyle changes and, if needed, taking medications.

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Sense of Mother Calling in Child rearing and Child Psychological Wellbeing: A Mediating Model Analysis

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ABSTRACT

Background: An increasing body of research highlights the significant role of calling in work and career domains. Nevertheless, very little correlational research has shown positive outcomes of parent sense of calling in child rearing context. To fulfill this gap, this study aimed to test the relationship between mother calling in child rearing and children's psychological well-being by mediating psychological well-being and satisfaction with being mothers of women teachers in Jalalabad Afghanistan.

Materials and Methods: The study participants included 250 mothers and 250 of their children who were selected by random sampling. The Questionnaires for mothers used in this study were: Subjective Sense of Calling in child rearing, Psychological Well-Being, and Parental Satisfaction. Children also completed the Psychological Well-Being. Methods for analyzing the data were: Bivariate correlation analysis, structural equation modelling for model fit indices, direct effect of the variables, and process macro bootstrapping for the indirect effect of variables.

Findings: The findings of this research showed the designed model had an excellent model fit. The direct path between women's sense of calling in child rearing and children psychological well-being, between mother psychological well-being and children psychological well-being. Between mother satisfaction with being mother and children psychological well-being were positive and significant as $p < 0.05$. Also, this research showed the indirect effects of mother calling mediated by psychological wellbeing and satisfaction with being of mothers on children psychological wellbeing are positive and significant.

Conclusion: The results suggested that in order to increase child psychological well-being, women should become more familiar with their role as calling in child rearing context.

Keyword: Mother Calling; Psychological wellbeing; Maternal Satisfaction

INTRODUCTION

Historically, calling refer to spread of religious belief but over the past decade, calling has been a hot topic for researchers in the career and workplace context (Elangovan et al., 2010; Dobrow, 2013; Dik & Duffy, 2009; Zhang et al., 2015) because of its relationship with positive individual outcomes, such as career success, work engagement (Xie et al., 2016), life satisfaction (Hagmaier & Abele, 2012; Hirschi et al., 2018; Duffy & Dik, 2013; Douglass et al., 2016; Chen et al., 2016), psychological well-being (Hirschi & Hermann, 2012; Coulson, 2011; Peterson et al., 2009), and job satisfaction (Park et al., 2016; Kim et al., 2018). People who feel calling in their work, actually they feel happier and more committed to the work and have inner enthusiasm to perform their work (Dik et al., 2019).

According Coulson et al. (2012b), the theory of sense of calling in child rearing is proposed in positive psychology. According to Coulson et al. (2012b), sense of calling in child rearing consists of three main components. The first component is life purpose: In this component, parents believe that one of the main reasons for human existence on earth is to become a parent. People with a sense of calling consider becoming a parent is a natural and ultimate goal in their lives. This specific goal forces parents to use appropriate and useful methods in raising their children. In this case, parents consider that rearing children makes up the core of their identity. The second component is awareness: It refers to conscious attention to the requirements of the parenting role and awareness of what parents are doing for their children all the time. In other words, parenting is considered as a person's main priority in life. Parents prioritize the needs of their children over their own needs, and all their worries and concerns are taking care of their children. They always think about their children. The third element is passion: This dimension indicates that parents have a high motivation to participate in the role of child rearing and related tasks. In this case, a parent enjoys parenting and does not see it as a tiring and exhausting activity, but loves being with his/her children (Coulson et al., 2012b).

Parents who consider raising a child as the natural purpose of life, they are having psychological well-being and their children have higher levels of life satisfaction (Coulson et al., 2012a). Having meaning and purpose in life are considered as the main factors of happiness and life satisfaction (Peterson et al., 2005). Research has shown that feeling calling in child rearing has positive consequences for parents. For example, Coulson (2011) showed that the sense of calling in child rearing is related to parental well-being and parental satisfaction. Duffy et al. (2012) also showed that those who felt a higher calling also had high levels of psychological well-being in life. People who identify and pursue meaningful goals with a sense of calling in the life domain and they successfully achieve their goals, as a result, they feel satisfied with life (Hall & Chandler, 2005). Calling continuously relate to outcomes such as meaning of work, career satisfaction, meaning of life, and life satisfaction (Duffy et al., 2017). In addition to the variable of calling, psychological well-being is one of the important variable of this study. Psychological well-being can be defined as a sense of cohesion and connection in life, emotional balance and overall life satisfaction. Psychological well-being can also be expressed as emotional and cognitive reactions to the perception of personal characteristics and abilities, efficient and effective interaction with the world, having a favorable relationship with the community and progressing over time. This condition can also include components such as life satisfaction, energy, and good morals (Dickerson, 2018). One of the most popular definitions of this concept is provided by Ryff (1989). He has considered six components for psychological well-being: Having a positive attitude towards oneself (self-acceptance), establishing warm and intimate relationships with others and the ability to empathize (positive relationships with others), feeling independent and being able to endure social

pressures (autonomy), having a purpose in life and giving meaning to it (purposeful living), the feeling of continuous growth (personal growth) and the individual's ability to manage the environment (mastery of the environment).

Maternal satisfaction with being mother is another important variable in this study. Salonen et al. (2011) defined parental satisfaction as the feeling of happiness and satisfaction associated with parenting. Parental satisfaction is related to parental competence, the more parents fulfill the tasks of raising children with consent and they are the more satisfied with their parenting (Kyriazos & Stalikas, 2018). Parents, who have low levels of satisfaction with being parents, use harsh punishment strategies and child abuse and neglect (Carpenter & Donohue, 2006). When parents feel calling, they are satisfied with their parenting role at a higher level, and as a result, they establish a good relationship with their children, which leads their children to psychological wellbeing (Coulson et al., 2012a). Mothers who are satisfied and worthy of being mothers also have a positive effect on their children psychological well-being and life satisfaction (Devito, 2010). A positive relationship between parent and child plays an important role in increasing children's psychological well-being and life satisfaction (Bireda & Pillay, 2017).

Due to the novelty of the concept of calling in child rearing, very limited research has been done on it, but these few studies show that the sense of calling in child rearing has positive consequences. The sense of calling in child rearing causes mothers to show more interest in their children and to communicate with them effectively. According to the concept of calling in child rearing, a deeper role and action can be considered for mothers' in upbringing children (Wrzesniewski et al., 1997).

Paying attention to what has been said about the importance of mother calling in child rearing, fewer correlational studies have been done in the world about it (Coulson, 2011). The casual relationship between maternal calling mediated by psychological wellbeing and maternal satisfaction of mothers' variables that explain the effects on child psychological wellbeing have not been explored. Thus, the present study might fulfill this research gap. Certainly the basic correlational nature could not determine causality (Coulson et al., 2011b). Based on the limited knowledge related to calling in child rearing context in the world, a model was designed and implemented to study this construct in Jalalabad Afghanistan. The aim of this research was the causal relationship between mother calling and children's psychological wellbeing mediated by psychological wellbeing and satisfaction with being mother of female teachers in Jalalabad, Afghanistan. The proposed model is shown in **Fig. 1.**

1. There is a direct and positive relationship between sense of mothers calling and their psychological wellbeing.
2. There is a direct and positive relationship between sense of mothers calling and their satisfaction with being mothers.
3. There is a direct and positive relationship between sense of mothers calling and their children psychological wellbeing.
4. There is a direct and positive relationship between the psychological well-being of mothers and the psychological well-being of their children.
5. There is a direct and positive relationship between the satisfaction of being a mother and the psychological well-being of their children.
6. There is an indirect relationship between sense of mothers calling and their children psychological well-being through the mothers' psychological well-being.

7. There is an indirect relationship between sense of mothers calling and their children psychological well-being through the satisfaction with being a mother.

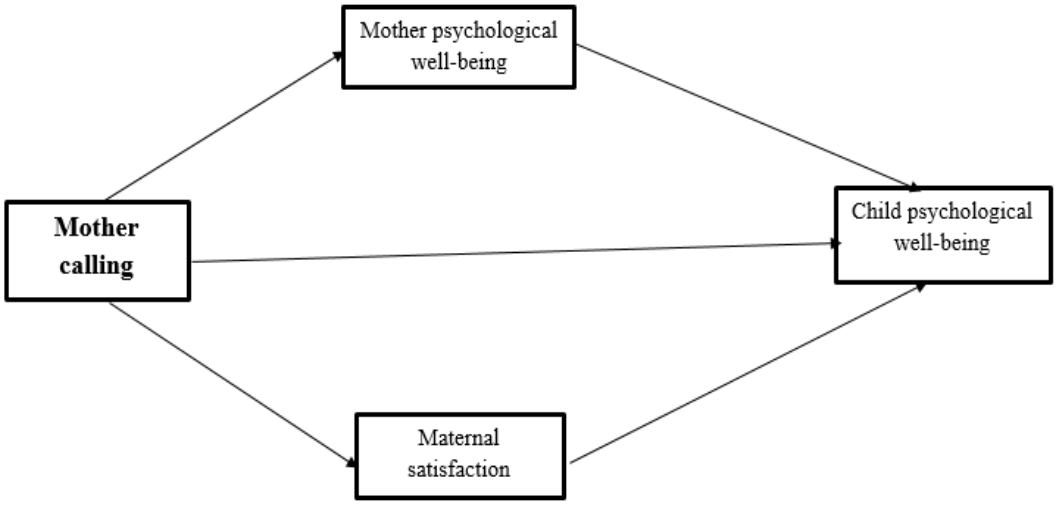

Fig. 1. Proposed model of research variables

MATERIALS AND METHODS

The statistical population of this study included all female teachers and one of their children aged 16 years or over in Jalalabad, Afghanistan. Preceding to the data collection, necessary permissions were obtained from the Education Department. Then, referring to each school, we explained the research objectives to participants and assured them that the data collected will be kept confidential and that they could withdraw from the study anytime they wanted to. This way, not only female teacher agreed to participate in the study, but also they promised to help the researcher with completing the questionnaire by one of their children. First we distributed the questionnaires to female teachers, and they filled them out in 30 minutes on average. After they completed the questionnaires, the questionnaire relating to their children were submitted to them to be completed by one of their children at home. A total of 300 questionnaires from female teachers and 300 questionnaires from their children were collected, of which 50 incomplete questionnaires were discarded and the final sample for testing the hypotheses was 250 female teachers and 250 of their children from 12 urban girls' schools by random sampling method.

The demographic result shows that a total of 168 female teacher participants in this study had two year diploma from teacher training colleges, 78 had bachelor's degree, and 4 were high school graduates. The majority of the female participants had more than 10 years of experience. Of these female teacher participants, 190 were between ages 36-50, and 60 were more than 50. In addition, the majority of the participating children in this study were females (154 girls, 96 boys).

Research tools

We used three different data collection tools in this study to collect data from female teachers and one questionnaire for their children. To know whether the questionnaires came from the same mother and child that were coded the same by giving numbers with abbreviated school name. Female teacher participants completed the Subjective Sense of Calling in child rearing Scale (SSCCS) (Coulson et al., 2012b), the Psychological Well-Being Questionnaire (PWB) (Ryff, 1989) and Parental Satisfaction Questionnaire (KPSQ) (Walter et al., 1985). Children also completed Ryff's (1989) PWB with a similar coding system. Each of them will be discussed

following in details.

Sense of Calling in child rearing Scale: In the present study, SSCCS developed by Coulson et al. (2012b) was used to assess the sense of mother calling. The scale includes 11 items and three dimensions of parenting calling, e.g. including 6 items of life purpose, 3 items of awareness and 2 items of passion in a 4-point Likert scale (strongly agree to strongly disagree). The Cronbach's alpha reliability of this scale has been reported for subscales as life purpose α =.83, awareness α =.75 and passion α =.57. The internal consistency score for the 11-items measure was α = .89 (Coulson et al., 2012b). In this study, the Cronbach's alpha reliability coefficient of this scale was α = .77. In order to evaluate the validity of the SSCCS in the present study, the method of confirmatory factor analysis (CFA) was used.

Goodness of model fit was assessed with several different indexes for example, the $\chi 2/df$, the comparative fit index (CFI), the incremental fit index (IFI), the Tucker–Lewis index (TLI) and the root mean square error of approximation (RMSEA). The $\chi 2/df$ <3 good; <5 sometimes acceptable; IFI and CFI >.95 excellent; >.90 standard; >.80 tolerated; RMSEA <.05 excellent; .05-.10 standard; >.10 not acceptable (Kline, 2011; Loehlin, 2004).With high correlation coefficients between item 7 and 9 of this scale two-way covariance was drawn. After applying the changes, the data were re-analyzed. And, fitness indicators show that the scale has an excellent validity (IFI= 1. 000, CFI = 1. 000, NFI= 0.913, TLI= 1.000, RMSEA = 0. 000, X^2/df = 0.892).

The Psychological Well-Being Questionnaire: To assess the psychological well-being of the mother, the short form 18-item Ryff's (1989) PWB was used in the present study. PWB includes six components: self-acceptance, positive relationships with others, autonomy, purposeful living, personal growth and mastery of the environment. Each dimension has three questions and the scores of these 6 factors are calculated as the overall score of psychological well-being. The answer to each of the 18 items is determined on a 6-point scale from strongly disagree (1) to strongly agree (6). Cronbach's alpha obtained in Ryff's study has been reported as: self-acceptance (α =. 86), positive relationships with others (α =. 91), autonomy (α = .93), purposefulness in life (α = .90), personal growth (α =. 87) and mastery of the environment (α =. 90). This scale was initially performed on a sample of 321 people and the coordination coefficient between the scales was from .86 Up to .93, and the reliability coefficient of the retest after six weeks on a sample of 117 people between .81 Up to .86 has been reported. The correlation between the short form of Ryff PWB and the main questionnaire has been reported as0.70 to 0.89 (Ryff & Singer, 2003). In the present study, the internal consistency score for the 18-items measure was α = .93 for female teachers and as well as for their children. Likewise, in this study the preliminary result of CFA shows poor validity of the PWB, then between items 1 and 6, 9 and 12, 11 and 13, 14 and 16 of this questionnaire with high correlation coefficients two-way covariance was drawn. After applying the modification, the data were re-analyzed. The modified results for the female teachers was (IFI = 1.000, CF I = 1.000, NFI = 0.96, TLI = 1.000, RMSEA = 0.000, X^2/df = 0.990) which showed the excellent validity of the questionnaire.

Parental Satisfaction Questionnaire: In this study, the KPSQ that developed by Walter et al. in 1985 was used to measure parental satisfaction. This questionnaire consists of 3 items, where mothers express their satisfaction in the form of a 7-point Likert scale from extremely satisfied (7) to extremely dissatisfied (1). James et al. (1985) reported the reliability of this scale with Cronbach's alpha in the range of 0.78 to 0.85. In this study, the Cronbach's alpha reliability coefficient of this questionnaire was 0.79.

Data Analysis

Methods for analyzing the data were: Bivariate Correlation Analysis for mean, standard deviation and correlation between the research Variables, Structural Equation Modelling for model fit indices and direct effect of the variables, and Process Macro Bootstrapping for the indirect effect of the variables.

RESUTLS

Descriptive findings

Descriptive statistics for the four variables of this research such as mean, standard deviation and their inter correlations can be seen in **Table 1.**

Table 1: Mean, Standard Deviation and Correlation between research Variables

Variable	*M*	*SD*	1	2	3	4
Mother calling	40.34	2.64	1			
Mother's psychological well-being	92.98	10.02	.59**	1		
Satisfaction with being a mother	18.41	1.85	.51**	.59**	1	
Children's satisfaction with life	94.04	9.27	.68**	.78**	.66**	1

****p < 0.05**

According to **Table 1**, there are Positive correlations among all variables. For example, between the independent variable of mothers calling in child rearing with the mediator variables of psychological well-being and maternal satisfaction with being mother; between the independent variable of mothers calling in child rearing with dependent variable of child psychological well-being; and also between mediators (psychological well-being and maternal satisfaction of mothers) and dependent variable (children's psychological well-being) were positively correlated ($p < 0.01$).

Table 2: Fitness Indices of the Proposed General Model and the Modified Model

Fit indicators	CFI	IFI	TLI	RMSEA	*df*	x^2/df	x^2
Proposed model	.83	.83	.82	.06	1417	2.012	2850.604
Modified model	.97	.97	.96	.03	1160	1.180	1368.299

Based on the primary results in **Table 2**, CFI = 0.83, IFI = 0.83, and TLI = 0.82 show that the proposed model needs to be modified. According to the suggestions provided by the 24 version of AMOS for example, (between items 7 and 9) of mother calling (between items 9 and 12, 11 and 13) of mother's psychological well-being, and (between items 1 and 6, 9 and 12, 14 and 16) of child's psychological well-being due to high correlation coefficients two-way covariance was drawn between them. After applying the modification, the data were re-analyzed. The modified results showed that the fitness indices reached an excellent model fit. As displayed in Table 2 and Figure 2, CFI = 0.97, IFI = 0.97, and TLI = 0.96, and RMSEA =0.03

According to the result of **Table 3**. The parameters related to the direct effects of the variables on each other in the modified model of the present study show that all direct paths are positive and significant (p< 0. 05).

Table 3: Direct Standardized Coefficients between Research Variables in the Modified Model

Paths	Effect	C.R.	P
Mother calling to Mother psychological well-being	.812	5.966	.001
Mother calling to Mother satisfaction	.750	5.978	.001
Mother calling to Child psychological well-being	.542	3.501	.001
Mother psychological well-being to Child psychological well-being	.282	2.961	.003
Mother satisfaction Child psychological well-being	.211	2.591	.005

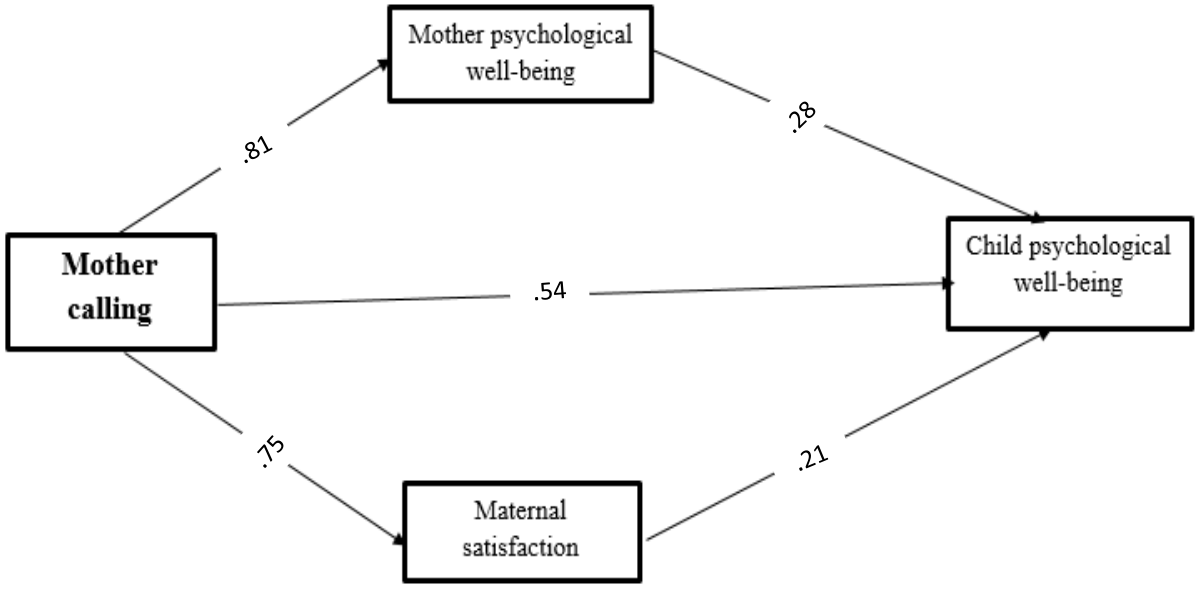

Fig. 2: Modified pattern and path coefficients

Indirect effect testing

The process macro for SPSS (Model 4) bootstrapping method was used to test the indirect effect of mother calling mediated by psychological well-being and maternal satisfaction with being mother on child psychological well-being. As Hayes (2018) proposed for multiple mediations model, the analysis was performed with 95% confidence intervals (CI) and 5,000 bootstraps sample for percentile bootstrap. Process macro approach was the most suitable for analyzing this study because it does not require assumptions of normality. If the bootstrap's lower level CI and upper level of CI does not contain zero, then it is considered significant.

Table 4: Bootstrap completely standardized indirect effects Results

Paths	Effect	SE	95% CI	
			LL	UL
Total indirect effect, sum of all below	.4076	.0532	.3152	.5230
Mother calling → Mother psychological well-being →Child psychological well-being	.2811	.0619	.1805	.4215
Mother calling →Mother satisfaction →Child psychological well-being	.1265	.0310	.0596	.1802

As can be seen in **Table 4,** the completely standardized indirect effects of mother calling mediated by psychological wellbeing and maternal satisfaction of mothers on children psychological wellbeing paths are positive and significant, because the lower and upper level of the CI does not include zero, so it can be said that the selected mediators were able to explain the relationship between antecedent and outcome variables in a good way.

DISCUSSION

Explanation of research hypotheses

There is a direct and positive relationship between sense of mothers calling in child rearing and their psychological wellbeing. The results of data analysis showed that the mothers sense of calling in child rearing have positive and significant effect on their psychological wellbeing. Therefore, hypothesis 1 of the research was confirmed. This finding is in line with the results of Coulson (2011) and Duffy et al. (2012).

Basically, the sense of calling in child rearing make these mothers evaluate parenting as a positive, useful and valuable task. In this case, they do not consider parenting to be the cause of their physical and mental exhaustion, but they consider doing this work as a duty and a kind of success in life. This positive attitude towards parenting can lead to a positive self-evaluation, and in this way, their psychological well-being increases. It can be said that these mothers with a sense of calling consider child rearing the main purposeful factor in their life, they experience intimate and warm relationships with their children with a lot of trust, a positive feeling in doing daily activities, they live for the present and plan the future well, which increases their psychological well-being by doing such a process. People, who see their lives as purposeful and meaningful, live longer, are healthier, and happier than those who do not see the meaningful life due to their motivational and self-regulating nature (Vail & Routledge, 2020). Gazica and Spector (2015) found that people who have high sense of calling in their jobs have better physical and psychological health than people who do not feel calling.

There is a direct and positive relationship between sense of mother calling in child rearing and their satisfaction with being mother. The results of the analysis of the present research have shown that the mothers' sense of calling in child rearing have a significant positive effect on their satisfaction with being mothers. Therefore, hypothesis 2 of the present study was confirmed. This finding is similar with Coulson (2011) results, who stated that there is a significant positive relationship between parenting calling and their satisfaction with parenthood.

One of the main reason for the increase in satisfaction with being mothers with the sense of the calling in child rearing is that probably these mothers consider parenting as their natural purpose. In this way, they may establish a proper relationship with their children, as a result of which they are satisfied with their parental role and their children's behavior. It can be said that these mothers, with a sense of calling, consider raising children as a supreme task. So, this work gives them inner satisfaction and satisfaction, and this increases their satisfaction with being mothers. According to the theory of life, becoming a parent is a basic goal in the surface of earth and the arrival of a child is considered the success of parents, which make them feel proud and satisfied. Parenthood is central to a meaningful and fulfilling life, and the lives of those without children are emptier, less rewarding, and lonelier than those of parents (Hansen, 2012). The positive theory related to the satisfaction of being a parent states that children provide the economic, self-determination, emotional and social needs of parents. Therefore, the birth of children increases the satisfaction of parents.

There is a direct and positive relationship between sense of mothers calling and their children psychological wellbeing. The results of data analysis showed that the mothers sense of calling in child rearing have positive and

significant effect on their children psychological wellbeing. Therefore, hypothesis 3 of the research was confirmed. This finding is similar with the results of Coulson (2011). The core reason for increasing children's psychological well-being with mothers calling is that these mothers feel motherhood is the ultimate goal of life and a key part of their identity. The more mothers relate the sense of calling in child rearing to the ultimate goal of life, the more time they spend in caring and raising for their children, which will increase their children's psychological well-being. Hall and Chandler (2005) state that a sense of calling means that people understand the purpose of their lives. Mothers who feel calling in child rearing have specific goals for raising children. For example, they consider raising children as their ultimate goal and their contribution to the world. Therefore, it is possible for them to communicate with their children in appropriate ways that are enjoyable and satisfying for their children.

There is a direct and positive relationship between the psychological well-being of the mother and the psychological well-being of the children. The results of the present research have shown that the psychological well-being of the mothers have a positive and significant effect on the psychological well-being of their children. Therefore, hypothesis 4 of the current research was confirmed. This finding is consistent with the research of Coles and Cage (2022), Dickerson (2021), and Khurshid et al. (2016). These researchers stated that the psychological well-being of mothers shows a strong and inseparable relationship with the psychological well-being of their children. These mothers may have warm relationships with their children due to their psychological well-being. They would properly manage their children's daily life needs, appropriately plan the future of their children, consider the values that are important for their children, and might always try to improve their children's lives. Another reason is that women who have more psychological well-being may pass these psychological well-being characteristics to their children naturally. Newland (2015) also stated that parents' well-being and life satisfaction have a positive effect on children's emotional and behavioral health. Mother-child relationships are a good model for increasing children's well-being. Positive parental behavior, such as mother's warmth and sensitivity, has many benefits for children's behavioral and emotional health (Doty & Mortimer, 2018).

There is a direct and positive relationship between the satisfaction of being a mother and the psychological well-being of their children. The results of the present research showed that the satisfaction with being mothers has a significant positive effect on the psychological well-being of children. Therefore, hypothesis 5 of the present study was confirmed. These findings are consistent with the research of Richter et al. (2018) and Newland (2015). Satisfaction with being a mother causes mothers to spend the appropriate time with raising their children. This allocating enough time of mothers in child rearing might increase the chance of their children's psychological well-being. Women who are more satisfied with their motherhood, they convey their satisfaction and positive emotions to their children. They interact well with the family, educate children better, prioritize their children, and upgrade their children healthier (Diener, 2011). Mothers who have satisfaction and competence in motherhood have a positive effect on the psychological well-being and life satisfaction of their children (Devito, 2010). In fact, the well-being of the family is the main basis for the healthier lives of children. The well-being and satisfaction with being a mother affects the emotional and behavioral health of their children (Newland, 2015).

There is an indirect relationship between sense of mother calling and child psychological well-being through the mother psychological well-being. The results of the analysis of the present research have shown that the sense of mother calling in child rearing has an indirect relationship with the psychological well-being of their children through the psychological well-being of the mother. Therefore, hypothesis 6 of the current research is confirmed.

These findings are consistent with the results of existing studies (Wrzesniewski et al., 1997; Coulson, 2011; Duffy et al., 2012). These researchers have shown that people who have a sense of calling might also have psychological well-being. People who have a sense of calling in their work, their level of psychological well-being would be better and absenteeism from work would decrease (Wrzesniewski et al., 1997). Newland (2015) defines "family well-being" as the foundation of parenting development and child well-being. Therefore, the way parents treat their children and participate in the joint activities affects children's development. Mother's characteristics, such as well-being and life satisfaction, affect children's emotional and behavioral health. Droogmans et al. (2010) indicated that happy parents are good models for their children. Families and especially mother-child relationships are of fundamental importance in the child's well-being. Positive parental behavior, such as mother's warmth and sensitivity, has many benefits for children's psychological well-being (Doty & Mortimer, 2018).

There is an indirect relationship between sense of mother calling and child psychological well-being through the satisfaction with being a mother. The results of this research showed that the sense of mother calling in childrearing have an indirect relationship with the psychological well-being of their children through the satisfaction of being mother. Therefore, hypothesis 7 of the current research is confirmed. Satisfaction with being a mother is a partial mediator in the relationship between sense of mother calling and children's psychological well-being. This finding is consistent with the results of the researches of Coulson (2011), Coulson et al. (2012). When mothers with a sense of calling consider parenting as a natural and final goal in life, it is likely that they would be satisfied with their role as parents at a higher level, and as a result, they will establish a proper and warm relationship with their children, which might increase their children's psychological well-being. Satisfaction with being mother cause mothers to spend the appropriate time in children rearing, this allocation of sufficient time in to children rearing might increases the child Psychological well-being. Satisfaction with motherhood has a positive and significant relationship with reducing children's depression. Women who are more satisfied with their motherhood convey their satisfaction and positive emotions to their children. They interact well with the family, raise children better, prioritize their children and raise their children better (Diener, 2011). Mothers who have satisfaction with being mothers and capability also they have a positive effect on the psychological well-being and life satisfaction of their children (Devito, 2010). Newland (2015) stated that family well-being is the basis of parents' satisfaction, as well as children's well-being and life satisfaction. Mother's characteristics, such as well-being and satisfaction, may also affect children's psychological well-being.

Limitation of the study

The present research contains a number of limitations. First, this study was conducted in one city, so in generalizing the results to other cities should be considered. Second, since the tools used in this study were questionnaires, and thereby the limitations of these tools should be considered, including the fact that despite the necessary emphasis and explanation by the researchers, some participants may not provide honest answer instead give a superficial and inaccurate answer. Third, the research design is cross-sectional, and the caution should be considered in interpreting the findings based on cause-and-effect relationships.

Theoretical suggestions

It is suggested that this research be repeated in other cities so that the comparison between the findings of this research and other findings in other cities leads to a better understanding of the studied phenomena and leads to better conclusions in the field of sense of calling in child rearing.

It is suggested that the model of the current research should be conducted in longitudinal studies as well. Comparing the findings of this cross-sectional study with longitudinal findings leads to a better understanding of the studied phenomena and leads to better conclusions in the field of calling in child rearing.

It is suggested to study the effect of a parent calling in child rearing on other variables of parent such as, parenting methods.

It is suggested to investigate the effect of a parent calling in child rearing on other children-related variables such as academic performance.

It is suggested to use other variables for sense of calling in child rearing such as the father's role.

Practical suggestions

According to the results of this research and the effect of mother calling in child rearing on the psychological well-being of their children, it is suggested that governmental and non-governmental organizations plan providing a training program to increase the sense of calling in women to learn necessary skills and able the mothers for reaching the needs of their children with awareness and passion.

According to the result of this research that shows mother psychological well-being has a direct effect on children's psychological well-being, it is suggested to take appropriate steps toward educating women to increase their psychological well-being.

According to the result of this research that showed mother satisfaction with being a mother has a direct effect on children's psychological well-being, it is suggested to take appropriate steps toward educating women to increase their satisfaction with being mothers.

CONCULSION

The findings of the present study showed that the designed model about the relationship between sense of calling in child rearing and child psychological well-being mediated by psychological well-being and satisfaction with being mothers in female teachers had excellent fit indices. In addition, all direct and indirect paths of mothers calling to children's psychological well-being were positive and significant. It means that the six hypotheses of this study are confirmed. The result of the present study shows that women's sense of calling in child rearing have positive effects on their psychological well-being as well as their satisfaction with being mothers. Additionally, the mother's calling can directly increase children's psychological well-being. Of course, this increase was also done indirectly mediated with psychological well-being, and satisfaction with being mothers. The mediation model of this study confirms that women with sense of calling have double effects on their children psychological well-being. They understand their actual parenting roles that increase their psychological wellbeing and satisfaction with being mothers. They contribute and increase their children’s psychological wellbeing.

According to the results of this study, it is suggested that women should become more familiar with their roles as having calling in child rearing to increase their psychological well-being and satisfaction with being mother as well as their children’s psychological well-being.

Conflicts of Interest

The authors declare no conflict of interest.

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Genetic Variations of Two Elite Tomato Varieties Yield and its Parameters in Nangarhar, Afghanistan

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ABSTRACT

Background: Tomato is considered as one of the most import crop in food industry due to its great health benefits in all over the world; especially in Afghanistan as it has been significant rule on boosting of balance of trade. This Study indicated the field experiment was conducted to evaluate the potential productivity of two elite tomato varieties, CXD_222 and Roma_VF, in Nangarhar, Afghanistan.

Materials and Methods: The experiment was conducted in a Randomized Complete Block Design (RCBD) with four replications in 2018 and 2019. Yield and yield parameters were measured at the red-ripe maturity stage.

Findings: The results revealed that CXD_222 produced significantly higher yield than Roma VF. Branch number were not significantly different; however, CXD_222 had significantly higher number of fruits per plants than Roma_VF. CXD_222 produced 3.72 and 3.88 kg tomato in 2018 and 2019, respectively whereas the yield was 2.5 and 2.63 kg in Roma_VF for the respective years. There was a strong correlation between fruit number per plant and fruit yield in both varieties which suggests that higher productivity in CXD_222 is solely due its capacity of producing higher number of fruits.

Conclusion: This study indicates that CXD_222 is a viable alternative to the long-used Roma_VF tomato variety.

Keywords: Tomato Yield; CXD_222; Roma_VF; Genetic Variation; Nangarhar

INTRODUCTION

Cultivated tomato (Solanum Lycopersicum L.) belongs to the Solanaceae family and is classified in the Lycopersicon section of Solanum. Tomato is one of the important globally consumed crop. More than 141.4 x 10^6 tons of tomato was harvested worldwide in 2009 (Olander et al., 2013). It is characterized to be available year-round and has significant health benefits. Tomato was first domesticated by the Native Americans and there is a possibility that the origin of tomato is Mexico. No one really knows how the current big fruit size occurred through evolution because its domestication happened in pre-historic times. It appears; however, that the current big size of the tomato is due to efforts of the Native Americans who were looking for mutated plants with big fruits.

Several factors including variety, sunlight, temperature, pollinators, water supply, nutrients and others determine the yield and health benefits of tomato (Dorais, 2005). In 2004, It was reported that tomato was cultivated on about 7,940 ha land and produced up to 85000 tons harvestable yield in Afghanistan (Abbas et al., 2012). The Food and Agriculture Organization (FAO) stated that tomato accounted up to 16% of horticulture crops in Afghanistan (Masini & Giordani, 2016). For having large quantity of water (Abraham et al., 2011), this plant is very sensitive to several factors and can easily perish (Nasrin et al., 2008). Moreover, poor cultural practices and post-harvest management can cause great losses (Rahman & Hossain, 2005).

Although over 80 percent of population is engaged in agriculture, Afghanistan is not self-sufficient in terms of tomato production; therefore, it relies on neighboring countries especially Pakistan to meet the tomato demand of the people. This is largely due to the long-lasting civil war that has destroyed agricultural infrastructures and research centers (Gulab et. al., 2020).

A number of factors challenge the production of tomato. The biggest challenge comes from the lack of infrastructures and research (Gulab et al., 2020). Currently, farmers rely on the seeds and seedling that are available in market or they use the seed of crops they harvest in previous season. Poor seed and seedling quality thus pose another challenge toward greater yield production in the country. To overcome this challenge, a study was required to test the productivity of recently imported tomato varieties. The Campbell varieties were reported to be the highest yielding genotypes in a study conducted in Balkh, Afghanistan (Mark, 2006) whereas the Roma_VF which is the widely grown variety of tomato had poor yield performance. The yield performance of these varieties, however, has not been checked in Nangarhar, Afghanistan which is famous for vegetable gardening. The study to assess the genetic yield potential of CXD_222 and Roma_VF tomato varieties in a stress-free environment in Nangarhar, Afghanistan and as study estimates the effect of variables as well compare the two varieties.

MATERIALS AND METHODS

Planting materials

Seeds were acquired from a trusted seed supplier in the market. Small seeding trays were filled with 2/3 sandy loam soil and 1/3 compost. The seeds were sown in these trays in spring 2018 and 2019. For a faster growth, we dissolved 20 g of urea in water and applied in a square meter. The trays were stored in a tunnel shaped plastic green house and were irrigated every other day. We used the Random Complete Block Design with four replications where each plot was 3 X 3 m. After the unfolding of 3rd true leaf (5 weeks after sowing), the seedlings were transplanted to field with already prepared furrows. The soil type was clay loam in the field with

a PH of 7.5. The centers of the furrows were 50 cm apart and each row was roughly 15 cm high. Planting space between seedlings in a row was 50 cm. An 80:60 $kgha^{-1}$ratio of Urea and DAP was applied to avoid nutrient problems. Total DAP and half of Urea was applied during transplanting and the remaining half of the urea was applied 30 days after transplanting. The seedlings were pruned slightly in order to avoid bushy plants and fruit setting close to the ground. We irrigated the field every week. Pesticides were used as necessary.

Yield Measurement

A two square meter area in the center of each plot was fenced with a string. This area then divided by two equal sections in order to increase the sampling size and reduce the variability. Border plants were not used in this study because they can be affected by several factors. Tomato were collected as soon as they reached the red-ripe maturity stage. Total yield per plant was the sum of all the fruits we harvested from the same plant. Every time the tomato was harvested, they were weighed and recorded. We measured the weight of four tomatoes in each harvested batch. The number of fruit bearing branches were also recorded after the fruit was harvested. The average value of these parameters per each square meter was used as the final observation for statistical procedures.

Statistical Analysis

We used Two-way analysis of variance (ANOVA), Tukey's test of multiple comparison (Tukey HSD), and Pearson's correlations test to validate the data. Two-way ANOVA was used between the different varieties, sowing years, and variety and sowing year interaction. We used Pandas, Numpy, Scipy and Pingouin libraries in python and Agricolae package (Mendiburu, 2015) in R for statistical procedures.

RESULTS

We observed significant difference in the yield performance of these two varieties; CXD_222 produced 3.72 kg and Roma_VF produced 2.5 kg fruit in square meter area in 2018, respectively. A slight increase in fruit yield was observed in both varieties in 2019 where CXD_222 had a fruit yield of 3.88 kg and Roma_VF produced 2.63 kg tomato. There was significant difference in yield per land area and per plant between the two varieties **(Fig. 1).**

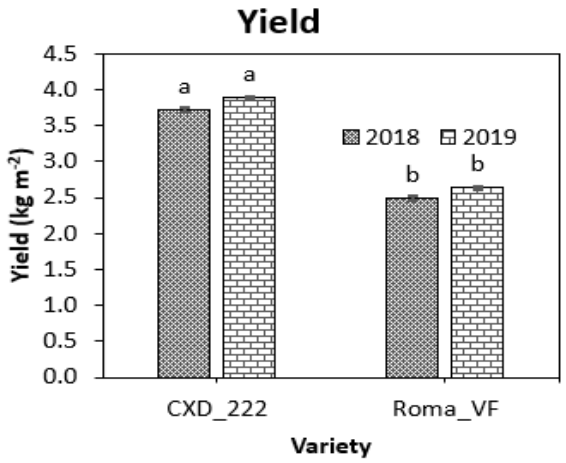

Fig.1. Average tomato yield in two elite tomato varieties. Bar show the mean of 16 observations. Standard error of sample is used to indicate the dispersion of the data from the mean. Letter on the top of each bar indicate level of significance.

. In 2018, CXD_222 produced significantly heavier fruits than Roma_VF whereas the difference was not significant between the two varieties in 2019. In 2018, the average fruit size of CXD_222 and Roma_VF was 98.31g and 97.54 g, respectively. A steep drop in the fruit size of CXD_222 was observed in 2019 whereas the fruit size of Roma_VF increase nearly two-fold. The average fruit size for CXD_222 and Roma_VF was 97.54 and 97.73 g, respectively. Although the Roma_VF produced heavier tomato in 2019, there was no significant difference between them **(Fig. 2).**

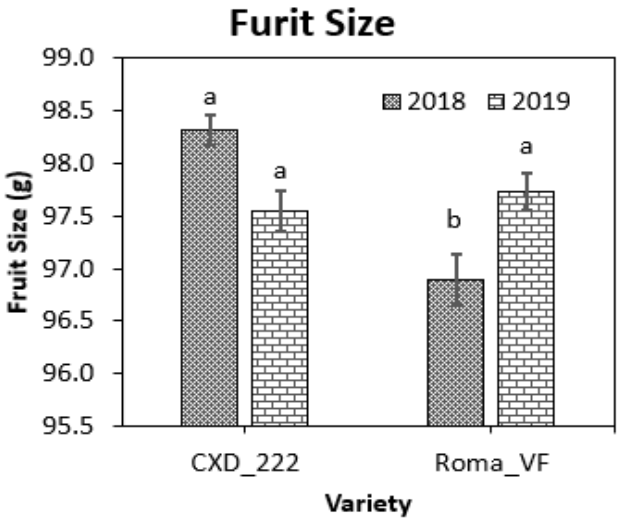

Fig. 2. Average fruit size is in gram. Bars show the mean of 16 observations. Standard error of sample is used to indicate the dispersion of the data. Letter on the top of each bar indicate level of significance between the varieties.

The fruit number per plant was observed and analyzed to be significantly higher in CXD_222 variety than Roma_VF in both growing years. An average, CXD_222 produced 9.47 and 9.95 tomato per plant in 2018 and 2019, respectively. On the contrary, Roma_VF had an average number of 6.45 and 6.74 tomato per plant. Comparing 2018, the fruit number was higher, but not significant for both varieties in 2019 **(Fig 3).**

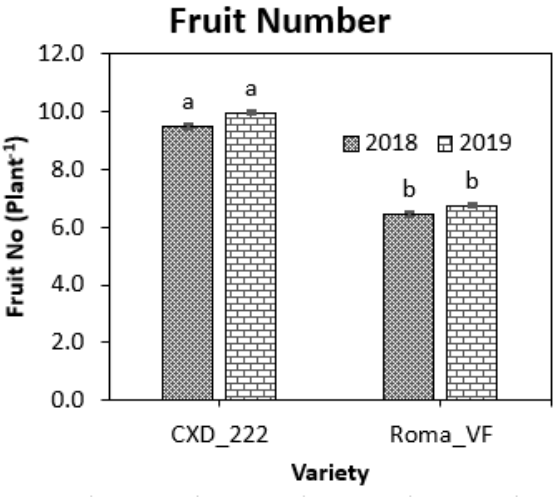

Fig.3. Average fruit number per each plant. Bars show the mean of 16 observations. Standard error of sample is used to indicate the dispersion of the data. Letter on the top of each bar indicate level of significance.

The following table summarizes some of the key yield parameters and statistical significance in both varieties. As can be seen, yield in square meter and yield per each plant was significantly higher in CXD_222 than Roma_VF in both years. The fruit size was higher in CXD_222 in 2018, but the difference was negligible in 2019. There was no significant difference in branch number between the two varieties. Fruit number per plant and fruit number per branch; however, remained significantly higher in CXD_222 than Roma_VF. Yield of fruit number per branch and fruit number per plant was higher in 2019 than 2018. The varietal and yearly interaction was seen in fruit size and branch number per plant, but it was not significant in other yield parameters.

Table 1. Analysis of variance on yield, fruit size, fruit number, branch number and fruit number per branch

Factor			Yield m^{-2}	Yield (g $plant^{-1}$)	Fruit Size (g)	Fruit No ($plant^{-1}$)	Branch NO ($plant^{-1}$)	Fruit No ($Branch^{-1}$)
Mean	Year (Y)	2018	3.11	778.44	97.60	7.96	2.91	2.74
		2019	3.26	815.01	97.63	8.34	2.90	2.87
	Variety (V)	CXD_222	3.80	951.57	97.93	9.71	2.92	3.33
		Roma_VF	2.56	641.91	97.31	6.59	2.89	2.28
	ANOVA	V	0.000***	0.000***	0.001**	0.000***	0.52*	0.000***
		Y	0.000***	0.000***	0.84^{ns}	0.000***	0.73^{ns}	0.003**
		V x Y	0.36^{ns}	0.43^{ns}	0.000***	0.16^{ns}	0.025*	0.35^{ns}

***= $\rho < 0.000$, **$\rho = 0.01$, * = $\rho < 0.05$, n.s.= not significant.

As **Fig. 4** and **Table 2** showed, the yield enhancement in both varieties was due to higher fruit set. Correlation matrix in a pooled data also showed a strong correlation between fruit size and yield but our analysis (data not shown) revealed that it was because of the higher fruit size of Roma_VF. This means that CXD_222 produced higher number of fruits in a plant. Therefore, it had yield superiority over Roma_VF. The fruit number was Relatively smaller in 2018 and it was due to the bigger fruit size. In fact, the cross-species fruit size had contribution to the yield enhancement in 2018, but the difference was negligible in 2019. Roma_VF produced smaller and fewer fruits in 2018 which could be due to continues hot weather that might have stressed the plant. With the ideal conditions of 2019; however, the fruit size was higher than CXD_222 although not significant. This indicates that if external factors do not play their role, the genetic yield potential in CXD_222 is solely to higher fruit setting rate (Fruit Number).

The final outcome of ANNOVA analysis of these variables consist, the fruit number outcome. There has been high association between the fruit size, fruit number and branch number. Furthermore, fruit number p value is 0.003, there is significant difference between other variables. Statistical test will be 0.003<0.005. Null hypothesis is rejected but there is significant difference between the other variables.

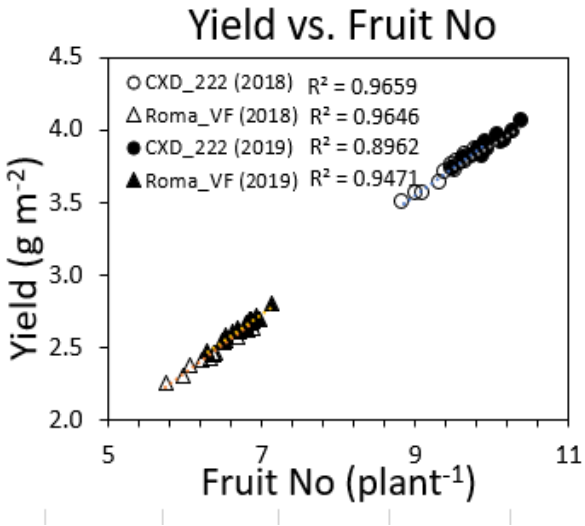

Fig.4.correlation between fruit No and Yield. Each correlation is drawn between 16 observations.

Table 2. Correlation between yield parameters. Correlation coefficients is drawn from an n=64 sample size. Cross-species and cross-year difference is not taken into consideration because the trend for yield remained the same for both years.

Correlation in Stacked Data

	Yield	Fruit Size	Fruit No	Branch No
Fruit Size	0.34**			
Fruit No	0.99***	0.30*		
Branch No	0.049	0.02	0.049	
Fruit branch^{-1}	0.97***	0.30*	0.97***	-0.16

*** = ρ < 0.000, ** = ρ < 0.001, * = ρ < 0.05

DISCUSSION

Variations in the genetic yield potential of tomato, with or without treatments, have been studied in several studies. Although great discrepancies exist in their results, recent release tomato varieties usually performed better than the old varieties (Amundson et. al., 2012; Gulab et al., 2020; Helyes et al., 2012; Tanksley, 2004). This is largely due to the selection and breeding of tomato genome for hunting highly productive genotypes (Tanksley, 2004). We conducted a study about two tomato varieties that are currently used in Afghanistan and around the world. The results revealed that CXD_222 which is one of the elite genotype from the Campbell variety produced significantly higher yield and was consistent in our two years long research. The average yield potential of CXD_222 for 2018 and 2019 was 38.0 metric tons in a ha. On the contrary, the Roma_VF produced 25.67 metric tons in a ha. Our study is consistent with the finding of Mark, (2006) but the magnitude at which

we produced tomato was drastically higher. He reported 27.7 tons of yield in a Hectare (ha) for the CXD_222 and 18.8 tons for Roma_VF which is indeed not as good as in our findings. This difference could be related to the better cultural practices and management techniques we applied in our research.

ANOVA test in Table 1 showed the interaction of year and variety in fruit size as well as branch number. This means that the fruit size and branch number per plant are not stable factors and are subjected to changes in different seasons. It has been well established in nearly all crops that a higher number of fruit production is usually associated with smaller fruit size and thus the smaller fruit size in CXD_222 in 2019 was compensated with higher number of fruit setting rate. It appears that the year and variety interaction was due to the hot weather conditions in 2018 that has affected Roma_VF. A similar strange correlation existed between the fruit size and Fruit number and fruit number in branch, but this was only significant when the data of both varieties for both years were combined. For each genotypes, however, the correlation was only significant in 2019 for Roma_VF. Since there was no consistency between both years, this correlation can be safely ignored.

To the best of our knowledge, this is the first and best study that compares the genetic yield potential of two elite tomato varieties in Nangarhar, Afghanistan. No study has reported yield potential of 38.06 metric tons in a Hectare (ha) in Afghanistan which suggests our study was a breakthrough in seeking the path for higher tomato productivity. Although the yield is astonishing on the country level, it is still far lower when compared with tomato productivity in countries such as the United States, Israel, Greece, France, Chile and others. Further research is required in order to improve the cultural practices of tomato for yield optimization.

CONCLUSION

This study was conducted to evaluate the genetic yield potential of two elite tomato varieties in Nangarhar, Afghanistan. The CXD_222 variety showed significantly higher yield than Roma_VF. Studied parameters revealed that the yield enhancement in CXD_222 was due to higher number of fruit production per plant.

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The Use of Indirect Calorimetry in Nutrition Therapy and Its Impact on Clinical Outcomes in Critically ill Patients: A review

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ABSTRACT

Adequate nutritional support is an essential element for achieving favorable outcomes in critically ill patients, which requires an accurate evaluation of energy needs to avoid under or overfeeding. Energy requirements of critically ill patients can be assessed either by Predictive equations (PEs) or by indirect calorimetry(IC) measurements. However, assessment of energy expenditure (EE) is a challenging task in intensive care unit patients because EE during critical illness is widely variable and the current PEs are inaccurate to determine the caloric requirement in these patients. Currently, IC is considered a gold standard for measuring EE as recommended by guidelines. Despite being the most accurate method of measuring EE, adequate data are lacking to validate the beneficial effects of IC-guided nutrition therapy on clinical outcomes in critically ill patients. Because of this, the actual clinical benefits of IC are poorly appreciated, and it is still an underutilized tool among dietitians in clinical practice. Therefore, in many centers, PEs are commonly used instead. The purpose of this review is to summarize the findings of recent studies regarding IC-guided nutrition therapy and its impact on clinical outcomes in critically ill patients.

Keywords: Nutrition therapy; Critical Illness; Indirect Calorimetry; Energy Expenditure; Predictive equations

INTRODUCTION

Critical patients in the Intensive Care Unit (ICU) are at higher risk of malnutrition (Yeh et al. 2015). Which is significantly associated with poorer clinical outcomes such as higher infection rate, increased morbidity, and mortality, longer hospital stay, increased health care costs, and reduced quality of life (Heyland et al., 2011; Sioson et al., 2017; Yeh et al., 2015). Combined with the higher prevalence of malnutrition in ICU (approximately 40 to 80%) (Lew et al. 2017; Wang et al. 2017). Hyper catabolism associated with critical illness may have further deleterious effects on outcomes when patients receive inadequate or inappropriate nutrition support (Delsoglio et al. 2019; Krishnan et al., 2003; Oshima et al., 2017).

The role of nutritional therapy during critical illness has been a focus of great interest in recent years, which is recognized as an essential component in the management of critically ill patients (Lambell et al., 2020; De Waele et al., 2019). Several published studies and two updated international clinical guidelines have shown that nutrition therapy has a positive impact on critically ill patients who meet their defined goals of caloric feeding (McClave et al. 2016; Singer et al. 2019). However, an inappropriate energy assessment can contribute to under- or overfeeding, resulting in deleterious effects (Heyland et al., 2011; Wichansawakun et al., 2014). Therefore, an accurate determination of EE is crucial in critically ill patients to optimize nutritional support and prevent negative effects from inappropriate feeding (Espinoza et al., 2016; Rattanachaiwong and Singer 2018; Wichansawakun et al., 2014). The provision of energy and protein is considered an integral part of optimal nutritional therapy (Ridley, Gantner, and Pellegrino 2015). However, the optimal amount of energy and protein required by critically ill patients to reduce morbidity and mortality is controversial (Wang et al., 2017). Some observational studies have shown that underfeeding or caloric debt is associated with adverse clinical outcomes in critically ill patients (Alberda et al., 2009; Elke et al., 2014; Heyland et al., 2011; Nicolo et al., 2015; Wei et al., 2015). In contrast, other studies have suggested better short-term outcomes in patients receiving low caloric intake (Arabi et al., 2010; Krishnan et al., 2003). “Besides this, the findings of the study by Zusman et al. (2016) suggested that both underfeeding and overfeeding appear to be harmful to critically ill patients (Zusman et al., 2016). Therefore, determination of energy requirements has vital importance as prescribed targets are used to guide nutrition delivery (Stapel et al., 2018; Tatucu-Babet, Ridley, and Tierney, 2016). However, accurately determining energy needs in critically ill patients is difficult because the effects that disease, injury, and stress have on REE are often varied and unpredictable”(Espinoza et al., 2016; Singer and Singer 2016; De Waele et al., 2019). “In clinical practice, PEs that estimate EE are the most commonly used method due to their ease of application; however, these equations have repeatedly proven inaccurate (Tatucu-Babet, Ridley, and Tierney 2016; Waele et al., 2016; Zusman et al., 2018). Importantly, these equations are not generally validated in those with higher nutritional risk, and its inaccuracies increase in obese, elderly, most severely unwell, and more malnourished populations (Reeves and Capra, 2003; Tatucu-Babet et al., 2016) and can lead to underfeeding or overfeeding (Ladd et al., 2018). Therefore, to feed critically ill patients adequately, an exact estimate of caloric goals is required, which is ideally performed using IC (Zusman et al., 2016; Gonzalez-granda et al., 2018; Oshima et al., 2019).

IC is currently the gold standard for measuring REE in critically ill patients (Gonzalez-granda et al., 2018; McClave et al., 2003; Oshima et al., 2017; Sioson et al., 2017). IC is a non-invasive method that precisely measures REE by measuring the volume of O2 (VO2) consumed, and the volume of CO2 (VCO2) produced and then, by using the Weir equation, REE calculates as follows (Espinoza et al., 2016; Das Gupta et al., 2017; Haugen

and Li, 2007; Oshima et al., 2017; Stapel et al., 2018):

REE (kcal/day) = [(3.9 x VO2) + (1.1 x VCO2) – 61] x 1440.

Both the European (ESPEN) and American (ASPEN/SCCM) clinical practice guidelines recommend the use of IC to measure EE (McClave, et al. 2016; Singer et al. 2019). However, despite recent technological advancement in metabolic carts, these recommendations have poorly implemented in practice to date (Lev, and Singer 2010; Oshima et al. 2017) and many ICU clinicians still apply weight-based formulas to calculate EE (Singer and Singer 2016; De Waele et al. 2019). whereas the use of IC to guide nutrition therapy is limited yet, and its impact on clinical outcomes is not entirely proven. This narrative review aims summarize the recent findings on IC guided nutrition therapy and its impact on clinical outcomes in critically ill patients and provide recommendations for clinical practice.

MATERIALS AND METHODS

The literature search was conducted in electronic databases, i.e., PubMed and Google Scholars." All randomized Studies included in this narrative review were conducted on critically ill patients who were adults (≥18 years old), mechanically ventilated, admitted to ICU, with more than 48 hr stay in the ICU.

In all included studies, IC was used to guide nutrition therapy in the intervention group and compared to a standard care group where a predictive equation was used to estimate energy requirement in adult critically ill patients. For understanding the impact of IC on clinical outcomes, "studies must report the percentages of energy received and at least one of the relevant clinical outcomes such as mortality, infectious complications, length of ICU, and hospital stay or duration of mechanical ventilation as primary or secondary outcomes. Non-randomized studies and Studies in non-critically ill patients who were not incubated were excluded."

Effects of indirect calorimetry-guided nutrition therapy on clinical outcomes

Despite the guidelines recommendations, for indirect calorimeter has been given little attention in the clinical practice. To date, only five randomized controlled trials have investigated the impact of IC-guided nutrition therapy on clinical outcomes and compared to energy delivery using weight base equations. Generally, all studies have shown conflicting results regarding the impact of IC on clinical outcomes in critically ill patients. However, some benefits of IC were shown in 2 trials.

Firstly, Saffle study in 1990 compared the effectiveness of IC guided enteral nutrition to enteral nutrition guided by Curreri formula in burn patients. This study found no differences in-hospital mortality, hospital LOS between the group that received IC-guided enteral nutrition and the group that received enteral nutrition guided by Curreri formula (RR 1.33, 95% CI 0.24, 7.26, p=0.74*; Saffle, Larson, and Sullivan 1990). On the other hand, Singer et al. (2011), in the pilot TICACOS study, randomized 112 patients to IC guided enteral nutrition (study group) and enteral nutrition determined by a weight-based formula (control group). Supplemental PN was used to achieve the energy targets in the study group when necessary." The mean target EE between IC and SC groups was (1976 versus 1838kcal), mean energy delivered was higher in the IC-guided group compare to SC group (2086 vs 1480kcal, respectively). "The study found a trend toward reduced hospital mortality in-hospital mortality in patients that received IC guided enteral nutrition compared to patients that received enteral nutrition determined by a weight-based formula (32.3% in study group vs 47.7% in the control group, p = 0.058)." However, the

number of mechanical ventilation days (16.1 ± 14.7 vs 10.5 ± 8.3 days, p = 0.03) and length of ICU Stay (17.2 ± 14.6 vs 11.7 ± 8.4 days, p = 0.04) were significantly increased in the intervention group compared to the control group. No differences were observed in ICU mortality between both groups (Lev et al. 2011). Furthermore, recently, Landes et al. (2016), randomized 27 patients to the physician-directed control group and to the IC-directed study group the results of this study showed that delivery of nutrition in both groups was suboptimal, with all patients receiving only 82.0 ±15.4 % of caloric requirements and there were no differences between groups regarding outcome (duration mechanical ventilation, healing of pressure sores) (Landes et al. 2016).. In another more recent published and slightly larger trail (EAT-ICU) assigned 203 patients to receive either IC-guided nutritional support or a simple weight-based equation. They found no difference in mortality, duration of ICU., infectious complications, and quality of life at six months (Lange et al. 2017). Similarly, in another pilot study (ONCA Study) by Gonzalez-granda et al. (2018), 40 mechanically ventilated patients were randomized into a group in which their energy requirements were determined by IC (IC group) and a group in which energy needs were calculated with a weight-based formula as standard care (SC group). The finding of this study showed that the IC group achieved 98% of their energy goal, whereas the SC group reached only 79% of the energy target." No statistically significant differences were observed between groups in the primary outcome of change in bioelectrical impedance phase angle (related to nutritional status and prognosis). Besides, a shorter length of ICU stay was observed in the IC group than in the SC group (13±8 vs 24±20 days, $P < 0.05$). In contrast, a non-significant increase in-hospital mortality was reported in the IC group while no differences in ICU mortality, hospital LOS, and duration of MV were observed between the two groups (Gonzalez-granda et al. 2018).

Table 1: Randomized control trials on IC guided nutritional therapy

First Author, Year Country	Saffle, 1990 (Anon)	Singer et al., 2011 (Israel)	Landes et al., 2016 (United State)	Allingstrup et al., 2017, (Denmark)	Gonzalez-Granda et al., 2018, (Germany)
Study Design	Prospective single-center RCT	Prospective single-center RCT	Prospective single-center RCT	Prospective single-center RCT	Prospective single-center RCT
No of hospital ICU., population	49 burned patients	130 MV patients General-ICU	27 long term MV patients	203 MV patients Medical ICU	40 MV patients Medical ICU
Age, years (mean±SD)	IC: 29.2±1.9 SC: 36.8± 1.9	IC: 59±18 SC:62±17	IC:72±7 SC: 74±10	IC:62±16 SC:65±17	IC: 57±16 SC: 56±14
BMI (kg/m^2) (mean±SD)	NR	IC: 27.8±6.3 SC: 27.4±7.3	IC: 25.3±6.4 SC: 25.7±7.5	IC: 22.7±4.5 SC: 22±3.8	IC:27.8±6.2 SC:25.0±4.3
APACHE II Score	NR	IC:22.1±7.4 SC:22.4±6.8	IC: 34.7±12.0 SC: 38.7±13.4	NR	IC: 27.1±7.0 SC: 28.9±8.3
SOFA	NR	IC: 6.4±2.9 SC: 6.6±3.5	NR	IC: 8(6-11)* SC: 8(5-10)	IC: 12.1±3.3 SC: 11.4±3.0
Mean energy requirement (kcal/day)	IC: 2764±97.7 SC: 3913±96.7	IC: 1976±468 SC: 1838±468	IC: 1976.2±481.1 SC:2067.33±340.8 IC: 86.5%±12% SC: 77%±18%	IC: 2069(1816-2380) SC: 1887(1674-2244)	NR
Mean energy delivered (kcal/day)	IC:3530±134.1 SC:3490±132.1	IC: 2086±460 SC: 1480±356 IC: 21(32) SC: 31(48)	NR	IC: 1877(1567-2254) SC: 1061(745-1470)	IC:98%±8% IC:98%±8%
Hospital Mortality N(%)	IC: 3(11.6) SC: 2(8.7)	IC: 16(25) SC: 17(26)	NR	NR	IC: 5(25) SC: 3(15)
Mortality (ICU) (%)	NR	IC:17±15 SC: 12±8	NR	NR	IC: 3(15) SC: 3(15)
LOS (ICU), Days (mean±SD)	NR	IC: 16±15 SC: 11±8	IC: 49±22 SC: 46±31	IC:7(5-22) SC:7(4-11)	IC:13±8 SC:24±20
Duration of MV, Days (mean±SD)	NR	IC: 34±23 SC: 32±27	NR	NR	IC:9±8 SC:10±5
LOS (Hospital) (days),(mean±SD	IC: 48.8±4.5 SC: 48.5±5.2			IC:30(12-53) SC:34(14-53)	IC:31±24 SC:40±23

Abbreviations: IC, indirect calorimetry; SC, standard care; NR, not reported; MV, mechanical ventilation; LOS, length of stay; ICU, intensive care unit.

The recent data from RCTs have shown conflicting results regarding the benefit of using IC to guide nutrition therapy over PEs on clinical outcomes in critically ill patients. Singer at al. (2011) reported a non-significant reduction in-hospital mortality in the IC group while in the study by Gonzalez-granda et al. (2018) showed a non-significant increase in in-hospital mortality compared to a standard care group. Meanwhile, no differences in ICU mortality and hospital LOS were found between IC and SC groups in any study. Furthermore, TICACOS is the

only study that revealed an increased in the mean duration of MV in IC versus PEs groups. Besides, similarly to other outcomes, mixed findings were reported for LOS-ICU. A significant reduction in LOS-ICU was shown by Gonzalez-Granda et al. (2018) while Singer at al. (2011) and Allingstrup et al. (2017) conversely found a significant increase in LOS-ICU. As well as compare to other studies, only Singer at al. (2011) investigated outcome related to infectious complications the study demonstrated a significant increase in infection rate with a trend for an increased incidence of VAP (ventilator-associated pneumonia) in the study group (27.7 vs 13.8%; p = 0.08). No studies investigated muscle wasting during ICU stay in critically ill patients.

In summary, due to the contradictory findings in these recent RCTs, the impact of IC guided nutrition therapy on clinical outcomes has not been fully observed. Although across all studies, the IC guided group achieved higher calorie intake compared to the SC group. Which indicates that energy delivery close to measured EE only can be achieved when IC is used, thereby minimizing negative energy balance and the risk of inadequate or excessive energy delivery. It could be a good reason for its routine use for measuring EE in nutrition therapy. On the other hand, with recent advances in technology, indirect calorimeters are now easier to operate, more portable, and affordable. Unfortunately, still, it is an underutilized tool among dietitians in critical care settings and conventionally is used in patients in whom altered EE is suspected, or the nutritional support based on PEs fails to respond. The routine implementation of this device in clinical practice will not be justified until further data is available to support the current guidelines recommendations.

Conclusion and Recommendation

The use of IC to direct nutritional care is limited, and its benefits are poorly appreciated in clinical practice because there is still a lack of conclusive data to show the clinical benefits of nutritional therapy guided by IC compare to PEs. In this regard, the results of existing RCTs had not resulted in greater clarity, as both benefits and harm have been demonstrated when IC guided nutrition therapy, which is not justifiable for its routine use in clinical care settings. Moreover, it also declared from the recent findings that IC could only effectively guide nutrition when the measured calorie administered, which needs an effective strategy to deliver the prescribed calories and optimize the advantages of nutritional support.

In most countries data regarding the impact of IC guided nutrition therapy on clinical outcomes is still lacking and is less commonly used in practice. It is suggested that respective institutions adopt IC and pay attention to the usage and implementation of this device in their routine feeding protocol to optimize nutrition therapy. This review will assist in developing a better understanding of the influences of IC on clinical outcomes in critically ill patients. Further research, specifically adequately powered multicenter RCTs, is also needed to investigate the impact of the IC guided nutrition therapy on important clinical outcomes such as LOS. LMV, infection complications, and mortality.

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Optimizing Planting Density and the Use of Potassium Fertilizers for the Prospect of Enhancing Yield of Field Grown 'CXD_222' Tomatoes

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ABSTRACT

Background: Potassium fertilizer and optimum planting density are important factors for maximizing fruit yield in field grown tomatoes. We conducted a field study to evaluate the effects of planting density and potassium fertilizers on the yield performance of CXD_222 tomatoes.

Materials and Methods: We evaluating the fruit yield of CXD_222 tomatoes in NP + 50 (nitrogen and phosphorus in a 50 x 50 cm planting pattern), NP + 40 (nitrogen and phosphorus in a 40 x 40 cm planting pattern), NPK + 50 (nitrogen, phosphorus, and potassium in a 50 x 50 cm planting pattern), and NPK + 40 (nitrogen, phosphorus, and potassium in a 40 x 40 cm planting pattern).

Findings: Wider spacing (NPK +50 and NP + 50) resulted in significantly heavier fruits per plants and produced more fruits per plants. Narrow spacing; however, produced significantly higher yield per square meter. The effect of potassium fertilizer was obvious in a sense that planting space treatments with potassium fertilizers produced significantly higher yield than the corresponding treatments without the application of potassium fertilizer. The highest yields of 5.2 and 4.75 kg m^{-2} were achieved in NPK + 40, NPK+50 and NP + 40, respectively whereas bigger and more fruits per plants were harvest from NPK + 50 and NP + 50, respectively.

Conclusion: This study indicates that optimizing planting space and the use of potassium fertilizers will significantly enhance fruit yield.

Keyword: Potassium Fertilizer; Planting Density; Yield; CXD_222: Tomatoes

INTRODUCTION

Tomato is the fourth most important fresh-market vegetable after potato, lettuce, and onions. It is rich in nutrients and offers several health benefits including weight loss, healthy skin, regulated blood pressure, diabetes prevention, and a healthy heart. Despite the significant health advantages, tomato was considered poisonous in the U.S around 200 years ago because it is related to the nightshade family (Mariya et al., 2020). Even though over 80% of its population is engaged in agriculture, Afghanistan is not self-sufficient in terms of tomato production; therefore, it relies on neighboring countries especially Pakistan to meet the tomato demand of the people. This is largely due to the long-lasting civil war that has destroyed agricultural infrastructures and research centers (Gulab et. al., 2020).

Several factors including variety, sunlight, temperature, pollinators, water supply, nutrients, and others determine the yield and health benefits of tomatoes (Dorais, 2007). In 2004, It was reported that tomato was cultivated on

about 7,940 ha land and produced up to 85,000 tons of harvestable yield in Afghanistan (MAIL, 2012). The Food and Agriculture Organization (FAO) stated that tomatoes accounted for up to 16% of horticulture crops in Afghanistan (GULAB et al., 2020). Because of having a large quantity of water (Gastelum et al., 2011), tomato is very sensitive to several factors and can easily perish (Nasrin et al., 2008). Moreover, poor cultural practices and post-harvest management can cause great losses (Rahman & Hossain, 2005).

Snyder (2007) states that plant density and pruning methods are important practices for achieving higher yield in tomatoes. He recommends a 4-4.3 ft-2 area for each tomato plant so it can grow optimally. Putting this to in-row and between row spacing, his recommended spacing is 13.7-15.7 and 4 ft, respectively. Similarly, field and greenhouse-grown tomatoes have shown different responses to different plant densities. Yield per plant was improved but yield per land was reduced in greenhouse-grown cherry tomato when the plant spacing was increased from 11.8 to 19.7 inches (Charlo et. al., 2007). High yield was achieved in heated greenhouses as the plant density was increased (Saglam and Yazgan, 1995). Although Kemble and colleagues (1994) didn't find any difference between fruit yield at 12 and 30 inches in-row spacing, higher yield in field-grown tomatoes was obtain with smaller in-row spacing (Santos et. al., 2010).

The yield response of tomato to animal manure and chemical fertilizers is well established (Ramyabharathi et al., 2014). Chapagain and Wiesman (200) reported that the use of Potassium (K) increases the growth of tomato plants and thus it results in higher fruit production. Potassium is essential to plant growth in several ways. As a major nutrient, it plays a great role in growth and development, activation of enzymes, energy consumption, photosynthesis, nutrient translocation, and water uptake (Havlin et. al., 2005). A yield enhancement of 35.55% over the control was reported in Ahmad and colleagues (2015) study when they applied a higher dose of 120 kg K2O/ha. Prajapati and Modi (2012) revealed that K improves the quality, disease resistance, and shelf-life of fruits. It was also reported that 375 kg ha of K2O resulted in 27.44% and 101.23% increase of total solids and soluble solids, respectively when compared to the control one (Javaria et. al., 2012).

In a previous study, we tested the seed yield of CXD_222 and Roma_VF in the field condition and found that the CXD_222 produced a significantly higher yield than the Roma_VF. In that study we used 50 x 50 cm planting space and did not use K. As stated in the literature, optimum planting space and K are extremely effective for fruit yield production in tomatoes. We hypothesized that reducing the planting space from 50 x 50 cm to 40 x 40 cm and the application of K fertilizers will improve yield in tomato. Therefore, the objective of the current study was to evaluate the yield potential of CXD_222 tomato variety with two planting densities and K fertilizer.

MATERIALS AND METHOD

Planting materials

A high yielding tomato variety, CXD_222, was chosen for this study. Seeding trays were filled with compost and sandy loam soil. Two seeds per hill were sown in the trays and were kept in a greenhouse in Sarkhroad, Nangarhar, Afghanistan. A 20 g m Urea was also used during seeding to assure the maximum growth rate of the seedling. The seedlings were then thinned to one after the first true leaf expanded. As soon as the seedling expanded the third true leaf, they were transplanted in the field. A randomized complete block design (RCBD) was assigned with two replications. The distance between the two replications was 500 m due to lack of land availability. Each plot was 3 x 12 m long. Two planting density patterns with and without K fertilizer were used

in each block which means each block had four plots. Our treatments consisted of NP + 50 (nitrogen and phosphorus in a 50 x 50 cm planting pattern), NP + 40 (nitrogen and phosphorus in a 40 x 40 cm planting pattern), NPK + 50 (nitrogen, phosphorus, and potassium in a 50 x 50 cm planting pattern), and NPK + 40 (nitrogen, phosphorus, and potassium in a 40 x 40 cm planting pattern). An 80:60:150 kg ha-1 ratio of Urea and DAP and K2O was applied. Total DAP and K2O, and half of N were applied during seedbed preparation. The remaining N was applied 30 days after transplanting. To avoid fruit setting close to the ground and have erected stems, with pruned the plants as they grew. Plants were irrigated on a regular basis.

Yield Measurement

We selected ten plants in the center of each plot for measuring yield and yield attributes. The fruit was harvested at the red-ripe maturity stage. Only the marketable tomatoes were used in the final yield measurements. Fruit weight per plant (FWPP) was the total of all fruits harvest from a single plant. Single fruit weight (SFW) was measured as the average weight of a single fruit in each plant. Fruit number per plant (FNPP) was measured as the number of all harvestable fruits per single plant.

Statistical Analysis

We used a two-way analysis of variance (ANOVA), tukey's test of multiple comparisons (Tukey, LSD), and Pearson's correlations test to validate the data. Two-way ANOVA was used between treatments, blocks, and treatment and block interaction. The reason for using a test between blocks and the interaction between block and treatments was due to the distance between two blocks. We used Pandas, Numpy, Scipy, and Pingouin libraries in python programming language and Agricolae package (Mendiburu, 2015) in R statistical language for the tests.

RESULTS

Plants with wider spacing and K applied (NPK + 50) resulted in significantly greater yield per plant which was followed by NP + 50. Total yield per plant was 1063.61 g and 969.25 g for NPK + 50 and NP + 50 treatments, respectively. In dense plants, the total yield per plant was 836.99 g and 760.37 g for NPK + 40 and NP + 40, respectively and the difference was highly significant among all groups. Fig.1 shows the influence of treatment on the yield performance of a single plant.

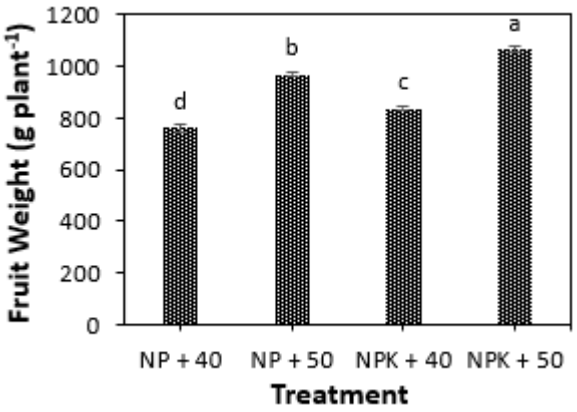

Fig.1. Fruit weight per plant (g). Each data point is the mean of n = 20 observations. Letters on the bar show level of significances.

We observed a similar pattern in fruit number per plant (FNPP) and single fruit weight (SFW). Winder spacing generally produced significantly more fruits per plant. The same thing happened in the case of K fertilizers. Tomatoes with K applied produced significantly more fruits than those without K. NPK +50 produced 10.53 fruits per plant and was followed by NP + 50 by producing 9.83 fruits per plant. In the narrow spacing, NPK + 40 produced significantly more fruits than NP + 40. Fruit number in NPK + 40 and NP + 40 was 8.75 and 8.08, respectively **(Fig. 2).** The weight of single fruit (SFW) also differed between the treatments. Wider spacing produced heavier fruits than narrow spacing. The difference was; however, significant only with K fertilizer treatment (NPK + 50). In narrow spacing, NPK + 40 produced heavier fruits than NP + 40, but the difference was not significant **(Fig. 3).**

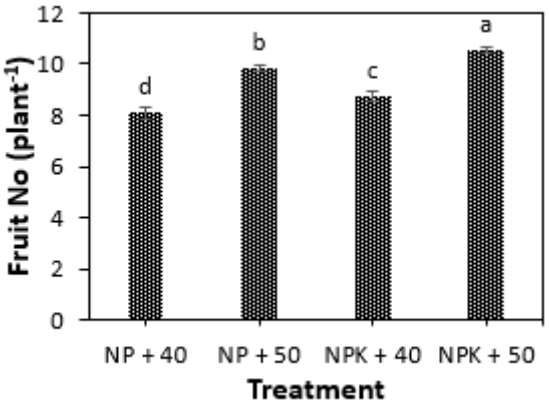

Fig. 2. Average number of fruits/plant (FNP). Each data point is the mean of n = 20 observations. Letters on the bar show level of significance.

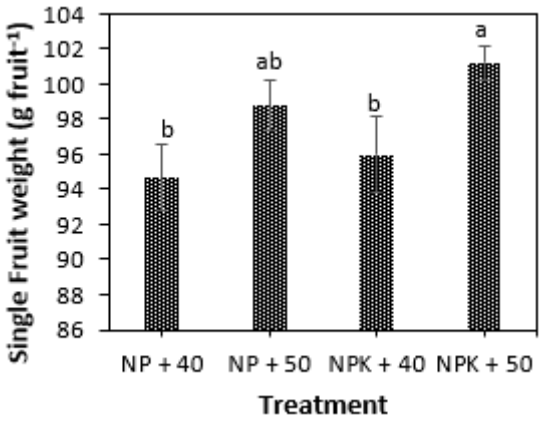

Fig. 3. Single fruit weight (SFW [g]). Each data point is the mean of n = 20 observations. Letters on the bar show level of significance.

As Fig. 4 reveals, the pattern of yield per square meter was the opposite of other measured yield parameters. Narrow spacing produced a significantly higher yield per square meter than the wider spacing. Moreover, yield in plots where K fertilizer was applied was significantly higher than those without K. NPK + 40 produced the highest

yield (5.2 kg m-2) in a square meter and was followed by NP + 40 (4.75 kg m-2). In wider planting space, NPK + 50 and NP + 50 yielded 4.25 and 3.87 kg m-2 tomatoes, respectively and the difference was significant.

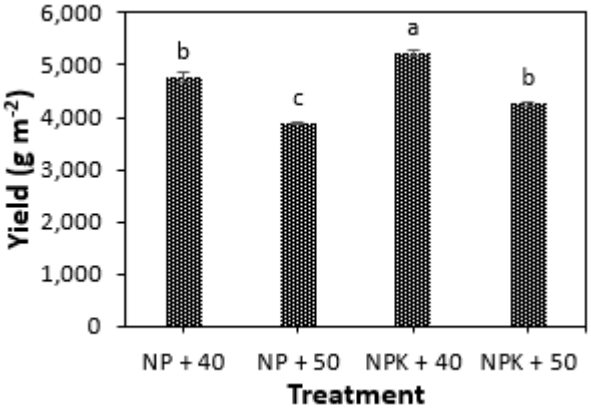

Fig. 4. Average yield/m2 in g. Each data point is the mean of n = 20 observations. Letters on the bar show level of significance.

The difference among treatments, between blocks, and the interaction of blocks and treatments are shown in the following table. A significant difference was observed among the treatments in all four yield parameters. We did not observe any significant difference between blocks and the interaction of blocks and treatments. As stated previously, the reason for the ANOVA test between blocks was to see if the location that was around 500 apart would affect the yield performance or not. Furthermore, the result showed that there is no significant difference among the blocks and treatments (P > 0.05), (Table. 1).

Table 1. Analysis of variance (ANOVA) on yield, single fruit weight, Fruit weight per plant and fruit number per plant

Factor			Yield (kg m^{-2})	SFW (g)	FWPP (g $plant^{-1}$)	FNPP ($plant^{-1}$)
Mean	Treatment (T)	NPK + 50	4.25	101.2	1064	10.53
		NP + 50	3.88	98.8	969	9.83
		NPK + 40	5.23	96.0	837	8.75
		NP + 40	4.75	94.6	760	8.08
	Block (B)	BLK1	4.54	97.8	910	9.30
		BLK2	4.52	97.5	905	9.30
	ANOVA	T	0.000***	0.002**	0.000***	0.000***
		B	0.44^{ns}	0.76^{ns}	0.33^{ns}	1.00^{ns}
		T X B	0.64^{ns}	0.96^{ns}	0.60^{ns}	0.84^{ns}

SFW = Single Fruit Weight; **FWPP** = Fruit Weight Per Plant; **FNPP** = Fruit No Per Plant
***= $\rho < 0.000$, **$\rho = 0.01$, n.s.= not significant.

Table 2 summarizes the correlation of yield parameters. With increasing planting density, the single fruit weight dropped significantly which could be due to competition for nutrients and other resources. In dense canopies,

competition for resources increase, and the imbalance of source and sink might have caused smaller fruit. The same applied in the case of fruit number per plant. Plants with sparse canopy resulted in more fruits per pant and thus the correlation between planting density and FNPP was strongly negative. A very strong correlation between yield per square meter and planting density suggests that yield per unit land area is strongly connected with planting density. Interestingly, the SFW and FNPP have a negative but insignificant correlation. The reason this correlation is not strong enough can be justified using K fertilizer and relatively wider spacing. As can be inferred from the literature, the smallest planting space (40 x 40 cm) we used in our experiment might be relatively wider for achieving further yield increases per unit land area.

Table 2. Coefficient of correlation. Stars indicate the significance and strength of the correlation.

Coefficient of Correlation				
	SFW	**FWPP**	**D**	**FNPP**
FWPP	0.41***			
D	-0.38***	-0.91***		
FNPP	-0.06^{ns}	0.88***	-0.80***	
Yield (m^{-2})	-0.28*	-0.60***	0.87***	-0.52***
Yield (ha^{-1})	-0.28*	-0.60***	0.877***	-0.52***

SFW = Single Fruit Weight; **FWPP** = Fruit Weight Per Plant;
FNPP = Fruit No Per Plant; **D** =Planting Density
*** = $\rho<0.000$, ** = $\rho < 0.001$, * = $\rho < 0.05$

DISCUSSION

The effect of planting density and K fertilizers in tomato crop has been explored in many studies. Our study revealed that wider planting space significantly increased fruit weight per plant, fruit number per plant, and single fruit weight. On the contrary, fruit yield per square meter was significantly higher in plants with narrow planting space.

Our results are consistent with the finding of Papadopoulos and Ormrod (1990) who stated that fruit weight per plant decreased while yield per square meter increased with narrow planting space. It can be explained by the smaller interplant and inter-row spacing that causes competition between plants (Fery and Janick, 1970). Since narrow spacing results in sparse canopies, light interception, and CO2 fixation by the lower leaves of a plant increase accordingly which ultimately increases yield per plant (Papadopoulos and Ormrod, 1990). Similarly, yield per plant was improved but yield per land was reduced in greenhouse-grown cherry tomato when the plant spacing was increased from 11.8 to 19.7 inches (Charlo et. al., 2007). High yield was achieved in heated greenhouses as the plant density was increased (Saglam and Yazgan, 1995). Although Kemble and colleagues (1994) didn't find any difference between fruit yield between 12 and 30 inches in-row spacing, higher yield in field-grown tomatoes was obtained with smaller in-row spacing (Santos et. al., 2010).

The yield response of tomato is well established to animal compost and chemical fertilizers. (Ramyabharathi et al., 2014). In this study, we found that K application increased all yield parameters irrespective of planting space. These findings are in alignment with the findings of Chapagain and Wiesman (200) who reported that the use of Potassium (K) increases the growth of tomato plants and thus it results in higher fruit production. A yield enhancement of 35.55% over the control was reported when a higher dose of 120 kg K2O/ha was applied (Ahmad

et. al., 2015). The use of K fertilizers increased the size of fruits especially in soil with low to medium fertility (Perkins-Veazie and Robert, 2003). Amjadet l., (2014) reported that the use of K significantly increases fruit diameter which could be attributed to the role of K in enhancing photosynthesis (Havlin et. al., 2005).

Our study suggests that if the fruit size is not of importance, reducing the planting density from 50 x 50 cm to 40 x 40 will significantly increase fruit yield in a unit land area. Using K, on the other hand, will not only improve tomato fruit size, fruit number per plant, and fruit weight per plant, it will also increase the total yield. We found this study as one of the biggest milestones towards increased tomato productivity in Sarkhroad, Nangarhar, Afghanistan.

CONCLUSION

We conducted this study to show the effect of planting density and K fertilizer on the yield and yield attributes of tomato in Nangarhar, Afghanistan. Less dense plants (wider spacing) increase the FNPP, SFW, and FWPP, however, yield per unit land area was not promising. Dense plants (narrow spacing) resulted in reduced FNPP, SFW, FWPP whereas yield per square meter was significantly increased. The application of K fertilizer affected all measured parameters irrespective of planting density, suggesting that if planting space is reduced and K fertilizer applied, the productivity would improve.

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Limitations of ocular care facility use amongst fifty period aged as well as greater population inside western Afghanistan. A descriptive research

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ABSTRACT

Background: Visual impairment and blindness from ocular diseases are important public health difficulty in developing countries, including Afghanistan. Evidence recommends that poor uptake of available eye services by potential beneficiaries is a major barrier for achieving a global reception to eye services. This research was performed to observe the limitation to use ocular care facilities between people elderly 50 life-span and greater inside Eye Department of Nangarhar University Teaching Hospital, Nangarhar-Afghanistan.

Materials & Methods: The information of this descriptive research was accumulated 794 recently recorded patients who came for receiving ocular care facilities during 1-June-2020 to 20 December- 2020. Information was analyzed with IBM SPSS (version 21). Descriptive statistics of the variables were tabulated in frequency chart with percentile, bivariate analysis of the variables were carried out utilizing chi-square tests, p-value less than 0.05 considered as notable.

Findings: Inside the Out Patient Department (OPD), from 800 recently recorded patients, 794 approve (99.3%) and finished meeting following ocular investigation. The ordinary limits to ocular health facility use were reported at the moment that 'issue un sensed' by 77,3%., accompanied by 'un cash to move' 12,0%, as well as 'no one to help' 11.2%, 'this is extremely away' 2.6%, ' this is from Allah,[s] view' 0.9%, ' no moment for turning to ' 0.8%, as well as 'unable move (according of the other illnesses) 0.6% properly .

Conclusion: Major part of the participants have never used the ocular care facilities due to an alert of the issues. In fact, a few preventable deprive of sight ocular illnesses which are persistent in genius and progress extremely unhurriedly without recognizing the issue inside the ocular, leading to absolute vision loss prior to searching for care, for the reason subordinate avoidable measures and society based ocular keeping safe advancement timetable including ocular keeping safe instruction inside the society are approved. Health advancement stages compulsory to incorporate community based health education as well as to observe the ways of making ocular care facilities inexpensive inside rustic area.

Keyword: Ocular care; limitations; Use; Afghanistan

INTRODUCTION

There are 287 million humans accompanied with sight deterioration (SD) globally (245 million little sight as well as 40 million visionless). From little vision 61% as well as visionless 81% human were elderly 50 years and greater than (Mariotti, 2012.). Inside developing nations, SD and visionless cause economic, community and general health difficulties (Shahriari et al., 2007). SD is differently spread in the WHO areas, the little widespread presence is perceived inside the America as well as inside Europe (30 and 32.7 instances per 1001 people properly), despite the highest widespread presence is perceived inside the WHO Eastern Mediterranean (EMR) at 41.5/1001, and South-East Asia Area (except India) at 49.3/1001 people (Resnikoff S et al., 2004.; Pararajasegaram, 1998). Additionally, almost 90% of sight deterioration population are living inside the developing nations (West & Sommer, 2001; Mariotti, 2012). Approach to the curative and preventive ocular facilities are seriously barriers in these nation due to lack of the facilities or un equal division (Yan et al., 2019). Globally, the principle cause of SD are Untreated Refractive Error (URE) at 44%, followed by lens opacity 32%. Further etiologies consist of glaucoma two percent, retinal anomaly by diabetes (RD), Trachoma, Age-related macular degeneration (AMD), as well as opacities of cornea (OC) 1% each. Unclear etiologies of sight deteriorations are 18% (Mariotti, 2012).

Inside Nangarhar, the prevalence of SD was 21.7% (20-25%, CL 95%) between fifty+ people and the most popular etiology was opacity of lens (51.8%), followed by incorrected refractive error (26.9%) as well as glaucoma (8.6%) which are usually preventable if facilities were used at the right time (Abdianwall & Güçiz Doğan, 2018). From 34 districts, 12 districts have ocular care facilities. These facilities were inside districts center alone. The ocular care facilities slightly reachable to people living inside the rural area of the province, mainly living distant regions. Although, the government department of health accompanying with Non-government organizations (NGOs) established outreach surgical ocular Camp on short-term basis. Vision 6/6, was established as a partnership among world health organization and global Organization for the prevention of blindness. This initiative is encouraging prevention of avoidable blindness as well as sight deterioration, based on illnesses control, human resource development , as well as technology and infrastructure (World Health Assembly, 2009). Ocular care use is little even inside developing nations since some limitations for example societal beliefs, less awareness, and no reachable and cheap ocular care facilities. World health survey performance in 70 nations all over the world in 2002-2003 specified that lonely 18% (95% CI = 17 - 19) adult had ocular problem, checked in the previous year. The grade of ocular check in the previous year in low, lower middle, upper middle , as well as money making nations were 10%, 24%, 22%, as well as 37% properly (Vela et al., 2012). Inside the developing nations, the need for resources (tools, infra structure and trained staff) provision was given higher priority compared to use of health facilities (Fletcher et al., 1999; Gnyawali et al., 2012). However, existing ocular care facilities are underused by the potential facilities users (Brilliant et al., 1991; Courtright et al., 1995; Venkataswamy & Billiant, 1981). Such as , South Africa a society rural survey discovered that only 39% of the people checked their eyes in five years or more in spite of the reachable and cheap ocular facilities (Oduntan & Raliavhegwa, 2001). It explains that besides providing funds to the facilities, it is important to increase awareness among the people to use the ocular facilities more often. In addition, neither ocular facilities are enough in number, nor they are used by the people in full capacity.

The limitation to use ocular care facilities are not only the lack of awareness about treatment accessibility, but it is also a cultural and traditional phenomenon. Therefore, this research was planned to show the usual limitation among 50 years or more elderly at Ocular Ward, Nangarhar University Teaching Hospital.

MATERIALS AND METHODS

This research was hospital based descriptive research, performed inside Ocular Ward, Nangarhar University Teaching Hospital from 1-6-2020 to 20 -12-2020.

The population of this research was composed of 50 year or older people who visited the hospital for ocular care facilities. Along the duration of this research, 800 population coming to search for ocular care inside Poly Clinic, Department of eye for the first time. Population elderly 50 years as well as greater than, who lived in the district of Nangarhar Province, recently recorded as well as recent coming to this department , had collaboration with the researcher as well as approve taking part in this research were involved. Population, from other districts as well as not approved the conversation were ruled out from this research. The investigation paper comprised of two parts. In the first part, a few population-related as well as individual nature of the individual were registered. In the second segment, a few investigations as well as nature related to limitations of ocular facilities utilization took place. The questionnaire was made ready as well as implicated in local language. Additionally, the aim of this research was expressed to each contributor and a written agreement was taken. Information was analyzed using IBMM SPSS statistics 21version software program. The discovers were presented by using the marginal chart with number and percentile. The discreptive statistics of central measure for example percent and mean plus were given. For determining relation among two categorical variabes , chi-squre test was used while for identifying the relation between two numerical variables student's T test was used . In bivariate analysis, all unconventional variables with p-value less than 0.05 considered as significant.

RESULTS

Between 1-6- 2020 to 20 -12-2020, 800 ocular patients, seeking ocular care were asked to take part in this research. A part of 800 patients 794 (99.4%) population approved taking part. from members, 37.7% from Jalalabad town on the other hand remaining from various parts of region. Males taking part was a little greater (53.7%) than females. nearly 1/5(18.5%) from members have been elderly 66 years and greater than. Women were youngers, average of the women age were =57.2. ±6 on the other hand this is 59.8±7 years for their male counterparts ($p<0.001$). The percentage of educated members 36.9% and this is more than twice as much greater between man 48.4% than the woman (23.6%). Of the members, 36.1% told of that them being in good health condition as well as 22.4% told of having fine idiomatic state. Any other population-based nature of the members by sex exhibited in **Table 1.**

Table 1. Population-based nature of members

Natures	Gender						p-value
	Man		Women		entire		
	N	%	N	%	n	%	
Life time							
50-54	106	24.9	128	34.8	234	29.5	0.005
55-59	118	27.7	102	27.7	220	27.7	
60-64	104	24.4	81	22.0	185	23.3	
65,+	98	23.0	57	15.5	155	19.5	
Educated	52	12.2	26	7.1	78	9.8	
Un educated	220	51.6	281	76.4	501	63.1	<0.001
Elementary class	38	8.8	30	7.8	68	8.5	
Middle class	24	5.8	14	3.5	38	4.8	
Senior class	80	2.10	22	5.5	103	12.6	
Post graduated	10	2.4	0	0	11	1.4	
One self-reported fitness condition							
Fine	159	37.1	126	34.1	287	36.1	0.001
Fair	243	56.3	186	51.3	425	53.5	
Broke	29	6.6	54	15.7	82	10.3	
Married condition							
Now married	367	86.2	231	62.8	598	75.s	<0.001
Now un marital	59	13.8	137	372	196	24.7	
Present home							
City	161	37.8	138	37.5	299	37.7	0.932
Rustic	265	62.2	230	62.5	495	62.3	
One self -reported SES[1]							
Not bad	96	22.5	82	22.3	178	22.4	<0.001
Fair	242	56.8	165	44.8	407	513	
Not good	88	20.7	121	32.9	209	26.3	
Job condition							
No	256	60.1	47	12.8	303	38.2	<0.001
Yes	170	39.9	321	87.2	491	61.8	
Total[3]	426	53.7	368	46.3	794	100	

[1]Socio-economic Status, [2]Percentages were measured from the numera of members whom were unhailed from the identical area (n=578), [3]Row percent; others are column percent's

This research discovers that the most usual limitation described by members was 'issue not perceived', which is 77.4 %. 2nd usual limitation was discovered 'no cash to go' 12.0% ; 'no one to go with' 11.2%, 'it is distant' 2.6%, ' it is from Allah' 0.9%, ' no time for checkup' 0.8%, and 'cannot go' (since other illnesses) 0.6% . The limitation of not using ocular facilities with its frequency, percentage, and gender is shown in **Table 2.**

Table 2. Distribution of members belonging to the limitations of not using ocular facilities and their gender

Go to eye doctor	Gender				
	Women		Male		Entire
	N	%	n	%	N
Cause of un visiting doctor [1]					
Issue not perceived	264	71.7	350	82.2	614
Poverty	5e	14.7	41	9.6	95
No one to go with	54	14.7	35	8.2	89
It is distant	14	3.8	7	1.6	21
It is from allah side	2	0.5	5	1.2	7
Lack of time	0	0	6	1.4	6
Unable to go (other illnesses)	2	0.5	3	0.7	5

[1]More than one answers, percent's were measured separately from the numeral of members whom have not met an eye specialist up to now (entire .794, man=425, woman=369)

DISCUSSION

The overall literacy rate among the target population was found to be 36.9%% (48.4% in man and 23.6%% in woman). Education level in this research is little greater than general education level in our country which is 31.4 % (44.5%% in man as well as 16% in woman) (Afghanistan, 2012). The cause for higher education rate in this research might be as a result of enrollment of 36.0% of members from Jalalabad City, the center of Nangarhar Province. Cities usually have greater safety conditions, educational institutes, and schools for both men and women. In addition, the economic condition of the cities is comparatively better than the rural areas. Our country is one of the nations which has lowest education level, it is 3rd in number after Burkina Faso and Southern Sudan in group of top 10 nations which has worst education level in the globe (Kristina, 2013). Among women, the percentage of education is 51.6 % that is very low in comparison to men 76.4% with ($p < 0.001$). The large space among man and woman related to education could be because of some reasons for example male dominant society norms (ignoring women schooling), fewer female school in close proximity, little request for education especially for woman because of society limitation as well as early marriage of women (Hanemann, 2012).

In this research, the largest limitation is not recognizing the issue (77.7%), which is indicative of less information about their ocular vision whether it was normal, ill, or how could the illness be avoided and cured. A research which was performed inside Nigeria evaluated the limitation of necessary not sensed as 33%, which is less than our research at 77.7% (Ebeigbe & Ovenseri-Ogbomo, 2014). The cause for the large percentage of issue not sensed could be less education of having impaired sight, less perception about the existence of ocular facilities as well as possibility of therapy and avoidance of preventable blindness. In addition to less education rate of the members, the lack of access to ocular care announcement in the media might have expanded the issue of not recognizing ocular illnesses. The second most usual limitation was the financial issue at 12.0%. This issue does not belong to the price of ocular care facilities directly, due to health facilities including ocular care are free of

cast to people inside Afghanistan (Hamidi & Jayakody, 2015) . This just belong to the transport system, food, and accommodation. Ocular care facilities are only provided inside the central area, ill people from far region have to stay in a hotel at least for two nights. Outcome of the research is in agreement with the discussion performed inside Michigan, that as well discovered the transport system charge as limitation to ocular care use (Elam & Lee, 2014).

The third limitation says 'no body to go with'. Sight deterioration or aged people necessary to have a person to go to for ocular care facilities for avoidance of other non-aimed condition. This issue is very deep for women illnesses due to even healthy females were not permitted culturally to go out lonely. The limitation of no one to go with is larger in this research correlated with the research performed in Nigeria, which estimated as 8.3% (Ebeigbe & Ovenseri-Ogbomo, 2014). The cause could be the reachability of ocular care facilities. In this research area, ocular care facilities are present in centeral area of the province that is remote to the villages around to it. So, men and women with low vision are unable to go to ocular care facilities lonely. Other limitation expressed to use by members during research such as less of time as well as more health issue supported by the outcome of research performed (du Toit et al., 2006; Grimes et al., 2011; Ocansey et al., 2013; Ubah et al., 2013). Men and women followed the same pattern without any important differences belong to the limitation of ocular care use. Due to of safety limitations, this research was performed inside health center environment, disregarding population up to now have not utilized ocular care facility. A population based on cross-sectional research with enough sample size and sampling method is important for the better discovering of the limitation of ocular care use in the society level. Almost, eight out of ten members expressed that no sense of the ocular issue as a reason for not using of the ocular care. In fact, some illnesses of the ocular are persistent, slowly developed and without any warning sign leading to blindness which are preventable with simple surgical, medical or physical interference. Hence, society based on ocular care developing plan including ocular care instruction for growing realization inside the society, secondary preventive measures (screening of blindness, ocular illnesses) and strengthening already existed outreach plans; are suggested.

CONCLUSION

A great number of people have never used the ocular care facilities because of not realizing the issue. Other common barriers are 'issue un sensed'., accompanied by 'un cash to move' as well as 'no one to help' 'this is extremely away' ' this is from Allah view' ' no moment for turning to ' as well as 'unable move (according of the others diseases) properly. Really, some preventable blindness ocular diseases are persistent in nature as well as progress gradually without realizing the issue inside the ocular, giving rise to somewhat sight loss, or complete visual lose before searching cure for it.

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Chemistry Related to Biology and Medicine

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ABSTRACT

Reviewing several facets of Fenton Chemistry's involvement in biology and medicine. There is growing indication that a number of Fenton and Fenton-like reactions can result in the formation of both the OH radical and ferryl $[Fe(IV)=O]^{2+}$. There are a few examples of hydroxyl radical generation that is unrelated to metals. The wood-decaying fungus that causes white rot and brown rot serve as examples of extracellular Fenton reactions. Numerous studies have been published in this area ever since Fenton chemistry and biomedicine were initially linked. Understanding and advancing this topic would be aided by a thorough exposition of the principles of Fenton chemistry and a synopsis of its representative applications in cancer therapy. The current state of Fenton chemistry is then examined, and a few pertinent illustrative instances are provided. Additionally, the current methods for further improving the efficacy of chemotherapy dynamic therapy under the direction of Fenton chemistry are highlighted. The combination of biomedicine and Fenton chemistry or a larger range of catalytic chemistry techniques is given with future possibilities being especially significant. Recently developed reactive oxygen species (ROS) engineered nano catalytic medicines in cancer therapy based on the Fenton reaction, defined as chemical dynamic therapy (CDT), have been extensively studied and made rapid progress. However, the complexity and heterogeneity of tumors reduce the Fenton reaction's ability to oxidize molecules effectively. To increase the effectiveness of CDT and conventional therapeutic approaches, numerous modified tactics, including the Fenton-like reaction and other reactions, are being investigated. This study highlights current developments in the development and use of Fenton nanocatalysts that use the Fenton or modified Fenton reaction for CDT. Also highlighted is the catechol-driven Fenton reaction's natural and useful use.

Keywords: Reactive O_2 Species; Redox Cycling; Oxidative Stress; Free Radicals; Carcinogenesis; Fenton Reaction and Chemo Dynamic Therapy

INTRODUCTION

All aerobic cells produce free radicals and other reactive O_2 and N_2 species, which remain recognized to take part in an extensive range of biological and metabolic processes. In addition to free radicals like superoxide radical anion ($O^{\bullet-}$), carbon-dioxide radical anion ($CO_3^{\bullet-}$), hydroperoxyl radical ($HOO^{\bullet}$), hydroxyl radical ($HO^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), and alkoxyl radical ($RO^{\bullet}$), the ROS designation also includes non-radicals like hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), and ozone (O_3). Since H_2O_2 is a significant ROS in living organism, maintaining the aforementioned equilibrium can take a variety of physiological and pathological effects. Additionally, H_2O_2 can undergo a Fenton or Fenton-like reaction to yield reactive $HO^{\bullet}$ radicals or the intermediary $[Fe(IV)=O]^{2+}$ (Carter et al., 2022).

H_2O_2, and other ROS oxidants have been linked to elderly, as well as serious human illnesses like cancer, heart disease, Alzheimer's, and other associated neurological diseases. Alternatively, new data suggest that H_2O_2 has a physiological function in cellular signal transduction as a second messenger. The production of ROS may result from exposure to several noxious hazard elements, including more or less xenobiotics, infectious agents, contaminants, Ultra violet light, cigarette smoke, and radiation (Halliwell and Gutteridge, 2015). On the other hand, ROS are constantly produced in minor amounts during usual cellular practices, along with RNS like nitrogen monoxide ($^{\bullet}NO$) and nitrogen dioxide ($^{\bullet}NO_2$), as well as non-radicals like peroxynitrite anion ($ONOO^-$), peroxy-nitrous acid (ONOOH), nitrosoperoxycarbonate anion ($ONOOCOO^-$), nitronium cation ($^+NO_2$), and dinitrogen trioxide (N_2O_3) Endogenously generated ROS and RNS play a vital role in a multiplicity of biological processes, making them vital to life. The significant role Fenton chemistry plays in physiological and pathological processes in living organisms is a highly important fact. The first chemical mechanisms used by Nature to generate ROS are likely the Fenton and Fenton-like reactions. This method results in the making of the most sensitive species, such as hydroxyl radicals (Daniel et al., 2006; Yeung et al., 2019; Valko et al., 2006).

PROGRESS OF FENTON CHEMISTRY

Knowledge of key factors affecting Fenton/efficiency is about the Fenton-like reaction has received a great deal of attention from researchers Optimize the Fenton chemistry. In this section, for the systematic development of Fenton chemistry, Details including morphological adjustments and facets Synthesis of Fenton/Fenton-like catalysts, monatomic Fenton/ Construction of Fenton-like catalysts, double reaction centers Fenton/Fenton-like catalysts that use electrons to improve Fenton/Fenton-like efficiency, in situ generation of H_2O_2 Fenton/Fenton-like reaction and during introduction a physical field for support (Tang et al, 2021).

THE BASIC INFFORMATION ABOUT FENTON CHEMISTRY

H. J. H. Fenton demonstrated that the arrangement $Fe(II)\text{-}H_2O_2$ displays severe oxidation effects to various organic acids in his seminal study titled "Oxidation of tartaric acid in presence of iron" more than 110 years past (Prousek, 2007). Later, it turns out that this mixture, recognized as the Fenton reagent, is a commanding oxidizer for a range of organic substrates (Koppenol, 2022). The presence of the hydroxyl radical ($HO^{\bullet}$) in the Fenton reaction has been hypothesized forty years later. The coordinated ferrous ion (LFe^{2+}), which is oxidized by H_2O_2 to produce LFe^{3+}, $HO^{\bullet}$ radical, and HO^- ions (Fenton reaction, Ia reaction), or an oxoiron (2+)

complex, is now preferred as an inner-sphere electron-transfer mechanism (Ib reaction) (Deguillaume et al, 2005).

$$H_2O_2 + LFe^{2+} \longrightarrow LFe^{3+} + HO^{\bullet} + OH^- \quad \text{(Ia)}$$

$$LFe{=}O^{2+} + H_2O \quad \text{(Ib)}$$

It is believed that during the anaerobic stage of life on Earth, iron became firmly entrenched as a bio-essential element. Enzymes involved in electron-transfer reactions primarily include it. Additionally, iron has harmful effects. The breathing thing takings excessive care to store iron in secure complicated arrangements as a result. Similar to other transition metals, the Fenton reaction may be the cause of iron toxicity. The availability of a functioning metal redox-cycling mechanism is a crucial component for Fenton chemistry action in Nature because quantity of iron in biological systems is frequently relatively low (Gozzo, 2001). In biological systems, the superoxide radical anion ($O^{\bullet -}$) serves as a reducing agent. A crucial metabolic process that leads to numerous biological responses is the reduction of $Fe(II)$ to Fe (II). As the result, the superoxide-driven Fenton reaction is a crucial biological event (Pryshchepa et al., 2022).

$$LF^{3+} + O^{\bullet -} \longrightarrow LFe^{2+} + O_2 \quad \text{(II)}$$

$$H_2O_2 + LFe^{2+} \longrightarrow LFe^{3+} + HO^{\bullet} + OH^- \quad \text{(Ia)}$$

Now, the iron is coordinated with the accessible biological ligand by LFe^{3+} or LFe^{2+} (Gutteridge and Bannister, 1986). Coordinated $Fe(III)$ is condensed by superoxide to $Fe(II)$ in the initial step, that is required for subsequent Fenton reaction. Iron reactivity in the Fenton reaction and, consequently, ultimate oxidative harm to biological systems, are governed by iron coordination (Bloot et al., 2021). The hydroxyl radical, also known as ferryl, is the reactive byproduct of the Fenton reaction, as was already mentioned. Therefore, the chemistry of these responsive intermediates is primary focus of Fenton chemistry. Ferrell or hydroxyl radicals' significant reactivity is effectively demonstrated interactions with biological substrates in their atmosphere. According to the ($HO^{\bullet}$) radical's responses can remain divided into the following categories (Engelmann et al., 2003):

i) Reactions happening with perception of H_2.

$$R\text{-}H + HO^{\bullet} \longrightarrow HO^- + ArH^{\bullet +} \quad \text{(III)}$$

ii) addition reactions

$$ArH + HO^{\bullet} \longrightarrow HO\text{-}ArH^{\bullet} \quad \text{(IVa)}$$

$$R_2C{=}{=}{=}CR_2 + HO^{\bullet} \longrightarrow HO\text{-}C(R)_2\text{-}C(R^{\bullet})_2 \quad \text{(IVb)}$$

iii) oxidation reactions

$$ArH + HO^{\bullet} \longrightarrow HO^- + ArH^{\bullet +} \quad \text{(Va)}$$

$$M^{n+} + HO^{\bullet} \longrightarrow HO^- + M^{(n+1)+} \quad \text{(Vb)}$$

Both inorganic and organic chemicals have been used to study the ferryl ion reactivity (Engelmann et al, 2003; Loegager et al., 1992). The actions of the hydroxyl radical, or H-abstraction, are extremely similar to the

reactions of organic molecules in terms of their reaction processes. What is ferryl's nature and function within the biological system? The unswerving $HO^{\bullet}$ radical production from H_2O_2+Fe_2^{+} appears to be the utmost widely recognized arrangements, particularly at low pH, in spite of the lengthy history of the ferryl ion intermediary being projected in the Fenton reaction. Even so, the ferrell charged atom intermediate is frequently planned in procedure of Fe_2^{+} multiplexes with H_2O_2 , in the response of H_2O_2 within the existence of biological substrates, and in porphyrin complexes (Cui et al., 2022; Dong et al., 2020; Barbusiński, 2009; Prousek, 2007; Yin et al., 2018). The two-e^- oxidized heme (compound I), which is typically a ferrous - porphyrin radical cation $[Por^{\bullet+}\ Fe(IV)=O]$, is produced by peroxidases and catalases when they counter with H_2O_2 ($\sim 10^7 M^{-1}\ s^{-1}$) and is then reduced by the substrate to form the peroxidase compound II, $Pro\ Fe(IV)=O$. Horseradish peroxidase (HRP) was used to identify the porphyrin iron hydrogen peroxide, also known as $Pro\ Fe(III)\text{-}OOH$ or compound O (Yin et al., 2018; Chen et al., 2022). For instance, myoglobin (Mb) incubation with H_2O_2 results in the slow $\sim 10^2\ M^{-1}\ s^{-1}$ conversion of the Fe^{3+} heme to a ferryl heme [$Pro\ Fe(IV)=O$], which is comparable to compound II in HRP. Contrarily, peroxidases and catalases easily form compound I from H_2O_2 ($\sim 10^7\ M^{-1}\ s^{-1}$) (Yin et al., 2018).

The main oxidizing intermediates in enzymatic processes are compound I species. H_2O_2's second oxidizing equivalent, $[Por^{\bullet+}\ Fe(IV)=O]$, is connected to the porphyrin cation radical. The hydrogen abstraction/oxygen reflection apparatus has been put forth for cytochrome P450 substrate oxidation (Chen et al., 2022).

$$Fe{=}O + H\text{-}C \longrightarrow \{Fe...O...H...C\} \longrightarrow Fe + HO\text{-}C \qquad \text{(VI)}$$

There has been using of the modified Fenton system (Fe^{2+}-H_2O_2-CH_3CN). The procedures of H_2O_2 reactions with catalases and peroxidases appears to be more widely accepted than that of reactions with ferrous and ferric salts. In the initial stage, both enzymes produce compound I. (VII reaction) (Chen et al., 2022).

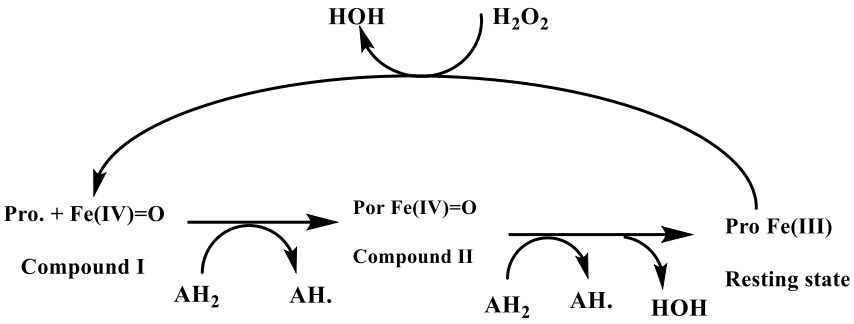
$$Peroxidase/Catalase + H_2O_2 \longrightarrow Compound\ I + H_2O \qquad \text{(VII)}$$

Catalases undergo a further reaction with H_2O_2 after forming chemicals.

$$Compound\ I + H_2O_2 \longrightarrow Catalase + O_2 + H_2O \qquad \text{(XIII)}$$

The water association in the peroxidase active site was used to explain this reactivity discrepancy. Water prevents H_2O_2 from gaining access. Compound II and the porphyrin radical cation are where compound I is reduced by organic substrates for peroxidases. In contrast to catalases, which rapidly release water from the active site, water is kept in the active site to allow H_2O_2 to prolix into the active site in the heme pocket (Jones, 2001).

For instance, HRP, a plant peroxidase with heme as a prosthetic group, uses H_2O_2 to catalyze the oxidation of a range of substrates. It has been determined that the process shown in Scheme 1 where chemicals 1 and 2 stand in for the ferryl intermediates and AH_2, the HRP substrate is how enzymatic reactions in an aqueous buffer often develop (Neelwarne and Rudrappa, 2013).



Scheme 1. Composite I is returned to its ferric latent state also through two successive one-electron transfer procedures from the peroxidase substrate or through two-electron oxidation events linked to the transfer of oxygen to the substrate (for example, R-S-R R-SO-R). As a two-oxidation corresponding overhead the inactive ferrous state, compound I participates in the catalytic turnover of HRP with H_2O_2 (Dunford, 2002). Biology places a high value on the involvement of ferryl intermediates in enzymatic Fenton-like reactions (Carter et al., 2022; Halliwell and Gutteridge, 2015), and the procedure of the response between heme-containing peroxidase and catalase enzymes with H_2O_2 is thus fully understood (Dunford, 2002).

MORPHOLOGY AND FACET REGULATION FENTON/FENTON-LIKE CATALYSTS

High physical and chemical properties of materials Based on morphology and facets for different Surface atom arrangement and coordination greatly affects Fenton/Fenton-like efficiency reaction. This section presents our current progress Fenton/Fenton-like morphology and facet regulation Catalysts and their mechanisms are discussed in detail (Tang et al, 2021).

The structure of a material determines its properties. The same atoms on different exposed facets have different chemical substances .A property that greatly affects catalytic activity (Jung et al, 2009; Hollingsworth, 2022). For example, Zhang's group Hematite Fenton catalyst system containing H_2O_2 and ascorbateion, the exposed face shows comparatively higher activity due to different amounts of iron cations contained in facet and facets (Xing et al, 2018). These are triple under adjusted (Fe_{3uc}) and 5-fold under coordinated (Fe_{5uc}), respectively. Fe_{5uc} sites show more steric hindrance Effect of reaction of H_2O_2 and acerbate on surfaces. The Fe3uc site has a higher affinity for ascorbate, Better catalysis of the face (Tang et al, 2021).

CLASSIFICATION AND PROPETIES OF FENTON REACTION IN CHEMO DYNAMIC THERAPY (CDT)

The unique properties of TME (H_2O_2 over expression and weak acidity) are able to induce the Fenton reaction in the presence of a Fenton catalyst. This specific Fenton reaction within tumor tissue only produces locally abundant toxic OH to induce pathological effects, resulting in tumor-specific therapeutic efficacy without significant side effects on normal tissues. Make it possible. The rapid development of the intratumoral Fenton reaction in cancer therapy is spurring the emergence of versatile therapeutic modalities (Meng et al., 2020).

FENTON TESPONSE IN CDT

The Fenton reaction and its cousins have been discovered for over 100 years, and this catalytic reaction has been extensively studied in versatile applications such as water purification. It is believed that OH produced by the Fenton reaction can convert organic pollutants into harmless substances (carbon dioxide, water, etc.) Cancer cells are usually composed mostly of organic matter, which triggers the Fenton reaction, which can destroy natural bio-molecules (DNA, proteins, lipids, etc.) and kill cancer cells. Fortunately, relatively high levels of H_2O_2 within tumor cells provide sufficient reactants for the Fenton reaction (Meng et al., 2020).

However, most Fe species in the human body are bound to some specific proteins. Few available free iron ions can be used to induce apoptosis or ferroptosis in the Fenton reaction (Liu et al, 2019). Therefore, various nanomedicine with ROS modulating properties have been designed for biomedical applications. Mainly, Fe-based Fenton nanocatalysts such as Fe_3O_4 and α-Fe_2O_3 can release iron ions. Iron ions can be tuned to convert endogenous H_2O_2 into highly toxic OH through the catalytic Fenton reaction, which fights cancer and interferes with cancer cells by reducing oxidative stress (Fan et al, 2019; Dixon and Stockwell, 2014). Inaddition, OH has a very short half-life (10-9 seconds), so that it damages only surrounding DNA, proteins, or lipids in situ and not distant areas (Zhao et al, 2019). Therefore, it is suitable to deliver Fe-based Fenton nanocatalysts to cancer cells to trigger intracellular Fenton reaction for cancer therapy with high therapeutic efficiency and low side effects (Meng et al, 2020).

BIOCHEMISTRY AND MEDICINE USING FENTON CHEMISTRY

Metal ions play numerous structural and functional roles in nature. Metal ions play a critical role as biological catalysts in electron-movement process as well as the activation and movement of small molecules like dioxygen. The assembly of the metallic, including the shapes of the multipart and the type of ligands connected to the metallic, and the situation of the metal composite are the two main determinants of the behavior of metal ions in biological structures (Halliwell and Gutteridge, 2015; Daniel et al., 2006; Yeung et al., 2019).

Iron is a crucial component of several proteins involved in the metabolism or transport of oxygen. Additionally, it needs to be stored, moved through the figure, and prepared available for the creation of iron proteins. Iron's capacity for redox cycling is a crucial component of how it works. Approximately 4.5 g of iron makes up a typical adult male human. Ferritin and hemosiderin are two important proteins that hold the iron in cells. It is primarily found in enzymes involved in electron transport in bacteria. For instance, Escherichia coli has about 10^6 ions of iron in per cell. The availability of numerous redox states is a second noteworthy characteristic of metallic ions with regard to their capacity to conduct biological oxidations. The oxidation states of iron that are physiologically significant are typically +2 and +3 (Dunford, 2002).

The availability to produce various ROS is linked to the process by which oxygen exhibits its toxicity, despite the fact that oxygen is necessary for living things. The one-electron reduction of molecular oxygen occurs step by step and is best described as below:

$$O_2 \longrightarrow O_2^{\bullet -} \longrightarrow H_2O_2 \longrightarrow HO^{\bullet} + H_2O \qquad (IX)$$

Water is end product of the lessening of oxygen. Few highly sensitive ROS, such as hydroxyl radicals, have the potential to harm a variety of biological target molecules, including DNA, proteins, and lipids. For these reactions, Fenton chemistry is crucial due to the bio-molecules it strongly reacts with.

The hydroxyl radical is arguably the ROS that can harm biological systems the most (Nordberg and Arnér, 2001; Pryor, 2006; Toyokuni, 2020).

REACTIVE N_2 AND O_2 SPECIES AND THEIR REACTIONS

All aerobic organisms produce and break down ROS and RNS, which result in oxidative stress, a pathogenic condition that impairs normal cellular activity. ROS are increasingly being shown to be used by cells in a variety of physiologically important ways, with intercellular waving and redox parameter. Since its discovery as a signaling molecule, nitric oxide (•NO) has gained widespread recognition as a controller of transcript cause activity and another factors that affect gene countenance. Similar intracellular effects are produced by H_2O_2, hypochlorous

acid ($HOCl$), and superoxide radical anion (Pryor, 2006). On the other hand, it has been demonstrated that ROS are linked to a wide range of adverse events, including radiation, inflammation, carcinogenesis, and reperfusion wound. The very rampant transition-metal ion in human bodies, iron, could function a catalyst to increase ROS production under pathological circumstances. Carcinogenesis is linked to iron excess. Additionally, a novel notion known as "genomic regions sensitive to the Fenton reaction" was developed as a result of the possibility that the Fenton reaction could result in oxidative harm at a certain region of the genome in vivo (Willson, 1977). The primary ROS created is superoxide radical anion, which function with some other molecules to produce secondary ROS, primarily by enzyme- or metal-catalyzed reactions, such as the hydroxyl radical (Fenton reaction). Through a process involving superoxide dismutase, superoxide is depleted (SOD).

$$2O_2^{\bullet -} + 2H^+ + SOD \longrightarrow H_2O_2 \longrightarrow O_2 \qquad (X)$$

SOD Enzymes four remits of extent faster this response within living arrangement. SOD Enzymes cooperate with H_2O_2 oxidizing enzymes like catalases and glutathione peroxidases in biological systems (Toyokuni, 2020). The majority of switching metals have complex redox and coordination chemistry that is intimately connected to production of numerous unrestricted radicals. Fenton chemistry is a key component of the main methods by which transition-metal ions activate oxygen. As a result, the redox form of the cell is kept strictly in physio-logical bounds. Superoxide releases Fe^{2+} ions from biological components that store iron when under stress. The Fenton reaction, which creates reactive hydroxyl radicals, can involve the released Fe^{2+} ions (Willson, 1977).

$$M^{n+} + H_2O_2 \longrightarrow M^{(n+1)+} + HO^{\bullet} + HO^{-} \qquad (XI)$$

$$M^{(n+1)+} + O_2^{\bullet -} \longrightarrow M^{n+} + O_2 \qquad (XII)$$

HYDROXYL (OH) FREE RADICALS AND METAL-UNBIASED PRODUCTION

A frequent species in cellular metabolism is $X^{\bullet -}$, a single-electron lessening intermediary of several radical anions. Superoxide radical anions (O^{o-}), semiquinone radical anions (Q^{o-}), and various one-electron reduced 2^{o-} xenobiotics , such as RNO_2 radical anions, are the major forms of these chemicals in connection to ROS generation (Carter et al., 2022; Halliwell and Gutteridge, 2015; Daniel et al., 2006; Yeung et al., 2019). Typically, the radical anion intermediate $A^{\bullet -}$ is generated when neutral molecule A receives one electron. In living systems, electron-transfer reactions are crucial metabolic processes. The electron adduct $A^{\bullet -}$ will initially form in a system containing the distinct solutes A, B, C, and D, each one of that reacts with e^-s at speeds close to dispersal control and which are exist in concentrations like $[A]>>[B]>>[C]>>[D]$. Nevertheless, following electron-transfer procedures could take place (Winterbourn, 1981):

$$A^{\bullet -} \longrightarrow B^{\bullet -} \longrightarrow C^{\bullet -} \longrightarrow D^{\bullet -} \qquad (XIII)$$

This illustration accurately depicts the actual condition in living biosystems. Stable radical anions can typically serve as an electron source for various biological electron-transfer processes. However, some of these radical anions quickly transform into free radicals and anions (e.g., halogenated xenobiotics). As a result, after undergoing a one-electron reduction, either quinone (Q) or oxygen (O_2) produce the fairly steady semi Quinone radical anion ($Q^{\bullet -}$) or superoxide radical anion ($Q^{\bullet -}$), correspondingly. On the other hand, when HCl_4 is reduced by one

electron, it produces $HCl_4^{\bullet -}$ radical anion, that instantly divide into the $\dot{C}Cl_3$ radical and Cl^- ion. Contingent on their like-mindedness for electrons, certain radical anions including $O^{\bullet -}$, semiquinone ($Q^{\bullet -}$), and $RNO^{\bullet -}$ can participate in the redox-cycling of transition metals or redox-decompose HOX molecules (HOOH, HOCl, HOONO, HOSCN, ROOH, etc.) by single-electron transferal to contribute to Fenton chemistry (XIV–XV reactions) (Saran et al., 1999).

$$O_2^{\bullet -} + HOX \longrightarrow O_2 + HO^{\bullet} + X^- \qquad (XIV)$$

$$Q^{\bullet -} + HOX \longrightarrow Q^{+} + HO^{\bullet} + X^- \qquad (XV)$$

$$RNO_2^{\bullet -} + HOX \longrightarrow RNO_2 + H\dot{O} + X^- \qquad (XVI)$$

Where X^- is an ion of HO, Cl, NO, or SCN. The examples provided make it abundantly evident that dissociative electron transfer frequently consequences in the creation of radical anions within biological systems. The generation of hydroxyl radicals is crucial from the perspective of Fenton chemistry. A very significant metal-independent source of the hydroxyl radical within phagocytosis, in addition to the Fenton-like reaction (17 reaction), is the interaction of superoxide with HOCl (XIII reaction) (Valko et al., 2006; Saran et al., 1999).

$$Fe^{2+} + HOCl \longrightarrow Fe^{3+} + HO^{\bullet} + Cl^- \qquad (XVII)$$

$$O_2^{\bullet -} + HOCl \longrightarrow O_2 + HO^{\bullet} + Cl^- \qquad (XVIII)$$

A significant amount of $HO^{\bullet}$ radicals will be produced by reaction 27 ($k_{27}=10^7\ M^{-1}\ S^{-1}$), which is crucial for the formation of ROS during phagocytosis. Since Adriamycin exhibits this behavior, its reduction by xanthine oxidase in the presence of nitrogen offers a practical technique to continuously produce semi Quinone ($Q^{\bullet}$). Additionally, hydroxyl radicals were produced when Adriamycin was combined with xanthine oxidase and xanthine under N_2 in the presence of H_2O_2 (Williamson and Davison, 2020).

$$Q^{\bullet -} + H_2O_2 \longrightarrow Q + HO^{\bullet} + HO^- \qquad (XIX)$$

This reaction doesn't require $O^{\bullet -}$ or a metal catalyst. The properties of this reaction suggest that it might play a significant role in the way that Adriamycin exerts its anticancer effects (Williamson and Davison, 2020). Adriamycin semi Quinone and H_2O_2 appear to react quickly, with the primary result being the hydroxyl radical. Ubiquinol, or reduced coenzyme Q, exhibits a similar pattern of behavior (Zhu et al., 2002). In the investigation of lipid peroxidation, the ubisemiquinone intermediary was discovered as a first reaction byproduct of ubiquinol. Hydroxyl radicals were produced as a result of semiquinone and H_2O_2 simultaneously forming (S reaction). It was discovered that ubisemiquinone interacts with together cumol (CumOOH) and lipid (LOOH) hydroperoxides in addition to inorganic peroxides like H_2O_2. Alkoxyl radicals, which are potent catalysts for lipid peroxidation, were reaction products of the reductive homolytic cleavage (Koppenol, 2022).

$$CumOOH/LOOH + Q^{\bullet -} \longrightarrow CumO / LO^{\bullet} + Q + HO^- \qquad (XX)$$

Semi Quinones ($Q^{\cdot-}$) have been discovered to interact with several substances existing during oxidative strain (O_2, H_2O_2, LOOH, HOX), showing that $Q^{\cdot-}$ may start a range of prooxidative processes. The second example illustrates the metal-independent hydroxyl radical generation from H_2O_2 and tetrachloro-1, 4-benzoquinone (TCBQ), a cancer-causing byproduct of the commonly used wood preservative penta-chlorophenol (Koppenol, 2022). This work employed electron spin resonance (ESR) trapping. It's interesting to note that 2, 5-Dichloro and 2-Chloro-1, 4-Benzoquinone were more effective at generating hydroxyl radicals than TCBQ . In contrast, H_2O_2 1, 4-benzoquinone, a non-halogenated Quinone, and the methyl-substituted Quinones 2,6-dimethyl and tetra-methyl-1, 4-benzoquinone did not produce any hydroxyl radicals (Haugland et al., 1990). The result that hydroxyl radical is formed by TCBQ and H_2O_2 from side to side a metal-independent process is well supported by a comparison investigation using ferrous ions and H_2O_2, the traditional Fenton system. The fact that the addition of H_2O_2, which was accompanied by the production of hydroxyl radicals, significantly reduced the $TCSQ^{\cdot-}$ ESR signal is another crucial indicator of such a process. These findings imply that H_2O_2 is directly reacted with by the $TCSQ^{\cdot-}$ semi Quinone radical anion, reducing it to a hydroxyl radical. It has already been suggested that a metal-independent Fenton reaction can theoretically occur when a Quinone/semi Quinone pair has a lessening potential of between -330 and +460 (Metosh-Dickey et al., 1998). These processes can be carried out thermodynamically and not needing metal ions for catalysis. The Quinone/semi Quinone pair for 2-chloro, 2,5-dichloro, and TCBQ , where the lessening capacities are, respectively, -100, +60, and +250 mV, may very well be examples of this. Contrarily, the 2, 6-dimethyl- and tetramethyl-1, 4-benzoquinone reduction potentials of -430 and -600 mV, correspondingly, is outside of this range, and hydroxyl radical production hasn't been noticed either (Williamson or Davison, 2020). It has fascinating biological ramifications because chlorinated quinines could react with H_2O_2 to form hydroxyl radicals in a metal-independent manner. For instance, many commonly used chlorinated aromatic compounds, including the well-known priority environmental pollutants 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid (2,4-D), can be metabolized in vivo to chlorinated Quinone's , that will have poisonous effects through the generation of hydroxyl radicals (Okado-Matsumoto and Fridovich, 2000).

Xenobiotic are broken down by a variety of enzymes and by a number of different mechanisms. Enzymes could make a xenobiotic radical anion through one-electron transfer, which can start a chain reaction that can produce further radicals that can damage cells. This is an instance of the enzyme glucose oxidase, which has the ability to catalyze the single-electron lessening of numerous kinds of xenobiotic, including 1, 4-naphthoquinone (1,4NQ) and 4-nitropyridine-N-oxide (4NPO), leading to the production of radical anion products (Okado-Matsumoto and Fridovich, 2000). The enzyme glucose oxidase couples oxygen reduction to glucose oxidation, producing H_2O_2 without any observable one-electron reduced intermediates (e.g., superoxide). Both $1,4NQ^{\cdot-}$ and $4NPO^{\cdot-}$ radical anions are identified by ESR when 4NPO and 1,4NQ where gestated collected at an equimolar concentration in the existence of glucose oxidase and glucose. The spectrum of the $4NPO^{\cdot-}$ is heavily dominated by the signal of $1,4NQ^{\cdot-}$. After being added, the ferric ion was converted to the ferrous ion and participated in the Fenton reaction, which produced a hydroxyl radical from H_2O_2. However, the likelihood of hydroxyl radical generation by the

interaction of $Q^{\bullet-}$ or $RNO^{\bullet-}$ radical anions with H_2O_2 cannot be firmly dismissed [Saran et al., 1999; Benov and Beema, 2003).

$$1,4NQ^{\bullet-} + H_2O_2 \longrightarrow 1,4NQ + HO^{\bullet} + HO^- \qquad (XXI)$$

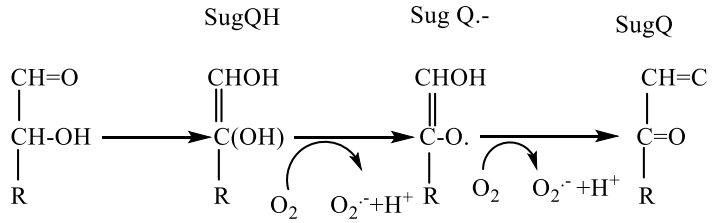
$$4NPO^{\bullet-} + H_2O_2 \longrightarrow 4NPO + HO^{\bullet} + HO^- \qquad (XXII)$$

Following is a description of how any appropriate radical anion $X^{\bullet-}$ participates in this kind of metal-free Fenton-like reaction (XXIII reaction).

$$X^{\bullet-} + H_2O_2 \longrightarrow X + HO^{\bullet} + HO^- \qquad (XXIII)$$

For a better knowledge of the mutagenic and carcinogenic characteristics of several significant pollutants for the environment, more research is required to describe the metal-independent hydroxyl radical generation.

Sometimes, a surprising issue with the carcinogenic potential of short-chain sugars is other illustration of how Fenton chemistry works in life. It is true that the short-chain reducing sugars can cause mutagenesis. The fundamental mechanisms of their activity involve the generation of (HO^-) radicals by metal-independent or -dependent Fenton-like processes. When non enzymatic glycosylation first begins, short-chain sugars including glycol aldehyde, glyceraldehyde, and dihydroxyacetone are formed. Such molecules tau-isomerize to enediols because the cyclization process cannot block the carbonyl groups on their carbon atoms (SugQH). One way in which their air oxidation produces the superoxide is in the final generation of H_2O_2 (Benov and Beema, 2003; Jay et al., 2006). On the other hand, enediols produce a semi Quinone-like intermediate ($SugQ^{\bullet-}$) after transferring one electron to oxygen, and a,-dicarbonyl (SugQ) after transferring a second electron (2 Scheme).



Scheme 2

$$2O_2^{\bullet-} + 2H^+ \longrightarrow 2HOO^{\bullet} + H_2O_2 + O_2 \qquad (XXIV)$$

Superoxide, which yields oxygen, can cause the one-electron oxidation slowly, while H_2O_2, which yields superoxide, can cause it more quickly (XXIV reaction). DNA can sustain damage from short-chain carbohydrates. Their oxygen-dependent mutagenesis impact is prevented by SOD. This shows that $O^{\bullet-}$ is essential for the mutagenicity generated by short-chain sugars. There are now two techniques to produce hydroxyl radicals: I by metal-free hydroxyl radical synthesis (XXV reaction) (Jay et al., 2006).

$$SigQ^{\bullet-} + H_2O_2 \longrightarrow SugQ \longrightarrow HO^{\bullet} + HO^- \qquad (XXV)$$

Either I by superoxide, which releases $Fe(II)$ from the [$4Fe\text{-}4S$] clusters, or (ii) by the Fenton reaction. We do know that oxidative stress brought on by diabetes mellitus might speed up the development of atherosclerosis (Hammel et al., 2002).

CONCLUSION

Due to the comparable chemistry of the ($HO^{\cdot}$) radical and ferryl involvement in Fenton chemistry, differentiation between them is not as significant in biology. More significant, however, is our understanding of the role that Fenton chemistry plays in biological systems, particularly in pathological processes like carcinogenesis, neurological disorders, atherosclerosis, etc. Knowing the kind of Fenton reaction that takes place in the specific biological system is also crucial. As one of the emerging cancer treatment modalities, CDT is characterized by high therapeutic efficiency in inhibiting cancer growth and negligible side effects on healthy cells/tissues. Although CDT and CDT-based combination therapies represent an active research frontier, some of the above-mentioned unresolved issues should be exploited in the coming years. Through joint efforts of chemists, materials scientists and biologists, nanocatalytic medicine with specific tumor targeting and on-demand anti-tumor efficacy will soon reach clinical application. Finally, it may be argued that while the Fenton reaction has been crucial to biology for the duration that life has existed on Earth, its significance for diseases associated with contemporary civilization is relatively recent.

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Prevalence of Ascaris Lumbricoides in School Children of Nangarhar Province, Eastern Afghanistan

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ABSTRACT

Backgroud: The rising major health concern with high rate of prevelence is Ascariasis in developing countries. The fundamental objective of present research was the parasitological investication estimating the prevelence of Ascaris lumbricoides based on age and sex among students of urban (Jalalabad City) and rural (Khogyani) schools in eastern, Afghanistan.

Materials and Mehtods: For accomplishment of research objective, totally 400 samples were obtained from students of selected schools and further procedure applied by formal-ether concentration technique. The entire population of 400 students in two groups of ages; 7-10 years and 10-13 years are chosen. 200 samples were collected from Jalalabad city school and the remaining 200 samples were collected from a local school in Khogiani district of Nangarhar province. Descriptive analysis of obtained data was performed using the R x64 3.3.1 version.

Findings: The results reviewed that in Urban areas schools twenty three were found positive and infection rate was 11.5%. In Urban areas schools children's, the infection was higher in 7-10 years old than 10-13 years old. The results documented that disease pattern in male and female children's was higher in males compared to females. Whereas, in rural areas schools 28, were found positive and infection rate was 14%. Furthermore, the occurrence of Ascariasis in rural areas schools children's, was higher in 7-10years old than 10-13 years old.

Conclusion: The recorded high contamination rate in young male children indicate to emphasize on better and improved sanitation and should be educated on the mode of infection and epidemiology of the parasite. The findings may also stimulate the development of customised strategies for the improved control and prevention of Ascaris infection Nangarhar.

Keywords: Ascarislumbricoides; Rural; Urban; Schools; Children

INTRODUCTION

Ascariasis infection is a major health problem with up to 1.2 billion people infected worldwide . A high rate of prevalence is observed in third world countries (Kucik et al, 2004).

Asciariasis is caused by Ascarislumbricoides,an intestinal nematode (round worm). Ingesting of infective eggs in contaminated food and water causing infection of human and fecally contaminated hands also infect human. After being ingested, in the circulation hatches where they are carried to the heart and lungs(Andrade et al, 2001). A few worm infection might be symptomless (Denham et et al, 1984),Worm masses of Ascaris L. can cause obstruction (Mosawi et al, 2019) or perforation of the intestine and occasionally obstruction of the bile ducts and pancreatic ducts (Dangana et al., 2011).Ascariasis effects may also contribute substantially to child morbidity when associated with malnutrition, pneumonia, enteric diseases and vitamin A deficiency (De Silva et al, 1997). It has also been associated with also been associated with stunted growth (Fernando et al, 2002). Harold et al. (1983) reported that Ascariasis can occur at any ages, mostly prevalent in the children of 5 to 9 years of age. Schoolchildren and young adults are more vulnerable. In male and female, the incidence is almost the same; due to poor hygiene and soil pollution causing the poor classes in urban and rural areas are most affected by the parasite. Infection is a household affair, the family being the unit of dissemination, infected children, provides the chief source of soil contamination by their indiscriminate defecation in door yards and earthen-floored houses, where the resistant eggs remain viable for long periods. By using adequate latrine, the soil can be prevented from becoming faecally pollution of Ascariasis. Avoiding the use of untreated human faeces as fertilizer and treating infected individuals are a part of a controlled program. It is also possible to be controlled by preventing eggs from being ingested by washing the hands before eating, avoiding eating of uncooked vegetables, green salads and fruits which may be contaminated with Ascaris eggs from polluted soil (Seo, 1983). In tropical countries where warm, wet climate enhances the transmission of the infection, infection occurs with highest prevalence (Holland et al, 2014). Fecal contamination is one of the most serious environmental health problems in poor countries (Ostan et al, 2007).

Intestinal parasitic infections negatively affect the health and development of a high proportion of school-age children. (Ezeamama et al, 2005). Although the major focus has been on prevalence of intestinal helminthes infection, fewer studies have investigated the socio economic effects of transmission of intestinal helminthes and namely Ascaris. It has been reported that the lack of standard toilets and education, occurrence of diarrhea, lower socio-economic status, inadequate disposal of human excreta and the level of sanitation in households are related to Ascariasis is a major health concern in Afghanistan with a 36% rate of transmission (Pullan et al, 2010). Factors such as limited access of Afghan people to clean drinking water, sanitary standard toilets and health services put Afghanistan in a high risk of ascariasis. According to a previously performed study, ascariasis is the most prevalent intestinal infections in eastern Afghanistan, especially among children (Korzeniewski et al, 2014).

Likewise, Afghan farmers use human feces as fertilizer for agricultural products such as vegetables, if not cleaned properly, in turn can cause Ascariasis. Urban residents and especially school children are considered as highly susceptible population against ascariasis. The factors such as polluted water and high population has put cities in risk of infection as well. Similar to cities, children in rural areas and remote towns are in the first line of the risk. This purpose of this study is to find the prevalence of ascariasis in Nangarhar province, Afghanistan. Also the information about the key factors affecting the infection in school children urban and rural areas. To our

knowledge, this is the first study conducted on this population and area about the prevalence of ascariasis. parasitoses (Cooper et al, 1988).

Research Objectives

The research was conducted in order to accomplish the given objectives:

- Study of age and sex in school children infected by Ascariasis.
- Comparative study of Ascariasis in children in urban and rural societies.
- Giving awareness to school children about Ascariasis.

MATERIALS AND METHODS

Sample

The overall population of 400 children were studied in two age groups; 7-10 yrs. and 10-13 yrs. 200 samples were collected from Jalalabad city school and the remaining 200 samples were collected from a local school in Khogiani district of Nangarhar province.

Fecal sample collection

Data was collected through direct interview schedule and recorded in a questionnaire which included information about age, sex, weight, height and socio-economic situation. Stool samples were collected in clean and dry wide mouthed container. The schedule is prepared maintaining relevance with the objective of the study. Before launching the survey, the questionnaire was be pre- tested and improved accordingly.

Fecal samples examination

Samples were transported to laboratory and was studied by flotation and sedimentation methods and through light microscope. The lab examination was performed in parasitology laboratory of veterinary science faculty of Nangarhar University.

Laboratory Investigations

Microscopy

Macroscopically checked the stool samples to observe the odor, color, presence of mucus and/ or blood. Microscopically examined the stool samples after collection in 24 hours. Eggs and larvae of the parasite examined using multiple approaches. The stool samples were concentrated using the formal-ether concentration technique and examined for the presence of Ascaris eggs by direct smears using normal saline and iodine solutions. Besides, sodium nitrate and zinc sulphide floatation techniques, Biermann and stool egg counting techniques were adopted to investigate and count worm eggs and larvae

Data Analysis

The obtained data were subjected to descriptive statistical analysis using the R x64 3.3.1 software.

RESULTS

A total of 400 fecal samples were examined for the presence of Asarislumbricoides eggs. 200 from Urban and 200 from rural areas. In Urban areas schools 23, were found positive and infection rate was 11.5% (**Table 1).**

Table. 1: Age and sex wise prevalence of Ascaris Lumbricoides in Urban areas, Schools:

		Number of Sample	Positive	Infection Percentage
Age	7-10 years	100	14	14
	10-13 years	100	9	9
Sex	Male	120	18	15
	Female	80	5	6.25

Age and sex wise prevalence

In Urban areas schools children's, the infection was higher in 7-10 years old than 10-13 years old (Table. 1). An analysis of disease pattern in male and female children's showed that infection was higher in males compared to females (Table. 1). In rural areas schools 28, were found positive and infection rate was 14% **(Table. 2).**

Table. 2: Age and sex wise prevalence of Ascaris Lumbricoides in rural areas, Schools:

		Number of Sample	Positive	Infection Percentage
Age	7-10 years	100	17	17
	10-13 years	100	11	11
Sex	Male	154	22	14.2
	Female	46	6	13

Age and sex wise prevalence

The occurrence of Ascariasis In rural areas schools children's, was higher in 7-10years old than 10-13 years old. The prevalence of Ascariasis was higher in male than females (Table. 2).

DISCUSSION

The common occurrence of Ascaris lumbricoides infection in developing countries carrying the hardest hit of the associated morbidity. A performed parasitological research in 2002 by German Armed Forces health service recorded in Kabul, among 217 local workers from the international military base, 64% were infected with intestinal helminths and protozoa, with Ascaris lumbricoides predominance observed 22.1% (Scheid and Thomas, 2004). WHO performed screening examination of stool samples taken from 1001 children aged 8-15 years in four provinces of Afghanistan, the results demonstrated the occurrence of intestinal helminthiasis in 47% of Children with the 41% predominance of Ascaris lumbricoides (Gabrielli et al., 2005).

Afghanistan is high risk country due to contaminated water and soils, many other etiologies are committed in transmission of Ascaris lumbricoides, as the health sector still rely on non-governmental organization's aids, the lack of health service workers leads to problems with epidemiological control in contaminant areas. The deficiency of basic drugs and medical equipment, chronic infective and parasitic disease vectors and migration to different societies are among the major contaminating factors (Wallace et al., 2002; Korzeniewski, 2009).

The essential matter of life, water contamination with pathogenic agents is common and 31% of Afghan households have access to safe drinking water. Meanwhile, about 5-7% of the Afghan community has to basic standard toilets (UNEP, 2008).

The poverty in Afghan society observed more than half of Afghan population (WHO, 2015). Due to limited diagnostic and therapeutic facilities of health services, Afghans are often treated by a trial and not proper method using a very poor assortment of pharmaceuticals. The low awareness of hygiene and lack of disease prevention strategy in Afghan society make easier spreading of infectious disease. Possibly even 90% of Afghan people may be infected with more than one parasite (Korzeniewsk et al., 2014). In literature, it has not been will recorded the information concerning infections caused by intestinal parasites in Afghanistan such as Ascaris lumbricoides, Giardia intestinalis, Entamoeba histolytica and so on (McCaw and Delay, 1985). This study results are in accordance to (Korzeniewski et al., 2017) findings, which carried out in Afghanistan. This study will represent the first findings of prevalence of A. lumbricoides infection among school children in eastern Afghanistan. Prevalence observed here is likely to approach the true prevalence of A. lumbricoides infection among children, in contrast to other prevalence studies, where generally only one microscopic technique is used. This approach led to several important observations concerning the presence of the parasite in the target population of Nangarhar province.

CONCLUSION

The results of study demonstrated a relatively high prevalence of A. lumbricoides infection among school children in Nangarhar Province with potential health consequences. Based on the results and investigations, more attention should be given to awareness, treat the infected people and training should be conducted to school children on the mode of infection and epidemiology of the parasite.

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Conflict of interest

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