VOLUME 01 - ISSUE 01 : AUGUST, 2022

NU IJB

NANGARHAR UNIVERSITY

INTERNATIONAL JOURNAL OF BIOSCIENCES

Contact : nuijb@nu.edu.af | nuijb.nu.edu.af

Address : Nangarhar University | Kabul-Jalalabad Highway | Daronta | Nangarhar | Afghanistan


بسم الله الرحمن الرحيم

Nangarhar University

Vice Chancellor in Academic Affairs

Nangarhar University International Journal of Biosciences

(NU IJB)

NU Press, NU Campus, Kabul-Jalalabad Highway, Nangarhar, Afghanistan.

Email: nuijb@nu.edu.af
Website: www.nuijb.nu.edu.af

Chancellor's Message

Prof. Dr. Khalil Ahmad Behsoodwal
Chancellor, Nangarhar University

It is obvious that academia is consisted of providing education and doing research. Academicians strive to solve the existing problems of community and the country at large, on the basis of research in order to put it on the track of development.

Nangarhar University, the second largest academic institution in Afghanistan, has recently achieved fundamental success in the third phase of Quality Assurance and is a highly reputable public university at the country level. Therefore, Nangarhar University considered it necessary to have an international online journal beside its domestic printed journals. So, as a result of the continuous efforts of the university's dedicated staff and professors, an international journal entitled "Nangarhar University International Journal of Biosciences" (NUIJB) has been created after completing its legal requirements and processes. I would like to congratulate the university community and fellow countrymen for this journal. Apart from being an income source, NUIJB facilitates the exchange of research accomplishments, and opinions with other countries around the world. NUIJB is definitely going to contribute a lot in solving other academic issues and paving the way for their development; expanding the knowledge of professors, individuals of opinions, and interested people; establishing cooperation among the domestic as well as foreign academicians; strengthening the motivation of research among the academic institutions; and paving the way for solving complicated academic issues. Finally, I would like to express my sincere gratitude to the founding committee of this journal and humbly request our respected professors to help the assurance and continuation of NUIJB through submitting their strong, reliable, and error-free articles to NUIJB.

Great wishes for a peaceful and self-sufficient Afghanistan!

Regards,

Prof. Dr. Khalil Ahmad Behsoodwal
Chancellor, Nangarhar University

Message of Editor-In-Chief

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

On behalf of our editorial team, I would like to offer a word of thanks to our readers, contributors, authors, editors and anonymous reviewers, all of whom have volunteered to contribute to the success of the journal and also for its mission to improve the quality of care and research in the form of publication in the sector of Biosciences. We are publishing our journal four time a year with a particular emphasis on quality, safety and better outcomes of research. I am equally elated to inform you all that NUIJB has been contributing tremendously to improve the quality of research and education in the medical, veterinary and agricultural sector by publishing its issues. An enormous amount of work has been done towards the development of this journal in the past days.

NUJIB is dedicated to the rapid dissemination of high quality research papers. We know that only advances in Medical, Agricultural, and Veterinary Sciences can help us in confronting the challenges of the 21st century, and to capitalize on the promises ahead. We welcome contributions that can demonstrate near-term practical usefulness, particularly contributions that take a multidisciplinary /convergent approach because many of the real world problems are complex in nature.

Finally, we encourage contributions from the scientific communities to ensure a continuity of a successful Biosciences journal. Authors, reviewers and guest editors are always welcome. We also welcome comments and suggestions that could improve the quality of the journal.

Thank you. We hope you will find NUJIB more informative in the future endeavor.

Regards,

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

Nangarhar University International Journal of Biosciences (NUIJB)

Editorial Team

Editors Of Veterinary Sciences

Prof. Mohammad Bayer Dermal
Veterinary Pharmacology and Toxicology | Faculty of Veterinary Science | Nangarhar University | Afghanistan
bayer500@yahoo.com

Prof. Glamazdin Igor Gennadievich
Food Production | Faculty of Veterinary Medicine and Zoo-technology | Moscow State University of Food Production Technologies|Moscow |Russia
glamazdin@mgupp.ru

Associate Professor Asadullah Hamid
Department of Pre-Clinic | Faculty of Veterinary Science | Kabul University | Afghanistan
pyarookhil@yahoo.com

Dr. Waseem Ali Vistor
Basic Veterinary Medicine | Sindh Agriculture University | Tandojam | Pakistan
drwaseemvisto@gmail.com

Editors Of Medical Sciences

Assistant Prof. Abdul Ghafar Sherzad
Department of Biochemistry | Faculty of Medicine | Nangarhar University | Afghanistan
ghafarsherzad11@gmail.com

Prof. Mohammad Haroon Rahimi
Department of Public Health | Faculty of Medicine | Nangarhar University | Afghanistan
haroonrahemi@yahoo.com

Assistant Prof. Abdul Azeem Rasouli
Ddepartment of ENT | Faculty of Medicine | Nangarhar University | Afghanistan
azeem284@gmail.com

Prof. Dr. Wrishmeen Sabawoon
Public Health | Epidemiology and Biostatistics | Tokyo University | Tokyo | Japan
wsabawoon786@gamil.com

Prof. Dr. Mohammad Azim Azimee
Department of Biochemistry | Faculty of Medicine | Nangarhar University | Afghanistan
azimazimee2017@gmail.com

Associate Prof. Mohammad Haris Abdianwall
Department of Epidemiology | Hacettepe University | institute of Health Science | Turkey
haris_abdianwall@yahoo.com.tr

Prof. Dr. Bilal Ahmad Rahimi
Clinical Tropical Medicine | Mohidol University | Bangkok | Thailand
drbilal77@yahoo.com

Assistant Prof. Gulbuddin Gulab
Department of Horticulture| Agriculture Faculty | Nangarhar University | Afghanistan
malyargulab@gmail.com

Disclaimer

The views expressed in the manuscript/text by the author(s) are their own and NUIJB, NU Publishers or Editor do not take any responsibility for the same.

In this issue, texts are copied from the soft copies provided by the author(s). hence printing errors/omissions if any are regretted.

Editorial Committee
NUIJB, 2022

Contents

Title	Page No
Toxic Effects of Norfloxacin on Cartilage Tissues of Broiler Chicken Darmel Mohammad Bayer*[1], Stanikzai Peer Mohammad[1], Omari Palwasha[1], Dost Asadullah[1], Memlawal Redwanullah[2], Rahmani Mohammad Malyar[3], Halim Mahshed	1
Effects of Different Planting Densities and Planting Spaces on the Growth and Yield Attributes of Rice under Irrigated Condition Wafa Imran Khan[1, 2], Kakar Kifayatullah [2, 3]*	6
Effects of Supplemental Japanese Pepper Seed on Thermoregulation, and Blood Monoamines in Heat Exposed Broiler Chicks Maroof Khushdil[1, 2]*, Oka Takao[1, 2], Himmat Jamaat Khan[2, 3] Fujihara Mika[2, 3], Bungo Takashi[1, 2]	13
Evaluation of Norfloxacin Acute Toxicity in Five Day old Broiler Chicken Darmel Mohammad Bayer*[1], Waziri Mohammad Younus[3], Nassary NoorAgha[3], Rahmani Mohammad Malyar[3], Omari Palwasha[1], Halim Mahshed, Khalili Fazal Akbar[3], Ahmady Huma[3], Muhammad Bilawal Arain[4]	22
Clinical Profile of COVID-19 Patients in Nangarhar University Teaching Hospital Hadi Saifullah[1], Del Del Aqa[1], Shirzay Aimal[1]	30
Pathological changes of Aortic Valve Calcification in Experimental Animal Models Sherzad Abdul Ghafar[1, 2]*, Behsodwal Khalil Ahmad[3], Azimee Mohammad Azim[2], Muhibullah Shinwari[4], Imran Zafarzai[5], Zaheer Shafiullah[2], Arash Nemat[1, 6], Osama Alsarhan[1], Qingchun Zeng[1, 7]*	37

Nangarhar University
International Journal of Biosciences

Received: 05 Apr 2022
Accepted after revision: 05 May 2022
Published Online: 19 Aug 2022

ORIGINAL ARTICLE Vol 1, Issue 1 (2022) https://nuijb.nu.edu.af

Toxic Effects of Norfloxacin on Cartilage Tissues of Broiler Chicken

Darmel Mohammad Bayer*[1], Stanikzai Peer Mohammad[1], Omari Palwasha[1], Dost Asadullah[1], Memlawal Redwanullah[2], Rahmani Mohammad Malyar[3], Halim Mahshed

[1]Department of Clinic, Veterinary Sciences Faculty, Nangarhar University, Afghanistan
[2]Department of Para Clinic, Veterinary Sciences Faculty, Nangarhar University, Afghanistan.
[3]Department of Pre Clinic, Veterinary Sciences Faculty, Nangarhar University, Afghanistan
*Corresponding author: bayer500@yahoo.com

ABSTRACT

Background: Norfloxacin is reported to have a wide distribution in the body. It penetrates well into tissues of the genito-urinary tract and crosses the placenta as well and it has relatively high concentrations when it gets into bile. After the withdrawal of enrofloxacin by the U.S. FDA for its use in poultry, the importance of norfloxacin is getting increased and already some veterinary formulations are introduced by authorized companies to the market. Besides, its wide usage for various bacterial infections, new and unrecognized toxicities have emerged; where the most important finding from the pre-clinical evaluation of flouroquinolone was arthropathogenic potential in young animals. This study was conducted to investigate the toxic effects of norfloxacin on the cartilaginous tissues of broiler chickens.

Materials and Methods: The norfloxacin powder was obtained from Trichem laboratories in Bangalore. For better dissolving 1gr 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. The experiment was carried out under hygienic conditions and standard management. One-week-old broiler chickens were procured from a reputed hatchery and divided into five groups; each containing six chickens. The study was conducted for 28 days.

Findings: In the result of the histopathological study, chondrocytes were swollen with degeneration, infiltrated neutrophils, and improper ossification. The chondrocytes degeneration around the blood vessels was also noticed. The lesions were supported by the biochemical finding of ALP (Alkaline phosphatase). On the 21st day of treatment. There was a significant increase in ALP values of groups IV, and V. On the 28th day of treatment the mean serum ALP was significantly increased in groups III, IV, and V birds ($P < 0.05$, $P < 0.01$, $P < 0.01$) respectively. They were compared to group I birds.

Conclusion: It was concluded that norfloxacin has a toxic effect on the cartilaginous tissues of chickens at the dose of 333 and 1100 mg/kg orally.

Keywords: Norfloxacin; Toxicity; Chondrocytes degeneration; Broiler chicken; Cartilage; Alkaline phosphatase

INTRODUCTION

Research into fluoroquinolone antibacterial has led to the discovery of several compounds with greatly improved potency, spectrum, bioavailability, and clinical efficacy (Ball, 1989). Norfloxacin is reported to have a wide distribution in the body. It penetrates well into tissues of the genito-urinary tract and crosses the placenta as well and relatively high concentrations were achieved in bile (Anadón et al., 1995). After the withdrawal of enrofloxacin by the US FDA for its use in poultry, the importance of norfloxacin is getting increased and already some veterinary formulations are introduced by authorized companies to the market.

Besides its wide usage for various bacterial infections, new and unrecognized toxicities have emerged; the most important finding from the pre-clinical evaluation of flouroquinolone was arthropathogenic potential in young animals (Stahlmann et al., 1990). For many years, quinolones induced cartilage toxicity in skeletally immature animals represented indisputable contraindication for the use in young animals. Apart from these, other organs like the kidney and liver have also been stated as the possible targets of quinolone toxicity (Christ, 1990). Corrado et al. (1987) reported toxicosis including cartilage erosion in juvenile dogs and crystalluria in dogs given 50-300 mg/kg norfloxacin for 20 weeks.

Fluoroquinolone antibacterial agents have been reported to induce tendon lesions in juvenile rats. The toxic potentials of 10 fluoroquinolones on the Achilles tendon were compared in juvenile rats. The toxic potential was differentiated: fleroxacin and pefloxacin were the most toxic, with the lowest toxic dose being 100 mg/kg; lomefloxacin, levofloxacin, and ofloxacin were the second most toxic, with the toxic dose being 300 mg/kg; sparfloxacin and enoxacin were the third most toxic, at 900 mg/kg, while norfloxacin, ciprofloxacin, and tosufloxacin showed no toxicity, even at the high dose of 900 mg/kg (Kashida & Kato, 1997). Chyský et al. (1991) reported that there were clear species and drug differences in quinolone effects on cartilage. When individual compounds were compared, pefloxacin caused slightly more transient articular adverse effects than ciprofloxacin Primary injury to juvenile cartilage may be from the direct action of these compounds or metabolites on the chondrocyte. The repression of normally secreted proteolytic inhibitors (Glynn, 1977).

According to Bell (1960) chickens 5-6 weeks of age with no visible lesions, the alkaline phosphatase ranged from 150-520 units. The levels increased in diseases of osseous origin. In the chick, Surendranathan &Nair (1981) reported normal blood glucose and alkaline phosphatase concentration of 153.61±2.15 and 2.08.98 ± 4.32 units respectively.

MATERIALS AND METHODS

A study was conducted to investigate the toxic effect of norfloxacin on the cartilaginous tissue of broiler chickens. The norfloxacin powder was obtained from Trichem laboratories in 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 the prepared stock solution was used for further dilution.

The experiment was carried out under hygienic conditions and standard management one one-week-old broiler chicken was 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 28 days.

Groups	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 VI (Vehicle)	Vehicle

The cartilage tissue from the Achilles tendon was collected on day 29 and was subjected to Histopathology and the blood samples were collected from the jugular vein on days 7, 14th, 21st, and 28 for biochemical analysis.

RESULTS

The birds who received med and high doses were showing difficulty in walking during the fourth week. The lesions recorded in chondrocytes for the high dose group were infiltration of neutrophils, improper ossification, degeneration around the blood vessels, and hemorrhage between bone and cartilaginous tissues. The same lesions in mild form were observed in cartilage tissue of broiler chickens received a 333 mg/kg dose of norfloxacin but no lesions were observed in chickens received 111mg/kg of norfloxacin **(Fig. 1).**

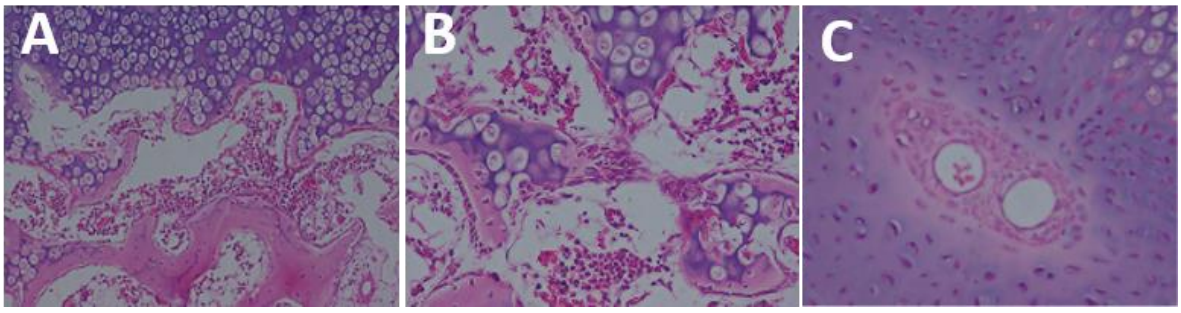

Fig. 1. Effects of norfloxacin on the Histomorphology of cartilage and chondrocytes. The results showed hemorrhage between the cartilaginous plates (Plates1-3) and chondrocytes were swollen with degeneration, infiltration of neutrophils and improper Ossification **(A&B)**. The chondrocytes degeneration around the blood vessels was also noticed **(C)**, (H & E 500X).

The lesions were supported by biochemical finding of ALP (Alkaline phosphatase)

On the 21st day of treatment I, II, III, IV, and V was 229.71±24.40, 221.51±10.00, 321.80±21.16, 635.03±22.35, 802.62±29.93 respectively. There was a significant increase in ALP values of groups IV, and V. On the 28th day of treatment the mean serum ALP level of groups I, II, III, IV, and V were 217.67±25.03, 216.79±32.43, 282.41±18.13, 561.80±32.00, 845.45±39.42 respectively. It was significantly increased in groups III, IV, and V birds (P <0.05, P <0, 01, P <0, 01) respectively compared to group I birds. The ALP levels of control and different treated groups are given in **Table 1.**

Table 1. Effects of Norfloxacin on ALP (U/dl) level of experimental broilers in repeated dose 28 day oral Toxicity study

Groups	Day 7	Day 14	Day 21	Day 28
Group I (control)	222.32±19.87	212.10±7.33	229.71±24.40	217.67±25.03
Group II (Vehicle)	228.03±7.93	259.75±20.51	221.51±10.00	216.79±32.43
Group v (0.11g/kg)	280.82±21.03	332.10±25.10	321.80±21.16	382.41±18.13*
Group IV (0.333g/kg)	460.57±28.13	532.53±32.25*	635.03±22.35***	561.80±32.00***
Group III (1.1g/kg)	528.15±62.43	773.64±54.71*	802.62±29.93***	845.45±39.42***

Data is presented as Mean±SE (n=6).

*, **, ***: Means are different in the same row at significance level of ***P<0, 01, **P<0, 01, *P<0, 05.

DISCUSSION

The results of the study indicate that the birds received med and high doses of norfloxacin were showing difficulty in walking during the fourth week. At the same time, histopathological lesions **(**infiltration of neutrophils, improper ossification, degeneration around the blood vessels, and hemorrhage between bone and cartilaginous tissues) were recorded in chondrocytes of high dose groups. The findings correlate with a finding (Stallmann, 1990): arthropathogenic potential in young animals (Christ et al., 1990): cartilage toxicity in skeletally immature animals Corrado et al. (1987): cartilage erosion in juvenile dogs but our study finding in term of med dose isn't within finding of (Kashida & Kato, 1997) where norfloxacin, ciprofloxacin, and tosufloxacin showed no toxicity, even at the high dose of 900 mg/kg in juvenile rats. This deference may be due to species variation and the opinion is getting supported by the findings of Chysky et al. (1991) who reported that there were clear species and drug differences in quinolone effects on cartilage. In this research, lesions were supported by the biochemical finding of ALP (Alkaline phosphatase) much with the finding of Bell (1960) who show that ALP getting increased in diseases of osseous origin.

CONCLUSION

From the present study it was concluded that the norfloxacin has toxic effect on the cartilaginous tissue of chickens at the dose of 333 and 1100 mg/kg orally.

Conflict of Interest

No, conflict of interest among all authors

REFERENCES

Anadón, A., Martinez-Larrañaga, M. R., Diaz, M. J., Fernandez, R., Martinez, M. A., & Fernandez, M. C. (1995). Pharmacokinetics and tissue residues of norfloxacin and its N-desethyl-and Oxo-metabolites in healthy pigs. Journal of veterinary pharmacology and therapeutics, 18(3), 220-225.BALL, P., 1989. Adverse reactions and interaction of fluoroquinolones. J. Clin. Invest. Med., 12: 28-34.

Ball, P. (1989). Adverse reactions and interactions of fluoroquinolones. Clinical and Investigative medicine. Medicine Clinique et Experimental, 12(1), 28-34.

Bell, D. J. (1960). Tissue components of the domestic fowl. 4. Plasma-alkaline-phosphatase activity. Biochemical Journal, 75(2), 224.CHYSKY, V., KAPILLA, K. and HULLMAN, R., 1991. Safety of ciprofloxacin in children: Worldwide clinical experience based on compassionate use. Emphasis on Joint Evolution Infection, 19: 289-296.

Chyský, V., Hullmann, R., Schacht, P., Kapila, K., Echols, R., & Arcieri, G. (1991). Safety of ciprofloxacin in children: worldwide clinical experience based on compassionate use. Emphasis on joint evaluation. Infection, 19(4), 289-296.

Christ, W. (1990). Central nervous system toxicity of quinolones: human and animal findings. Journal of Antimicrobial Chemotherapy, 26(suppl_B), 219-225.

Corrado, M. L., Struble, W. E., Peter, C., Hoagland, V., & Sabbaj, J. (1987). Norfloxacin: review of safety studies. The American journal of medicine, 82(6), 22-26.CHRIST, W., 1990. Central nervous system toxicity of quinolones: human and animal findings. J. Antimicrob. Chemother, 26: 219-225.

Glynn, L. E. (1977). Primary lesion in osteoarthrosis. The Lancet, 309(8011), 574-575.STAHLMANN, R., KUHNER, S., SHAKIBAEI, M., SCHWABE, R., FLORES, J., EVANDER, S. A. and VAN SICKLE, D. C., 2000. Chondrotoxicity of ciprofloxacin in immature beagle dogs; immunohistochemistry, electron microscopy, drug plasma concentrations. Arch. Toxicol., 73: 31-44.

Kashida, Y., & Kato, M. (1997). Characterization of fluoroquinolone-induced Achilles tendon Toxicity in rats: Comparison of toxicities of 10 fluoroquinolones and effects of anti-inflammatory compounds. Antimicrobial agents and chemotherapy, 41(11), 2389-2393.

Surendranathan, K. P., & Nair, S. G. (1981). Effects of thiamine and/or riboflavin deficient diets on the haematology of chicks. Indian veterinary journal.United States Pharmacopia Dispensing Information Update. US Pharmacopial Convention, 1987, 4: 61-63.

Stahlmann, R., Kühner, S., Shakibaei, M., Schwabe, R., Flores, J., Evander, S. A., & Van Sickle, D. C. (2000). Chondrotoxicity of ciprofloxacin in immature beagle dogs: immunohistochemistry, electron microscopy and drug plasma concentrations. Archives of toxicology, 73(10), 564-572.

Nangarhar University
International Journal of Biosciences

Received: 10 Mar 2022
Accepted after revision: 05 Apr 2022
Published Online: 19 Aug 2022

ORIGINAL ARTICLE Vol 1, Issue 1 (2022) https://nuijb.nu.edu.af

Effects of Different Planting Densities and Planting Spaces on the Growth and Yield Attributes of Rice under Irrigated Condition

Wafa Imran Khan[1, 2], Kakar Kifayatullah [2, 3]*

[1]Agricultural Research Institute of Afghanistan, Ministry of Agriculture, Irrigation, and Livestock, Kabul 1001, Afghanistan; imrankhan_wafa@yahoo.com

[2]Graduate School for International Development and Cooperation, Hiroshima University, Hiroshima 739-8529, Japan

[3]Faculty of Agriculture, Nangarhar University, Nangarhar 2601, Afghanistan

*Correspondence: kifayatullahkakar@gmail.com

ABSTRACT

Background: Rice is a staple food for majority of the world's population. Biotic and abiotic factors can affect its growth, yield and quality attributes. An open field experiment was conducted during the rice-growing period from March to October 2015 to identify the effects of different planting densities and planting spacing on the growth attributes and yield performances of a high tillering capacity Indica rice variety (IR-28).

Materials and Methods: The experiment was conducted under lowland condition in the research farm of Tsukuba International Center, Japan with four different planting densities and three replications in a randomized complete block design. Four different planting densities were selected as high density (15x20cm), standard density (15x30cm), medium density (25x25cm) and low density (30x30cm). Rice growth traits including plant length, tiller number, SPAD value and leaf color; yield and yield components consisting of panicle number per unit land area, spikelet number per panicle, ripening ratio, and 1000 grain weight were compared.

Findings: The greatest grain yield was obtained from high planting density which was 6.5 ton per ha and the lowest (5.8 ton/ha) was from medium planting density. Low planting density increased plant length, tiller number per hill, SPAD value (chlorophyll content), leaf color beside panicle number per hill, spikelet number per panicle and percentage of ripened grain.

Conclusion: This study clearly elucidated the effects of planting density on rice crop and helps farmers in the achievement of optimum yield. Thus, to obtain a satisfactory yield, planting density must be considered based on soil type and production requirement.

Key words: Growth; Yield; Planting Density; Planting Space; Rice

INTRODUCTION

Rice is among the important cereal crops which involved in deity of more than 50% of the world population (Jeon et al., 2010; Kakar et al., 2019a). Its growth, yield and grain quality is affected by several factors including planting densities which limit and decline rice productivity. Dense cultivated crops may face with competition for temperature, solar radiation, moisture and soil fertility which affects their growth, yield and grain quality performance (Bozorgi et al., 2011). Planting density plays a crucial role on growth, yield and grain quality of rice plant through influence of growth parameters such as tillers, panicles and spikelet numbers (Wu, 1998).

Bozorgi et al. (2011) reported that dense rice cultivation increases growth traits, yield, and yield components in contrast to medium or low planting densities. Dense rice cultivation also raises the number of tiller on unit land area (Huang et al., 2013). However, Fukushima et al. (2011) mentioned that high planting density decreased the total number of leaf on the main culms and the number of spikelets per panicle. Baloch et al., (2002) observed that low planting density increased panicle number per hill, ripened grains percentage, 1000-grain weight, and consequently grain yield per hill.

Every plant has its own requirement of optimum planting density (Baloch et al., 2002). Plant characters, growth duration, planting date and method, soil fertility and condition, environmental factors, and agronomical practices are important factors which affect crop optimum planting density (Bozorgi et al., 2011). Wider spaces between crops increased the performance of individual crops and lead them to get enough nutrients and solar radiation for a better photosynthetic process (Asmamaw, 2017). High planting density increased yield, but produces less amount of spikelet per panicle and shorten panicle length (Kakar et al., 2019b; Uddin et al., 2011).

It is well understood that rice tillering capacity is affected by nitrogenous fertilizer and planting density (Fagada, 1971). Therefore, planting density should be in mind when paddy rice is cultivated, as it influence both upper and lower parts of the rice plants (Baloch et al., 2002). Many researchers have described that high planting density optimizes rice grain yield, but little is known regarding Indica rice cultivars especially IR-28. To address the issue, this experiment was conducted to understand the relationship of growth and yield components of IR-28 with different planting densities and planting spaces under the irrigated condition and to find out the optimum planting density for growth and yield of IR-28 rice cultivar.

MATERIALS AND METHODS

Site selection and experimental design

This experiment was conducted at the research field of Tsukuba International Center (TBIC), Ibaraki prefecture, Japan during the rice-growing season from March to October 2015 under irrigated condition. The experiment was one factorial randomized complete block design with four treatments of planting densities and three replications. Four different planting densities were as high (15x20cm; 33.3 hills per m^2), standard (15x30cm; 22.2 hills per m^2), medium (25x25cm; 16 hills per m^2) and low (30x30cm; 11.1 hills per m^2). Total research area was 24 m^2 in which 12 plots were isolated individually based on randomization. Soil characters of the research farm are listed in **Table 1**.

Table 1. Soil characteristics of the experimental farm

Soil characteristics	Portion
pH	5.8
Total Nitrogen	4.70 g kg^{-1}
Total Carbon	49.6 g kg^{-1}
P_2O_5	1042 mg kg^{-1}
K_2O	156 mg kg^{-1}

Plant materials and field preparation

High tillering capacity Indica rice cultivar IR-28 was chosen as the main crop. Seeds were selected using a salt solution of specific gravity 1.13 and then washed with clean water to remove remaining salts. Seeds were disinfected using a fungicide (Benlate-T) for 24 h and then dried for 24 h; disinfected seeds were soaked in fresh water for four days and left at room temperature for one day to sprout. 50 g pre-germinated seeds were sown at nursery trays (60cm x 30cm) on 15th April 2015 using commercial soil. 31 days-old rice seedlings at 3.5 plant ages in leaf number were transplanted by hands on 15th May 2015 at three seedlings per hill and different planting densities were applied based on treatments. Herbicide was used at five days after transplanting to diminished weeds growth and attacks.

Experimental field was plowed and puddle using power tiller and leveled manually. Ahead of plowing 40 kg/ha N, 90 kg/ha P and 50 kg/ha K fertilizers were applied as a basal dressing. The sources of fertilizers were urea (46-0-0), single superphosphate (0-17.5-0) and potassium chloride (0-0-60), respectively. Water was kept at 5-10 cm during growing season. Physical and chemical weeding was conducted at different growing stages using hand weeding and herbicide application. Plants were harvested at the end of October 2015 to measure yield components and total grain yield.

Data collection and measurements

Data of plant growth parameters were recorded on plant length, tiller number, SPAD value, and leaf color at three growing (vegetative, reproductive and ripening) stages. Ten and 60 hills were respectively selected from each plot to evaluate growth and yield parameters using the method described by Hoshikawa, 1997. Plant length was recorded from the surface of soil the till top of the plant; tiller number was simply counted with hands. SPAD value and leaf color were recorded by SPAD (Konica-Minolta 502 Plus, Tokyo, Japan) and leaf color chart (IRRI, Philippines), respectively.

Selected hills were picked out for yield and its components analysis, panicles were cut from rachises. To determine the percentage of ripened grain, separated spikelets were added in fresh water to remove unfilled grain and then filled grains were weighted. 1000 grain weight was measured based on counting and weighing 1000 grains. Grains were dehusked applying a small impeller hulling machine (FC2K, Ohtake Corporation, Japan) to measure brown rice yield. Data were analyzed using one-way ANOVA by SPSS 13.0 software (IBM Corporation).

RESULTS

Growth performance

Significant differences were observed among treatments in term of plant length, number of tillers per hill and unit land area, SPAD value and leaf color ($p < 0.01$). Increased in planting density, decreased plant length, tiller number per hill, SPAD value and leaf color, but increased tiller number per unit land area (**Table 2**). Besides, low planting

density enhanced plant length, tiller number per hill, SPAD value and leaf color. Tiller numbers per hill and per unit land area showed opposite values among treatments, increased tiller number per hill reduced tiller number per unit land area and follow the same order in all treatments. SPAD value follows a similar trend as leaf color and had a positive correlation.

Table 2. Effects of different planting densities and planting spacing on growth parameters on rice plant

Treatments	Plant length (cm)	Tiller No. hill^{-1}	Tiller No. m^{-2}	SPAD value	Leaf color
High	99.2 d	9.8 d	325.0 a	30.6 d	3.5 c
Standard	103.7 b	12.7 c	281.5 b	32.1 c	4.1 b
Medium	100.6 c	14.1 b	225.3 d	33.4 b	4.1 b
Low	106.1 a	22.6 a	250.9 c	33.7 a	4.3 a
Significant	**	**	**	**	**

** indicates a significant difference at p<0.01 probability level. Different letters in the same column mean significant difference at p<0.05 probability level.

Yield components

Paddy grain yield and its components are summarized in **Table 3**. There were significant differences in terms of panicle number per unit land area, spikelet number per panicle, the percentage of repined grain, 1000 grain weight and paddy grain yield. Increased in planting density, raised panicle number per unit land area, ripened grain ratio and paddy grain yield. Moreover, spikelet number per panicle was completely opposite with panicle number per unit land area and exhibited negative correlation. The highest number of spikelets per panicle was observed in low planting density and the lowest was in high planting density. Rice grain yield also followed the same order as paddy yield and was higher at high planting density compare to other treatments (**Fig. 1**).

Table 3. Effects of different planting densities and planting spacing on yield and yield components

Treatments	Panicle No. m^{-2}	Spikelet No. panicle^{-1}	Ripened grain ratio (%)	1000 grain weight (g)	Paddy grain yield (ton ha^{-1})
High	317.2 a	82.6 c	85.6 a	29.0 b	6.5 a
Standard	250.3 b	105.8 b	76.2 c	29.5 a	6.0 b
Medium	215.3 c	117.5 a	84.4 b	28.7 c	5.8 c
Low	207.3 d	117.9 a	85.0 ab	28.6 c	5.9 bc
Significant	**	**	**	**	**

** means a significant difference at p<0.01 probability level. Different letters in the same column mean significant difference at p<0.05 probability level.

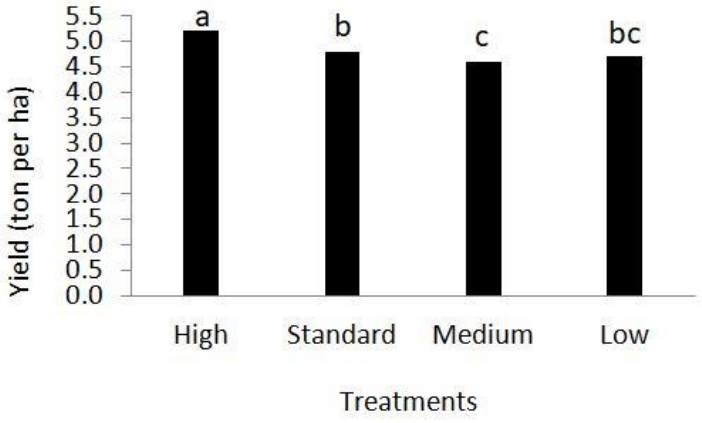

Fig. 1. Rice grain yield in different treatments of planting densities, different letters indicate significant difference at $p < 0.05$ probability level.

DISCUSSION

Among morphological characteristics in rice plant, the most affected characteristic by planting density in this experiment was tiller number per hill and per unit land area which is also reported by Akita (1992) that tiller is affecting by planting density. Low planting density increased tiller number per hill after transplanting till harvesting in contrast with other treatments, more space between crops in low planting density may provide a great condition for their better growth. In high planting density, the space between crops was less which leads the way for competition among them. Therefore, the value of leaf color was decreased and showed that less nitrogen is available for crops as reported by Lin et al. (2009).

Effects of planting density apparently direct on growth and yield performance of rice plant. Akita (1992) reported that yield is calculated from four components which are panicle number per hill, spikelet number per panicle, the percentage of ripened grain and 1000 grain weight. In high planting density; tiller number per hill was lower but tiller number per unit land area was higher, resulted to produce more panicle per unit land area. It is true because the number of plants per unit land area was higher in high planting density treatment. Panicle number per unit land area and paddy yield have a positive correlation; as panicle number is increased, grain yield also increased (Asmamaw, 2017; Sheieh, 1997).

Reduced in planting density caused to increase spikelet number per panicle, highest spikelet number per panicle was observed in low planting density which was 117.9. Increased in spikelet number per panicle in low planting density is due to more space between crops in this density which lead the way for proper nutrient supply and easy solar light penetration to the lower parts of the crops as was also reported by Uddin et al., (2010 and 2011). In this experiment, grain ripening ration had negligible difference among treatments and was ranged from 76.2 to 85.6 percent. The weight of 1000 grain is not correlated with planting density (Akita, 1992; Kakar et al., 2019c). In our experiment, the weight of 1000 grain also was not affected by planting densities; the same results were found by Asmamaw, (2017). The results revealed that paddy yield and number of panicle per unit land area had a positive correlation. High yield was obtained at high planting density as we as high panicle number per unit land area; identical results were cited by Mobasser et al., (2007) and Chandrankar et al., (1981).

CONCLUSION

To obtain significant yield and decrease input cast, planting density must be a focal point when rice plant is cultivated. Every cultivar within a species has its own requirement of nutrients and cultivation system including planting density. Planting density had significant effects on plant length, number of tiller per panicle and unit land area, SPAD value, leaf color, number of spikelet per panicle, ripened grain ration, 1000 grain weight, and paddy and rice grain yields. The yield of rice plant was more attributed with number of panicle per unit land area. The yield was not significant among treatment, but little seedlings were used in low planting density. Therefore, we would like to recommend low planting density for IR-28 rice cultivar.

Acknowledgements

The authors would like to express sincere appreciation and gratitude to the Japan International Cooperation Agency (JICA) and the Tsukuba International Center (TBIC) staff for their technical and financial support to this research. Dr. Tetsu Watanabe, Dr. Shinzo Koizumi, Mr. Yoshiaki Umemiya, Mr. Mitsuo Numata, Dr. Mitate Yamada, and Mr. Toshio Fujii are highly acknowledged for their active and constant cooperation during this research.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Akita, K., Tanaka, N. (1992). Effect of planting density and planting patterns of young seedlings transplanting on the growth and yield of rice plant. Japan Journal of Crop Science, 61, 80-86.

Asmamaw, B. A. (2017). Effects of planting density on growth, yield and yield attributes of rice (Oryza sativa L.). African Journal of Agricultural Researches, 35, 2713-2721.

Baloch, A. W., Soomro, A. M., Javed, M. A., Ahmed, M. (2002). Optimum plant density for high yield in rice (Oryza sativa L.). Asian Journal of Plant Science, 1, 25-27.

Bozorgi, H. R., Faraji, A., Danesh, R. K., Keshavarz, A., Azarpour, E., Tarighi, F. (2011). Effect of plant density on yield and yield components of rice. World Applied Science Journal, 12, 2053-2057.

Chandrankar, B. L., Khan, R. A. (1981). Optimum spacing for early medium and late duration tall indica rice cultivars. Oryza, 18, 108-109.

Fadaga, S. O. and De Datta, S. K. (1971). Leaf area index, tillering capacity, and grain yield of tropical rice as affected by plant density and nitrogen level. Agronomy, 63, 503-506.

Fukushima, A., Shiratsuchi, H., Yamaguchi, H., Fukuda, A. (2011). Effects of nitrogen application and planting density on morphological traits, dry matter production and yield of large grain type rice variety bekoaoba and strategies for super high yielding rice in the Tohoko region of Japan. Plant Production Science, 14, 56-63.

Hoshikawa, K. (1989). In: The Growing Rice Plant, an anatomical monograph: Panicle, Flower and Grain. First Ed. Nobunkyo, Tokyo, Japan, 280-281.

Huang, M., Yang, C., Ji, Q., Jinang, L., Tan, J., Li, Y. (2013). Tillering responses of rice to plant density and nitrogen rate in a subtropical environment of southern China. Field Crops Research, 149, 187-192.

Jeon, W. T., Seong, K. Y., Lee, J. K., Oh, I. S., Lee, Y. H., Ok, Y. S. (2010). Effects of green manure and carbonized rice husk on soil properties and rice growth. Korean Journal of Soil Science and Fertilizer, 43, 484-489.

Kakar, K., Xuan, T. D., Haqani, M. I., Rayee, R., Wafa, I. K., Abdiani, S., Tran, H. D. (2019a). Current situation and sustainable development of rice cultivation and production in Afghanistan. Agriculture, 9(3), 49-56.

Kakar, K., Nitta, Y., Asagi, N., Komatsuzaki, M., Shiotsu, F., Kokubo, T., Xuan, T. D. (2019b). Morphological analysis on comparison of organic and chemical fertilizers on grain quality of rice at different planting densities. Plant Production Science, 22(4), 510-518.

Kakar, K., Xuan, T. D., Abdiani, S., Wafa, I. K., Noori, Z., Attai, S... Tran, H. D. (2019c). Morphological observation and correlation of growth and yield characteristics with grain quality and antioxidant activities in exotic rice varieties of Afghanistan. Agriculture, 9(8), 167-178.

Lin, X. Q., Zhu, D. F., Chen, H. Z., Cheng, S. H., Uphoff, N. (2009). Effect of plant density and nitrogen fertilizer rates on grain yield and nitrogen uptake of hybrid rice (Oryza sativa L.). Journal of Agriculture, Biotechnology and Sustainable Development, 1, 44-53.

Mobasser, H. R., Delarestaghi, M. M., Khorgami, A., Tari, B. D., Pourkalhor, H. (2007). Effect of planting density on agronomical characteristics of rice (Oryza sativa L.) varieties in North of Iran. Pakistan Journal of Biological Science, 18, 3205-3209.

Sheieh, Y. J. (1977). Effect of planting density on community photosynthesis and on yielding components of rice plants. Botanical Bulletin of Academia Sinica, 18, 153-168.

Uddin, J., Hasan, M., Ahmed, S., Hasan, M. (2010). Effect of spacing on morphology and yield response of different aroma rice cultivars under costal high land ecosystem. Indian Journal of Agricultural Research, 44, 251-258.

Uddin, J., Ahmed, S., Harun, O. R., Hasan, M., Asaduzzaman. (2011). Effect of spacing on the yield and yield attributes of transplanted aroma rice cultivars in medium lowland ecosystem of Bangladesh. Journal of Agricultural Research, 49, 465-476.

Wu, G., Wilson, L. T., McClung, A. M. (1998). Contribution of rice tillering to dry matter accumulation and yield. Agronomy, 90, 317-323.

Nangarhar University
International Journal of Biosciences

Received: 07 Mar 2022
Accepted after revision: 05 Apr 2022
Published Online: 19 Aug 2022

ORIGINAL ARTICLE Vol 1, Issue 1 (2022) https://nuijb.nu.edu.af

Effects of Supplemental Japanese Pepper Seed on Thermoregulation, and Blood Monoamines in Heat Exposed Broiler Chicks

Maroof Khushdil[1, 2*], Oka Takao[1, 2], Himmat Jamaat Khan[2, 3] Fujihara Mika[2, 3], Bungo Takashi[1, 2]

[1]Department of Animal Science, Agriculture Faculty Nangarhar University, Nangarhar Afghanistan

[2]Laboratory of Animal Behavior and Physiology, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan

[3]Department of Basic Science, Medical Faculty Nangarhar University, Nangarhar Afghanistan

[4]Wakayama Prefectural Kihoku Livestock Hygiene Service Center, Wakayama 640-8483, Japan

Corresponding author: khmaroof@gmail.com

ABSTRACT

Background: This study was carried out to assess the effects of enriched feed with Japanese Pepper Seeds (1 and 2%) on plasma monoamine and thermoregulation in broilers.

Materials and Methods: A day old male broiler chicks were obtained from hatchery (Fukuda, Japan), and were kept in cages with floor of wire-mesh; 3 broilers in each cage. The surrounding heat was controlled at 30 ± 1 ^{0}C for three days in the beginning of experiment, and gently lowered to 26 ± 1 ^{0}C until broilers reached 11 days of age.

Findings: The study result indicated that after 6 days of feeding, their feed intake and body weight gain were not significantly different among groups of control and supplemented with Japanese pepper seeds. However, feed conversion ratio decreased significantly with feed of 1% Japanese pepper seeds against 2% in Japanese pepper seeds broilers ($P<0.05$). Subsequently, all groups were subjected to high heat at 38^o C, for 3 hours with water but not feed. The tendency was in wing drop and panting during temperature exposure groups ($P>0.05$). With acute heat stress, the temperature of all groups was elevated. The effects of time and Japanese pepper seeds were significant ($P<0.05$) in temperature exposed broilers. The interaction between time and Japanese pepper seeds were measured to reflect a tendency of significance ($P<0.01$). The affinities were measured in rectal temperature of control group and 2.0% group of Japanese pepper seeds broilers to reduce after 2h, although they kept advancing in 1% group of Japanese pepper seeds. There were no significant differences in the level of plasma monoamines in 5- HT, Ad and NA among groups ($P>0.05$), although the level of DA in plasma in 2% Japanese pepper seeds in broilers was lower than control one ($P<0.05$).

Conclusion: Present investigation recommend that Japanese pepper seeds affect thermoregulation through the catecholaminergic system in broilers. Nevertheless, it may have adverse effects under long period high temperature in chicks.

Keywords: Broiler; Japanese pepper seed; Thermoregulation; Plasma Monoamines; High temperature

INTRODUCTION

Many of plant components gained the attention of nutrition specialist, which develop as anti-nutrition ingredients or elements, which should be eliminated or avoided, or their effects need to be improved. Nevertheless, even though, among the materials, that are concede to this class, it is mostly found that, in a proper status, plants components may have good effects. For example the ability of anti-carcinogenic properties of phyto-oestroneges (Migas & Krauze-Baranowska, 2015) and glucosinolates (Nugon-Baudon & Rabot, 1994), the anti-oxidative and anti-atherogenic effects of disuphide and allyl thiosulphinate and the potential coccidiostatic action of artmisin (Allen et al., 1999). While these possibly useful effects have reported mostly with health of human, it is possible, that some of components will be of worth in animal diet such as fit. Thermally stressed environment is a major concern in modern poultry farming. Various strategies reported to alleviate the negative effect of high temperature in broilers (Yahav et al., 1995; Sahin et al., 2003), but the badly behaved has not yet been explained. The nutritional manipulations are one of the countermeasures to cope with heat stress, and there have been in particular many investigations about supplementation of anti-oxidative compounds, such as vitamins (Takahashi et al., 1991; Lin et al., 2002). As shown in Table 1 in Chapter 1, JPS contains relatively much tocopherols (0.045 mg/g), and it is expected to negative effects of oxidation stress in animals. The present was investigated to explain the effects of Japanese pepper seeds on plasma monoamines and thermoregulation in high temperature exposed broilers.

MATERIALS AND METHODS

Animals in Experiments

Male broilers (Chunky) at the age day old were obtained from hatchery (Okayama, Fukuda Hatchery, and Japan). Day old broilers were kept in cages (wooden) with floor of wire-mesh (18x25x20 cm) at density population of 3 broilers in each cage. Broilers were kept with 24 hours lighting in a room. The surrounding heat was controlled at 30±1 ° C for three days in the beginning of experiment, and gently lowered to 26±1 ° C until broilers reached 11 days of age. They had free access to a commercial starter feed (3100 kcal/kg of AME_n and 22% CP; Kobe, Japan, Nichiwa Sangyo Co. Ltd.) and water throughout the pre-experimental duration. The hold of broilers performed due to guidelines of the Animal Experiment Committee of Hiroshima University.

Body weight and feed intake

At day 4 of age, the broilers were placed in order into experimental sets based on body mass so the normal body weight was as similar as likely for each treatment. The broilers were kept separately in cages (13x25x25 cm) during experiment, had free access to water, and feed until the experiment ended. Commercial starter diet (basal diet) was fed to control group and the other groups were fed with ground Japanese pepper seeds added basal diet at 1.0 or 2.0%. The added level was determined at (5% Japanese pepper seeds feed may stimulate secretin of adrenaline, Japanese pepper seeds level was fixed to be less than half). Japanese pepper seeds was taken from Japanese pepper farm from prefecture of Wakayama, Japan. Feed intake and body weight gain measured in each day until the broilers were at age of 11 days.

Heat exposure treatment

Afterward of last determination of feed intake and body weight gain, all birds (11 days old) exposed to heat stress at 38 ° C for 3 hours with water but not feed (experimental groups in the thermo-neutral zone were not set, because supplemental JPS had no effect on rectal temperature in previous experiments. Rectal temperature was measured hourly during the heat exposure test. During the heat test, the beginning times of excesses performances in wing drop and panting (Etches et al., 2008) in broilers were visually recorded and monitored. All broilers were bled at

the end of the test by cardiac puncture and all samples were collected in heparinized tubes, and for 15 minutes centrifuged. Collected plasma samples were kept at -20 ° C until analyzed.

Blood parameters

The levels of NEFA (non-esterified fatty acid), glucose, TG (triglyceride), total cholesterol, HDL (high density lipoprotein), LDL (low-density lipoprotein cholesterol), LA (lactic acid), calcium, ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were determined by Beckman Coulter AU480 biochemistry analysis automatic system (CA, USA), which contain reagents prepared by producer.

Plasma monoamines were tested by the method of Terao et al. (2008), which used HPLC (high-performance liquid chromatography. Separation of monoamines were attained using a 5-µm reversed-phase Octadecyl-Silica column (Kyoto, Japan, CA-5 ODD; Eicom Co.). Column heat retained at 25 ° C by a thermocontroller (Tokyo Japan, TSK CO-8000; Tosoh Co.). The solvent transfer system (Tokyo 105 Japan, TSK CCPD; Tosoh Co.) enclosed 20 µM Na_2EDTA and 2.5 mM 1-octanesulfonic acid sodium salt (SOS), and 12% methanol in a 0.1 M NaH_2PO_4"0.1 M Na_2HPO_4= 1000:85 (0.1M phosphate buffer solution). The pH of the buffer was adjusted to 3.5 with H_3PO_4. The buffer was filtered and degassed by degasser (Tokyo Japan, TSK SK-8022; Tosoh Co.) and then the flow rate adjusted to 150 µL/min. The electrochemical detector (Tokyo Japan, TSK EC-8020; Tosoh Co.) was set at 900 mV and peak height were measured using a computer integrator. All values were corrected for real recovery based on the extraction rate of the inner slandered isopropanol.

Statistical analysis

Collected data were examined via accessible package of StatView (SAS Institute, Version 5, Cary, USA, 1998). For numbers with normal distribution, ANOVA and Tukey-Kramer test were performed. If data were not normally distributed, they were subjected to Kruskal-Wallis variance analysis and Steel-Dwass post-hoc procedures. To compare rectal temperature at the different time-points, a linear to define the dose-response relations at separately time period. Changes were stated significant at $P < 0.05$, and a value of $P < 0.01$ was measured to reveal a tendency to significance.

RESULTS

Effects of Japanese pepper seeds on feed consumption, body weight and feed conversion ratio.

Table 1 showed the effects of supplement JPS on BW (body weight gain), feed consumption and feed FCR (conversion ratio) in broilers. Feed consumption and BW gain were none-significantly changed ($P > 0.01$) among Japanese pepper seeds and control one. Nevertheless, FCR decreased significantly in broilers fed with 1.0% Japanese pepper seeds feed compared to 2.0 % Japanese pepper seeds ($P < 0.05$) in broilers.

Table 1. Effect of Japanese Paper seed on body weight and feed intake in broiler checks

	Control (5)	1.0 % JP (5)	2.0 % JP (5)	P value
Initial BW (4 d)	67.5±0.98	66.6±0.75	66.7±0.43	0.665
Final BW (11 d)	207.4±9.08	215.7±8.16	198.7±8.01	0.393
BW gain (g)	140.0±8.75	149.1±8.27	132.0±8.28	0.388
Feed intake (g)	175.0±10.31	176.6±8.86	165.7±8.68	0.679
FCR	1.25±0.030 [a]	1.19±0.007 [b]	1.26±0.018 [a]	0.048

JP: Japanese paper seeds, BW: body weight, FCR: Feed conversion ratio. Values are Mean±SE of the number of checks in parentheses. Means with different letters are significantly different at $p < 0.05$.

Effects of JPS on body temperature and behavioral response during heat challenge

Fig. 1. Indicated the result of JPS supplemented feed on tendency in wing-drop or panting throughout high temperature test. Bot heat dissipation behaviors were not significantly different between the Japanese pepper seeds ($P > 0.01$) and control one.

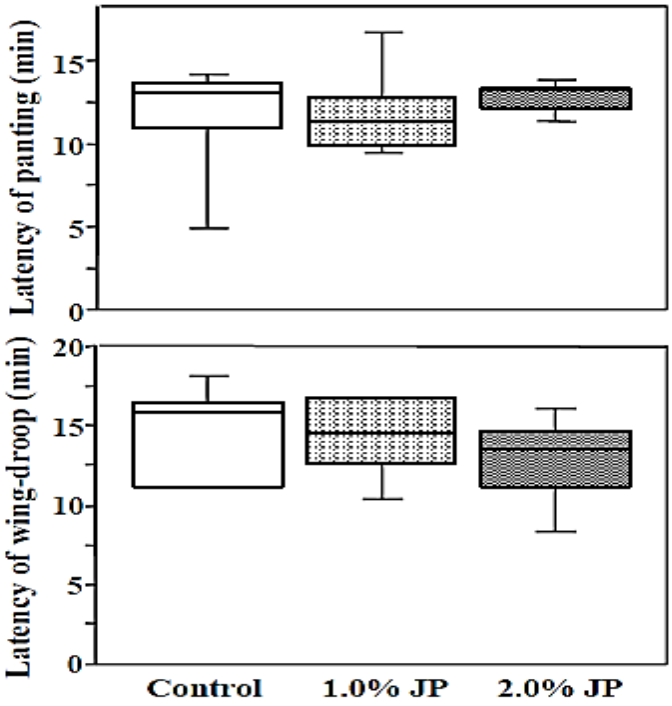

Fig. 1. Effects of Japanese pepper seed on thermoregulation behavior (panting and wing-droop) in heat exposed checks. Box plots show the median (center line) and the interquartile range from the 25th to the 75th percentile. Whiskers above and below the box indicate the 10th and 9th percentiles and circles indicate outliers.

The result of JPS supplemented feed on rectal temperature in heat-exposed chicks publicized in **Fig. 2** number 2. Before the high temperature exposure, rectal heat in control, 1.0 or 2.0% JPS chicks was 40.7±0.1, 40.8±0.1, 40.9±0.1 ^{o} C, respectively. The temperature each group were increased by high temperature. The effect of feed and time were significant in high temperature exposed broilers ($P<0.05$, $P<0.0001$). An association between feed and time was measured to return a tendency to significance ($P<0.01$). Rectal temperature at 2 and 3 h heat exposure indicated quadratic changes (2 h: y= 41.78 (SE 0.135, $P<0.0001$) +1.370 (SE 0.345, $P=0.0018$) X -0.630 (SE 0.166, $P=0.0025$) X^2, $R2=0.497$, $P=0.0065$; 3 h: y=41.60 (SE 0.241, $P<0.0001$) + 1.570 (SE 0.614, $P=0.0251$) X-0.750 (SE 0.295, $P=0.0292$)X^2, $R2=0.245$, $P=0.0735$). There were trends for rectal temperature of control ones and 2.0% Japanese Pepper Seeds broilers group the reduction after 2 h though that of 1.0% Japanese pepper seeds reserved advancing.

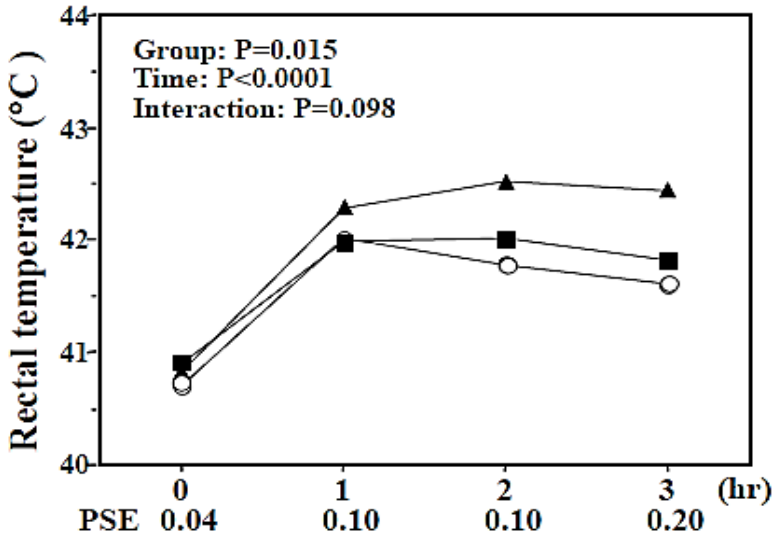

Fig. 2. Effects of Japanese pepper seed on rectal temperature of heat exposed checks during 3h. PSE, pooled SE, open circles; control, solid triangles; 1.0% JP, Solid Square; 2.0% JP. The number of checks in each group was as follows; control 5; 1.0% JP, 5; 2.0% JP, 5.

Effect of JPS on blood parameters after warm air challenge

The effect of supplemental JPS on blood parameters after high temperature stress revealed in **Table. 2**. Non-significant differences were find in all blood parameters (AST, ALT, and glucose, NEFA, T-Cho, TG, HDL, LDL, LA, Ca and IgA) among the groups (P > 0.01).

Table 2. Effects of Japanese pepper seed on plasma parameters in heat exposed chicks

	Control (5)	1.0 % JP (5)	2.0 % JP (5)	P-value
AST (U/L)	167.1±7.6	157.5±6.9	1667.6±4.6	0.484
ALT (U/L)	3.64±0.40	3.88±0.39	3.90±0.87	0.944
Glucose (mg/dL)	235.9±10.2	240.1±11.2	226.0±5.2	0.559
NEFA (mEq/L)	681.3±98.2	903.7±57.6	815.8±132.9	0.327
T-Cho (mg/dL)	130.6±8.6	123.8±8.4	130.9±3.4	0.738
TG (mg/dL)	16.5±1.9	13.9±0.8	17.1±0.6	0.209
HDL (mg/dL)	90.4±5.8	83.7±6.0	88.6±2.4	0.635
LDL (mg/dL)	13.0±1.4	12.9±1.3	15.3±0.6	0.296
LA (mg/dL)	25.1±5.6	25.4±2.5	28.0±3.7	0.863
Ca (mg/dL)	8.46±0.43	8.62±0.25	8.45±0.35	0.931
IgA (mg/dL)	2.30±0.13	2.14±0.09	2.12±0.10	0.458

JP: Japanese pepper seeds, AST: Aspartate aminotransferase, ALT: alanine aminotransferase, NEFA: Non-esterified Fatty acid, T-Cho: Total cholesterol, TG: Triglycerides, HDL: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein cholesterol, LA: Lactic acid, Ca: Calcium, IGA: Immunoglobulin A. Values are means± SEM of the numbers of chicks in parentheses.

Table 3 explained the result of Japanese pepper seeds in plasma monoamines in heat-exposed broilers. Although, significantly difference were not found in Ad, 5-HT and N A between groups ($P > 0.01$), the amount of DA in plasma in 2% Japanese pepper seeds broilers was not high then control one ($P < 0.05$).

Table 3. Effects of Japanese pepper seed on plasma monoamines in heat exposed chicks

	Control (5)	1.0 % JP (4)	2.0 % JP (5)	P-value
NA (ng/mL)	8.33±2.41	9.08±3.35	5.78±1.62	0.617
Ad (ng/mL)	6.27±1.24	20.46±8.29	10.98±5.60	0.229
DA (ng/mL)	1.36±0.61 ^{a}	0.44±0.36 ^{ab}	0.22±0.06 ^{b}	0.046
5-HT (ng/mL)	14.92±10.20	3.08±1.60	6.18±3.30	0.463

JP: Japanese pepper seeds, NA: noradrenalin, AD: adrenalin, DA: dopamine, 5-HT: serotonin. Values are means ± SEM of the number of chicks in parentheses. Means with different letters are significantly different at $P < 0.05$.

DISCUSSION

A sequence of metabolic and physiological changes in broilers occurred such as painting, increasing of body heat, alkalosis in respiration, through which metabolic status bring out and reduce level of plasma triiodothyronine (Deyhim & Teeter, 1991). Growth promoters are expecting to increase growth rate and improve gut health ensuing in better-feed consumption and reducing FCR (feed conversion ratio) (Visek, 1978). Birds outside of normal temperature may suffer physiological changes, such as decrease feed intake and egg production. Fowl is delicate to heat as related ecological challenges such as heat stress. Heat stress elevate steroidogenesis, which effects tissues and metabolism. Understand and control of environment is threat to success poultry production and welfare (Lucas & Marcos, 2013). However, the current study indicates that there is no effect of JPS on feed consumption and body weight gain supplemented as feed additives, but FCR was significantly lower in 1% JPS compare to other groups (**Table 1**). Deduction in FCR in 1% JPS might be because of positive phenolic outcome of JPS as antioxidant which improved FCR, improved intestinal mucosal lining and improved digestion. Our study is supported that enhancement in FCR might be due to the active ingredient of medicinal plant which increase fat metabolism and energy utilization (Smeets & Lejeune, 2005). This may effect of herbal plant and enhance the digestive system function in assimilation of feed, and role of the polyphenol that possessed antioxidant activities (Papoutsi et al., 2005). This may effect of herbal plant and enhance the function of digestive system in adjustment of feed, and character of the polyphenol that keep antioxidant activities (Papoutsi et al., 2005). Plants and phytogenic property may control and enhance the growth of various pathogenic and nonpathogenic species of bacteria in chick's gut, which may affect to a better digestion and consumption of feed, results in enhanced FCR (Bedford, 2000). Plants derived feed additives may have helpful effect on digestion and increase absorption volume of gastric mucosa (Gonzalez et al., 1998). Supplementation of JPS as feed extracts might have helpful effect on digestion of feed and further active on nutrition, and due to deviations in intestinal ecosystem and absorption of digestive materials, thus FCR decreased in 1% JPS.

In the present study plasma Dopamine significantly lowered in 2% JPS compare to control and 1% JPS (**Table 3**). High doses of JPS decreased Dopamine level in broiler chicks. It could be support of JPS and removal of adverse influence of high temperature on adrenal lipid peroxidation at cell membrane (Edens & Siegel, 1975).

The reduction in plasma Dopamine could be due to effect of JPS tocopherol on heat stress, α-tocopherol of plants decrease the adverse effect of high temperature in broilers (Young et al., 2003), the inclusion of medicinal plant in the ration lessened the synthesis of corticosterone hormone and prevented their negative effect (Kutlu & Forbes, 1993). Phenylalanine and tyrosine are precursor of dopamine, epinephrine and norepinephrine. Phenylalanine first converted to tyrosine and serves as precursor of epinephrine, norepinephrine and dopamine. Epinephrine and norepinephrine stimulated glucose uptake of muscle, and norepinephrine activity stimulated by stress (Choochote et al., 2009).

Under heat stress, addition of JPS as feed additives in broilers diets prevent negative effect of corticosteroid hormone by decreasing their synthesis, which improved performance of broiler chicks under heat stress. Dopamine is highly correlated with faster and stronger adaptation of chicken to heat stressors (Hester et al., 1996a, 1996b). The effect of group (feed) and time were significant in high temperature exposed broilers ($P<0.05$) is shown in **Fig 2**. Supplementation of the diet with JPS improved group (feed) and time of heat stressed may be due to decreasing the synthesis of corticosteroid hormones and prevented their adverse effects. The significant in feed and time interaction indicated that JPS had more strong positive effect in heat stress group then non-heat stressed broilers. Supplementation of feed with acidifiers has the ability to increase chicken presentation under high teperature situation (Daskiran et al., 2004).

CONCLUSION

In conclusion, the present investigations recommend that Japanese pepper seeds distress temperature regulation through catecholaminergic system in broilers, although, it make possible thermoregulation through the catecholaminergic system in chicks but it may convert the opposing effect under the long time high temperature in chicks.

Acknowledgment

The investigation was helped through Promotion and Enhancement of Afghan Capacity for Effective Development (PEACE) from JICA (Japan International Cooperation Agency).

Conflict of Interest

No, conflict of interest among all authors

REFERENCES

Allen, M. J., Shan, X., Caruccio, P., Froggett, S. J., Moffat, K. G., & Murphey, R. K. (1999). Targeted expression of Truncated glued disrupts giant fiber synapse formation in Drosophila. Journal of Neuroscience, 19(21), 9374-9384.

Bedford, M. (2000). Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. World's Poultry Science Journal, 56(4), 347-365.

Choochote, W., Chaithong, U., Kamsuk, K., Jitpakdi, A., Tippawangkosol, P., Tuetun, B., & Pitasawat, B. (2007). Repellent activity of selected essential oils against Aedes aegypti. Fitoterapia, 78(5), 359-364.

Daskiran, M., Teeter, R.G., Vanhooser, S.L., Gibson, M.L. and Roura, E. (2004). Effect of dietary acidification on Mortality rates, general performance, carcass characteristics and serum chemistry of broiler exposed to cyclic high ambient temperature stress. Journal of Applied Poultry Research, 13: 605-613.

Devhim, F. And Teeter, R.G. (1991). Sodium and potassium chloride drinking water supplementation effects on Acid-base balance and plasma corticosterone in broilers reared in thermoneutral and heat-distressed environment. Poultry Science, 70: 2551-2553.

Edens, F.W. and Siegel, HS. (1975).Adrenal responses in high and low ACTH response lines of chickens during acute heat stress. General Comparative Endocrinology, 25: 64-73.

Gonzalez. R., Dunkel, R., Koletzko, B., Schusdziarra, V. and Allescher, H. (1998). Effect of capsaicin containing red pepper sauce suspension on upper gastrointestinal motility in healthy volunteers. Digestive Diseases and Science, 43: 1165-1171.

Hesters, P.Y., Muir, W.M., Craig, J.V. and Albright, J.L. (1996a). Group selection for adaptation to multiple-hen cages: Hematology and adrenal function. Poultry Science, 75: 1295-1307.

Hesters, P.Y., Muir, W.M., Craig, J.V. (1996b).Group selection for adaptation to multiple-hen cages: Production traits du ring heat and cold exposure. Poultry Science, 75: 1308-1314.

Kutlu, H.R. and Forbes, J.M. (1993).Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. Livestock Production Science, 36: 335-350.

Lin, H., Decuypere, E., Sang, J.L. Yie, Y.M. and Yany, R.M. (2002). Effect of dietary Supplemental levels of vitamin a on the egg production and immune response of heat stressed laying hens. Poultry Science, 81: 458-465.

Lucas, J.L. and Marcos, H.R. (2013). Impact of heat stress on poultry production. Animals, 3:356-369.

Migas, P. and Krauze-Baranowska, M. (2015). The significance of arbutin and its derivatives in therapy and cosmetics. Phytochemistry Letters, 13: 35-40.

Nugon-baudon, L. And Sylvie, R. (1994). Glucosinolates and glucosinolate derivatives: Implications for protection against chemical carcinogenesis. Nutrition Research Reviews), 7, 205-231

Papoutsi, Z., Kassi, E., Tsiapara, A., Fokialakis, N., Chrousos, G.P. and Moutsatsou. P. (2005). Evaluation of estrogenic/antiestrogen activity of ellagic acid via the estrogen receptor subtypes ERα and ERβ. Journal of Agricultural Food Chemistry, 53: 7715-7720.

Sahin, K., Sahin, N. and Kucuk, O. (2003). Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32 º C). Nutrition Research, 23: 225-238.

Smeets, A.J. and Lejeune, M.P. (2005). Sensory and gastrointestinal satiety effects of capsaicin on food intake. International Journal of Obesity, 29: 682-688.

Takahashi, K., Akiba, Y. and Horiguch, M. (1991). Effects of supplemental ascorbic acid on performance, organ weight and plasma cholesterol concentration in broilers treated with propylthiouracil. British Poultry Science, 32: 545-554.

Visek, W.J. (1978). The mode of growth promotion by antibiotic. Journal of Animal Science, 46: 1447-1469.

Yahav, S., Goldfeld, S., Plavnik, I. and Hurwitz, S. (1995). Physiological response of chickens and turkeys to relative humidity during exposure to high ambient temperature, Journal of Thermal Biology, 20: 245-253.

Young, J.F., Stagsted, J., Jensen, S.K, Karlsson, A.H. and Henckle, P. (2003).Ascorbic acid, Alpha-tocopherol, and organo supplements reduce stress induced deterioration of chicken meat quality. Poultry Science, 82: 1343-1351.

Nangarhar University
International Journal of Biosciences

Received: 07 March 2022
Accepted after revision: 05 Apr 2022
Published Online: 19 Aug 2022

ORIGINAL ARTICLE Vol 1, Issue 1 (2022) https://nuijb.nu.edu.af

Evaluation of Norfloxacin Acute Toxicity in Five Day old Broiler Chicken

Darmel Mohammad Bayer*[1], Waziri Mohammad Younus[3], Nassary NoorAgha[3], Rahmani Mohammad Malyar[3], Omari Palwasha[1], Halim Mahshed, Khalili Fazal Akbar[3], Ahmady Huma[3], Arain Muhammad Bilawal[4]

[1]Department of Clinic, Veterinary sciences Faculty, Nangarhar University, Afghanistan
[2]Department of Para Clinic, Veterinary sciences Faculty, Nangarhar University, Afghanistan
[3]Department of Pre Clinic, Veterinary sciences Faculty, Nangarhar University, Afghanistan
[4]Department of Veterinary Pharmacology, Sindh Agriculture University, Tando Jam
Corresponding author: bayer500@yahoo.com

ABSTRACT

Background: Norfloxacin is a second generation fluoroquinolone, used widely against sensitive organisms. After the withdrawal of enrofloxacin by the U.S. FDA for its use in poultry, the importance of Norfloxacin is getting increased and already some veterinary formulations were introduced by pharmaceutical companies in the market. The present study was undertaken to evaluate the acute toxicity of Norfloxacin IP, a member of fluoroquinolone in five day old broiler chicken.

Materials and Methods: Healthy 60 COBB broiler a day old chicks, with an average body weight of 45±5g were acclimatized to the laboratory condition for five days. Six groups; each group consisting 10 experimental birds were used for determining LD_{50} safety value. Clinical observations were made. The tissue samples from concerned organs of all the birds were collected for histopathological study.

Findings: The maximum dose of Norfloxacin, where no mortality occurred was 1.4 g /kg. The dose at which 100 percent mortality observed was calculated to be 1.8 g/kg /body weight. The clinical signs of toxicity observed before death were dullness, excitability, and drowsiness, circling, oozing of fluid from mouth and gasping for breath with beaks wide open. The calculated LD_{50} value for Norfloxacin was found to be 1.55 g/kg for 24 h observation period. The histopathological findings observed in concerned organs were: hemorrhage, congestion and tubular epithelium degeneration and necrosis in kidney, Hemorrhage, edema and infiltration of few inflammatory cells in heart. Congestion of vessels and dilatation of sinusoidal space were observed. Perivascular hepatocytes were degenerated with infiltration of few inflammatory cells on liver and sever lymphocytolysis in spleen.

Conclusion: Norfloxacin can be classified as a moderately toxic drug according to Global Harmonization System (GHS) for classification of toxic compounds as per given protocol.

Key words: $LD_{50;}$ Acute toxicity; Broiler chicken; Lesions; Norfloxacin

INTRODUCTION

Fluoroquinolones a class of antimicrobials which is getting widespread acceptance for the treatment of various bacterial infections due to their broad spectrum antibacterial activity against most of the sensitive gram-positive and gram–negative aerobes, *Mycoplasma* spp. and *Rickettsia* spp at very low concentration (Brown, 1996).

Norfloxacin is a second generation fluoroquinolone, primarily used in the treatment of urinary tract infections, however later clinical studies have shown the efficacy of Norfloxacin for a variety of gram-negative infections including pneumonia, CNS infections, prostatitis and septicemia (Mascellino et al., 1988). Norfloxacin usage in the treatment of gastrointestinal infections caused by *Esherichia coli*, *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. were also reported (Braunwald et al., 1987). Beside its wide usage for various bacterial infections, new and unrecognized toxicities have emerged; most important finding from pre-clinical evaluation of the flouroquinolone was arthropathogenic potential in young animals due to Ciprofloxacin toxicity (Stahlmann et al., 2000). Apart from these, other organs like kidney and liver have also been stated as the possible targets of quinolone toxicity (Christ, 1988). Norfloxacin is second-generation fluoroquinolone compound featuring a fluorine atom at position-6 and piperazinyl substituent at position-7 of the quinolone nucleus (Wentland, 1990). Clinically, fluoroquinolones are generally well tolerated, but CNS disorders including confusion, hallucination, anxiety, nervousness and seizures have been reported in two per cent of human population (Anastasio et al., 1988; Christ, 1990). Sachan (1998) conducted an acute oral toxicity study of pefloxacin on day old broiler chicks and reported LD_{50} of 1025 mg/kg for pefloxacin and also observed toxic CNS clinical signs such as excitability followed by dullness after single oral administration of pefloxacin. Zou and Wang (2007) reported the oral LD_{50} 3458 mg/kg for Norfloxacin nicotinate in 5-week-old chickens and toxic clinical signs observed in chicks were salivation, diarrhea and nervousness.

The flouroquinolones can cross the blood brain barrier and have been proposed as alternative for the treatment of CNS infections not responding to other drugs (Scheld, 1989). Fluoroquinolone antibiotics were found to induce central nervous system excitatory side effects, including anxiety, nervousness, hallucination, and even seizures on rare occasions (Christ, 1990). Domagala (1994) had pointed out that the substitution at the seventh position of fluoroquinolones greatly influences their efficacies and toxicities. For example, central nervous system effects and interactions with theophylline and nonsteroidal anti-inflammatory drugs were reported to be directly influenced by the substitution at the seventh position in chemical structure. A study was conducted by Zhang et al. (2003) regarding neurotoxicity and toxicokinetics of Norfloxacin on free moving rats in which the epileptiform discharges appeared in all Norfloxacin groups with different latent periods, accompanied with limb twitching and colonic and tonic seizures and relative power of EEG increased. The effect of ciprofloxacin and Norfloxacin treatments on the behavior of rats in the open-field, elevated plus maze, elevated zero maze, feeding latency and social interaction tests were respectively observed. In the result of the ratio between open arm and enclosed arm time and entries also indicated that both ciprofloxacin and Norfloxacin treated rats showed anxious behavior in comparison with control rats in all the parameters studied. Ciprofloxacin and Norfloxacin treatments caused significantly enhanced feeding latencies in comparison to control treatment in the novel environment. The consumption of feed in Norfloxacin treated group was 50.29±1.84 in control group 67.57±1.53 which showed a significant increase when compared to control group (Sen et al., 2007). The quinolones bore both an acidic group (carboxylic acid) and a basic group (tertiary amine). This association gave them amphoteric properties. Their lipid

solubility was low except between pH 6 and 8, within this range they had low water solubility and was proven to precipitate under more acidic conditions. Due to this property crystalluria had been observed in man and animals (Ball, 1986). Corrado et al., (1987) noted crystalluria in dogs after administration of norfloxacin at the dose of 50-300 mg/kg for 20 weeks. As the primary rout for excretion of fluoroquinolones was kidney so for high urine concentration and their poor water solubility at acidic pH had caused the formation of crystals in the urinary tract, where the crystals were thought to be responsible for the renal lesions (Ball, 1986). This reason is based on the fact that lesions of the kidney following fluoroquinolone exposure have never been observed without the presence of crystals but crystals have been observed without evidence of renal lesions. Moreover crystals were also present at lower concentrations than those producing the lesions (Vancutsem et al., 1990). After the withdrawal of enrofloxacin by the US FDA for its use in poultry, the importance of Norfloxacin is getting increased and already some veterinary formulation are introduced by reputed pharmaceutical companies in the market. In view of the above facts the present study was undertaken on Norfloxacin in broiler chicken with the following objective. This study was conducted to evaluate the acute toxicity of Norfloxacin IP a member of fluoroquinolone class antimicrobials in broiler chickens.

MATERIALS AND METHODS

Experimental birds: Sixty unsexed COBB breed of broiler day old chicken with an average body weight of 45±5g were procured for the study from a local farm. They were housed in carton boxes. Plastic mesh was used during the primary days of experiment to avoid any discomfort and any annoyance from the insects, mosquitoes etc. Standard management and hygienic conditions were maintained during the period of the experiment. The chicks were allowed to acclimatize with laboratory conditions for 5 days before starting the experiment

Feed and water consumption: Standard feed free from any antibiotics and drugs including coccidiostats procured from is prepared to the birds' ad libitum, Water was given ad libitum.

Drug administration: The norfloxacin yellowish white powder was not soluble in water. One gram norfloxacin was first added to 0.25 ml of acetic acid and 2 ml of 50mmol/L acetate buffer pH 4.5 then stirred until the drug was completely dissolved. The pH of the drug was checked and was solution was pH=4.5. This stock solution was used for further dilution. Fifty mmol / L Acetate buffer was prepared in the following method. Acetic acid 50mmol/L and 50 mmol / L of sodium acetate were prepared then the acetic acid solution was added to the solution of sodium acetate to makeup the pH 4.5. In acute toxicity study the required dose of norfloxacin drug was dissolved in solvent, by addition required amount of distilled water to change the fixed dose concentration and administered through oral rout by using modified infant stomach tube guage No-8. The drug was administered in the solution form directly to the crop of bird through oral rout. For sub-acute toxicity study 1 g norfloxacin IP powder was dissolved in 0, 25 ml of acetic acid and 2ml of acetate buffer that was used as vehicle and the last volume was 3ml that had the concentration of 0.333 g/m. The solution was prepared fresh every day before administering to the birds.

Study procedure: The acute oral toxicity for norfloxacin was conducted in five day old broiler chicken according to the OECD (Organization for Economic Cooperation and Development) guidelines 420.

Preparation of the birds: Healthy 60 COBB breed of broiler day old chicks with an average body weight of 45±5g were adapted to the laboratory condition for five days prior to experiment.

Experiment design: Six groups of birds each group of experimental birds consist 10 experimental birds were used for determining LD_{50} safety value by random selection based on body weight.

Dose selection: The dose to be selected was derived after conduction of preliminary study according to OCDE guideline. Six doses were selected for determining LD_{50} value.

Administration of the dose: Experimental birds used for acute toxicity safety study were given a single oral toxic dose of norfloxacin IP. It was administered directly into the crop using through oral rout by thin modified infant feeding plastic tube gauge No-8 attached to a one ml syringe and the volume of norfloxacin IP solution was maintained up to one ml per experimental bird dilution of with distilled water. Feed was withheld for 12 h before drug administration and offered 6 h after drug administration. Water was provided *adlibitum*

The groups detail dose of norfloxacin IP per kg body weigh concentrations are given in the Table below.

Table 1. The details regarding dose of Norfloxacin administered for each group

No	Groups	No.of Birds	Dose (g/kg)	Concentration after dilution water with distilled
1	Group I	10	1.4	3.1461
2	Group II	10	1.5	3.1761
3	Group III	10	1.6	3.2041
4	Group IV	10	1.7	3.2304
5	Group V	10	1.8	3.2553
6	Group VI	10	1.9	3.2788

Birds general clinical observation: During the observation period birds were carefully observed for effects on skin, face, eyes, mucous membranes, circulatory and respiratory systems, autonomic nervous change as salivation, central nervous system effects as tremors and convulsions, changes in the level of activity, reactivity to handling or sensory stimuli, and altered strength, health conditions, gait, posture, and mortality. The LD_{50} was calculated as per the graphical method described by ProStat software Pearl River. NY 10965. USA.

Collection of organs: The birds, gross morphological changes, were recorded during autopsy. The representative tissue samples from organs such as liver, kidney, spleen, intestine, heart, pancreas, brain of birds were collected for histopathological study. The collected organs were fixed in 10 per cent neutral buffered formalin (Anderson, 1997). The liver, spleen, kidney, heart, lung, intestine, brain of five microns thickness and stained with Haematoxylin and Eosin stain (Luna, 1968).

RESULTS

LD_{50} of norfloxacin: The toxicity study of Norfloxacin was carried out in the present study. From the present study it was found that the maximum dose of Norfloxacin where no mortality occurred was at 1.4 g /kg. The dose at which 100 per cent mortality was observed was calculated to be 1.8 g/kg /body weight.

The calculated LD_{50} value for Norfloxacin was found to be 1.55 g/kg. For confirmation, 10 additional birds were challenged with the dose of 1.55 g/kg and among these birds 50 per cent mortality was found during the 24 h

observation period. Fig 1. The details related to dosing of Norfloxacin, grouping, number of experimental birds and mortality percentage is given in **Table 1.**

Table 1. Dose of Norfloxacin and mortality percentage acute toxicity study

No	Groups	No of birds in each group	No of birds in each group	Concentration after dilution water with distilled	Mortality percentage
1	Group I	10	1.4	3.1461	0
2	Group II	10	1.5	3.1761	30
3	Group III	10	1.6	3.2041	70
4	Group IV	10	1.7	3.2304	70
5	Group V	10	1.8	3.2553	100
6	Group VI	10	1.9	3.2788	100

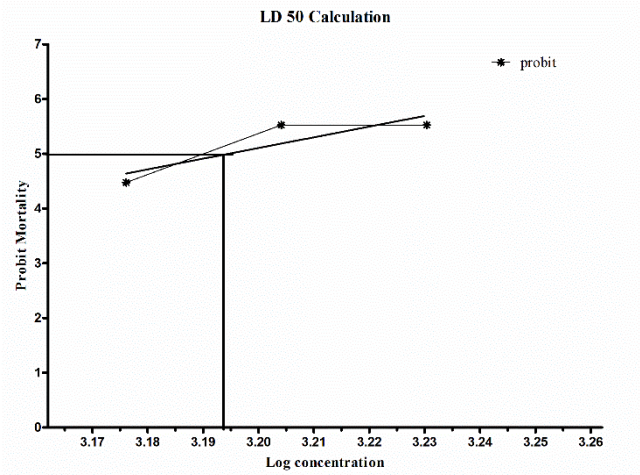

Fig. 1. The graph showing LD_{50} of norfloxacin LD 50 of norfloxacin by probit analysis. The corresponding log 10 dose of probit value 5=3.192. Antilog of 3.192=1549. Therefore, LD 50 is 1549 mg/kg or 1.549/kg

Clinical observations: Some of the clinical signs of toxicity observed before death were dullness, drowsiness, and excitability, circling, oozing of fluid from mouth and gasping for breath with beaks wide open.

Histopathology: Postmortem was conducted on dead experimental birds immediately after death. The histopathological findings observed (acute toxicity 1.8g/kg) in concerned organs were: hemorrhage, congestion and tubular epithelium degeneration and necrosis in kidney (Plate 1), Hemorrhage, edema and infiltration of few inflammatory cells in heart (Plate 2). Congestion of vessels and dilatation of sinusoidal space. Perivascular hepatocytes degeneration with infiltration of few inflammatory cells on liver (Plate, 3) and sever lymphocytolysis in spleen (Plate 4).

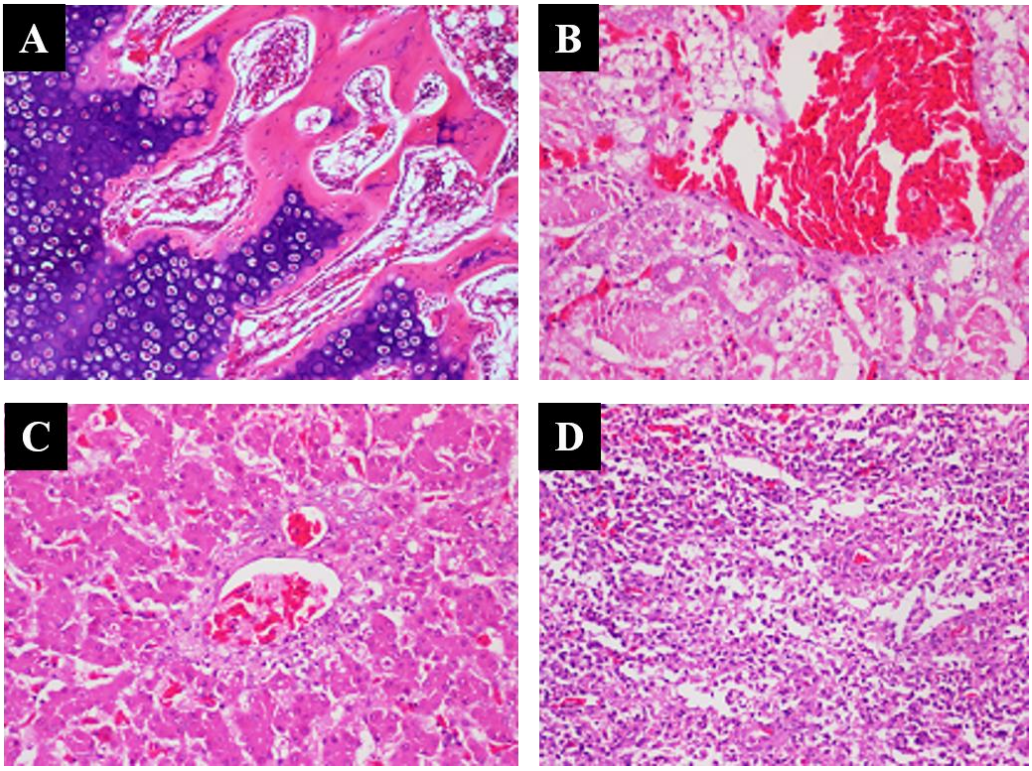

Fig. 1. Plate 1. Acute toxicity (1.8g/kg), Kidney: hemorrhage, congestion and tubular epithelium degeneration and necrosis -H&Ex500 **(A)**. Plate 2. Acute toxicity (1.8g/kg) Heart: Hemorrhage edema and infiltration of inflammatory cells H&Ex500 **(B).** Plate 3.Acute toxicity (1.8g/kg) Liver: Congestion of vessels and dilatation of sinusoidal space. Perivascular hepatocytes degeneration with infiltration of few inflammatory cells - H&Ex500 **(C),** and Plate 4. Acute toxicity (1.8g/kg) Spleen: Sever lympholysis and depletion H&Ex500 **(D).**

DISCUSSION

The results obtained for toxicity studies are discussed here. The literature available on Norfloxacin toxicity is scanty hence, for discussion other fluoroquinolones were also considered. The calculated LD_{50} value of Norfloxacin was 1.55 g/kg body weight. The symptoms of toxicity observed were dullness, drowsiness, excitability making continuous sound, circling, oozing of fluid from mouth and gasping of breath .The dose at which 100 per cent mortality was observed was calculated to be 1.8g/kg and in the maximum dose of 1.4 g /kg body weight where no mortality occurred. So Norfloxacin can be classified as per given protocol a moderately toxic drug according to Global harmonization system (GHS) for classification of toxic compounds. Zhou and Wang, (2007) reported LD_{50} of Norfloxacin nicotinate to be 3458 mg/kg for 5 week old broiler chicken. The symptoms observed were salivation, diarrhea, and nervousness. In the present study the estimated LD_{50} value for Norfloxacin was found to be 2 to 3 fold lesser then the LD_{50} reported by Zhou and Wang, (2007). The observed variation in LD_{50} in the present study has been attributed to the difference in age and body weight of birds. Further, it is also reported that higher values of LD_{50} can be expected in adult birds. the presumption can be further supported by the fact that metabolism and excretion of xenobiotics are compromised by under developed microsomal enzyme system, membrane permeability and hepatic and renal clearance capabilities in young animals

(Clarence et al., 1991). The observed CNS toxicity signs like circling, neuromuscular in-coordination and ataxia in the present acute toxicity study may be due to crossing of flouroquinolones into CNS as Blood brain barrier is also not fully developed in young birds (Scheld, 1989). Further, there are reports where in Fluoroquinolones may induce central nervous system excitatory side effects, including anxiety, nervousness, hallucination, and even seizures on rare occasions in many species (Christ, 1990). Zhang et al. (2003) found neurotoxi effects of Norfloxacin on free moving rats in which the epileptiform discharges appeared in all Norfloxacin groups with different latent periods, accompanied with limb twitching and clonic tonic seizures and the relative power of EEG increased.

CONCLUSION

We can conclude that: the dose at which 100 per cent mortality was observed was calculated to be 1.8g/kg and in the maximum dose of 1.4 g /kg body weight and the calculated LD_{50} value for Norfloxacin was found to be 1.55 g/kg where no mortality occurred. So it can be classified as a moderately toxic drug according to Global harmonization system (GHS) for classification of toxic compounds

Conflict of Interest

No, conflict of interest among all authors

REFERENCES

Anderson-Mackenzie, J. M., Hulmes, D. J. S., & Thorp, B. H. (1997). Degenerative joint disease in poultry—differences in composition and morphology of articular cartilage are associated with strain susceptibility. Research in veterinary science, 63(1), 29-33.

Anastasio, G. D., Menscer, D., & Little Jr, J. M. (1988). Norfloxacin and seizures. Annals of Internal Medicine, 109(2), 169-170.

Ball, P. (1989). Adverse reactions and interactions of fluoroquinolones. Clinical and Investigative medicine. Medecine Clinique et Experimentale, 12(1), 28-34.

Ball, P. (1986). Ciprofloxacin: an overview of adverse experiences. Journal of antimicrobial chemotherapy, 18(Supplement_D), 187-193.

Braunwald, E., Fauci, A. S., Kasper, D. L., Hauser, S. L., Longo, D. L., & Jameson, L. (2001). Harrison's Principles of Internal Medicine 15th. NY: McGraw-Hill Book Company.

Brown, S. A. (1996). Fluoroquinolones in animal health. Journal of veterinary pharmacology and therapeutics, 19(1), 1-14.

Christ, W. (1990). Central nervous system toxicity of quinolones: human and animal findings. Journal of Antimicrobial Chemotherapy, 26(suppl_B), 219-225.

Merck, R. (1991). The Merck veterinary manual. na.

Corrado, M. L., Struble, W. E., Peter, C., Hoagland, V., & Sabbaj, J. (1987). Norfloxacin: review of safety studies. The American journal of medicine, 82(6), 22-26.

Domagala, J. M. (1994). Structure-activity and structure-side-effect relationships for the quinolone antibacterials. Journal of Antimicrobial Chemotherapy, 33(4), 685-706.

Finney, D. J. (1971). Statistical Method in Biological Assay, 2nd edn (Griffin, London, 1964); D. Colquhoun, Lectures on Biostatistics.

Luna, L. G. (1968). Manual of histologic staining methods of the Armed Forces Institute of Pathology.

Mascellino, M. T., Lorenzi, A., Bonanni, M., & Iegri, F. (1986). Antimicrobial activity of norfloxacin in enteric and urinary tract infections: combined effect of norfloxacin with aminoglycosides, tetracycline and chloramphenicol. Drugs under experimental and clinical research, 12(4), 319-323.

SACHIN, A. (1998). Toxicity Studies of Pefloxacin Fluoroquinolone in Broiler (Doctoral dissertation, University of Agricultural Sciences, GKVK).

Scheld, W. M. (1989). Quinolone therapy for infections of the central nervous system. Reviews of Infectious Diseases, 11(Supplement_5), S1194-S1202.

Ball, P. (1986). Ciprofloxacin: an overview of adverse experiences. Journal of antimicrobial chemotherapy, 18(Supplement_D), 187-193.

Sen, S., Jaiswal, A. K., Yanpallewar, S., & Acharya, S. B. (2007). Anxiogenic potential of ciprofloxacin and norfloxacin in rats. Singapore medical journal, 48(11), 1028.

Vancutsem, P. M., Babish, J. G., & Schwark, W. S. (1990). The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. The Cornell Veterinarian, 80(2), 173-186.

Wentland, M. P. (1990). Structure-activity relationships of fluoroquinolones. The new generation of quinolones, 1-43.

Wolfson, J. S., & Hooper, D. C. (1985). The fluoroquinolones: structures, mechanisms of action and resistance, and spectra of activity in vitro. Antimicrobial agents and Chemotherapy, 28(4), 581-586.

Zhang, L. R., Li, X. T., Tang, W. L., Wang, Y. M., Cheng, N. N., & Chen, B. Y. (2006). Changes in brain interleukin-1β following the coadministration of norfloxacin with biphenylacetic acid in rats. European journal of pharmacology, 543(1-3), 21-26.

ZHOU and WANG. (2007). Study on the acute toxicity of Norfloxacin nicotinate in chickens.Journal of Huazhong, 22 (4): 372

Nangarhar University
International Journal of Biosciences

Received: 07 Apr 2022
Accepted after revision: 07 May 2022
Published Online: 19 Aug 2022

ORIGINAL ARTICLE Vol 1, Issue 1 (2022) https://nuijb.nu.edu.af

Clinical Profile of COVID-19 Patients in Nangarhar University Teaching Hospital

Hadi Saifullah[1], Del Del Aqa[1], Shirzay Aimal[1]

[1]Department of Internal Medicine, Faculty of Medicine, Nangarhar University. Afghanistan
*Corresponding author: saifullahhadi6@gmail.com

ABSTRACT

Background: The coronavirus COVID-19, causing severe acute respiratory syndrome and patients' mortality in considerable proportion, has affected 220 countries. There was no description of the clinical profiles such as demography (sex, age), and co-morbidities of COVID-19 patients in Eastern Region of Afghanistan, comprising Nangarhar, Nuristan, Kunar, and Laghman provinces. A vast majority of patients from these areas refer to Nangarhar University Teaching Hospital for health issues. Therefore, the researchers compiled a descriptive profile of the patients from this particular area.

Materials and Methods: It was a descriptive record based study of Medical Ward, Nangarhar University Teaching Hospital. The number of profiled patients was 50, with age over 18 years old. The patient's samples were sent to Nangarhar Public Health Hospital for confirmation in Real-Time Reverse Transcriptase Polymerase Chain Reaction Assay for SARS-CoV-2 examination.

Findings: Out of 50 COVID-19 affected patients, 68% were females and 32% were males. The mean age was (53±17). According to the clinical profiles, 8% had no symptoms and most common ones were fever (80%), cough (60%) and dyspnea (10%). The major comorbidities were respiratory disease (56%), hypertension (38%), Diabetes Mellitus (16%), Heart Failure (12%), obesity (10%) and chronic kidney disease (4%).

Conclusion: The study concluded that the event was more common in females and aged persons than males and young patients. Fever was obviously common among all the identified patients. COVID-19 was severe in patients with respiratory diseases and hypertension.

Key words: COVID-19; Demography; Clinical profiles; Comorbidities; Afghanistan

INTRODUCTION

In the middle of March 2003, the severe acute respiratory syndrome (SARS) was initially identified as a global threat. SARS was originally identified in Guangdong Province, China, in November 2002.The World Health Organization stated that the final human chain of SARS transmission during that pandemic has been broken (https://covid19.who.int). The SARS coronavirus (SARS CoV), the etiological agent, was thought to be an animal virus that recently broke through the species barrier to humans when ecological shifts or alterations in human behavior increased opportunities for human exposure to the virus and virus adaptation, allowing human-to-human transmission (Meena et al., 2020). On December 31, 2019, the China Health Authority informed the WHO of multiple instances of pneumonia with unknown causes in Wuhan City, Hubei Province, central China. Since the cases were first reported on December 8, 2019, majority of the patients had either worked at or resided close to the Huanan Seafood Wholesale Market. However, some earlier cases had no connection to this market. 2019-nCoV was the initial short name for the novel Coronavirus given by the WHO (https://covid19.who.int), it was discovered in a patient's throat swab sample. The pathogen was eventually given the name severe acute respiratory syndrome coronavirus 2 (SARSCoV-2) by the Coronavirus Study Group, and the illness was given the term coronavirus disease 2019 (COVID-19) by the World Health Organization. World Health Organization (WHO) has determined that the SARS-CoV-2 outbreak qualified as a Public Health Emergency of International Concern as well as the WHO named the corona virus disease-19 (COVID-19) a pandemic on April 25, 2021 (https://covid19.who.int). The corona virus is extremely contagious and spreads by airborne droplets, close contact, coughing, sneezing, and cuddling (Meena, et al, 2020). World Meter-real Time World Statistics, there were 164,886,821 confirmed cases of SARS-CoV-2 worldwide, 143,811,583 recovered cases, and 3,418,430 fatalities (https://covid19.who.int) as well as WHO (2021) stated that 31.6 million People call the South Asian nation of Afghanistan home, and 71.5% of them reside in rural areas (SAEED & Mir, 2020) . Mousavi et al. (2020) stated that the first COVID-19 case in Afghanistan was discovered on February 24, 2020, in a resident of Herat Province who had recently returned from Iran.

On March 22, 2020, it was reported that a 40-year-old man had died in Balkh Province's Chimtal District as a result of COVID-19. According to Tolonews (2020), two fresh instances in the Kabul Province had their status as foreign diplomats confirmed (https://www.worldometers.info).Total confirmed cases in Afghanistan to far are 64,122, recovered cases are 55,118, and deaths are 2,762 (SAEED & Mir, 2020). Since the presence of COVID-19 has been confirmed in more than 220 nations, it is clear that SARS-CoV-2 is spreading swiftly over the world and is expected to cause significant illness and mortality if the spread is not immediately halted. This could potentially have significant worldwide socioeconomic repercussions and put a heavy burden on health care resources (Bollinger & Ray, 2021). The SARS-CoV-2 is caused by COVID-19, according to (Stuart, 2021), also they claimed that when a virus's genes are altered, or mutated, other viruses emerge. According to Ray, RNA viruses like the coronavirus have a tendency to alter over time. It is neither novel nor surprising for viruses to evolve, including the coronavirus responsible for the COVID-19 pandemic.

Ray, (2020) claims that since it was first identified in China, the SARS-CoV-2 coronavirus has changed into a number of other species. He states that in southeastern England, a single coronavirus mutant was discovered. That variety, now known as B.1.1.7, quickly became the most common coronavirus in the UK with regard to new COVID-19 cases in December. It has taken over as the main coronavirus strain in a number of countries.

In areas like California, Brazil, and other places, several varieties have emerged. B.1.351, a coronavirus variant first discovered in South Africa, has the potential to reinfect patients who have already recovered from other coronavirus variants. Additionally, several of the coronavirus vaccines that are being developed may only be somewhat effective. However, several immunizations currently being tested appear to provide defense against life-threatening illness in patients infected with B.1.351 (Coleman et al., 2021). The Covid variant known as B.1.617, which is allegedly more contagious in India, is allegedly being decoded by researchers from all over the world, according to (Joshi, 2021). Soni et al, (2020) stated that the COVID-19 is a very contagious condition. Its initial symptoms are similar to those of SARS and include fever, coughing, and tiredness. The same patient also displays additional symptoms such as fatigue, nasal congestion, myalgia, a sore throat, and diarrhea.

A systematic review was conducted on 19,584 COVID-19 patients (median age, 52 years; 47.5 percent female; 29.4 percent Hispanic) who passed away or were sent home during the study period. In the group, 31.1 % had diabetes, 50.4 % had hypertension, 14.3 % had heart failure, 18 % had coronary artery disease, and 5.6 % had end-stage renal disease (Baradaran, 2020). Hossain et al, (2020) conducted at a private hospital in Dhaka, Bangladesh, the main symptoms of the patients they enrolled were fever (88 percent), cough (81 percent), dyspnea (58 percent), and exhaustion (50 percent). Diabetes (54%) and Hypertension (48%) affected about half of the patients (47 percent).

There are currently no documented effective treatments for this virus, according to (Meena et al., 2020). However, the COVID-19 patients in Afghanistan's Eastern Zone's demographics, clinical features, and co-morbid diseases have not yet been identified. We therefore sought to explain these traits in COVID -19 confirmed individuals for this particular site.

MATERIALS AND METHODS

With the second coronavirus wave, in Afghanistan, preventive measures were seriously taken in late 2020. Afghan Ministry of Public Health took many steps to prevent coronavirus spreading, including commissioning Focal Points in hospitals so that suspected COVID-19 patients can be excluded from the OPDs and IPDs. Internal Medicine Ward of Nangarhar University Teaching Hospital was commissioned as a Focal Point for the Eastern Region by Nangarhar Public Health Department.

This Focal Point, first, registered suspected COVID-19 patients according to the format the researchers had arranged, consisting Patient’s introduction, contact number, demographics (age, sex), clinical profiles (fever, cough, and dyspnea) and comorbidities (respiratory diseases, chronic kidney disease, chronic liver disease, Diabetes Mellitus, Hypertension and obesity). After registering, the sample would be taken from the throat of patient, and sent to the public health hospital lab for confirmation. After 48 hours, the result of Polymerase Chain Reaction (PCR) were detected. Then, the patients’ demographics, clinical profile, and comorbidities were evaluated and the confirmed patients were to be transferred to reference COVID-19 hospital.

The study design was descriptive, ran from 1.12.2020 to 30.3.2021 over 50 confirmed COVID-19 patients. It was conducted in Internal Medicine Ward of Nangarhar University Teaching Hospital. The study included the patients with age 18 years and older. Ethical approval of the study has been gotten from the hospital research board.

RESULTS

This study was conducted on 50 COVID-19 affected patients came to Nangarhar university teaching hospital for different reasons. Females were more commonly affected (68%) than males (32%). The mean age at presentation was 51±67 years. The most common symptoms were fever (80%), cough (60%) dyspnea (10%), sore throat (10%),

myalgia (8%), and diarrhea (6%). The major comorbidities were respiratory disease (56%), systemic hypertension (38%), diabetes mellitus (16%), Heart Failure (12%), obesity (10%), chronic kidney disease (4%), chronic liver diseases (2%). Eleven patients (22%) had no co-morbidities as shown in **Fig. 1.**

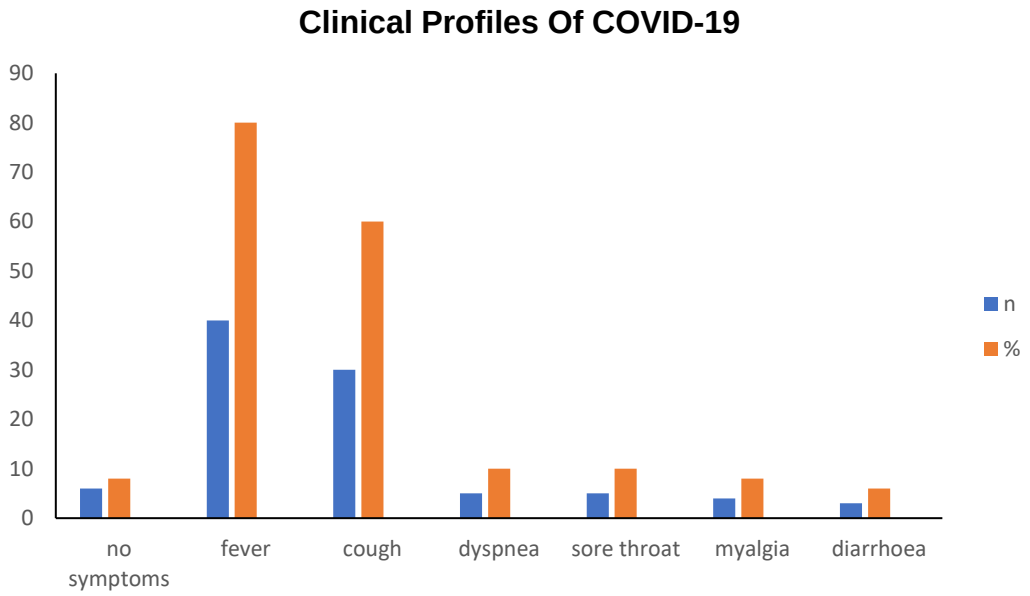

Fig. 1. Clinical Profiles of COVID-19 in Nangarhar university teaching hospital for different reasons

Table 1. Ages, sex and co-morbidities distribution

Age distribution	Female			Male		
	N (%)	Comorbid (+)	Comorbid (-)	N (%)	Comorbid (+)	Comorbid (-)
60≤	14(28%)	12(24%)	2(4%)	10(20%)	9(18%)	1(2%)
40-59	10(20%)	8(16%)	2(4%)	2 (4%)	1(2%)	1(2%)
18-39	10(20%)	9(18%)	1(2%)	4(8%)	4(8%)	0(0%)
Total	34(68%)	29(58%)	5(10%)	16(32%)	10(20%)	2(4%)

Table 2. Comorbidities of COVIDE-19

Comorbidities	Female		Male	
	Present	Absent	Present	Absent
Respiratory Diseases	18 (36%)	16 (34%)	11 (22%)	5 (10%)
Hypertension	12 (24%)	22 (44%)	6 (12%)	10 (20%)
Diabetes	7 (14%)	27 (52%)	1 (2%)	15 (30%)
Heart Failure	4 (8%)	30 (60%)	3 (6%)	13 (26%)
Obesity	3 (6%)	31 (62%)	2 (4%)	14 (18%)
Chronic Kidney Disease	1 (2%)	33 (66%)	1 (2%)	15 (30%)
No co-morbidities	9 (18%)		2 (4%)	
Multiple factors	10 (20%)		6 (12%)	

DISCUSSION

Our aims were to describe the demography, clinical profile and comorbidities of COVID-19 patients in the Eastern Region of Afghanistan. In our study, the mean age of population was 51±67 years, which is quite similar with the findings of other studies: 53 years (Hossain, 2020), 47.5 years (Budhiraja et al., 2020), 51years (Baradaran, 2020). We found that females were more commonly affected than males (68% vs 32%). While in most international studies males were predominantly affected by COVID 19. The result of a meta-analysis conducted on 33 studies found that the men were more affected (55%) than women (Barek at al., 2020). Hossain (2020), also observed greater percentage of male (65%) than female (35%) affected with COVID-19. She reported "Similar male preponderance in other studies, 73% of the first reported study of China or 63% of a study in DMCH, Bangladesh". The clients of Nangarhar University Teaching Hospital are mostly females; therefore, the females were more predominant in this study. We found that fever was obvious symptom (80%) followed by cough (60%) dyspnea (10%), sore throat (10%), myalgia (8%), and diarrhea (6%), which is in agreement with other international studies. In a study conducted in Dhaka fever (88%) with respiratory symptoms like cough (81%) & dyspnea (58%) topped the list, followed by fatigue (50%) (Hossain, 2020). Chen N et al. found that "Patients had clinical manifestations of fever (82 [83%] patients), cough (81 [82%] patients), and shortness of breath (31 [31%] patients), muscle ache (11 [11%] patients), confusion (nine [9%] patients), headache (eight [8%] patients), sore throat (five [5%] patients), rhinorrhea (four [4%] patients), chest pain (two [2%] patients), diarrhea (two [2%] patients), and nausea and vomiting (one [1%] patient)". (Ratti Ram, 2020). Patients with respiratory diseases (56%) and hypertension (38%) were more affected in this study. An Indian Experience said that the major co-morbidities in covid-19 patients were hypertension (23.7%), diabetes without (15.4%), and with complications (9.6%) (Budhiraja et al., 2020). A large US study of 5,700 hospitalized patients revealed an overall hypertension rate of 56%, similar to hypertension rates reported from China and Italy (50% and 49%, respectively) (Kulkarni et al., 2020). Our study was conducted in winter months, in this season the occurrence of respiratory diseases is more common and as we know the viral infections course is worse in the respiratory diseases affected people, therefore in under study individuals the chronic respiratory diseases were more prevalent. There was no fund for this research. Lab Examinations were free of charge by the government and examinations were not interfered with.

CONCLUSION

Our investigation showed that the event was more witnessed in females and aged than male and young patients. Fever was obvious. Comorbidities due to COVID-19 was common in patients with respiratory diseases and hypertension. We must give emphasis on early diagnosis, early isolation and early management of all COVID-19 patients to reduce transmission and mortality, thus to save mankind from this invisible enemy. As the obvious symptoms of COVID-19 were fever, cough & myalgia, therefore, the patients with such symptoms should be paid special attention to confirm this disease in time. Aged, hypertensive, and patients with chronic respiratory disease are more prone to covid-19, so these patients should be in the first line for preventive measures and screening. The government should also provide sufficient facilities for diagnosis of COVID-19 and private sector should be permitted play active role in early diagnosis and prevention of disease progression.

Acknowledgments

We are grateful to focal point of COVID-19 staff at Nangarhar University Teaching Hospital for collaborating with us on data collection and we also express thankfulness to our coworker for their continuous help in the completion of this research.

Conflict of Interest

No, conflict of interest among all authors

REFERENCES

Baradaran, A., Ebrahimzadeh, M. H., Baradaran, A., & Kachooei, A. R. (2020). Prevalence of comorbidities in COVID-19 patients: a systematic review and meta-analysis. Archives of Bone and Joint Surgery, 8(Suppl 1), 247.

Barek, M. A., Aziz, M. A., & Islam, M. S. (2020). Impact of age, sex, comorbidities and clinical symptoms on the severity of COVID-19 cases: a meta-analysis with 55 studies and 10014 cases. Heliyon, 6(12), e05684.

Budhiraja, S., Soni, A., Jha, V., Indrayan, A., Dewan, A., Singh, O., ... & Jha, S. (2020). Clinical Profile of First 1000 COVID-19 cases admitted at tertiary care hospitals and the correlates of their mortality: an Indian experience. MedRxiv.

Bollinger, R., & Ray, S. (2021). New variants of coronavirus: What you should know. John Hopkins Medicine. Retrieved February, 26, 2021.

Coleman, K. J., Stewart, C. C., Bruschke, C., Flores, J. P., Altschuler, A., Beck, A., ... & Ahmedani, B. K. (2021). Identifying People at Risk for Suicide: Implementation of Screening for the Zero Suicide Initiative in Large Health Systems. Advances in Psychiatry and Behavioral Health, 1(1), 67-76.

Hossain, H. T., Chowdhury, T., Majumder, M. I., Ava, A. R., Rahman, Q. A. A., Zahiruddin, M., ... & Islam, Q. T. (2020). Demographic and clinical profile of 190 COVID-19 patients in a tertiary care private hospital of Dhaka, Bangladesh: an observational study. Journal of Medicine, 21(2), 82-88.

Joshi, S. R. (2021). COVID 19 are we at the End of the Road in India-Pandemic to Endemic Journey. Journal of The Association of Physicians of India, 69, 11.

Kulkarni, S., Jenner, B. L., & Wilkinson, I. (2020). COVID-19 and hypertension. Journal of the renin-angiotensin-aldosterone system, 21(2), 1470320320927851.

Meena, R. R., Choudhary, K. D., Verma, A., (2020). COVID-19 clinical profile: A review based on current Evidence, International Journal of Medical and Health Research, Volume 6; Page No. 04-06.

Mousavi, S. H., Shah, J., Giang, H. T., Al-Ahdal, T. M., Zahid, S. U., Temory, F., ... & Huy, N. T. (2020). The first COVID-19 case in Afghanistan acquired from Iran. The Lancet Infectious diseases, 20(6), 657-658.

Soni, S. L., Kajal, K., Yaddanapudi, L. N., Malhotra, P., Puri, G. D., Bhalla, A., ... & Guru, R. R. (2021). Demographic & clinical profile of patients with COVID-19 at a tertiary care hospital in north India. The Indian journal of medical research, 153(1-2), 115.

SAEED, K. M. I., & Mir, K. (2020). Epidemiological Characteristics of Coronavirus disease 2019 (COVID-19) in Afghanistan. Glob Acad J Med Sci, 2(4), 31-40.

Tolonews. First Coronavirus Death Confirmed in Afghanistan. Available online at: tolonews.com/health/first-Coronavirus-death-confirmed-Afghanistan Worldometer-real time world statistics. Afghanistan COVID (2021). Available online at: https://www.worldometers.info

World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard. Available from: https://covid19.who.int , accessed on April 25, 2021.

Nangarhar University
International Journal of Biosciences

Received: 10 Mar 2022
Accepted after revision: 07 May 2022
Published Online: 19 Aug 2022

REVIEW ARTICLE Vol 1, Issue 1 (2022) https://nuijb.nu.edu.af

Pathological changes of Aortic Valve Calcification in Experimental Animal Models

Sherzad Abdul Ghafar[1, 2]*, Behsodwal Khalil Ahmad[3], Azimee Mohammad Azim[2], Shinwari Muhibullah[4], Zafarzai Imran[5], Zaheer Shafiullah[2], Arash Nemat[1, 6], Osama Alsarhan[1], Qingchun Zeng[1, 7]*

[1]Southern Medical University, Department of Cardiology, State Key Laboratory of Organ Failure Research, Nanfang Hospital, Guangzhou, Guangdong, People's Republic of China

[2]Nangarhar University, Faculty of Medicine, Department of Biochemistry, Jalalabad, Nangarhar, Afghanistan

[3]Nangarhar University, Faculty of Medicine, Department of Histopathology, Jalalabad, Nangarhar, Afghanistan

[4]Nangarhar University, Faculty of Medicine, Department of Physiology, Jalalabad, Nangarhar, Afghanistan

[5]Nangarhar University, Faculty of Medicine, Department of Forensic Medicine, Jalalabad, Nangarhar, Afghanistan

[6]Kabul University of Medical Sciences, Department of Microbiology, Kabul, Afghanistan

[7]Guangzhou Regenerative Medicine and Health Guangdong Laboratory, 5100050, Guangzhou, China

Corresponding author: qingchunzeng@smu.edu.cn, ghafarsherzad15@gmail.com

ABSTRACT

Calcific Aortic Valve Disease (CAVD) is a sluggish and progressive disease that comprises “early sclerosis, characterized by leaflet thickening without left ventricular outflow obstruction, to late stenosis with stiffened leaflets, obstructed flow and compromised cardiac function”. CAVD was formerly believed to afflict the tricuspid or congenitally bicuspid aortic valve and be a passive, senile, or degenerative disorder. However, recent investigations have demonstrated that this is a pathobiological activity that is active and heavily cell-mediated, which shares several risk factors with atherosclerosis. Numerous studies show that CAVD are not a normal aspect of aging and may be linked to certain risk factors. Nevertheless, no pharmacological therapy available to halt or arrest the development of CAVD in a clinically relevant way, and surgery is the only effective treatment option. As a result, there is an urgent scientific need to determine pathobiological mechanism of CAVD and to find new ways to treat CAVD. Animal models are developing as crucial instruments to this aim, assisted by the development of new models and greater knowledge of the efficacy of old models. In this review paper, we will present the most extensively utilized large and small animal models that were used to explore CAVD.

Keywords: Calcific aortic valve diseases; Aortic valve calcification; Aortic valve stenosis; Atherosclerosis; Experimental Animal Models

INTRODUCTION

CAVD is a serious global public health issue, in 2019; there were reportedly around 9,404,078 cases (male 5,027,261, female 4,376,817), of CAVD Globally, even with age standardization, global CAVD incidence, prevalence, and mortality increased 3.51-, 4.43-, and 1.38-fold from 1990 to 2019, respectively. In 2019, there were an estimated 126,827 patients (male 54,175 and female 72,652) died from CAVD globally. The highest rates of CAVD mortality were recorded in the USA, followed by Germany and Japan (248,256, 13,154, and 12,868, respectively), (Yi et al., 2021). According to studies, CAVD is presently the main reason for cardiac valve disease in both industrialized and developing nations. CAVD ranges from early sclerosis, which is described by thickening of the leaflets without obstructing the left ventricle's outflow, to late stenosis, which has rigid leaflets, obstructed flow, and compromised cardiac function (Gillis et al., 2017). CAVD is the most prevalent cause of aortic stenosis (AS) in adults and was previously thought to be a passive, senile, or degenerative process affecting a normal trileaflet or congenital bicuspid valve (Lilly & Braunwald, 2012), but over the past decade, a number of studies have revealed that a number of noteworthy molecular processes play a role in the emergence of this condition, and recent scientific findings have also shown that it is an active and highly cell-mediated pathobiological process (Le Quang et al., 2014). A 50% greater risk of cardiovascular mortality and myocardial infarction has been linked to sclerosis, whereas the prognosis for individuals with stenosis is quite dismal. (Sider et al., 2014). CAVD is the third most common cardiovascular disease in the western world after coronary artery disease and hypertension, accounting for half of all valvular heart disease (Scatena et al., 2018), and the main reason for transcatheter heart valve replacement or surgical heart valve replacement.(Hisamatsu et al., 2018). CAVD has similar risk factors to atherosclerosis: age, male gender, smoking, high cholesterol, hypertension, diabetes mellitus ,kidney failure, and bicuspid aortic valve (Scatena et al., 2018); see **Fig. 1**). According to current data, clinically significant atherosclerosis is not present in 50% of patients with CAVD. It is important to emphasize that mechanical damage induced by hemodynamic stress produced by continual leaflet opening and shutting is regarded to be a significant risk factor for aortic valve stenosis. The BCA, particularly adds to increased mechanical stress, has been observed to accelerate the course of AVS in younger individuals with a low risk of atherosclerosis. Additionally, the non-coronary leaflets is more susceptible to injury than other leaflets because to the greater mechanical stress caused by the lack of diastolic coronary flow. (Honda et al., 2014). Additionally, tissue prosthetic valves were seen to calcify prematurely, mimicking the natural course of bicuspid aortic valves. (Cohen et al., 2004). Aortic valve sclerosis (AVSc) affects 25 to 30 % of people over the age of 65, 40 % of patients over the age of 75 (Anselmo et al., 2018; Branchetti et al., 2013), and up to 75 % of patients over the age of 85. Severe AS affects the population over 75 years at a 3 % incidence rate (Parisi et al., 2015). Several research' data indicate that chronic inflammation is essential for both atherosclerotic calcification and CAVD. “This is demonstrated in human disease by the existence of macrophages ,T cells, sub endothelial oxidized low-density lipoprotein (LDL) deposits and α-Smooth muscle actin (α-SMA) positive cells, associated with late CAVD, these are found within primary lesions infrequently” (Alushi et al., 2020; Demer & Tintut, 2019; O’Brien, 2006; Sider et al., 2014);increased superoxide and hydrogen peroxide (Rajamannan et al., 2011); increased oxidative stress and reduced endothelial nitric oxide synthase(Miller et al., 2009); activation of complement (Helske et al., 2008); elevated expression of TNF-α, active mast cells, matrix metalloproteinases (MMP-1,-2,-3,-9) (Hakuno et al., 2010), interleukin-2 (IL-2), ACE, angiotensin II (Ang II), angiotensin II type-1 receptor (AT1R), and chymase (O'Brien, 2006), as well as valvular endothelial cells' (VEC's) expression of intracellular adhesion molecule-1

(ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) (Alushi et al., 2020), and E-selectin (Ghaisas et al., 2000). Inflammatory processes are related with, and may contribute to, the valve ECM changes associated with CAVD, including leaflet thickening, protein turnover, and fibrosis (Weisell, 2020); Proteoglycans and hyaluronan buildup, elastin fragmentation(Sider et al., 2014), and calcification (Rajamannan et al., 2011; Tanaka et al., 2005). Finally, valve stiffness or dysfunction are caused by an underdevelopment of the valve ECM, While many characteristics of human CAVD are clearly established (especially those of late-stage human CAVD), early sclerosis is little understood.

METHODS

Articles providing data on Experimental Animal Models of CAVD were found by searching PubMed with the following MESH terms for CAVD OR (Aortic Valve Sclerosis AND Aortic Valve Stenosis) AND Experimental Animal Models AND Atherosclerosis; added all terms with Boolean AND, OR operators. PubMed, Google Scholar, ScienceDirect.com, and the Cochrane Library databases were also searched for the above terms. Given the paucity of available updated material in the literature. The all-scientific information cited in this review were carefully selected, retrieved and reviewed.

1. Biology of the normal Aortic valve

The majority of people have a tricuspid aortic valve, which has three semilunar cusps and is situated where the left ventricular outflow tract and the aortic root converge. In order to sustain bidirectional blood flow from the left side of the heart to the systemic and coronary circulations, it is a flexible membrane that opens and closes more than 100,000 times per day (Hulin & Oury, 2018; Lerman & Alotti, 2015). In healthy individuals, the valve cusps are less than a millimeter thick and are surrounded by an endothelial layer composed of valvular endothelial cells (VECs) on both sides. The valve's interstitium is composed of three separate layers: fibrosa, spongiosa, and ventricularis as shown in figure 2. The predominant cell type identified here is valvular interstitial cells (VICs) (Rutkovskiy et al., 2017). To meet their functional requirements under these adverse conditions, the thin, flexible leaflets are organized in three different layers of extracellular matrix (ECM). (1) The lamina fibrosa on the aortic side of the leaflet, which constitutes the majority of the valve and also the load-bearing structure, is composed primarily of circumferentially aligned collagen fibers (type 1 collagen fibrils) that contribute the majority of the leaflet's mechanical strength; (2) the Spongiosa is located in the center of the leaflet. It consists of a loose mucopolysaccharides matrix that promotes movement between the fibrosa and ventricularis during leaflet motion and serves as a cushion against compressive stresses; and (3) collagen and radially aligned elastin encompass the ventricularis layer on that ventricular side, which contributes to leaflet flexibility by permitting changes in leaflet shape during opening and shutting (Goody et al., 2020; Hulin et al., 2018; Sider & Simmons, 2011). Isolated macrophages are often seen in the ventricularis or spongiosa of mature human aortic valve cusps but not in the normal fibrosa (O'Brien et al., 1996), All three layers are avascular with no cellular infiltrates and are innervated by adrenergic and cholinergic neural networks in normal conditions. The aortic valve must be repaired on a regular basis throughout one's life in order to remain flexible (Xu, et al., 2010). Valvular endothelial cells (VECs) and valvular interstitial cells (VICs), which maintain valve homeostasis and structural leaflet integrity, are among the biological components of the aortic valve, and VICs, the most common cell type in the heart valve, are important in the evolution of CAVD (Hjortnaes et al., 2015). Mesenchymal VICs, quiescent VICs, progenitor VICs, active VICs, and osteoblast VICs are the five types of VICs. During valve development, epithelial-mesenchymal transition generates mesenchymal VICs from endothelial cells of the endocardial cushion (Liu, 2007). The normal

valve contains quiescent VICs (qVICs), which maintain its normal structure and function (Liu, 2007) ,VICs that can multiply in reply to injury are known as progenitor VICs (pVICs) and during the inflammatory reaction to pathogenic stimuli like mechanical stress or lipids, pVICs are transformed to aVICs. The aVICs function as profibrotic cells and have myofibroblast features such as contractility, stress fibers, and the striated-muscle isoform of myosin heavy chain. Further processing of aVICs results in obVICs, which accelerate calcification (Liu, 2007). VECs create an endothelial monolayer on the surface of the heart valve and are special because they may undergo endothelial-mesenchymal transformation, and an important phase in developing valvulogenesis. (Hjortnaes et al., 2015).

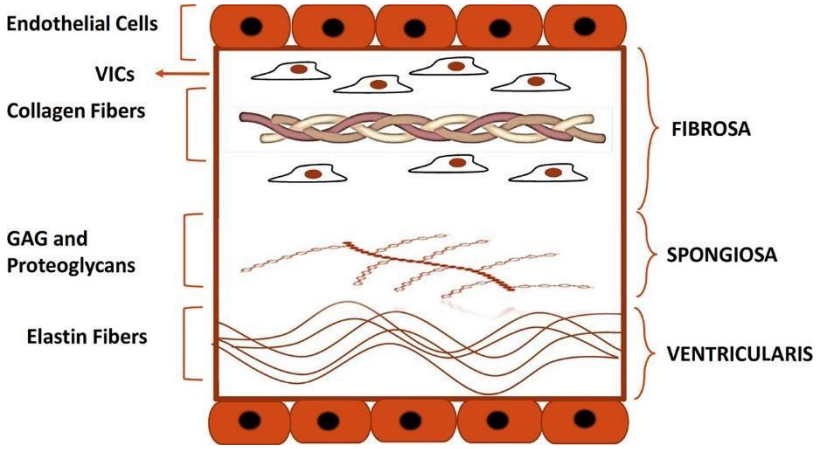

Fig. 1. Histological structure of the aortic valve. Depicted is the histological structure of the healthy aortic valve. Fibrosa, spongiosa, and, ventricularis are the three layers that make up the structure of a normal aortic valve. The Fibrosa layer is composed of type I and III collagen fibers and contains VICs. Spongiosa and ventricularis layers are respectively composed of GAG and proteoglycans and elastin fibers. Endothelial cells form a monolayer on each side of the cusp. GAG, glycosaminoglycans; VIC, valve interstitial cell (Alushi et al., 2020).

2. Pathobiology of CAVD

The initiation phase and the propagation phase are two separate stages in the pathobiology of CAVD. The propagation phase is characterized by fibrosis, calcification, and neoangiogenesis, while the early phase is characterized by endothelium damage, lipid accumulation, and inflammation. CAVD is carried on by endothelium dysfunction, which can be caused by turbulent flow with low shear stress (Dweck & Newby, 2012; Lindman & Mathieu, 2016; Ohukainen & Rysa, 2018). LDL, Lp (a), and proinflammatory cytokines such as monocytes and lymphocytes may enter the valve as a result of endothelial injury, when LDL and Lp (a) are oxidized and build up in the valve, inflammatory cells and valvular interstitial cells release inflammatory cytokines and chemokines. When macrophages ingest lipoproteins in the valve, foam cells are formed. The stimulation of valve interstitial cells (VICs) further mediates the fibro-calcific process, which is triggered by inflammation. Extracellular matrix remodeling is facilitated by activated VICs (aVICs), which increase collagen synthesis and disrupt the valve's natural structure. Osteoblast VICs (obVICs) are formed as the illness progresses, and they emit osteogenic markers. Proliferation occurs when calcification creates greater mechanical stress and injury that leads to even more calcification. This cycle repeats again.(Pawade & Dweck, 2015).

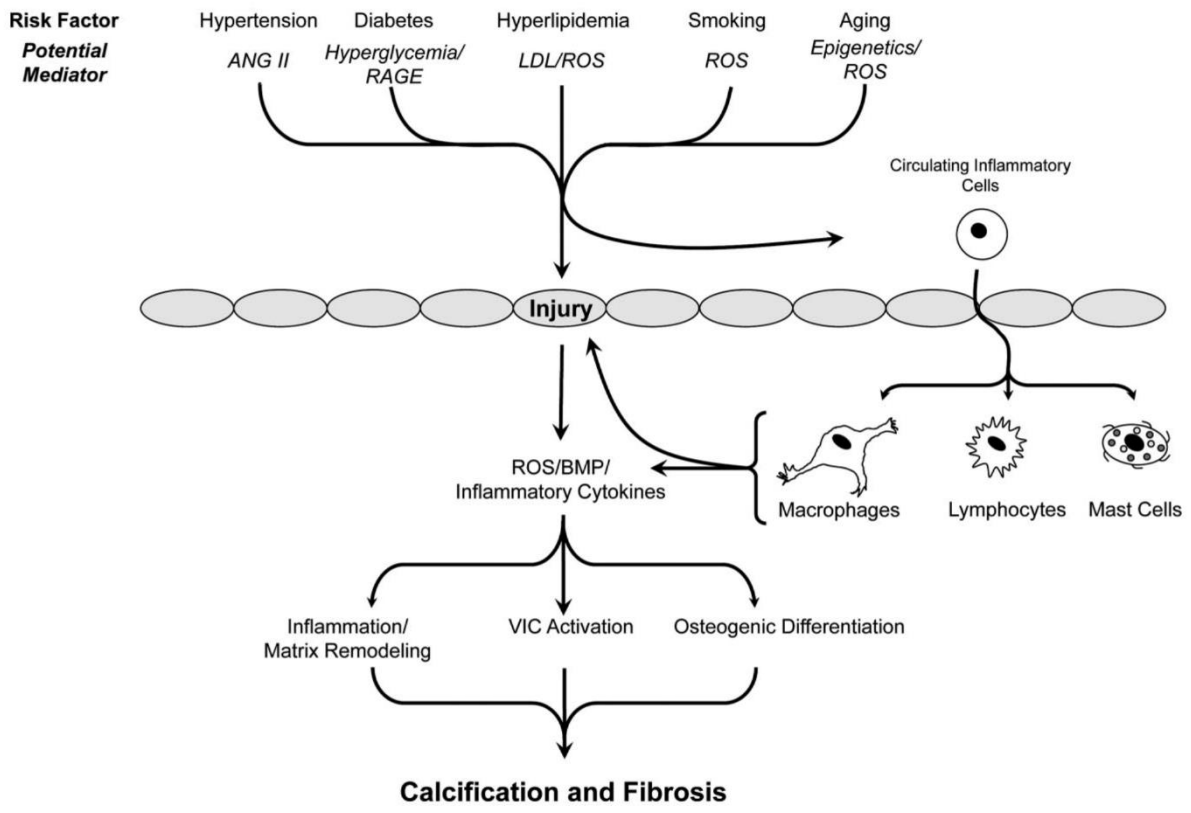

Fig. 2. Overview of risk factors and potential mechanisms that contribute to Calcification and Fibrosis of the aortic valve (Miller & Heistad, 2011).

3. CAVD Animal Models

There are several advantages of using animal models to study CAVD in vivo as well as assessing the outcomes of various therapy approaches. To be most successful, models should closely resemble real diseases and the circumstances under which genuine CAVD develops. The three animals that are most frequently used to model CAVD are swine, rabbits, and mice. Only swine have been proven to naturally acquire lesions with age, although this is a gradual process that is typically sped up by hypercholesterolemia brought on by diet. Some animals, including mice and rabbits, have not been found to naturally progress lesions but are reactive to diet- suggested hypercholesterolemia. Mice also need a genetic propensity to stimulate severe illness (Sider et al., 2011). Although no model can entirely replicate the intricacies present in human pathologies, they are crucial in analyzing disease mechanisms as well as novel diagnostic tools, preventions, and therapies (Chorro & López-Merino, 2009; Walters et al., 2012). This paper will reviews swine, rabbit and mouse models of CAVD, together with their advantages and drawbacks of the most usually used animal models of CAVD, which are summarized in **(Table 1-3).**

Table 1. Advantages and drawbacks of Mice models of CAVD

Advantages	Disadvantages
Low price and short generation time	Wild-type mice have a high resistance to the development of atherosclerosis
Extremely accessible	CETP activity in the plasma is absent
Easy to use and maintain	The vast majority of cholesterol is transmitted by HDL particles
Ridable reproduction	Because of their small size, collecting blood from mice is challenging due to the cutting up of tiny arteries
Standardized procedures for targeted genetic manipulation	It is required to use genetically manipulated mice (e.g., apoE-deficient, LRLD-deficient)
Availability of inbred strains and clearly defined genetics	In most vessels, there is no plaque rupture or luminal thrombosis.

(Fuster & Andrés, 2012)

Table 2. Advantages and drawbacks of the swine CAVD model

Advantages	Disadvantages
Lesion location, morphology and content are similar to human haemodynamics and pathogenesis	Its large size restricts its practical application
Cardiovascular anatomy and heart size are similar to human	To induce atherosclerosis, a toxic diet is required
Similar lipid metabolism, except for Apo II deficiency in swine	Purchase and maintenance costs are both high
For genetic manipulation, highly specified genotypes are required. The minipig variant is a less expensive option	Handling difficulty (except for minipig strains)
When fed an atherogenic diet, it can spontaneously acquire atherosclerosis at a faster rate than mice and rabbits	Atheroma development takes longer time in humans than in other animals
In comparison to smaller species, imaging techniques such as ultrasound, CT, and MRI are quite simple.	

(Lee et al., 2017; Leong & Jaarin, 2015; Leong et al., 2015; Fuster et al., 2012)

Table 3. Advantages and drawbacks of Rabbit models of CAVD

Advantage	Disadvantage
Lipoprotein metabolism is similar to that of humans (except for hepatic lipase deficiency in rabbits)	The development of hypercholesterolemia and atherosclerosis requires a highly abnormal diet
No particular needs, easy to manage and maintain	Long-term high-cholesterol eating causes massive inflammation and damage in the liver du low hepatic lipase activity
Similar lesion development morphology	Does not always respond to cholesterol in the diet
Because of its small size, it has a low maintenance cost and high availability	Human cardiovascular physiology: HDL being the major plasma lipoprotein, lack of Apo AII, decreased liver lipase activity
Clinical assessment is possible with larger arteries: Ultrasound and MRI can be used to identify plaque composition and vulnerability	Plaque lesion that is not human-like: Advanced lesion (e.g., fibrosis, haemorrhage, and ulceration) with increased fatty streak and macrophage rich foam cells are not visible
Response to dietary cholesterol is favorable Hyperlipidemic mutant strains are available Large enough to accommodate physiological research	Site of diverse predilection: Atherosclerotic plaque deposits preferentially in the aorta and iliac arteries

(Leong et al., 2015; Fuster et al., 2012)

3.1. Small Animal Models

Both the pathophysiology of the illness in small animal models and the evaluation of therapeutic approaches are quite interesting to investigate further. Rats and mice are very practical and manageable owing to their small size. Small animals are becoming more the focus of CAVD research (Roosens et al., 2013).

3.1.1. Mouse Models

The key benefits of this species are its short gestation and affordable housing and breeding costs. Due to their awareness of their genomes, ability to edit them, and capacity for rapid data collecting of genomic modification, mice are an interesting model system for studying multiple processes that are impacted during the development of cardiovascular illnesses. (Bostick & Duan, 2011; Doevendans, 1995).

3.1.1.1. Nutritionally and Genetically-Susceptible Mouse Models

To produce advanced CAVD, mice must be genetically manipulated and sometimes-nutritional intervention is required. The majority of the mice utilized are LDL receptor deficient (Ldlr/) mice (Roosens et al., 2013). Drolet et al. studied the effects of a four-month high-fat/high-carbohydrate (HF/HC) diet with low cholesterol on the development of early degenerative aortic valve stenosis (AS) in adult wild-type (WT) and low-density lipoprotein receptor-deficient (LDLr/) mice. Wild-type mice on an HF/HC diet developed mild metabolic syndrome (hypercholesterolemia, obesity, and hyperglycemia).This study suggests that various atherogenic factors, including obesity, hyperglycemia, and mild dyslipidaemia, may significantly contribute to the development of AVS and that treating isolated hypercholesterolemia alone is not the most effective strategy. (Drolet et al., 2006). Weiss et al. examined low-density lipoprotein receptor-deficient apolipoprotein B-100-only (LDLr-/-

ApoB100/100) elderly mice with hypercholesterolemia who were fed standard chow. At 20 months, LDLr-/-ApoB100/100 mice showed functionally significant severe AS on echocardiography, with a decrease in valve area (>50%) compared to controls. Additionally, animals with aortic stenosis had increased quantities of superoxide in their valve tissue, indicating the beginning of oxidative stress. This finding supports the previously established relationship between tissue oxidative stress and valve disease. (Weiss et al., 2006) see **Fig. 3**. Le et al. have established the type 2 diabetes mellitus susceptible LDLr/ApoB100/100/IGF-II mice model of CAVD. Mice fed a high fat/sucrose/cholesterol (HFSC) diet for six months developed severe AS, calcification and the development of inflammatory infiltrates as compared to control mice (mostly macrophages). Additionally, diabetic mice's aortic tissues showed overexpression of osteogenic genes like spp1, bglap, and runx2, as well as myocardial tissues demonstrated upregulation of hypertrophic genes like atrial natriuretic peptide, brain natriuretic peptide, and myosin heavy chain (anp, bnp, and mch) (Le Quang et al., 2014). A noteworthy finding of this study was the development of AS in 40% of nondiabetic LDLr/ApoB100/100/) mice and 80 % of diabetic LDLr/ApoB100/100/IGF-II mice after 6 months of the HFSC diet. It is significant to note that the proportion of LRKOB100 mice in the current study (40%) that developed AS was comparable to that seen in Weiss et al. (2017). (33 %) (Weiss et al., 2006). Although AS developed in this research in a significantly shorter amount of time (6 versus 20 months), this can be attributed to the use of a cholesterol-enriched diabetogenic diet (HFSC diet) as opposed to the conventional diet recommended by (Weiss et al., 2006), also explored male LDLr-/-:ApoB100/100 mice. In this study, the rats were randomly allocated to either the usual chow or the diabetogenic, procalcific diet group (NC). After 14 months on the diabetogenic diet, LDLr-/-ApoB100/100 mice exhibited calcification, thickened leaflets, and 77 % hemodynamically significant AS. Those fed normal chow (NC) revealed a 38 % incidence of AS, and very tiny valve calcification in contrast to LDLr-/-ApoB100/100 mice. T2DM and metabolic syndrome were also developed in diabetogenic, procalcific diet fed LDLr-/-ApoB100/100 animals compared to normal chow fed mice (Scatena et al., 2018). The "endogenously hyperlipidemic" ApoE-deficient (Apoe-/-) mouse is another genetically engineered and extensively used model, which facilitates receptor-mediated clearance of very-low density lipoprotein (VLDL) from the circulation. Previous study in Apolipoprotein E-deficient (Apoe-/-) mice revealed the effects of lipids on CAVD. They observed inflammatory responses that were similar to those seen in humans, with repeated apoptotic cell death, α-SMA, osteocalcin and chemokine expression, macrophage and T-cell accumulation, nodular calcifications, mild regurgitation, and significant rises of transvalvular velocity in the aortic valve of (Apoe-/-) mice (Tanaka et al., 2005). Zeadin et al. (2015) tested the special effects of the adipocytokine leptin on valvular calcification and lesion size in a new version of the Apoe-/-mice. Leptin-treated mice did not develop hypercholesterolemia or a change in the size of atherosclerotic lesions. Furthermore, leptin-treated animals had considerably enhanced valvular calcification and ALP-positive staining, which was linked to an increase in the expression of osteoblast-specific markers (osteocalcin (OCN) and osteopontin (OPN) (Zeadin et al., 2009). Srivastava et al. (2011) revealed "the effects of acrolein, a dietary aldehyde formed during inflammation and oxidative stress, on atherosclerosis." In this study, male Apoe-/-mice were fed (2.5 mg/kg/day) for 8 weeks in this study. Mice exposed to acrolein exhibited hypercholesterolemia, lipid and macrophage infiltration, and dramatically elevated E-selectin and PAI-1 levels after 8 weeks. These findings imply that acrolein stimulation of platelets and endothelial cells occurs in vivo (Srivastava et al., 2011). Medications such as rosuvastatin and lithium chloride have been demonstrated to have anti-inflammatory effects on the aortic valve of Apoe-/-mice fed high fat/cholesterol diets, as evidenced by a considerable reduction in

macrophage infiltration and expression of vascular cell adhesion molecule-1 (VCAM-1). (Choi et al., 2010; Monetti et al., 2007). In addition to nutritionally and genetically vulnerable mouse models, Honda et al. developed a unique mechanical wire injury model in which a spring guidewire is placed into the left ventricle of the heart under echocardiographic guidance through the right common carotid artery of C57BL/6 mice. After that, the wire is twisted to cause endothelium injury. The velocity of blood flow can be measu6red by echocardiography to indicate AVS development. When compared to sham mice, echocardiography revealed enhanced aortic blood flow velocity. Furthermore, valvular calcification, increased formation of reactive oxygen species, expression of inflammatory cytokines and osteochondrogenic factors, and increased expression of inflammatory cytokines (Honda et al., 2014). By changing the wire type, tip angle, and number of turnings, mild, moderate, or severe stenosis can now be induced (Niepmann et al., 2019). The aorta anatomy of mice differs from that of humans in that they lack a trilayer structure (Hinton Jr et al., 2008). As a result, it is important to replicate any findings from mice in human valves or human cells. When compared to a control group, Fujisaka et al. discovered that giving Male ApoE-null mice high-dose Ang II (1000 ng/kg/min) for 4 weeks resulted in aortic valve thickness, endothelial disruption, and enhanced myofibroblast infiltration. Furthermore, management with olmesartan, an Ang II type 1 receptor blocker, prevented these effects. In ApoE-deficient mice, olmesartan also reduced aortic diameter dilation.(Fujisaka et al., 2013). Rattazzi et al. (2018) examined the impact of warfarin and rivaroxaban on the emergence of aortic valve calcification in ApoE deficient mice. In this study, they were split into three groups as follows: Western-Type Diet (WTD) group, 3 mg of warfarin for the warfarin group, and 5 mg of rivaroxaban for the rivaroxaban group over an 8-week period. Animals given warfarin experienced greater aortic valve degeneration and calcification as compared to mice given rivaroxaban treatment. These changes included calcium deposition on the aortic valve leaflets. According to the results of this ground-breaking research, rivaroxaban had a decreased risk of aortic valve calcification development.(Rattazzi et al., 2018).

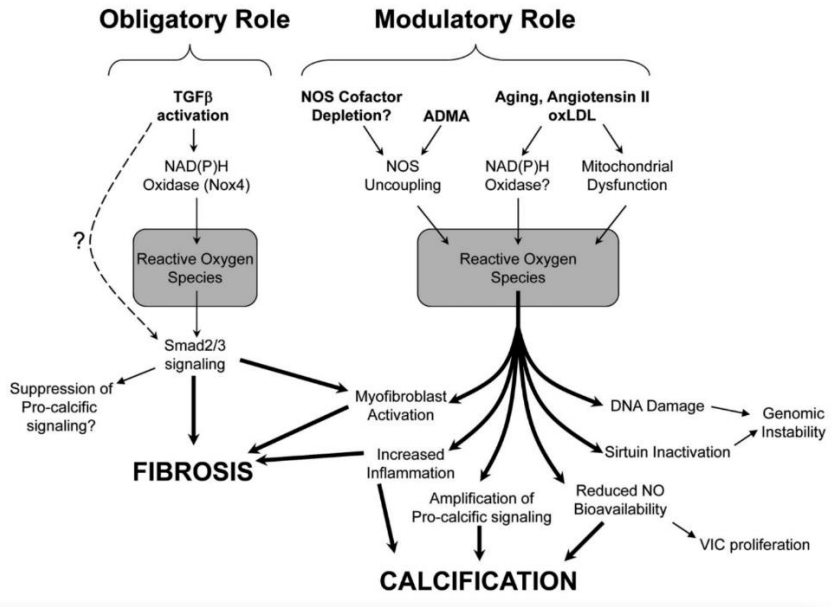

Fig. 3. Mechanisms whereby reactive oxygen species (ROS) may modulate pro-calcific and profibrotic signaling in calcific aortic valve stenosis (Miller et al., 2011).

3.1.1.2. Congenitally-Susceptible Mouse Models

According to various studies, "a congenital bicuspid aortic valve is associated with a significantly elevated risk of CAVD " (Hoffman & Kaplan, 2002).The Notch pathway has been linked to the formation of BAV and CAVD in humans, and a number of mouse studies have been conducted to investigate the functions of Notch and Notch effectors in aortic valve embryonic progress."Notch1 levels are greater in the developing mouse valve than throughout postnatal growth " (Garg et al., 2005). Notch1-null mice are embryonically fatal due to vascular abnormalities; however, mice that are Notch1 heterozygous (Notch1+/-) have five times the amount of calcium in their aortic valves compared to WT controls, but they do not have bicuspid valves (Nigam & Srivastava, 2009). In some investigations, no BAVs were observed in Notch1+/- mice(Nigam & Srivastava, 2009), while others showed rates as high as 6%, all without statistical significance when compared to WT incidence rates (Nus et al., 2011). Mice with VEC-specific homozygous deletion of Notch1 (post-endo-MT, utilizing Nfatc1enCre mice) showed aortic valve thickening, fibrosis, and proteoglycaneous aortic valve leaflet, with a 30% occurrence (Wang et al., 2017). Pleiotrophin (Ptn) and delta-like 1 homolog (Dlk1), a powerful Notch1 inhibitor, are ectopically generated in the endocardial cushions when periostin is missing throughout development. This resulted in a reduction in Notch1 signaling, a significant rise in Runx2, the main transcriptional regulator of osteoblast cellular proliferation, and aortic valve calcification (Tkatchenko et al., 2009). At 10 months of age, Postn-/- mice had an abnormal aortic morphology with Runt-related transcription factor 2 (runx2), osteopontin (OPN), and osteocalcin (OCN) expression, as well as significant valvular calcium deposition (Yoshioka et al., 2006). When compared to WT mice fed the same diet, Postn/ mice with a high-fat diet have reduced valve thickness, macrophage infiltration, myogenic differentiation, circumferential fibrosis, and MMP-2/3 increased expression, possibly reflecting a reduced ability of myofibroblasts and monocytes to adhere to and infiltrate the ECM (Hakuno et al., 2010).There is no correlation between the expression of periostin and chondromodulin-I (ChmI). Increases in valve thickness, lipid buildup, calcification, vascular endothelial growth factor-A (VEGF-A), and angiogenesis are all seen in older ChmI/ mice (Yoshioka et al., 2006). Human bicuspid valves have reduced expression of eNOS in the valvular endothelium (Aicher et al., 2007), and approximately (27-42%) of mice that are defective in endothelial nitric oxide synthase have RC/NC bicuspid aortic valves, which are thought to result from impaired, shear-stress and nitric oxide (NO)-dependent epithelial to mesenchymal transformation and reduction invasion of the endothelial cushion by mesenteric mesenchym (Fernández et al., 2009). In heterozygous Nos3+/- mice, no BAVs were found. By 6 months of age, Nos3-/- mice with BAVs develop fibrosis and leaflet calcification, but even by 18 months, Nos3-/- mice with normal tricuspid aortic valves (TAVs) were just fibrotic, not calcified, and function was unaffected (El Accaoui et al., 2014). A targeted deletion of Gata5 in mice led to BAV and hypoplastic heart development, according to a study. The majority of BAVs in humans are caused by the fusion of the right-coronary and left-coronary leaflets (R-L) or the right-coronary and noncoronary leaflets (R-N) (R-N)."The detected BAVs caused by fusion of the right-coronary and noncoronary leaflets, the subtype associated with more severe valve malfunction in people," the researchers concluded (Laforest et al., 2011). Gata5 controls eNOS and Notch signaling; Gata5-/-mice had substantially lower Nos3 and Notch ligand Jag1 transcription, and the mouse Nos3 promoter has several GATA binding sites. Adult Gata5-/-mice have considerably compromised. aortic valve function (Laforest et al., 2011). Epithelial growth factor receptor (EGFR) signaling pathways are known to control the development of the embryonic aortic valve in mice (Chen et al., 2000), and possibly humans (McBride et al., 2011).

3.2. Large Animal Models

The primary aims of using animal models are to advance human health and to make scientific discoveries that can be translated into practical applications. As they reveal disease characteristics similar to those in humans and provide mechanistic understanding into the biological and pathological processes, large animal models can help accomplish these objectives (Tsang et al., 2016). Larger animal models, as opposed to mice, are more costly to buy, feed, and care for under contemporary animal husbandry circumstances. Complex atherosclerotic lesions, on the other hand, take a longer time to form in mice than they do in humans. CAVD may be studied in large animals such as pigs and rabbits because of their structural and functional parallels to humans, making them useful models for preclinical and clinical investigations (Brousseau & Hoeg, 1999; Cimini et al., 2005; Fernández-Jiménez et al., 2015; Swinkels & Demacker, 1988).

3.2.1. Swine Models

In studies of atherosclerosis, swine are excellent models because they: 1) have comparable systemic hemodynamic factors and cardiac structure, serum cholesterol, lipid metabolism, and a akin genome in size and chromosomal structure to living beings, making swine models attractive for genomic studies; and 2) develop human atherosclerotic lesions on high-fat/high-cholesterol diet and develops naturally with high-fat diets to study CAVD. These characteristics emphasize the swine as an attractive model for studying CAVD and demonstrating the propensity for developing valvular lesions (Dixon et al., 1999; Gerrity et al., 2001; Sider et al., 2014; Skold & Ramsey, 1966). On the other hand, Pigs are not often used because of their large size. Mini pigs that have been genetically modified to have hyperlipidemia and atherosclerosis have just come on the market; they are less expensive to maintain than full-sized pigs. Its pathophysiological processes were examined in detail and found to be identical to human atherosclerosis, which is not the observed in mouse models. (Agarwala et al., 2013; Davis et al., 2014).

3.2.1.1. Nutritionally-Susceptible Swine Models

Research on CAVD has recently begun using Swine models, which have traditionally been employed in studies on atherosclerosis (Sider et al., 2011). According to certain studies, starting a diet early in life may be more successful in creating advanced illness (Gerrity et al., 2001). A swine model of early aortic valve sclerosis was studied by Sider et al, in this study, swine were fed either a typical diet or a high fat/cholesterol (HF/HC) diet for 2–5 months. The coronary aortic valve leaflets of swine given the HF/HC diet established thicker lesions on the aortic side and histologically opaque regions of proteoglycans, collagen, and elastin within the fibrosa layer, comparable to early human CAVD.(Sider et al., 2014). Recently, Go et al. investigated a model for morphological and mechanical changes in the aortic valve in female Yorkshire domestic pigs (Go et al., 2018). The animals were nourished a normal or a high-fat/high-cholesterol (HF/HC) diet for 16 weeks in this experiment. The control group was administered a normal swine feed with 14.5 % protein, 3% fat, and 3.3 Kcal/g of feed. The experimental group was given a high fat, high-fructose diet with 17 % protein, 20% fat, and 4.1 Kcal/g of feed. Aortic valve degradation and calcification were seen in pigs fed a high-fat diet for 16 weeks in this study, which is consistent with the findings of the previous research (Go et al., 2018). Through dietary intervention, a substantial link between endothelial phenotypic heterogeneity, regional CAVD susceptibility, and local hemodynamics was previously discovered in normal and hypercholesterolemic pigs.

The results show that aortic valve calcification largely affects the aortic side of the valve leaflets and is related to a side-specific elevation of eNOS and activated leukocyte adhesion molecule in endothelial cells (ALCAM)

(Guerraty et al., 2010 ; Simmons et al., 2005). Microarray and quantitative Real time polymerase chain reaction (qRT-PCR) were used to compare gene expression in aortic and ventricular side aortic Valve surface endothelial cells (VECs) from adult male swine. The aortic and ventricular VECs were revealed to have side-specific expression differences in this investigation (Simmons et al., 2005). Genes linked with vascular calcification and skeletal development, such as bone morphogenetic proteins, were shown to have increased expression on the aortic side of the valve (BMP-4). In the aortic side VECs, lower expression of proteins known to suppress ectopic calcification, such as osteoprotegerin (OPG), C-type natriuretic peptide (CNP), and chordin, was also found (an inhibitor of the osteoinductive activity of BMPs) (Simmons et al., 2005). Furthermore, "higher expression of antioxidative genes on the aortic side and the absence of differential expression of pro-inflammatory proteins on the aortic side suggests potential protection in the normal valve against lesion development and inflammation," according to the researchers (Butcher et al., 2011; Simmons et al., 2005). In swine, atherosclerotic plaque progress can be enhanced by combining a high-cholesterol diet with nearby formed vascular injury forced by a variety of methods, such as guide-wire-induced injury (De Smet et al., 1998; Granada et al., 2009), endovascular balloon inflation with or without stent deployment, (Thim et al., 2010), partial vessel ligation,(Ishii et al., 2006) in addition to percutaneous intramural injection of cholesteryl esters and human oxLDL after a 2-week balloon angioplasty (Granada et al., 2005; Granada et al., 2007). In addition to reducing the problems in care and high preservation costs linked with the use of swine models by shortening the study's duration, atherogenic diets and vascular injury protocols are also highly relevant models for translational research in the field of percutaneous interventions and cardiovascular imaging animal models (Fuster et al., 2012).

3.2.1.2. Genetically-Susceptible Porcine Models

In swine, naturally, occurring mutations have been used to establish non-diet-induced hypercholesterolemia models for CAVD. The LDLR and/or apolipoprotein genes exhibit mutations in these models. Among the most popular models are (Hasler-Rapacz et al., 1996; Hasler-Rapacz et al., 1998):(1) familial hypercholesterolemia with altered lipid profiles due to an LDLR mutation; In a recent investigation of Familial hypercholesterolemia (FH), a total of 21 female animals were evaluated:4 wild type (WT) swine, 3 one-year-old (1 yo) WT swine, 3 Rapacz FH (RFH) swine, 4 two-year-old (2 yo) adult RFH swine, and 5 three-year-old (3 yo) RFH swine Throughout the trial, the animals were fed a conventional swine diet (consisting of 75.8%, 14.7 percent, and 9.4% of daily calories from carbohydrates, protein, and fat, respectively). Adult RFH showed early signs of CAVD.A substantial thickening of the cusps as well as considerable extracellular matrix alteration, including proteoglycan enrichment, collagen disruption, and elastin fragmentation, lipid oxidation and macrophage infiltration have both increased. Mild aortic valve sclerosis was revealed on echocardiography. Valve microarrays from adult and juvenile RFH mice indicated considerable activation of inflammation-related genes, as well as various similarities and overlaps with atherosclerosis and human CAVD (Porras et al., 2015). (2) LDLR and ApoB mutations cause a second kind of familial hypercholesterolemia. (Grunwald et al., 1999). The Rapacz pig is a wild-type animal with a natural mutation in the ApoB and LDLR genes that was developed through selective breeding of high-cholesterol pigs (Davis et al., 1984). These pigs developed significant hypercholesterolemia, with LDL as the major circulating lipoprotein, within 2-4 years on a normal diet, which has been linked to the development of coronary atherosclerosis (Lee et al., 2017). Their use is restricted because of the considerable time it takes to develop complex atherosclerotic lesions, even when fed atherogenic diets (2–3 years), as well as their large size and weight (> 200 kg). The practice of smaller swine strains, such as the Yucatan mini pig, which progress

humanoid complicated lesions with profuse necrosis, lipid deposits, and significant calcification, reduces these issues in treatment and expensive maintenance costs (Barbeau et al., 1997; De Smet et al., 1998; Gal et al., 1990; Holvoet et al., 1998; Panepinto & Phillips, 1986; Reitman et al., 1982).

3.2.2. Rabbit Models

In 1908, Ignatowski wrote about the first evidence that experimental animals may produce atherosclerosis. He gave rabbits a high-protein diet that included meat, milk, and egg yolk, which caused atherosclerotic lesions to form on the aortic wall (Ignatowski, 1908). Animals including rabbits, mice, rats, guinea pigs, hamsters, birds, swine, dogs, and nonhuman primates have all been produced since then (Ignatowski, 1908).

3.2.2.1. Nutritionally-Susceptible Rabbit Models

Rabbits have also been used to investigate aortic valve disease in its various stages, from early to advance. A hypercholesterolemic diet is commonly used to induce pathogenesis. (Guerraty & Mohler, 2007) Capillary stenosis in rabbits was explored by Mourino-Alvarez and colleagues. Animals considering 2–2.5 kg were split into two separate groups: the control group received standard rabbit chow, while the pathological group received chow enhanced with 1 percent cholesterol and 50,000 IU/kg vitamin D2. The experiments lasted 12 weeks in each group. (Drolet, Couët, & Arsenault, 2008; Mourino-Alvarez et al., 2018) There was an increase in cholesterol in the diseased group, as well as thicker AVs on echocardiography. These features have been seen before in individuals with CAS (Akat et al., 2010; Kamath & Pai, 2008; Mourino-Alvarez et al., 2018).

Hematology showed considerable calcium deposits, a profusion of macrophages (RAM11-positive cells), and a profusion of -actin, a marker for smooth muscle cells including myofibroblasts-lactate dehydrogenase B chain (LDHB) and tropomyosin -1 chain (TPM-1) both revealed the similar pattern in plasma and tissue, whereas transitional endoplasmic reticulum ATPase (TERA) was upregulated in tissue and downregulated in both rabbit and human plasma (Mourino-Alvarez et al., 2018). The effect of a high-fat, high-cholesterol diet supplemented with vitamin D on the growth and development of heart valves as far back as 2003. The male New Zealand White rabbits utilized in this experiment were divided into the following groups: a control group fed regular food with no dietary supplements; b) animals fed food enriched with 0.5 percent cholesterol plus 50,000 IU of vitamin D2 per day; c) animals fed food enriched with 0.5 percent cholesterol plus 50,000 IU of vitamin D2 per day; and d) animals fed food enriched with 0.5 percent cholesterol plus 50,000 IU of vitamin D2 per day (Drolet et al., 2003), Group 1 exhibited no change in cholesterol levels after 12 weeks; however groups 2 and 3 had significantly higher levels. Vitamin D2 had a surprising effect on cholesterol levels, despite the fact that both cholesterol consumption and vitamin D2 levels were the same. In comparison to groups 1 and 2, calcium levels were somewhat higher in group 3. According to echocardiography, the Aortic valve area (AVA) decreased by 36%, the maximal gradient increased by 300%, and the mean gradient increased by 107% (all p 0.05) (Drolet et al., 2003).There was a clear correlation between vitamin D3 levels and the progress of AVS in a sample of chronic renal failure patients, as revealed by (Malergue et al., 997). After controlling for age, gender, overall creatinine clearance, raised calcium-phosphate product in individuals with adequate renal function was likewise linked with the severity of AVS (Mills et al., 2004). Eight weeks of treatment with vitamin D2 alone at 25,000 IU/4 days per week enhanced the amount of aortic valve stenosis (AVS) in male New Zealand white rabbits. An enhanced aortic valve backscatter (AVBS), enhanced transvalvular velocity, and higher pressure gradient were seen in rabbits treated with Vitamin D2. The calcification, lipid accumulation, and macrophage infiltration of the valves were all present. An increase in intravalvular thioredoxin-interacting protein (TXNIP) content was found in the endothelium. According to

histology results, early AVS in humans is associated with endothelial dysfunction and redox stress. Loss of nitric oxide regulation of TXNIP expression may lead to the development of AVS (Ngo et al., 2008). AVS therapy may also be tested on rabbits, which have shown to be a valuable tool. According to Rajamannan and colleagues (Galante et al., 2001), osteopontin and osteoblast gene markers (alkaline phosphatase, osteopontin, and osteoblast lineage-specific transcription factor (Cbfa-1) in the cholesterol-fed rabbits compared to the control rabbits were associated with an atherosclerosis proliferative valve lesion. Treatment with HMG CoA reductase inhibitors lowered the levels of all indicators except hsCRP (Jialal et al., 2001; Rajamannan et al., 2002). An animal model of Aortic valve sclerosis was used to test the effects of dietary modification and statin medication on the tissue response to therapy. Male New Zealanders were assessed in this study. White rabbits were fed a 0.25 % cholesterol-supplemented diet for six months in order to maintain cholesterol levels of 500 mg/dl, and they were then tittered (0.125–0.25 %). Six rabbits were fed as a control group. By 15 months, the cusps of five cholesterol-fed rabbits had thickened due to lipid deposition, macrophage infiltration, and osteopontin expression. The remaining cholesterol-fed rabbits were divided into four groups. Rabbits were fed food enriched with 0.125 % cholesterol. Standard chow was given to rabbits receiving only nutritional treatment, whereas rabbits receiving only statin medication got a pill containing atorvastatin 2.5 mg/kg every day (Hamilton et al., 2011; Rajamannan et al., 2005; Rajamannan et al., 2002; Stock, et al., 2005). Statin and dietary treatment rabbits received 2.5 mg/kg per day of atorvastatin calcium in regular chow for an additional 15 months, along with 0.125 % cholesterol-supplemented chow. Rabbit cusps showed a substantial rise in osteopontin expression, collagen deposition, lipid, macrophage infiltration, and osteopontin expression by 30 months on the atherogenic diet alone. Increases in CD3+ lymphocyte invasion and calcification were also noted. However, after statin therapy, osteopontin expression and immune cell infiltration significantly decreased in the valve cusps. Unfortunately, calcification and lipid retention persisted in all of the treated valves. We conclude that the cellular response to statin medication does not fully reverse the sclerotic process in established AVSc (Hamilton et al., 2011). Arishiro et al. discovered that taking ARB (olmesartan, 1 mg/kg/day) for the previous four weeks caused atherosclerotic changes in the aortic valves of rabbits fed a 1% cholesterol diet for eight weeks. Olmesartan treatment dramatically lowered lipid deposition, macrophage accumulation, osteopontin expression, angiotensin-converting enzyme, and alpha-smooth muscle actin-positive myofibroblasts, enhanced eNOS expression, and decreased messenger ribonucleic acid for Cbfa-1 mRNA production. Endothelial integrity was maintained and trans differentiation of valvular fibroblasts into myofibroblasts and/or osteoblasts in the valve leaflets was avoided on the lesion-prone aortic side of the valve (Arishiro et al., 2007).

3.2.2.2. Genetically-Susceptible Rabbit Models

Despite the fact that feeding rabbits a high-fat diet for the long term has unfavorable side effects and increases mortality owing to liver toxicity (Fuster et al., 2012), genetically engineered rabbits that induce spontaneous atherosclerotic lesions have been established. For instance, (1) An LDLR-deficient model is the Watanabe heritable hypercholesterolemic rabbit (WHHL) (Burnstock & Aliev, 1998; Rajamannan et al., 2005; Shiomi & Ito, 2009);(2) Rabbits from St. Thomas Hospital that have high cholesterol and triglycerides (Beaty et al., 1992); (3) rabbits with changed lipid profiles, such as induced human ApoB100 (Beaty et al., 1992) or Apo(a).(Fan et al., 2001). The Watanabe heritable hyperlipidemic (WHHL) rabbit (Watanabe, 1980), is the most widely used , and it was utilized in a CAVD investigation to show that atorvastatin reduces hypercholesterolemia-induced AV calcification, which is mediated in part via the Lrp5/-catenin pathway. This developmental mechanism could play

a role in the disease's signaling pathway (Caira, et al., 2005). Recently found a rabbit model of familial hypercholesterolemia and atherosclerosis, the Watanabe heritable hyperlipidemic (WHHLMI) rabbit, which is prone to myocardial infarction (Hara et al., 2018). The research demonstrated age-dependent progression of aortic valve sclerosis in (WHHLMI) rabbits fed normal chow or without a high-cholesterol diet or vitamin D supplement. This study used WHHLMI rabbits (Hara et al., 2018; Shiomi et al., 2003), aged 20 or 30 months, as well as control Japanese White rabbits, were assessed. WHHLMI rabbits that were 20 and 30 months old had comparable lipid profiles. In comparison to twenty-month-old WHHLMI rabbits, the aortic valve area and maximum transvalvular pressure gradient were much smaller in the thirty-month-old WHHLMI rabbits. A macroscopic study at 30 months revealed thickened and deteriorated valve leaflets. Histological analysis at 30 months showed thicker leaflets with calcified nodules. Real-time polymerase chain reaction (PCR) analysis of 30-month-old rabbits revealed elevated expression of molecules involved in calcification, including osteopontin (OPN), Sox9 (Caira et al., 2006), Bmp2, receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG), and transcription factor for osteoblast differentiation (Runx2) (Wirrig & Yutzey, 2011). WHHLMI rabbits may serve as useful in vivo models of early AS (Hara et al., 2018).

CONCLUSION

It is crucial to employ carefully chosen and well-given animal models in order to better understand the pathophysiology of CAVD. In order to better comprehend the pathophysiology of CAVD, it is crucial to use animal models that are well chosen and adequately administered. Human CAVD pathophysiology has been replicated in many animal models, allowing researchers to undertake studies that would otherwise be hard or impractical to carry out on patients themselves. New models and better knowledge of the value of current models have pushed animal model-based research to new heights in this discipline. Experimental models for this illness have been found in a wide range of animal species. In spite of the fact that several animal models are being employed, none of them should be called an excellent model of human illness. Translational research studies can be more readily applied to people since CAVD in swine carefully resembles the key morphological and biochemical aspects of human CAVD, hence findings from large animal models can be more simply extrapolated to humans. Researchers working with huge animals face a variety of significant challenges, including problems in handling and the high costs of maintaining them. Genetic advances have made it possible to develop mini pigs with human-like physiology and ease of handling that are more human-like than non-human primates, and that have anatomical and physiological characteristics that are strikingly similar to those of humans, including lipoprotein metabolism and atherosclerotic pathophysiology. There are several benefits to using small animals for experiments (e.g. simple handling and inexpensive cost), but they don't often acquire the advanced susceptible lesion that is typical of human patients with severe CAVD. The utilization of all currently available animal models will definitely continue to allow for significant advancements in CAVD research, which should lead to better CAVD treatment, prevention, and diagnosis.

Acknowledgments

The contributors to this work are gratefully thanked by the authors

Conflict of Interest

The authors have declared no conflict of interests exist regarding publication of this paper.

REFERENCES

Agarwala, A., Billheimer, J., & Rader, D. J. (2013). Mighty minipig in fight against cardiovascular disease. Science translational medicine, 5(166), 166fs161-166fs161.

Aicher, D., Urbich, C., Zeiher, A., Dimmeler, S., & Schäfers, H.-J. (2007). Endothelial nitric oxide synthase in bicuspid aortic valve disease. The Annals of thoracic surgery, 83(4), 1290-1294.

Akat, K., Kaden, J. J., Schmitz, F., Ewering, S., Anton, A., Klomfaß, S., . . . Ortlepp, J. R. (2010). Calcium metabolism in adults with severe aortic valve stenosis and preserved renal function. The American journal of cardiology, 105(6), 862-864.

Alushi, B., Curini, L., Christopher, M. R., Grubitzch, H., Landmesser, U., Amedei, A., & Lauten, A. (2020). Calcific aortic valve disease-natural history and future therapeutic strategies. Frontiers in Pharmacology, 11, 685.

Anselmo, W., Branchetti, E., Grau, J. B., Li, G., Ayoub, S., Lai, E. K., . . . Sacks, M. S. (2018). Porphyrin-Based SOD Mimic MnTnBu OE-2-PyP5+ Inhibits Mechanisms of Aortic Valve Remodeling in Human and Murine Models of Aortic Valve Sclerosis. Journal of the American Heart Association, 7(20), e007861.

Arishiro, K., Hoshiga, M., Negoro, N., Jin, D., Takai, S., Miyazaki, M., . . . Hanafusa, T. (2007). Angiotensin receptor-1 blocker inhibits atherosclerotic changes and endothelial disruption of the aortic valve in hypercholesterolemic rabbits. Journal of the American College of Cardiology, 49(13), 1482-1489.

Barbeau, M. L., Klemp, K. F., Guyton, J. R., & Rogers, K. A. (1997). Dietary fish oil: influence on lesion regression in the porcine model of atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology, 17(4), 688-694.

Beaty, T., Prenger, V., Virgil, D., Lewis, B., Kwiterovich, P., & Bachorik, P. (1992). A genetic model for control of hypertriglyceridemia and apolipoprotein B levels in the Johns Hopkins colony of St. Thomas Hospital rabbits. Genetics, 132(4), 1095-1104.

Bostick, B., Yue, Y., & Duan, D. (2011). Phenotyping cardiac gene therapy in mice. In Muscle Gene Therapy (pp. 91-104): Springer.

Branchetti, E., Sainger, R., Poggio, P., Grau, J. B., Patterson-Fortin, J., Bavaria, J. E., . . . Levy, R. J. (2013). Antioxidant enzymes reduce DNA damage and early activation of valvular interstitial cells in aortic valve sclerosis. Arteriosclerosis, thrombosis, and vascular biology, 33(2), e66-e74.

Brousseau, M. E., & Hoeg, J. M. (1999). Transgenic rabbits as models for atherosclerosis research. Journal of lipid research, 40(3), 365-375.

Burnstock, G., & Aliev, G. (1998). Watanabe rabbits with heritable hypercholesterolaemia: a model of atherosclerosis. Histology and histopathology, 13(3), 797-817.

Butcher, J. T., Mahler, G. J., & Hockaday, L. A. (2011). Aortic valve disease and treatment: the need for naturally engineered solutions. Advanced drug delivery reviews, 63(4-5), 242-268.

Caira, F. C., Stock, S. R., Gleason, T. G., McGee, E. C., Huang, J., Bonow, R. O., . . . Rajamannan, N. M. (2006). Human degenerative valve disease is associated with up-regulation of low-density lipoprotein receptor-related protein 5 receptor-mediated bone formation. Journal of the American College of Cardiology, 47(8), 1707-1712.

Chen, B., Bronson, R. T., Klaman, L. D., Hampton, T. G., Wang, J.-f., Green, P. J., . . . Neel, B. G. (2000). Mice mutant for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. Nature genetics, 24(3), 296-299.

Choi, S.-E., Jang, H.-J., Kang, Y., Jung, J. G., Han, S. J., Kim, H. J., . . . Lee, K.-W. (2010). Atherosclerosis induced by a high-fat diet is alleviated by lithium chloride via reduction of VCAM expression in ApoE-deficient mice. Vascular pharmacology, 53(5-6), 264-272.

Chorro, F. J., Such-Belenguer, L., & López-Merino, V. (2009). Animal models of cardiovascular disease. Revista Española de Cardiología (English Edition), 62(1), 69-84.

Cimini, M., Boughner, D. R., Ronald, J. A., Aldington, L., & Rogers, K. A. (2005). Development of aortic valve sclerosis in a rabbit model of atherosclerosis: an immunohistochemical and histological study. The Journal of heart valve disease, 14(3), 365-375.

Cohen, D. J., Malave, D., Ghidoni, J. J., Iakovidis, P., Everett, M. M., You, S., . . . Boyan, B. D. (2004). Role of oral bacterial flora in calcific aortic stenosis: an animal model. The Annals of thoracic surgery, 77(2), 537-543.

Davis, B. T., Wang, X.-J., Rohret, J. A., Struzynski, J. T., Merricks, E. P., Bellinger, D. A., . . . Rogers, C. S. (2014). Targeted disruption of LDLR causes hypercholesterolemia and atherosclerosis in Yucatan miniature pigs. PloS one, 9(4), e93457.

Davis, H. R., Vesselinovitch, D., & Wissler, R. W. (1984). Reticuloendothelial system response to hyperlipidemia in rhesus and cynomolgus monkeys. Journal of leukocyte biology, 36(1), 63-80.

De Smet, B., Van der Zande, J., Van Der Helm, Y., Kuntz, R., Borst, C., & Post, M. (1998). The atherosclerotic Yucatan animal model to study the arterial response after balloon angioplasty: the natural history of remodeling. Cardiovascular Research, 39(1), 224-232.

Demer, L. L., & Tintut, Y. (2019). Heart valve calcification. In Principles of Heart Valve Engineering (pp. 307-319): Elsevier.

Dixon, J. L., Stoops, J., Parker, J., Laughlin, M., Weisman, G., & Sturek, M. (1999). Dyslipidemia and vascular dysfunction in diabetic pigs fed an atherogenic diet. Arteriosclerosis, thrombosis, and vascular biology, 19(12), 2981-2992.

Doevendans, P. A. (1995). Strategies for studying cardiovascular diseases in transgenic and gene-trageted mice. Strategies in transgenic animal science., 107-144.

Drolet, M.-C., Arsenault, M., & Couet, J. (2003). Experimental aortic valve stenosis in rabbits. Journal of the American College of Cardiology, 41(7), 1211-1217.

Drolet, M.-C., Couët, J., & Arsenault, M. (2008). Development of aortic valve sclerosis or stenosis in rabbits: role of cholesterol and calcium.

Drolet, M.-C., Roussel, E., Deshaies, Y., Couet, J., & Arsenault, M. (2006). A high fat/high carbohydrate diet induces aortic valve disease in C57BL/6J mice. Journal of the American College of Cardiology, 47(4), 850-855.

Dweck, M. R., Boon, N. A., & Newby, D. E. (2012). Calcific aortic stenosis: a disease of the valve and the myocardium. Journal of the American College of Cardiology, 60(19), 1854-1863.

El Accaoui, R. N., Gould, S. T., Hajj, G. P., Chu, Y., Davis, M. K., Kraft, D. C., . . . Zimmerman, K. A. (2014). Aortic valve sclerosis in mice deficient in endothelial nitric oxide synthase. American Journal of Physiology-Heart and Circulatory Physiology, 306(9), H1302-H1313.

Fan, J., Shimoyamada, H., Sun, H., Marcovina, S., Honda, K., & Watanabe, T. (2001). Transgenic rabbits expressing human apolipoprotein (a) develop more extensive atherosclerotic lesions in response to a cholesterol-rich diet. Arteriosclerosis, thrombosis, and vascular biology, 21(1), 88-94.

Fernández-Jiménez, R., García-Prieto, J., Sánchez-González, J., Agüero, J., López-Martín, G. J., Galán-Arriola, C., . . . Ibáñez, B. (2015). Pathophysiology underlying the bimodal edema phenomenon after myocardial ischemia/reperfusion. Journal of the American College of Cardiology, 66(7), 816-828.

Fernández, B., Durán, A. C., Fernández-Gallego, T., Fernández, M. C., Such, M., Arqué, J. M., & Sans-Coma, V. (2009). Bicuspid aortic valves with different spatial orientations of the leaflets are distinct etiological entities. Journal of the American College of Cardiology, 54(24), 2312-2318.

Fujisaka, T., Hoshiga, M., Hotchi, J., Takeda, Y., Jin, D., Takai, S., . . . Ishizaka, N. (2013). Angiotensin II promotes aortic valve thickening independent of elevated blood pressure in apolipoprotein-E deficient mice. Atherosclerosis, 226(1), 82-87.

Fuster, J. J., Castillo, A. I., Zaragoza, C., Ibáñez, B., & Andrés, V. (2012). Animal models of atherosclerosis. Progress in molecular biology and translational science, 105, 1-23.

Gal, D., Rongione, A. J., Slovenkai, G. A., DeJesus, S. T., Lucas, A., Fields, C. D., & Isner, J. M. (1990). Atherosclerotic Yucatan microswine: An animalmodel with high-grade, fibrocalcific, nonfatty lesions suitable for testing catheter-based interventions. American heart journal, 119(2), 291-300.

Galante, A., Pietroiusti, A., Vellini, M., Piccolo, P., Possati, G., De Bonis, M., . . . Favalli, C. (2001). C-reactive protein is increased in patients with degenerative aortic valvular stenosis. Journal of the American College of Cardiology, 38(4), 1078-1082.

Garg, V., Muth, A. N., Ransom, J. F., Schluterman, M. K., Barnes, R., King, I. N., . . . Srivastava, D. (2005). Mutations in NOTCH1 cause aortic valve disease. nature, 437(7056), 270-274.

Gerrity, R. G., Natarajan, R., Nadler, J. L., & Kimsey, T. (2001). Diabetes-induced accelerated atherosclerosis in swine. Diabetes, 50(7), 1654-1665.

Ghaisas, N. K., Foley, J. B., O'Briain, D. S., Crean, P., Kelleher, D., & Walsh, M. (2000). Adhesion molecules in nonrheumatic aortic valve disease: endothelial expression, serum levels and effects of valve replacement. Journal of the American College of Cardiology, 36(7), 2257-2262.

Gillis, K., Roosens, B., Bala, G., Remory, I., Hernot, S., Delvenne, P., . . . Cosyns, B. (2017). Interaction of renal failure and dyslipidaemia in the development of calcific aortic valve disease in rats. Acta Cardiologica, 72(5), 537-546.

Go, J. L., Prem, K., Al-Hijji, M. A., Qin, Q., Noble, C., Young, M. D., . . . Lerman, A. (2018). Experimental metabolic syndrome model associated with mechanical and structural degenerative changes of the aortic valve. Scientific reports, 8(1), 1-11.

Goody, P. R., Hosen, M. R., Christmann, D., Niepmann, S. T., Zietzer, A., Adam, M., . . . Jansen, F. (2020). Aortic valve stenosis: from basic mechanisms to novel therapeutic targets. Arteriosclerosis, thrombosis, and vascular biology, 40(4), 885-900.

Granada, J. F., Kaluza, G. L., Wilensky, R. L., Biedermann, B. C., Schwartz, R. S., & Falk, E. (2009). Porcine models of coronary atherosclerosis and vulnerable plaque for imaging and interventional research. EuroIntervention, 5(1), 140-148.

Granada, J. F., Moreno, P. R., Burke, A. P., Schulz, D. G., Raizner, A. E., & Kaluza, G. L. (2005). Endovascular needle injection of cholesteryl linoleate into the arterial wall produces complex vascular lesions identifiable by intravascular ultrasound: early development in a porcine model of vulnerable plaque. Coronary artery disease, 16(4), 217-224.

Granada, J. F., Wallace-Bradley, D., Win, H. K., Alviar, C. L., Builes, A., Lev, E. I., . . . Kaluza, G. L. (2007). In vivo plaque characterization using intravascular ultrasound–virtual histology in a porcine model of complex coronary lesions. Arteriosclerosis, thrombosis, and vascular biology, 27(2), 387-393.

Grunwald, K. A., Schueler, K., Uelmen, P. J., Lipton, B. A., Kaiser, M., Buhman, K., & Attie, A. D. (1999). Identification of a novel Arg→ Cys mutation in the LDL receptor that contributes to spontaneous hypercholesterolemia in pigs. Journal of lipid research, 40(3), 475-485.

Guerraty, M., & Mohler, E. R. (2007). Models of aortic valve calcification. Journal of Investigative Medicine, 55(6), 278-283.

Guerraty, M. A., Grant, G. R., Karanian, J. W., Chiesa, O. A., Pritchard, W. F., & Davies, P. F. (2010). Hypercholesterolemia Induces Side-Specific Phenotypic Changes and Peroxisome Proliferator–Activated Receptor-γ Pathway Activation in Swine Aortic Valve Endothelium. Arteriosclerosis, thrombosis, and vascular biology, 30(2), 225-231.

Guerraty, M. A., Grant, G. R., Karanian, J. W., Chiesa, O. A., Pritchard, W. F., & Davies, P. F. (2011). Side-specific expression of activated leukocyte adhesion molecule (ALCAM; CD166) in pathosusceptible regions of swine aortic valve endothelium. The Journal of heart valve disease, 20(2), 165.

Hakuno, D., Kimura, N., Yoshioka, M., Mukai, M., Kimura, T., Okada, Y., . . . Kudo, A. (2010). Periostin advances atherosclerotic and rheumatic cardiac valve degeneration by inducing angiogenesis and MMP production in humans and rodents. The Journal of clinical investigation, 120(7), 2292-2306.

Hamilton, A. M., Boughner, D. R., Drangova, M., & Rogers, K. A. (2011). Statin treatment of hypercholesterolemic-induced aortic valve sclerosis. Cardiovascular Pathology, 20(2), 84-92.

Hara, T., Tsukada, N., Okano, M., Ishida, T., Hirata, K.-i., & Shiomi, M. (2018). Progression of calcific aortic valve sclerosis in WHHLMI rabbits. Atherosclerosis, 273, 8-14.

Hasler-Rapacz, J., Kempen, H. J., Princen, H. M., Kudchodkar, B. J., Lacko, A., & Rapacz, J. (1996). Effects of simvastatin on plasma lipids and apolipoproteins in familial hypercholesterolemic swine. Arteriosclerosis, thrombosis, and vascular biology, 16(1), 137-143.

Hasler-Rapacz, J., Ellegren, H., Fridolfsson, A. K., Kirkpatrick, B., Kirk, S., Andersson, L., & Rapacz, J. (1998). Identification of a mutation in the low density lipoprotein receptor gene associated with recessive familial hypercholesterolemia in swine. American journal of medical genetics, 76(5), 379-386.

Helske, S., Oksjoki, R., Lindstedt, K. A., Lommi, J., Turto, H., Werkkala, K., . . . Kovanen, P. T. (2008). Complement system is activated in stenotic aortic valves. Atherosclerosis, 196(1), 190-200.

Hinton Jr, R. B., Alfieri, C. M., Witt, S. A., Glascock, B. J., Khoury, P. R., Benson, D. W., & Yutzey, K. E. (2008). Mouse heart valve structure and function: echocardiographic and morphometric analyses from the fetus through the aged adult. American Journal of Physiology-Heart and Circulatory Physiology, 294(6), H2480-H2488.

Hisamatsu, T., Miura, K., Fujiyoshi, A., Kadota, A., Miyagawa, N., Satoh, A., . . . Ueshima, H. (2018). Serum magnesium, phosphorus, and calcium levels and subclinical calcific aortic valve disease: A population-based study. Atherosclerosis, 273, 145-152.

Hjortnaes, J., Shapero, K., Goettsch, C., Hutcheson, J. D., Keegan, J., Kluin, J., . . . Aikawa, E. (2015). Valvular interstitial cells suppress calcification of valvular endothelial cells. Atherosclerosis, 242(1), 251-260.

Hoffman, J. I., & Kaplan, S. (2002). The incidence of congenital heart disease. Journal of the American College of Cardiology, 39(12), 1890-1900.

Holvoet, P., Theilmeier, G., Shivalkar, B., Flameng, W., & Collen, D. s. (1998). LDL hypercholesterolemia is associated with accumulation of oxidized LDL, atherosclerotic plaque growth, and compensatory vessel enlargement in coronary arteries of miniature pigs. Arteriosclerosis, thrombosis, and vascular biology, 18(3), 415-422.

Honda, S., Miyamoto, T., Watanabe, T., Narumi, T., Kadowaki, S., Honda, Y., . . . Funayama, A. (2014). A novel mouse model of aortic valve stenosis induced by direct wire injury. Arteriosclerosis, thrombosis, and vascular biology, 34(2), 270-278.

Hulin, A., Hego, A., Lancellotti, P., & Oury, C. (2018). Advances in pathophysiology of calcific aortic valve disease propose novel molecular therapeutic targets. Frontiers in cardiovascular medicine, 5, 21.

Ignatowski, A. (1908). Influence of animal food on the organsim of rabbits. Izvest Imper Voennomed Akad St Petersburg, 16, 154-173.

Ishii, A., Vinuela, F., Murayama, Y., Yuki, I., Nien, Y., Yeh, D., & Vinters, H. (2006). Swine model of carotid artery atherosclerosis: experimental induction by surgical partial ligation and dietary hypercholesterolemia. American Journal of Neuroradiology, 27(9), 1893-1899.

Jialal, I., Stein, D., Balis, D., Grundy, S. M., Adams-Huet, B., & Devaraj, S. (2001). Effect of hydroxymethyl glutaryl coenzyme a reductase inhibitor therapy on high sensitive C-reactive protein levels. Circulation, 103(15), 1933-1935.

Kamath, A. R., & Pai, R. G. (2008). Risk factors for progression of calcific aortic stenosis and potential therapeutic targets. The International journal of angiology: official publication of the International College of Angiology, Inc, 17(2), 63.

Laforest, B., Andelfinger, G., & Nemer, M. (2011). Loss of Gata5 in mice leads to bicuspid aortic valve. The Journal of clinical investigation, 121(7), 2876-2887.

Le Quang, K., Bouchareb, R., Lachance, D., Laplante, M.-A., Husseini, D. E., Boulanger, M.-C., . . . Pibarot, P. (2014). Early development of calcific aortic valve disease and left ventricular hypertrophy in a mouse model of combined dyslipidemia and type 2 diabetes mellitus. Arteriosclerosis, thrombosis, and vascular biology, 34(10), 2283-2291.

Lee, Y. T., Laxton, V., Lin, H. Y., Chan, Y. W. F., Fitzgerald-Smith, S., To, T. L. O., . . . Tse, G. (2017). Animal models of atherosclerosis. Biomedical reports, 6(3), 259-266.

Leong, X.-F., Ng, C.-Y., & Jaarin, K. (2015). Animal models in cardiovascular research: hypertension and atherosclerosis. BioMed research international, 2015.

Lerman, D. A., Prasad, S., & Alotti, N. (2015). Calcific aortic valve disease: molecular mechanisms and therapeutic approaches. European Cardiology Review, 10(2), 108.

Lilly, L. S., & Braunwald, E. (2012). Braunwald's heart disease: a textbook of cardiovascular medicine (Vol. 2): Elsevier Health Sciences.

Lindman, B., Clavel, M., & Mathieu, P. (2016). lung, B., Lancellotti, P., Otto, CM, Pibarot. Calcific aortic stenosis. Nat Rev Dis Primers, 3(2), 16006.

Liu, A. (2007). Joag VR, Gotlieb AI. The emerging role of valve interstitial cell phenotypes in regulating heart valve pathobiology. Am J Pathol, 171, 1407-1418.

Malergue, M., Urena, P., Prieur, P., Guedon-Rapoud, C., & Petrover, M. (1997). Incidence and development of aortic stenosis in chronic hemodialysis. An ultrasonographic and biological study of 112 patients. Archives des Maladies du Coeur et des Vaisseaux, 90(12), 1595-1601.

McBride, K. L., Zender, G. A., Fitzgerald–Butt, S. M., Seagraves, N. J., Fernbach, S. D., Zapata, G., . . . Belmont, J. W. (2011). Association of common variants in ERBB4 with congenital left ventricular outflow tract obstruction defects. Birth Defects Research Part A: Clinical and Molecular Teratology, 91(3), 162-168.

Miller, J. D., Weiss, R. M., & Heistad, D. D. (2011). Calcific aortic valve stenosis: methods, models, and mechanisms. Circulation research, 108(11), 1392-1412.

Miller, J. D., Weiss, R. M., Serrano, K. M., & Brooks, R. M. (2009). Lowering plasma cholesterol levels halts progression of aortic valve disease in mice. Circulation, 119(20), 2693.

Mills, W. R., Einstadter, D., & Finkelhor, R. S. (2004). Relation of calcium-phosphorus product to the severity of aortic stenosis in patients with normal renal function. The American journal of cardiology, 94(9), 1196-1198.

Monetti, M., Canavesi, M., Camera, M., Parente, R., Paoletti, R., Tremoli, E., . . . Bellosta, S. (2007). Rosuvastatin displays anti-atherothrombotic and anti-inflammatory properties in apoE-deficient mice. Pharmacological research, 55(5), 441-449.

Mourino-Alvarez, L., Baldan-Martin, M., Sastre-Oliva, T., Martin-Lorenzo, M., Maroto, A. S., Corbacho-Alonso, N., . . . Alvarez-Llamas, G. (2018). A comprehensive study of calcific aortic stenosis: from rabbit to human samples. Disease models & mechanisms, 11(6).

Ngo, D. T., Stafford, I., Kelly, D. J., Sverdlov, A. L., Wuttke, R. D., Weedon, H., . . . Chirkov, Y. Y. (2008). Vitamin D2 supplementation induces the development of aortic stenosis in rabbits: Interactions with endothelial function and thioredoxin-interacting protein. European journal of pharmacology, 590(1-3), 290-296.

Niepmann, S. T., Steffen, E., Zietzer, A., Adam, M., Nordsiek, J., Gyamfi-Poku, I., . . . Kelm, M. (2019). Graded murine wire-induced aortic valve stenosis model mimics human functional and morphological disease phenotype. Clinical Research in Cardiology, 108(8), 847-856.

Nigam, V., & Srivastava, D. (2009). Notch1 represses osteogenic pathways in aortic valve cells. Journal of molecular and cellular cardiology, 47(6), 828-834.

Nus, M., MacGrogan, D., Martínez-Poveda, B., Benito, Y., Casanova, J. C., Fernández-Avilés, F., . . . de la Pompa, J. L. (2011). Diet-induced aortic valve disease in mice haploinsufficient for the Notch pathway effector RBPJK/CSL. Arteriosclerosis, thrombosis, and vascular biology, 31(7), 1580-1588.

O'Brien, K. D. (2006). Pathogenesis of calcific aortic valve disease: a disease process comes of age (and a good deal more). Arteriosclerosis, thrombosis, and vascular biology, 26(8), 1721-1728.

O'Brien, K. D., Reichenbach, D. D., Marcovina, S. M., Kuusisto, J., Alpers, C. E., & Otto, C. M. (1996). Apolipoproteins B,(a), and E accumulate in the morphologically early lesion of 'degenerative'valvular aortic stenosis. Arteriosclerosis, thrombosis, and vascular biology, 16(4), 523-532.

Ohukainen, P., Ruskoaho, H., & Rysa, J. (2018). Cellular mechanisms of valvular thickening in early and intermediate calcific aortic valve disease. Current Cardiology Reviews, 14(4), 264-271.

Panepinto, L., & Phillips, R. (1986). The Yucatan miniature pig: characterization and utilization in biomedical research. Laboratory animal science, 36(4), 344-347.

Parisi, V., Leosco, D., Ferro, G., Bevilacqua, A., Pagano, G., de Lucia, C., . . . Ferrara, N. (2015). The lipid theory in the pathogenesis of calcific aortic stenosis. Nutrition, Metabolism and Cardiovascular Diseases, 25(6), 519-525.

Pawade, T. A., Newby, D. E., & Dweck, M. R. (2015). Calcification in aortic stenosis: the skeleton key. Journal of the American College of Cardiology, 66(5), 561-577.

Porras, A. M., Shanmuganayagam, D., Meudt, J. J., Krueger, C. G., Hacker, T. A., Rahko, P. S., . . . Masters, K. S. (2015). Development of aortic valve disease in familial hypercholesterolemic swine: implications for elucidating disease etiology. Journal of the American Heart Association, 4(10), e002254.

Rajamannan, N. M., Evans, F. J., Aikawa, E., Grande-Allen, K. J., Demer, L. L., Heistad, D. D., . . . O'Brien, K. D. (2011). Calcific aortic valve disease: Not simply a degenerative process a review and agenda for research from the National Heart and Lung and Blood Institute Aortic Stenosis Working Group. Circulation, 124(16), 1783.

Rajamannan, N. M., Subramaniam, M., Caira, F., Stock, S. R., & Spelsberg, T. C. (2005). Atorvastatin inhibits hypercholesterolemia-induced calcification in the aortic valves via the Lrp5 receptor pathway. Circulation, 112(9_supplement), I-229-I-234.

Rajamannan, N. M., Subramaniam, M., Springett, M., Sebo, T. C., Niekrasz, M., McConnell, J. P., . . . Spelsberg, T. C. (2002). Atorvastatin inhibits hypercholesterolemia-induced cellular proliferation and bone matrix production in the rabbit aortic valve. Circulation, 105(22), 2660-2665.

Rajamannan, N. M., Subramaniam, M., Stock, S., Stone, N., Springett, M., Ignatiev, K., . . . Spelsberg, T. (2005). Atorvastatin inhibits calcification and enhances nitric oxide synthase production in the hypercholesterolaemic aortic valve. Heart, 91(6), 806-810.

Rattazzi, M., Faggin, E., Bertacco, E., Nardin, C., Pagliani, L., Plebani, M., . . . Pauletto, P. (2018). Warfarin, but not rivaroxaban, promotes the calcification of the aortic valve in ApoE−/− mice. Cardiovascular therapeutics, 36(4), e12438.

Reitman, J., Mahley, R., & Fry, D. (1982). Yucatan miniature swine as a model for diet-induced atherosclerosis. Atherosclerosis, 43(1), 119-132.

Roosens, B., Bala, G., Droogmans, S., Van Camp, G., Breyne, J., & Cosyns, B. (2013). Animal models of organic heart valve disease. International journal of cardiology, 165(3), 398-409.

Rutkovskiy, A., Malashicheva, A., Sullivan, G., Bogdanova, M., Kostareva, A., Stensløkken, K. O., . . . Vaage, J. (2017). Valve interstitial cells: the key to understanding the pathophysiology of heart valve calcification. Journal of the American Heart Association, 6(9), e006339.

Scatena, M., Jackson, M. F., Speer, M. Y., Leaf, E. M., Wallingford, M. C., & Giachelli, C. M. (2018). Increased Calcific Aortic Valve Disease in response to a diabetogenic, procalcific diet in the LDLr-/-ApoB100/100 mouse model. Cardiovascular Pathology, 34, 28-37.

Shiomi, M., & Ito, T. (2009). The Watanabe heritable hyperlipidemic (WHHL) rabbit, its characteristics and history of development: a tribute to the late Dr. Yoshio Watanabe. Atherosclerosis, 207(1), 1-7.

Shiomi, M., Ito, T., Yamada, S., Kawashima, S., & Fan, J. (2003). Development of an animal model for spontaneous myocardial infarction (WHHLMI rabbit). Arteriosclerosis, thrombosis, and vascular biology, 23(7), 1239-1244.

Sider, K. L., Blaser, M. C., & Simmons, C. A. (2011). Animal models of calcific aortic valve disease. International journal of inflammation, 2011.

Sider, K. L., Zhu, C., Kwong, A. V., Mirzaei, Z., de Langé, C. F., & Simmons, C. A. (2014). Evaluation of a porcine model of early aortic valve sclerosis. Cardiovascular Pathology, 23(5), 289-297.

Simmons, C. A., Grant, G. R., Manduchi, E., & Davies, P. F. (2005). Spatial heterogeneity of endothelial phenotypes correlates with side-specific vulnerability to calcification in normal porcine aortic valves. Circulation research, 96(7), 792-799.

Skold, B., Getty, R., & Ramsey, F. (1966). Spontaneous atherosclerosis in the arterial system of aging swine. American journal of veterinary research, 27(116), 257-273.

Srivastava, S., Sithu, S. D., Vladykovskaya, E., Haberzettl, P., Hoetker, D. J., Siddiqui, M. A., . . . Bhatnagar, A. (2011). Oral exposure to acrolein exacerbates atherosclerosis in apoE-null mice. Atherosclerosis, 215(2), 301-308.

Swinkels, D. W., & Demacker, P. N. (1988). Comparative studies on the low density lipoprotein subfractions from pig and man. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 90(2), 297-300.

Tanaka, K., Sata, M., Fukuda, D., Suematsu, Y., Motomura, N., Takamoto, S., . . . Nagai, R. (2005). Age-associated aortic stenosis in apolipoprotein E-deficient mice. Journal of the American College of Cardiology, 46(1), 134-141.

Thim, T., Hagensen, M. K., Drouet, L., Bonneau, M., Granada, J., Nielsen, L., . . . Falk, E. (2010). Familial hypercholesterolaemic downsized pig with human-like coronary atherosclerosis: a model for preclinical studies. EuroIntervention: journal of EuroPCR in collaboration with the Working Group on Interventional Cardiology of the European Society of Cardiology, 6(2), 261-268.

Tkatchenko, T. V., Moreno-Rodriguez, R. A., Conway, S. J., Molkentin, J. D., Markwald, R. R., & Tkatchenko, A. V. (2009). Lack of periostin leads to suppression of Notch1 signaling and calcific aortic valve disease. Physiological genomics, 39(3), 160-168.

Tsang, H., Rashdan, N., Whitelaw, C., Corcoran, B., Summers, K., & MacRae, V. (2016). Large animal models of cardiovascular disease. Cell biochemistry and function, 34(3), 113-132.

Walters, E. M., Wolf, E., Whyte, J. J., Mao, J., Renner, S., Nagashima, H., . . . Critser, J. K. (2012). Completion of the swine genome will simplify the production of swine as a large animal biomedical model. BMC medical genomics, 5(1), 1-11.

Wang, Y., Wu, B., Farrar, E., Lui, W., Lu, P., Zhang, D., . . . Yang, D. (2017). Notch-Tnf signalling is required for development and homeostasis of arterial valves. European heart journal, 38(9), 675-686.

Watanabe, Y. (1980). Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-rabbit): incidence and development of atherosclerosis and xanthoma. Atherosclerosis, 36(2), 261-268.

Weisell, J. (2020). Studies on calcific aortic valve disease: from experimental models to human disease. Itä-Suomen yliopisto,

Weiss, R. M., Chu, Y., Brooks, R. M., Lund, D. D., Cheng, J., Zimmerman, K. A., . . . Shao, J. Q. (2018). Discovery of an experimental model of unicuspid aortic valve. Journal of the American Heart Association, 7(13), e006908.

Weiss, R. M., Ohashi, M., Miller, J. D., Young, S. G., & Heistad, D. D. (2006). Calcific aortic valve stenosis in old hypercholesterolemic mice. Circulation, 114(19), 2065-2069.

Wirrig, E. E., & Yutzey, K. E. (2011). Transcriptional regulation of heart valve development and disease. Cardiovascular Pathology, 20(3), 162-167.

Xu, S., Liu, A. C., & Gotlieb, A. I. (2010). Common pathogenic features of atherosclerosis and calcific aortic stenosis: role of transforming growth factor-β. Cardiovascular Pathology, 19(4), 236-247.

Yi, B., Zeng, W., Lv, L., & Hua, P. (2021). Changing epidemiology of calcific aortic valve disease: 30-year trends of incidence, prevalence, and deaths across 204 countries and territories. Aging (Albany NY), 13(9), 12710-12732. doi:10.18632/aging.202942

Yoshioka, M., Yuasa, S., Matsumura, K., Kimura, K., Shiomi, T., Kimura, N., . . . Shin, H. (2006). Chondromodulin-I maintains cardiac valvular function by preventing angiogenesis. Nature medicine, 12(10), 1151-1159.

Zeadin, M., Butcher, M., Werstuck, G., Khan, M., Yee, C. K., & Shaughnessy, S. G. (2009). Effect of leptin on vascular calcification in apolipoprotein E–deficient mice. Arteriosclerosis, thrombosis, and vascular biology, 29(12), 2069-2075.

NU IJB

NANGARHAR UNIVERSITY

INTERNATIONAL JOURNAL OF BIOSCIENCES