International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; 8(5): 162-166

www.veterinarypaper.com
Received: 20-07-2023
Accepted: 23-08-2023

Sravathi Vemula
Ph.D Scholar, Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

RaviKumar Yadala
Assistant Professor and Head, Department of Veterinary Pathology, College of Veterinary Science, Rajendragar, Hyderabad, Telangana, India

AnilKumar Banothu
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Nagarjuna Gandham
MVSc Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Mounika Kamishetti
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Durga Veera Hanuman Donga
Ph.D Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Bhaskar Debbarma
Ph.D Scholar, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
RaviKumar Yadala
Assistant Professor and Head, Department of Veterinary Pathology, College of Veterinary Science, Rajendragar, Hyderabad, Telangana, India

Role of Naringenin on 5-Fluorouracil induced testicular toxicity in *Wistar* Rats

Sravathi Vemula, RaviKumar Yadala, AnilKumar Banothu, Nagarjuna Gandham, Mounika Kamishetti, Durga Veera Hanuman Donga and Bhaskar Debbarma

Abstract
The aim of current study was to assess potential antioxidant status Wistar of naringenin (NG) in mitigating testicular toxicity induced by 5-Fluorouracil (5-FU) in rats. The experimental rats were divided into four groups (n=6 each): Group I-(normal saline), Group II-(5-FU), Group III-(NG) at a dosage of 100 mg/kg body weight-orally and Group IV received both 5-FU+NG. On 28th day, we observed significant reduced in testicular weights in group-II rats. In addition, results exhibited a significant (p<0.01) reduction in antioxidant enzyme activities (SOD, CAT and GSH) accompanied by an increase in lipid peroxidation (LPO). However, Group IV rats, received both 5-FU and NG displayed a substantial (p<0.01) increase in the activities of CAT, GSH and SOD of testis along with a decrease in LPO levels. The administration of NG effectively restored the compromised antioxidant status. This study underscores ameliorative effect of NG against 5-FU-induced testicular toxicity.

Keywords: Naringinin, oxidative stress and catalase

Introduction
5-FU, an FDA-approved an antimetabolite used mostly colorectal and various solid tumors treatment, has been associated with testicular toxicity. It is the second most commonly used drug, but it associated with various organ toxicities [1]. This toxicity includes issues such germinal epithelial sloughing, cell death, the formation of multinucleated cells, atrophy of seminiferous tubules with halted spermatid development [2]. 5-FU, mostly associated with the inhibition of thymidylate synthetase (TS) which further responsible for formation of breaks in DNA and RNA strands which further stimulation of lipid peroxidation (LPO) [3]. In addition, oxidative stress with controlled levels are essential for processes like capacitation, hyperactivation and the acrosome reaction in mammalian sperm, which are critical for fertilization [2]. Imbalances in antioxidant levels are linked to idiopathic infertility, while an increase in pro-oxidants can lead to stress on spermatozoa [4]. Chemotherapeutic agents can induce oxidative stress in testis and potentially impact male fertility [5].
NG is a naturally available phytochemical, found in fruits like oranges, grapes and are generally considered safe as direct human food ingredients [6]. Studies have suggested that NG may have protective effect against experimentally induced testicular toxicity [7]. Additionally, NG have demonstrated the ability to improve antioxidant levels, sperm count and testosterone levels in cases of chemotherapy-induced testicular toxicity [8]. Therefore, the current study were conducted to investigate the potential effect of NG in mitigating testicular toxicity induced by 5-FU in rat models.

Materials and Methods
Experimental design
NG (CAS No. 10236-47) were bought from Sigma (SAC-St Louis, MO, USA) and Celon laboratories (Hyderabad) provided 5-FU. All the chemicals of biochemical analysis were of analytical grade and procured from HiMEDIA Lab. Private Limited and SRL Private Limited. Rat feed in pellet form was bought from Jeeva life sciences in Hyderabad.

Adult male *Wistar* rats (n=28, 300 gms, assigned four groups), were procured from Jeeva Life Sciences, Hyderabad, ISO 9001:2015 certification. The experimental protocol received approval from the Institutional Animal Ethics Committee (IAEC) under form number (3/25/C.V.Sc, Hyd. IAEC).

Table 1: Experimental design

Group	Treatment	Number
Group 1	Sham	6
Group 2	Toxic control-5-FU @ 20 mg/kg b. wt for initial 5 consecutive days-IP	6
Group 3	Ameliorative Naringenin @ 100 mg/ kg b. wt-orally	6
Group 4	Combination of both 5-FU+NG	6

After 28 days, rats were sacrificed, testes were collected and washed with ice cold normal saline and stored for -20 °C for enzyme activity.

Testicular weights

Experimental rats were sacrificed by cervical dislocation on 28th day of study. Then testis were excised and fat was trimmed and weights of testes from each animal were evaluated by using electronic balance

Analysis of oxidative stress parameters

Homogenate of testes tissue (10% w/v) was prepared in 10 mL of Tris HCl buffer (pH 7.2), centrifuged at 10,000 RPM for 20 min at 4 °C and were collected and used for antioxidant enzyme analysis. Total protein content of testis were measured as protocol given previously [9] with addition of Folin's phenol reagent.

Estimation of Superoxide (SOD)

SOD activity was assessed following Marklund and Marklund's method [10]. The resulting supernatant used for SOD activity determination. For the SOD assay, 0.5 ml of the supernatant was combined with 2 ml of pH 8.2 Tris-HCl buffer, 1.5 ml distilled water and 0.5 ml of 2 mm pyrogallol in pH 7.4 Tris-HCl buffer. The assay mixture included enzyme aliquots and the reaction was monitored by measuring the rate of pyrogallol auto-oxidation inhibition.

Estimation of Catalase (CAT)

Catalase (CAT) activity is determined following protocol given previouly [11], which is based on reduction in absorbance at 240 nm due to breakdown of H_2O_2. In brief, 0.2 ml of the tissue homogenate supernatant was mixed with 1 ml of 30 mM H_2O_2, and the reduction in absorbance at 240 nm was monitored over a 3-minute period.

Estimation of glutathione (GSH)

GSH (Glutathione) activity was determined according to the method developed [12]. This method relies on the reaction with 5, 5'-dithio-bis (2-nitrobenzoate) (DTNB or Ellman's reagent), resulting in the formation of a yellow-colored compound with absorbance measured at 412 nm.

Estimation of lipid peroxidation (LPO)

Lipid peroxidation (LPO) in the tissue homogenate was assessed using the method of Yagi [13] involving thiobarbituric acid (TBA). Malondialdehyde (MDA), the end product of lipid peroxidation, indicates oxidative stress intensity. MDA reacts with TBA, forming a pink-colored product.

Results

Effect of NG on Absolute Testicular weights

Mean values of absolute testicular weights (mg) in 5-FU treated group rats were significantly (p<0.05) reduced when compared to the control group rats, whereas NG and 5-FU combination group results in significant (p<0.05) improvement in the testis weight when compared to toxic group. However, the absolute testis weights of NG ameliorative group were nearly similar to that of sham (Fig. 1).

Effect of NG on lipid peroxidation and restoration of anti-oxidants

5-FU leads to significant (p<0.05) increase in the concentration TBARs (nmoles/mg protein) compared to normal sham group. Whereas combination of both agents resulted in a significant (p<0.05) reduction of the values as compared to toxic group 2 rats. There was no significant variation observed among the values of control and NG given group. The anti-oxidant capacity of tissues were measured in terms of SOD, GSH and Catalase. Furthermore investion, we observed the activity of SOD, GSH and Catalase (U/mg protein) in testes and noted that a significant (p<0.05) lower concentration in toxic 5-FU treated group. Interestingly, significant higher mean values of these enzymes were observed in NG and 5-FU treated group indicating that NG has anti-oxidant capacity. Whereas, the values of control sham and NG group-3, were similar to each other indicating safety of this compound. The mean values were depicted in fig. 2.

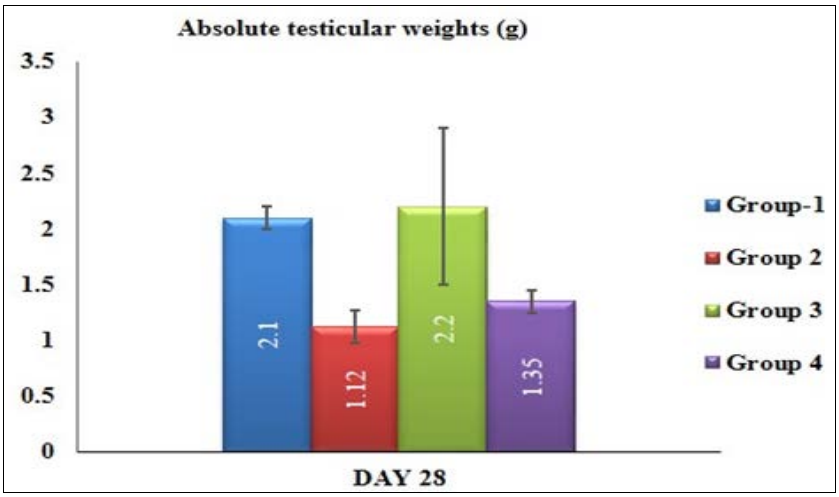

Fig 1: effect of NG on testicular weights (g)

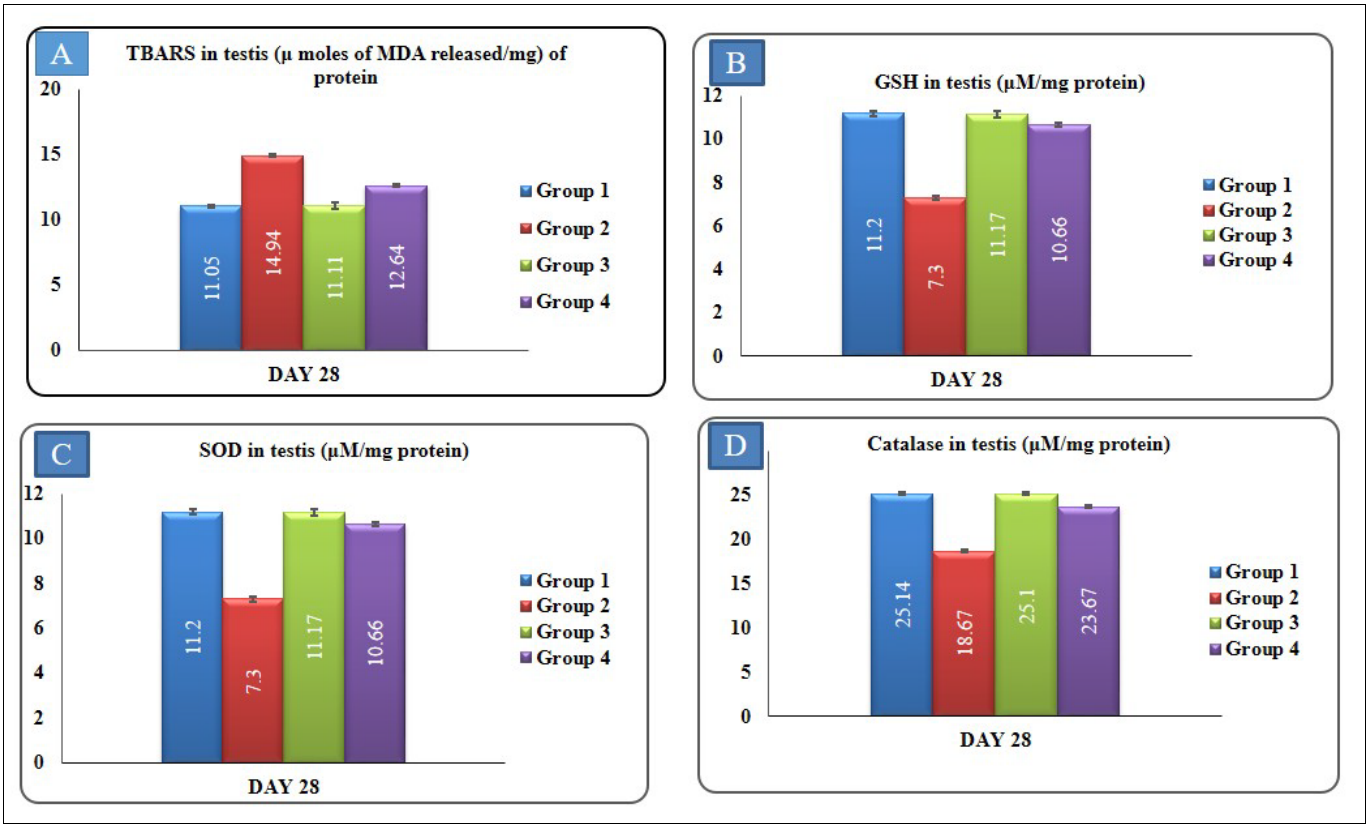

Fig 2: Effect of NG on oxidative stress parameters

Discussion

ROS significantly contribute to infertility by reducing sperm motility, viability and causing sperm abnormalities. The current study revealed significant decrease in the testicular weights in 5-FU treated rats and this effect is then probably mediated by the hormonal imbalance in serum cause tubular atrophy which leads to significant fall in sperm density and causing reduced weights in testes [14]. Other potential factor contributing to this phenomenon could be the reduction in ATP production [15]. Typically, 5-FU is converted into flouroacetate, which inhibits the Krebs cycle, ultimately leading to decreased energy production and a subsequent decrease in active spermatogenesis [16]. These findings align with previous studies. Interestingly, exposure of NG to 5-FU-treated showed maintained testicular weights which are co-incide with previous literature. Whereas treatment with NG alone showed a non-noticeable response and confirmed that it has the protective role and no observable toxic side effects in control group -3 rats [17].

Oxidative stress, a cellular phenomenon arising from an imbalance in levels of antioxidants and oxidants, particularly ROS and free radicals. The body employs a range of protective mechanisms involving antioxidants such as glutathione, vitamin E, ascorbic acid, alpha-tocopherol, as well as antioxidant enzymes like SOD, CAT and GSH to mitigate the damage caused by radicals during oxidative stress [18]. Spermatozoa's plasma membranes are rich in polyunsaturated fatty acids (PUFA) but have low concentrations of the scavenging enzymes in their cytoplasm, rendering them highly vulnerable to the oxidative stress-induced harm [19]. Oxidative stress also compromise integrity of the sperm nucleus DNA, hastening germ cell apoptosis and resulting in reduced sperm count [20-21]. Furthermore, it has the potential to significantly affect steroidogenic function of the Leydig cells, contributing to male infertility with a noticeable decline in semen quality [22]. GSH stands out as a potent antioxidant that plays a pivotal role in bolstering the defense against ROS and free radicals during exogenous toxic insults [20]. Abundantly present within cells, GSH exhibits the capability to engage with a variety of free radicals, thus contributing to its protective function. SOD is an enzymatic catalyst responsible for the dismutation of superoxide radicals, converting them into either H_2O_2 or molecular O_2. This enzymatic activity serves to mitigate the potentially devastating effects of superoxide anions, thereby minimizing their harm [23]. Furthermore, SOD plays a protective role by averting lipid peroxidation and effectively safeguarding against tissue necrosis, thereby providing critical defense mechanisms against oxidative damage. MDA is best indicator of the oxidative stress as it is end product of multichain unsaturated fatty acids and it is endogenous lipid peroxidation. In our study there is significant increase in TBARS level in group-2 toxic rats. In addition to that, 5-FU also significant decrease in antioxidants enzymes (SOD, GSH and CAT). It might be due to action of 5-FU PUFAs which makes more susceptible for oxidative stress along with reminiscents of 5-FU in seminiferous tubules which make more susceptible for oxidative stress which are accordance with previous literature [24, 25]. NG has direct antioxidant function involving the scavenging of free radicals, by stimulating endogenous antioxidant system and upregulating the expression of glutamyl cystinyl ligase, thereby increasing GSH levels. Its antioxidant effects are attributed to its hydroxyl (OH-) substituents, which exhibit strong reactivity against both ROS and reactive nitrogen species (RNS) by reducing NADPH activity. The hydroxyl radical (OH-) can transfer its hydrogen to free radicals, enhancing antioxidant capacity [26]. Additionally, NG elevates levels of vitamin C and vitamin E [27] and possesses lipophilic properties that allow it to bind to cell membranes, reducing the formation of free radicals and protecting these membranes [28]. Moreover, NG suppresses the increase in MDA, offering protection

against lipid peroxidation which are same findings observed in previous literature on testicular toxicity [29]. Based on results, we conclude that NG has anti-inflammatory and anti-oxidant properties of molecular mechanism in testicular toxicities (Fig.3)

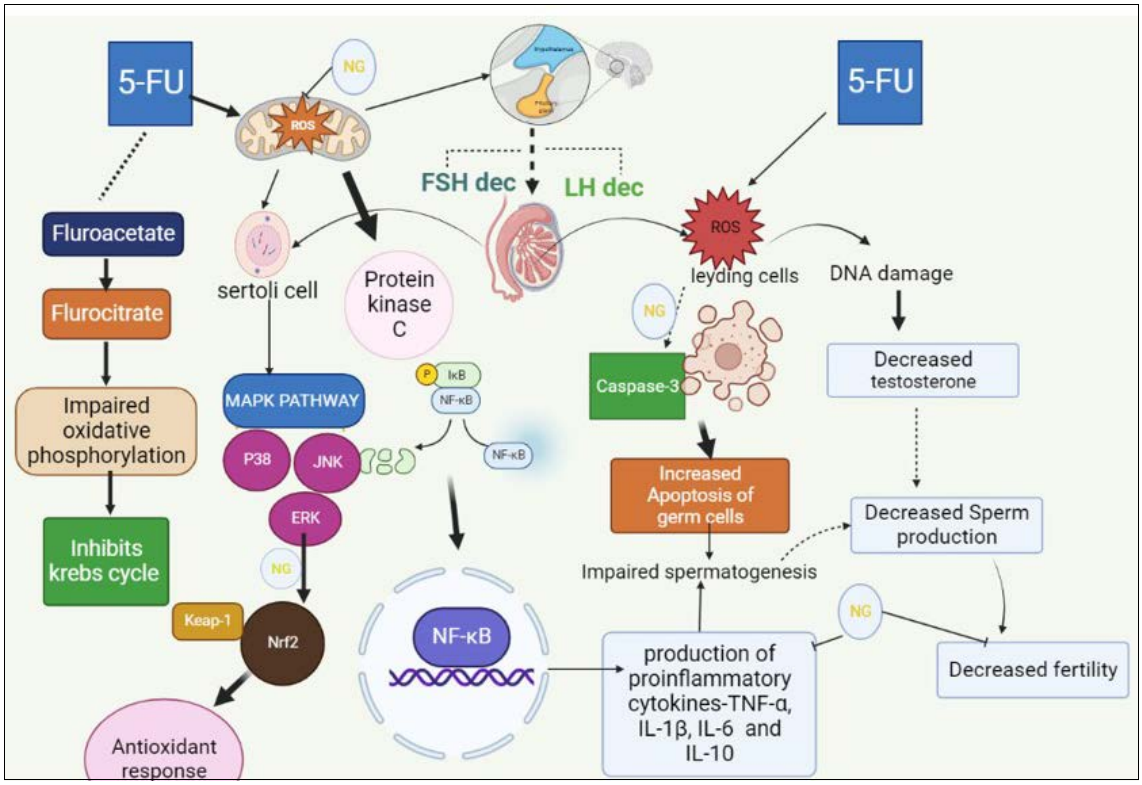

Fig 3: Molecular Mechanism of NG on testicular toxicity

Conclusion

In conclusion, the combination of NG and 5-FU treatment shows promise in mitigating complications, particularly inflammation, by harnessing their anti-oxidant action with gonadropic properties. However, further extensive research is imperative to elucidate the full extent of NG's benefits in reducing pathogencities of molecular mechanism and its associated pathogenicity across varying time intervals.

References

1. Badawoud MH, Elshal EB, Zaki AI, Amin HA. The possible protective effect of L-arginine against 5-Fluorouracil induced nephrotoxicity in male albino rats. *Folia Morphologica.* 2017;76(4):608-619.
2. Noordhuis P, Holwerda U, Van Der Wilt CL, Van Groeningen CJ, Smid K, Meijer S, *et al.* 5-Fluorouracil incorporation into RNA and DNA in relation to thymidylate synthase inhibition of human colorectal cancers. Annals of oncology. 2004;15(7):1025-32.
3. Inomata A, Matsumoto H, Horii I. Collaborative Work To Evaluate Toxicity On Male Reproductive Organs By Repeated Dose Studies In Rats: 18) Comparative 4 And 2 Weeks Oral Repeated Dosing Studies On Male Reproductive Organs In Rats Treated With 5-Fluorouracil. The Journal of Toxicological Sciences. 2000;25(Special Issue):179-186.
4. Taylor CT. Antioxidants and reactive oxygen species in human fertility. Environmental toxicology and pharmacology. 2001;10(4):189-98.
5. Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. Urology. 1996;48(6):835-50.
6. Moghaddam RH, Samimi Z, Moradi SZ, Little PJ, Xu S, Farzaei MH. Naringenin and naringin in cardiovascular disease prevention: A preclinical review. European Journal of Pharmacology. 2020;887(8):173-185.
7. Rai R, Jat D, Mishra SK. Naringenin ameliorates aluminum toxicity-induced testicular dysfunctions in mice by suppressing oxidative stress and histopathological alterations. Systems Biology in Reproductive Medicine. 2023;19:1-7.
8. Salehi B, Fokou PV, Sharifi-Rad M, Zucca P, Pezzani R, Martins N, *et al.* The therapeutic potential of naringenin: a review of clinical trials. Pharmaceuticals. 2019;12(1):11.
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent [J Biol Chem 0840^ 082, 154-164.
10. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European journal of biochemistry. 1974;47(3):469-74.
11. Sinha AK. Colorimetric assay of catalase. Analytical biochemistry. 1972;47(2):389-94.
12. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179:588-590.
13. Yagi K. Simple fluorimetric assay for lipid peroxides in blood plasma. Biochem. Med. 1976;15:212-216.
14. Takizawa S, Horii I. Endocrinological assessment of toxic effects on the male reproductive system in rats treated with 5-fluorouracil for 2 or 4 weeks. The Journal of toxicological sciences. 2002;27(1):49-56.
15. Muneoka K, Shirai Y, Yokoyama N, Wakai T, Hatakeyama K. 5-Fluorouracil cardiotoxicity induced by

alpha-fluoro-beta-alanine. International Journal of Clinical Oncology. 2005;10:441-3.
16. Miura K, Kinouchi M, Ishida K, Fujibuchi W, Naitoh T, Ogawa H, *et al*. 5-fu metabolism in cancer and orally-administrable 5-fu drugs. Cancers. 2010;2(3):1717
17. El-Sayyad IH, Hassan A. Effects of adriamycin, cisplatin, and 5-fluorouracil on the testes of albino rats. Br J Med Health Sci. 2013;1:45-62.
18. Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in the testes. Molecular mechanisms in spermatogenesis; c2008. p. 154-71.
19. Sikka SC. Oxidative stress and role of antioxidants in normal and abnormal sperm function. Frontiers in bioscience: a journal and virtual library. 1996;1:e78-86.
20. Waxman S, Scher BM, Hellinger N, Scher W. Combination cytotoxic-differentiation therapy of mouse erythroleukemia cells with 5-fluorouracil and hex methylene bisacetamide. Cancer research. 1990;50(13):3878-87.
21. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertility and sterility. 2003;79(4):829-43.
22. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford university press, USA; c2015.
23. Adedara IA, Farombi EO. Chemoprotection of ethylene glycol monoethyl ether-induced reproductive toxicity in male rats by kolaviron, isolated biflavonoid from Garcinia kola seed. Human & experimental toxicology. 2012;31(5):506-17.
24. Pavithra S, Preetha SP, Thangapandiyan M, Karthikeyan A. Role of fractionated pectin powder capped zinc oxide nanoparticle in 5-fluorouracil induced testicular oxidative stress in rats.
25. Yadav YC. Effect of cisplatin on pancreas and testes in Wistar rats: biochemical parameters and histology. Heliyon; c2019, 5(8).
26. Reddy TK, Nagaraju I, Kumar KH, Lokanatha V, Reddy CD, Jagetia GC. Cardioprotective effect of naringin in mice treated with doxorubicin. Planta Medica. 2008;74(03):49.
27. Rajadurai M, Prince PSM. Preventive effect of naringin on lipid peroxides and antioxidants in isoproterenol-induced cardiotoxicity in *Wistar* rats: Biochemical and Histopathological Evidences. Toxicology. 2006;228(3):259-268.
28. Fouad AA, Refaie MM, Abdelghany MI. Naringenin palliates cisplatin and doxorubicin gonadal toxicity in male rats. Toxicology mechanisms and methods. 2019;29(1):67-73.
29. Renugadevi J, Prabu SM. Naringenin protects against cadmium-induced oxidative renal dysfunction in rats. Toxicologist. 2009;256(2):128-134.