Trop J Nat Prod Res, December 2023; 7(12):5710-5714

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Efficacy of Prepubertal Administration of *Zingiber officinale* (Ginger) on Reproductive Hormones in Male Sprague-Dawley Rats

Babajide S. Johnson [1], Bakare A. Adeola[2]*, Olayinka T. Abudulkareem[3]

[1]*University hospitals of Bristol and Weston NHS foundation trust, Bristol England.*
[2]*Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria.*
[3]*Department of Anatomy, Faculty of Basic Medical Sciences, Kampala International University, Ishaka, Uganda.*

ARTICLE INFO

Article history:
Received 31 October 2023
Revised 10 November 2023
Accepted 30 November 2023
Published online 01 January 2024

ABSTRACT

There has been continuous interest in the use of ginger due to its antioxidant indices in the treatment of diseases. Considering the increase in the consumption of ginger also by the younger generation and while its antioxidant effect is considered a benefit, it is important to investigate its impact on other system-induced functions in the body. Therefore, this study was designed to determine the possible effects of prepubertal exposure of ginger on reproductive hormones. A total of 24 male prepubertal models were divided into three experimental groups of 8 pups each. Group A received distilled water while group B and C received low dose (250 mg/kg body weight) and high dose (500 mg/kg body weight) of ginger suspension daily for 14 days respectively. The antioxidant status was evaluated using serum biochemical assays while reproductive hormone profiles were determined by chemiluminescence immunoassay techniques. Data were analysed using Graph pad prism version 8.0. The estimations of glutathione, superoxide dismutase and catalase levels were significantly higher in the low and high dose groups while malondialdehyde estimations in both low and high dose groups showed a statistical significance lower level when compared with the control. There were significant higher levels of follicle stimulation hormone, luteinizing hormone, estrogen and testosterone in all high dose groups (β**P<0.01; (β***P<0.001) when compared with the control. It is evident that ginger has positive impact on reproductive hormones following prepubertal exposure. Therefore, the consumption of ginger is considered a safe agent against fertility function.

***Keywords*:** Ginger, Antioxidant, Reproductive hormones, Fertility, Sprague-Dawley rats

Introduction

Zingiber officinale is the generic name of ginger from the family *Zingiberaceae*. It commonly used in folk medicine for the treatment of diseases in both adult and children and this is based on its antioxidant property.[1] Gingerol and shogaol are major chemical agents present in ginger and have been shown to inhibit arachidonic acid release through the suppression of prostaglandin synthetase. Arachidonic acid becomes oxygenated to form free radical mediators. Therefore, ginger exert its effect by suppressing the production of arachidonic acid by the body cells which will subsequently reduce the activities of free radicals and inflammatory agents and finally inhibit the progression of diseases [2,3]

Children are predominantly susceptible to the health consequences of biochemical exposures, and this is one of the factors that contribute to the increasing rates of diseases in younger population.[4] Even though there is deep belief regarding the effectiveness of plant medications from ancient practice, scientific evidences are important to substantiate the possible side-effects that may come with them especially on other organs in the body. Further investigations specifically on evaluating the safety of these natural plant products on other functions exerted by the body should be of urgent demand. [5]

*Corresponding author. E mail: aabakare@unilag.edu.ng
Tel: 2348033691277

Citation: Johnson BS, Adeola BA, Abudulkareem OT. Efficacy of Prepubertal Administration of *Zingiber officinale* (Ginger) on Reproductive Hormones in Male Sprague-Dawley Rats. Trop J Nat Prod Res. 2023; 7(12):5710-5714. http://www.doi.org/10.26538/tjnpr/v7i12.50

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Considering the increase in the consumption of ginger also by the younger generation and while its antioxidant effect is considered as an advantage, it is imperative to check its impact on other system-induced functions in the body such as reproduction when adulthood is attained. Reproduction is a vital essence of life that ensures procreation, continuity and variation of species. Prepuberty is the period in a child's life before they start to develop adult sexual features. This period a sensitive window for male reproductive clock when spermatogenesis has not been staged but susceptible to agents that can disrupt endocrine regulations.[6] Male reproduction is under the control of synchronized hormone events through the hypothalamic-pituitary-gonadal axis. The hypothalamus produces gonadotropin releasing hormone that stimulates the anterior lobe of the pituitary gland to produce follicle stimulating hormone (FSH) and luteinizing hormone (LH).[7] FSH regulates the maturation of germ cells while LH excites the Leydig cells to secrete testosterone which is needed for the regulation of male sexual activities.[8] Estrogen secreted by the testes is responsible for controlling sexual desire and spermatogenesis.[9] All hormones must be kept under regulated levels and precise enough to exert events that are basis for establishing fertility. Even though there have been associated beneficial effects of *Zingiber officinale* on reproductive profiles in male rats, which were established with increased sperm parameters, testosterone, FSH and LH levels. [10-13] It is important to investigate its effect on reproductive indices in adulthood following a prepubertal exposure as a reproductive preparatory stage that is vulnerable. Hence, this study was designed to evaluate the possible effects of prepubertal exposure to ginger suspension on reproductive hormones in adulthood.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Collection of plant material and preparation of extract
Fresh ginger was sourced from Botany Department of University of Lagos in September, 2021 and was identified by Dr Nodza George with voucher number LUH8936. The extraction procedure was carried out using maceration method in the Department of Pharmacology, University of Lagos. Ginger was washed with clean water and dried. The dried ginger was grounded into powder, 5 g of the powder was added into 500 ml of ethanol and 500 ml of distilled water. The mixture was continuously stirred and then pass through Whatman filter paper to filter the mixture. The extract collected was concentrated in an oven and the final ginger extract was stored in the refrigerator throughout the course administration. [14]

Animal procurement, care and handling
Twelve female and six male adult Sprague-Dawley (SD) rats of weights ranging from 145 g to 170 g were purchased from the animal house unit of the University of Lagos. The animals were allowed to acclimatize for a duration of two weeks under standard laboratory housing condition in well-ventilated compartments with 12-hours light alternate with 12-hours dark periods. The rats were fed *ad libitum* with pelletized rat chow and clean water throughout the study duration. Ethical approval was sourced from the College of Medicine, University of Lagos (CMUL/ACUREC/11/22/1129) and the animals were handled according internationally accepted principles for laboratory investigations throughout the experiment .[15]

Stagging of estrous cycle, Mating, pregnancy and collection of neonates
The establishment of the phases of estrous cycle was done by daily microscopic evaluation of fresh vaginal smear each morning. The collected smear was viewed under a light microscope and microscopic evaluations of the types and population of cells were used to stage the phases of estrous cycle. [15] Three groups each of female rats on estrus phase when on heat and highly receptive to males were caged together with the opposite sex in the ratio of 3:1 for mating. Mating was confirmed by the presence of vaginal plug the next morning after couplation.[16] The pregnant animals were closely monitored throughout the duration of pregnancy till delivery. The neonates were born by the 21st day and allowed to stay with their mothers for nurturing.[17] The mothers were fed ad libitum with rat chow while the pups received breast milk from their mothers until when they are weaned to rat chow.

Experimental design
A total of 24 male pubertal rats at postnatal age of 28 day after delivery were obtained and given tail tag as identification mark. The pubertal rats were divided into three groups A, B and C of 8 pups each. Group A the control received distilled water only, group B was given 250 mg/kg body weight of ginger suspension and group C was given 500 mg/kg body weight of ginger suspension daily for 14 days through the oral route.[18]

Animal sacrifice and sample collection for biochemical analysis
The animals were left in fasting mood overnight following the last dose of administration. The next morning, the animals were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally). [19] Blood was collected from the apex of the heart into heparinized bottles, after which centrifugation was done at 3,000 rpm and the plasma content was kept frozen until needed for biochemical assay.

Antioxidant status
Total Antioxidant Capacity Assay (TAC) Kit was used to evaluate antioxidant levels. This was done according to the operation manual which uses reduction in ion antioxidant defence mechanism. TAC of Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Malondialdehyde (MDA) levels were measured by the required levels needed to make changes in absorbance rate.[20]

Hormone profile evaluation
Hormone profile of plasma FSH, LH, testosterone and estrogen were determined by the chemiluminescence techniques using kits and in accordance with standard protocols outlined by the kit's producer Autobio diagnostics Co., Ltd. Zhengzhou, China.

Statistical analysis
Data analysis was done using Graph pad prism version 8.0. The results obtained were presented as mean ± SD (Standard Deviation). Analysis of significant difference between the mean of the control group and treatment groups were determined using one-way analysis of variance (ANOVA) while multiple comparisons were done using Turkey's post hoc test with significance level at p-value≤0.05

Results and Discussion

Total antioxidant capacity (TAC)
The GSH levels in the ginger treated prepubertal animals showed significantly higher levels when compared with the control. Group C that received low doses of ginger (250mg/kg) showed a less significant value (β*P<0.05) while group B that received high doses of ginger (500mg/kg) showed a higher significant value (β***P<0.01) when compared with the control (Figure 1). In the same way, the serum analysis from group B that received high doses of ginger showed significant higher level in the estimation of SOD when compared with control group (β*P<0.05) (Figure 2). The estimation of catalase level in group B showed higher significant value (β**P<0.01) while group C showed less significance difference (α*P<0.05) when compared with the control group (Figure 3). There were significance reductions in malondialdehyde estimation of all ginger treated groups when compared with control. However, Group B showed a higher statistically significant reduction (β**P<0.01) when compared with control while Group C that received low dose of ginger showed less significant reduction (β*P<0.05) of MDA when compared with group B. (Figure 4).

Hormonal assay
FSH and LH levels were higher in both B and C groups that received high and low dose of ginger respectively when compared with the control group. However, the serum levels of FSH were significantly higher in group B (β**P<0.01), the high dose group while less significantly higher (β*P<0.05) in group C, the low dose group (Figure 5). The same expression was seen with LH levels with significant higher levels in group B (β**P<0.01) the high dose group and less significant higher (β*P<0.05) in group C (Figure 6). There were high significant differences in the mean values of serum estrogen and testosterone in group B and C when compared with the control group.

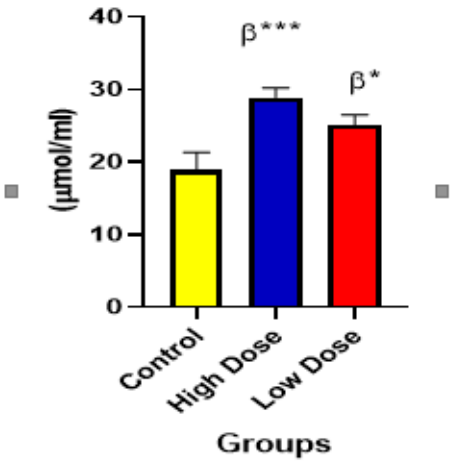

Figure 1: Showing Glutathione levels in prepubertal ginger treated male animals. (β*** =P<0.001/ more significant value; β* =P<0.05/less significant value)

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

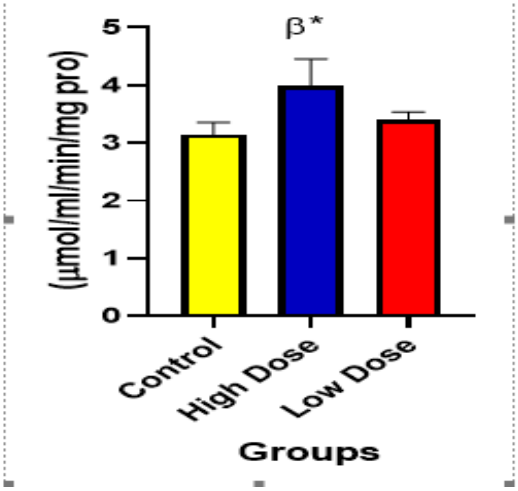

Figure 2: Showing superoxide dismutase levels in in prepubertal ginger treated male animals. (β* =P<0.05/less significant value)

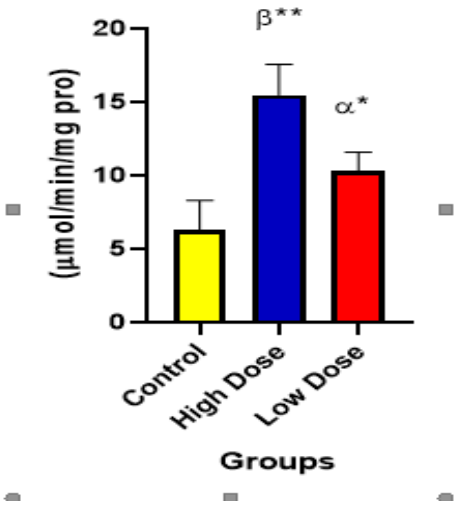

Figure 3: Showing catalase levels in prepubertal ginger treated male animals. (β** =P<0.01/more significant value; α* =P<0.05/less significant value)

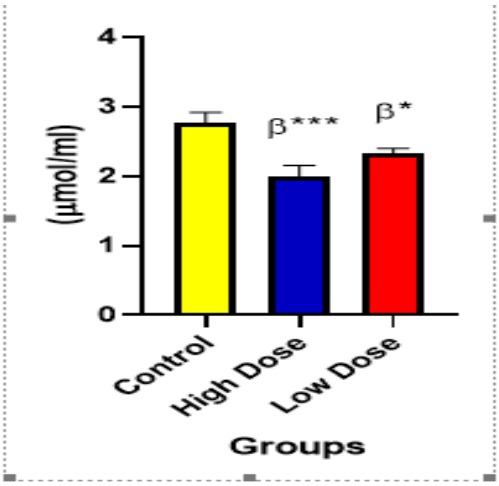

Figure 4: Showing malonaldehyde levels in prepubertal ginger treated male animals. (β*** =P<0.001/high significant value; β* =P<0.05/less significant value)

For the serum estrogen analysis, group B was significantly higher (β***P<0.001) while group C was less significantly higher (α**P<0.01) (Figure 7). Similarly, there was higher significant mean value of serum testosterone in group B when compared with group C (β***P<0.001) (Figure 8).

This current evaluation validates the antioxidant effect of ginger where its application in disease treatment is rooted from. As observed in this study, ginger showed positive effects on antioxidant indicators which include; GSH, SOD, CAH and MDA. It is well established in literature that the antioxidant capacity of ginger through the scavenging impact on free radicals subsequently suppresses the progressive activities in disease conditions such as; cancer, neurogenerative, cardiovascular, liver and kidney diseases. [21-25]

More of interest, ginger administration has been shown to improve sperm parameters such as; sperm count, sperm motility and structural configuration. This seems to be attributed to the strong antioxidant property due to its phenolic content and androgenic effect. The integration of the antioxidant and androgenic properties improves semen value and enhances fertility by hindering free radical actions through the destruction of oxidative chain reactions which in turns reduces oxidative stress damage on the testis.[26] Upon the establishment its antioxidant characterization, gonadotropins (FSH and LH) and sex hormones (testosterone) are the drivers of reproductive functions and thus exerts their actions to birth viable sperm cells.[27] The positive effects of prepubertal exposure of ginger on reproductive hormones in this present study establishes the androgenic property of ginger which also compliments its antioxidant capacity even when taken at a window period that is susceptible to endocrine manipulations. As observed in this study, ginger increases serum levels of FSH, LH, testosterone and estrogen levels in a dose-dependent manner. This result is supported by the finding from a study that showed that ginger extract in a dose and duration dependent manner increased reproductive hormones and sperm parameters.[28-30]

It has been established that the androgenic effect of ginger is targeted primarily to increase LH and FSH under the stimulation of hypothalamic gonadotropin releasing hormone. These actions are propagated through ginger actions of increasing cholesterol level, decreasing oxidative stress damage and reducing lipid peroxidation in the testes. [30] In male, LH enhances the maturation of primordial germ cell to become mature sperm cell and also stimulates the Leydig cells in the testes to produce testosterone. FSH regulates the development of primordial cells into mature germ cells and at the end, these regulated endocrine events will exert reproductive activities used to gauge fertility. [31] The consequential actions are projected as basis for the effect of ginger on improved semen quality.[28-31]

More importantly, the direct effect of the antioxidant property of ginger could also be a factor to consider. There are obvious differences between the plasma membrane of sperm cell and other cells in the body in-terms of lipid composition. The plasma membranes of sperm cells contain higher amount of polyunsaturated fatty acids which subject them to hazards, leading to oxidative stress mutilation. [32-33] Increased oxidative stress lowers LH, FSH, testosterone and estrogen productions. The degeneration of Sertoli cells and rupture of the blood-testicular barrier are also exhibited and these could disrupt the process of spermatogenesis and ultimately leading to a decrease in sperm count and consequently infertility. [34-36] Natural product with antioxidant property such as ginger has been shown to reduce free radicals, end oxidative chain reactions and ultimately improved reproductive actions.

Conclusion

Zingiber officinale manifested antioxidant activity which compliments its androgenic actions by increasing reproductive hormones following prepubertal exposure. This underscores its safety and benefit on male fertility.

Conflict of Interest

The authors declare no conflict of interest.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

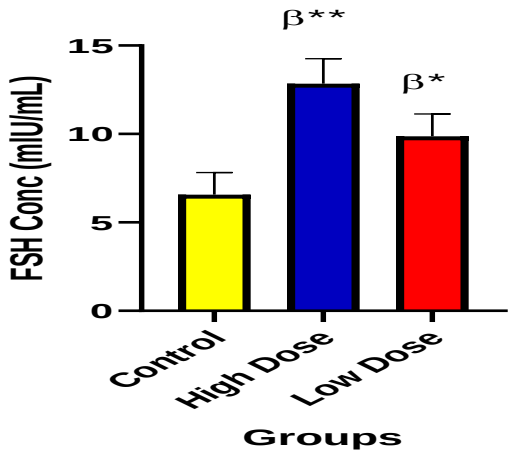

Figure 5: Showing FSH levels in prepubertal ginger treated male animals (β** =P<0.01//more significant value; β* =P<0.05//less significant value)

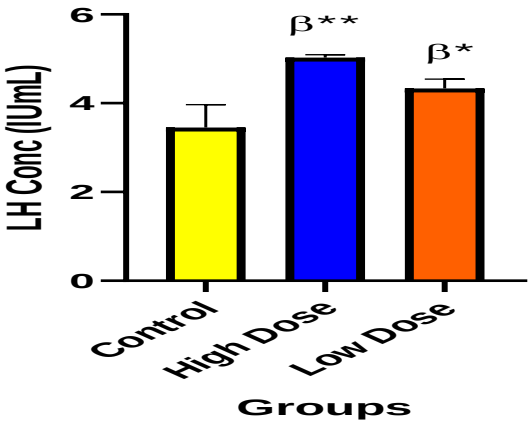

Figure 6: Showing LH levels in prepubertal ginger treated male animals (β** =P<0.01/more significant value; β* =P<0.05/less significant value)

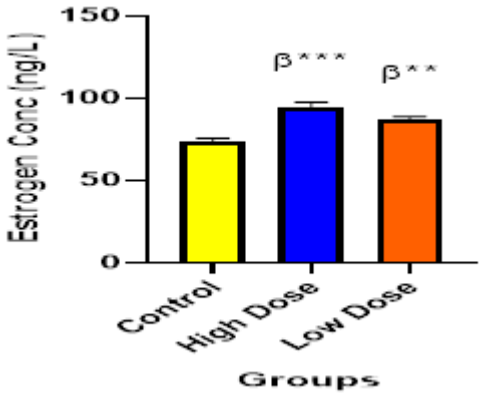

Figure 7: Showing estrogen levels in prepubertal ginger treated male animals (β*** =P<0.001/more significant value; β** =P<0.01/less significant value)

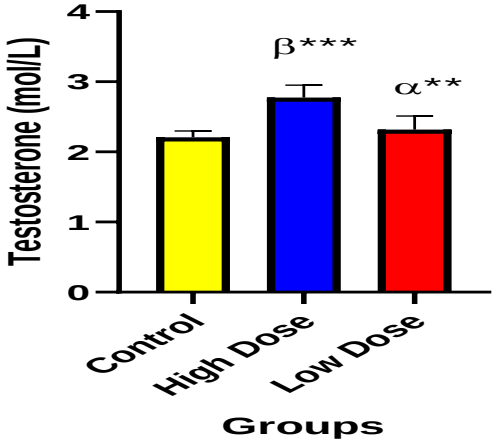

Figure 8: Showing testosterone levels in prepubertal ginger treated male animals (β*** =P<0.001/high significant value; α** =P<0.01/less significant value)

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.\

Acknowledgments

The authors are grateful for the support rendered by all staff of Department of Anatomy, Faculty of Basic Medical Sciences, University of Lagos, Nigeria.

References

1. Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T and Li HB. Bioactive Compounds and Bioactivities of Ginger (*Zingiber officinale* Roscoe). Foods. 2019; 8(6):185.
2. Warnalatha D, Mallikarjuna RP, Vishna DN, Madhu KB, Satyanarayana T and Jayaveera NK. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. J Eth pharma. 2010; 127, 2: 515-520.
3. Bellik Y. Total Antioxidant Activity and Antimicrobial Potency of the Essential Oil and Oleoresin of *Zingiber Officinale* Roscoe. Asian Pac. J. Trop. Dis. 2014; 4(1), 40–44.
4. Katie H and Julia M. Pediatric Chemical Exposure: Opportunities for Prevention. Journal of J Pediatr Health Care 2022;36, 1:27-33.
5. Ernst E and Schmidt K. Health risks over the Internet: Advice offered by "medical herbalists" to a pregnant woman. Wien Med Wochenschr. 2002;152(7-8):190–2.
6. Predieri B, Iughetti L, Bernasconi S and Street ME. Endocrine Disrupting Chemicals' Effects in Children: What We Know and What We Need to Learn? Int J Mol Sci. 2022;23(19):11899.
7. Ciccone NA and Kaiser UB. The biology of gonadotroph regulation. Curr Opin Endocrinol Diabetes Obes. 2009;16:321–7.
8. Oduwole OO, Huhtaniemi IT and Misrahi M. The Roles of Luteinizing Hormone, Follicle-Stimulating Hormone and Testosterone in Spermatogenesis and Folliculogenesis Revisited. Int J Mol Sci. 2021;22(23):12735.
9. Schulster M, Bernie AM and Ramasamy R. The role of estradiol in male reproductive function. Asian J Androl. 2016;18(3):435-40.
10. Jalil H, Azar M, Mamaghani H, Hosseinifar MA, Sadighi G, Farid D and Mahdi S. The influence of ginger (*Zingiber*

officinale) on human sperm quality and DNA fragmentation: A double-blind randomized clinical trial. Int J Reprod Biomed.2016; 14(8): 533–540.
11. Femi M, Adeniyi OS and Adesina A. Effects of *Zingiber Officinale* on Reproductive Functions in the Male Rat. Afri J Biomed Res. 2010; (11) 329 – 334.
12. Abdelfattah MG, Hussein MT, and Ragab SM. The effects of Ginger (*Zingiber officinale*) roots on the reproductive aspects in male Japanese Quails (*Coturnix coturnix japonica*). BMC Vet Res. 2023; 19, 34.
13. Waleed AM and Wisam SN. The effect of Ginger on semen parameters and serum FSH, LH & testosterone of infertile men. The Med J Tikrit University. 2012; 18- 22 (182): 322-329.
14. Sharif MF and Bennett MT. The effect of different methods and solvents on the extraction of polyphenols in ginger (Zingiber officinale). J Teknologi. 2016; 78:11-2.
15. ACUC. 2011. Guide for the Care and Use of Laboratory Animals. 8th Edition.
16. Marcondes FK, Bianchi FJ and Paula TA. ‘‘Determination of the estrous cycle phases of rats: some helpful considerations.’’ Braz. J. Biol., vol.2002; 62*(*4A):609-14.
17. Huda YH and Zuki AB. Reproductive characteristics of the female laboratory rat. Afri J biotech. 2012; Vol. 12(19): 2510-2514.
18. Ikpeme EV, Udensi OU, Ekerette EE and Okon UH. Potential of Ginger (*Zingiber officinale*) Rhizome and Watermelon (Citrullus lanatus) Seeds in Mitigating Aspartame-Induced Oxidative Stress in Rat Model. Res J Med Plant. 2016; 10(1):55-66.
19. Laferriere CA and Pang DS. Review of Intraperitoneal Injection of Sodium Pentobarbital as a Method of Euthanasia in Laboratory Rodents. J Am Assoc Lab Anim Sci. 2020;59(3):254-263.
20. Pillay CS, Eagling BD, Driscoll SR and Rohwer JM. Quantitative measures for redox signalling. Free Radic. Biol. Med. 2016; 96:290–303.
21. Stoilova I, Krastanov A, Stoyanova A, Denev P and Gargova S. Antioxidant activity
22. of a ginger extract (*Zingiber officinale*). Food Chem. 2007; Volume 102, Issue 3, Pages 764-770.
23. Yuki M, Hiroe K, Masashi H and Nobuji N. Antioxidant properties of gingerol related compounds from ginger. Bio-Factors. 2004; 21(1-4):293-296.
24. Zeng G, Zhang Z, Lu L, Xiao D, Zong S and He J. Protective effects of ginger root extract on Alzheimer disease-induced behavioral dysfunction in rats. Rejuv. Res. 2013, 16, 124–133.
25. Zhang F, Zhang J, Qu J, Zhang Q, Prasad C and Wei Z. Assessment of anti-cancerous potential of 6-gingerol (Tongling white ginger) and its synergy with drugs on human cervical adenocarcinoma cells. Food Chem. Toxicol. 2017; 109, 910–922.
26. Lai Y, Lee W, Lin Y, Ho C, Lu K, Lin S, Panyod S, Chu Y and Sheen L. Ginger essential oil ameliorates hepatic injury and lipid accumulation in high fat diet-induced nonalcoholic fatty liver disease. J. Agric. Food Chem. 2016; 64, 2062–2071.
27. Saberi H, Keshavarzi B, Shirpoor A, Gharalari FH and Rasmi Y. Rescue effects of ginger extract on dose dependent radiation-induced histological and biochemical changes in the kidneys of male Wistar rats. Biomed. Pharmaco. 2017; 94, 569–576.
28. Gholami-Ahangaran M, Karimi-Dehkordi M, Akbari JA, Haj SM and Ostadpoor MA systematic review on the effect of Ginger (*Zingiber officinale*) on improvement of biological and fertility indices of sperm in laboratory animals, poultry and humans. Vet Med Sci. 2021;7(5):1959-1969.
29. Akinyemi AJ, Adedara IA, Thome GR, Morsch VM, Rovani MT, Mujica LK, Duarte T, Duarte M, Oboh G and Schetinger MR. Dietary supplementation of ginger and turmeric improves reproductive function in hypertensive male rats. Toxicol Reps. 2015;13(2), 1357–1366.
30. Imani AM and Ainehchi N. Comparison of the effects of methotrexate and ginger extract on reproductive parameters in rats. Crescent Journal of Med and Bio Sc. 2014; 1(3): 103-109.
31. Morakinyo AO, Adeniyi OS and Arikawe AP. Effects of *Zingiber officinale* on Reproductive Functions in the Male Rat. Afr J Biomed Res. 2008; 11: 329-34.
32. Oduwole OO, Huhtaniemi IT and Misrahi M. The Roles of Luteinizing Hormone, Follicle-Stimulating Hormone and Testosterone in Spermatogenesis and Folliculogenesis Revisited. Int J Mol Sci. 2021;22(23):12735.
33. Ezike JC, Chima MU, Anugwo DN, Ezea J and Ewa EU. Effect of Ginger (*Zingiber officinale*) Rhizome Powder on Libido, Relative Organ Weight, Semen Quality and Histological changes in the Testes of Rabbit Bucks. Nig Agri J. 2023; 54(1):106-112
34. Osman A, Alsomait H, Seshadri S, El-Toukhy T and Khalaf Y. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. Reprod Biomed Online. 2015; **30**:120–127.
35. El-Shahat AE, Gabr A, Meki A and Mehana ES. Altered testicular morphology and oxidative stress induced by cadmium in experimental rats and protective effect of simultaneous green tea extract. Int J morphol. 2009; 27(3):757-764.
36. Monageng E, Offor U, Takalani NB, Mohlala K and Opuwari CS. A Review on the Impact of Oxidative Stress and Medicinal Plants on Leydig Cells. Basel J. 2023; 12(8):1559.
37. Roychoudhury S, Chakraborty S, Choudhury AP, Das A, Jha NK, Slama P, Nath M, Massanyi P, Ruokolainen J and Kesari KK. Environmental Factors-Induced Oxidative Stress: Hormonal and Molecular Pathway Disruptions in Hypogonadism and Erectile Dysfunction. Basel J. 2021; 10(6):837.