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AVOCADO (*PERSEA AMERICANA*) OIL MITIGATES AGAINST UNPREDICTABLE CHRONIC MILD STRESS-INDUCED CHANGES IN SPERM DNA PARAMETERS, GONADOSOMATIC INDEX, AND OXIDATIVE STRESS IN ADULT MALE WISTAR RATS

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ABSTRACT

Globally, infertility is a public health concern that affects couples with male factors and oxidative stress accounting for many of the cases. Chronic stress affects the reproductive system through the hypothalamic-pituitary-adrenal axis stimulation, which negatively causes disruption and impairment of spermatogenesis and testicular function. Avocado oil (AO) has rich antioxidant properties which are beneficial in cellular and tissue repair. This study evaluated the protective effects of avocado oil against testicular oxidative stress, sperm DNA concentration and purity, and gonadosomatic index distortion caused by unpredictable chronic mild stress (UCMS) in adult male Wistar rats. Thirty adults male Wistar rats were divided into six groups (n=5). Control (distilled water 1ml/kg), UCMS only, UCMS + AO 4.0ml/kg, UCMS + AO 8.0ml/kg, UCMS + AO 16.0ml/kg and UCMS + 150mg/kg Vitamin E. Daily oral administration of avocado oil and vitamin E was done 2 hours before induction of stress factors. Treatment lasted for 60 days after which the rats were sacrificed. Body weights were then evaluated. Body and testicular weight were used to evaluate gonadosomatic index (GSI), testicular homogenate for oxidative stress biomarkers (ROS, CAT and GSH) and semen sample for sperm DNA assay. The gonadosomatic index, oxidative stress biomarkers and sperm DNA parameters were significantly improved ($p<0.05$) following avocado oil administration at 16 ml/kg. In conclusion, avocado (*Persea americana)* oil has protective effects against unpredictable chronic mild stress-induced testicular dysfunction and improves the gonadosomatic index. This indicates its fertility potential in the management of male infertility especially the unexplained type mainly associated with stress.

Keywords: Avocado oil, gonadosomatic index, infertility, reactive oxygen species, spermatogenesis, sperm DNA parameters.

INTRODUCTION

Infertility is a public health concern as it affects about 10-20% of couples worldwide with associated psychological trauma and male factors result in 30-40% of the aetiology (WHO, 2018). The causes of male infertility are classified as pre-testicular, testicular and post-testicular. The pre-testicular and the testicular causes are mainly endocrine disorders originating from the hypothalamic-pituitary-gonadal axis that have adverse effects on spermatogenesis and post-testicular being mainly obstruction to the passage of semen in the male reproductive tract (Bardhi and Drakopoulos, 2021). Environmental stress which includes physical, chemical, biological, psychological

and social stress affects the reproductive system through the hypothalamic-pituitary-adrenal axis stimulation, which negatively disrupts hypothalamic-pituitary-gonadal function with impaired spermatogenesis and testicular function (Bardhi and Drakopoulos, 2021).

Unpredictable chronic mild stress (UCMS) simulates the symptoms of daily related stressors that humans undergo, and this has been reported to cause impaired spermatogenesis, decreased sperm concentration and motility, spermatozoa DNA damage and apoptosis (Zou *et al*., 2019; Choowong-in *et al*., 2021). Excessive stress generates a high amount of reactive oxygen species (ROS) that affect the testes resulting in infertility (Zou *et al*., 2019). It is estimated that approximately 25- 80% of infertile men have high levels of ROS in the semen and this lowers the antioxidant capacity of sperm (Benatta *et al*., 2020). Spermatozoa have abundant polyunsaturated fatty acids in their plasma membrane and cytoplasm and limited antioxidant and DNA repair capacity; thus, they are vulnerable to ROS attack (Benatta *et al*., 2020). Although sperm cells require minimal stress to achieve optimal physiological function, however, excessive stress result in pathological effects in spermatogenic functions mediated through androgen insufficiency, DNA damage, lipid peroxidation, and apoptosis with impaired reproductive function (Aitken *et al*., 2022).

Avocado is well-known for having a high nutritional value, rich in iron, potassium, phosphorus, lecithin, protein, fiber, polyunsaturated (linoleic and linolenic acids) and monounsaturated (oleic acid) fats, polyphenols, tocopherols, carotenoids, β-carotene, and vitamins A, C, D, and E (Akusu *et al*., 2021). Avocado oil has been proven to lower oxidative damage and ROS formation in a number of industrial applications, such as skin care products, polymers, and commercial vegetable oil for food preparation (Arancibia *et al*., 2017). Avocado oil has been reported to help with liver metabolism, blood cholesterol reduction, and diabetes management (Arancibia *et al*., 2017). Additionally, it has anti-inflammatory properties and has been proven to shield goat sperm spermatozoids from the harmful effects of free radicals during the cryopreservation process (Olamitibo *et al*. 2016). However, its use in the management of male infertility, especially the unexplained type is limited. Thus, the need for this study.

This study aimed to determine the protective effects of Avocado (*Persea americana)* oil against unpredictable chronic mild stress-induced testicular oxidative injury on sperm DNA parameters and gonadosomatic index in adult male Wistar rats.

MATERIALS AND METHODS

Materials

Wistar rats, Avocado oil, formal saline, normal saline solution (0.9 %w/vol), diazepam and ketamine, eosin stains, anaesthetic chamber, dissecting kits, vitamin E, spectrophotometer, weighing balance, plastic cages, sawdust, clean water, feeds, cannula, needle and syringe, cotton wool, standard bottles, dissecting board, bench centrifuge and other analytical reagents.

Methods

Ethical Approval

Ethical approval was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number: ABUCAUC/ 2023/056.

Avocado fruits collection and identification

The avocado fruits were purchased from major distributors in Sabon Gari Market, Zaria. They were identified by a Taxonomist in the department of Biological Science, Ahmadu Bello University, Zaria, a voucher number ABU0992 was assigned for identification.

Extraction of Oil from Avocado Fruit Pulp

The extraction of avocado (*Persea america*) oil was done at the National Research Institute for Chemical Technology (NARsICT), Zaria, Kaduna state, Nigeria, by cold-pressed mechanical method (Costagli and Betti, 2015) with modification according to Akusu *et al*. (2021).

Phytochemical Study

Photochemical screening and quantification of avocado (*persea americana*) oil for alkaloids, flavanoids, saponins, steroids, tannin, phenol and glycosides were done using standard methods as reported by Sofowora, (1993); Dubale *et al.* 2023) in the department of Pharmacognosy and Pharmaceutical Chemistry, Ahmadu Bello University, Zaria.

Experimental Design

Thirty (30) adults male Wistar rats of about 6 weeks weighing between 150g and 250g were sourced from the animal house of Human Physiology were given access to water and food ad libitum until the commencement of the experiment. They were divided into six (6) groups of five (5) rats each (n=5) as shown below. The dose of avocado oil was arrived at following LD50 determination and pilot study.

Group I: Normal control administered distilled water 1ml/kg.
Group II: Unpredictable chronic mild stress (UCMS) only;
Group III: UCMS + 4ml / kg Avocado (*persea americana)* oil;
Group IV: UCMS + 8ml / kg Avocado (*persea americana)* oil;
Group V: UCMS + 16ml / kg Avocado (*persea americana)* oil;
Group VI: UCMS +150mg/kg Vitamin E (Malmir *et al*., 2021).

Oral administration of the avocado oil and vitamin E was done daily, two hours before the commencement of stress procedure for a period of sixty (60) days. The unpredictable chronic mild stress factors were as follows: 2-hour damp sawdust (200ml of water in 100g sawdust); 24- hour food deprivation; 24-hour water deprivation; forced swimming for 5 minutes (each rat was placed in an inescapable transparent cylindrical tank filled with water (24^0C); and 6 hours restraint (rats were placed in a 100ml plastic tube with openings in both ends for breathing (Willner *et al*., 1997) as shown in table 1 below. Each stress procedure was commenced at 8.00 am daily.

Animal Sacrifice and Sample Collection

At the end of 60 days of administration of Avocado oil and stress procedure, each rat was weighed and then anaesthetized using intramuscular ketamine (10mg/kg) and diazepam (2mg/kg) (Mahmud *et al*., 2014). Following anaesthetic induction, the scrotal sac was dissected and the testes were harvested and weighed. The epididymis was carefully removed and the seminal fluids were obtained for sperm DNA parameter assay.

Table I: Unpredictable Chronic Mild Stress Procedure (Willner *et al*., 1997)

Groups	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Group II	Damp Sawdust	Restraint Stress	Forced Swim	Water Deprivation	Food Deprivation	Damp Sawdust	Restraint Stress
Group III	Food Deprivation	Damp Sawdust	Restraint Stress	Forced Swim	Water Deprivation	Food Deprivation	Damp Sawdust
Group IV	Water Deprivation	Food Deprivation	Damp Sawdust	Restraint Stress	Forced Swim	Water Deprivation	Food Deprivation
Group V	Forced Swim	Water Deprivation	Food Deprivation	Damp Sawdust	Restraint Stress	Forced Swim	Water Deprivation
Group VI	Restraint Stress	Forced Swim	Water Deprivation	Food Deprivation	Damp Sawdust	Restraint Stress	Forced Swim

Determination of Gonadosomatic Index (GSI)

The weight of the rats was taken at the onset of the experiment and sacrifice using a precision weighing scale and the gonadosomatic index (GSI) was calculated using the formula: Weight of testes x 100 /Body weight at sacrifice (Oyeyemi and Ajuwon, 2020).

Determination of the Biomarkers of Oxidative Stress

Assay of total reactive oxygen species (ROS) levels, reduced glutathione (GSH) level, and catalase (CAT) activities were determined using appropriate ELISA kits according to the manufacturer's manual (Shanghai Coon Koon Biotech Co., Ltd, China).

Sperm DNA Extraction, Quantification and Purity

The sperm DNA extraction, quantification and purity were carried out according to the method adopted by Darbandi *et al*., (2018). Quantity and purity of DNA extracted with this method were assessed using a Nano Drops ND-1000 spectrophotometer (PeqLab, Erlangen, Germany) which calculates the concentration of double stranded DNA (dsDNA). The DNA concentration and ratio of the 260/280 absorbance were automatically measured to define DNA purity. The ratio of absorbance at 260 nm and 280 nm was used to assess protein contamination.

Data Analysis

The data obtained were expressed as mean ± standard error of mean (SEM). The results were analysed by one-way analysis of variance (ANOVA) using SPSS version 23 and Tukey *post-hoc* test for multiple comparison. Data were represented in tables and figures. Values of $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Under chronic stress conditions, there is an imbalance between the oxidative and antioxidant systems of the reproductive system, and the excessive induction of oxidative stress triggers inflammation and distort the testicular microenvironment (Zou *et al*., 2019). Avocado oil contains rich nutritional constituents including phytochemicals (alkaloids, flavonoids, phenols, steroids) that are essential for normal body functions (Akusu *et al*., 2021). This is evident in our study with high level of phenol and flavonoid similar to a previous study by Akusu *et al*. (2021), The absence of saponin, tannin and oxalates in the avocado oil used in the present study explains its antioxidant activity (Akusu et al., 2021) (Table II).

Table II: Phytochemical Screening of Avocado Oil (Qualitative and Quantitative Assay)

Constituents	Qualitative Screening	Quantitative (%)
Alkaloids	+	2.7
Flavonoids	+	7.0
Phenols	+	8.5
Steroids	+	4.4
Triterpenes	+	3.5
Cardiac glycosides	+	4.8
Saponins	-	0
Tannins	-	0
Antraquinones	-	0

The phytochemical screening of Avocado oil was found positive for alkaloid (2.7%), flavonoid (7.0%), phenols (8.5%), steroids (4.4%), triterpenes (3.5%), cardiac glucosides (4.8%) and negative for saponins, tannins and antraquinones.

Phenol and flavonoids have been proven to have significant health benefits, particularly to the reproductive system (Almujaydil, 2023). The UCMS exposure significantly caused impairment in the testicular tissue evident by decreased gonadosomatic index possibly through testicular tissue degradation caused by induced oxidative stress. However, following treatment with Avocado oil, the gonadosomatic index (GSI) was significantly increased compared to the UCMS-only group in the 8 ml/kg and 16 ml/kg groups respectively ($p<0.05$) (Figure I). The gonadosomatic index is a good indicator of reproductive activities, and determines the reproductive potential of the male (Oyeyemi and Ajuwon, 2020). The improvement observed after avocado oil administration could be due to a reduction in oxidative damage in the testes and elevation in the testosterone level owing to the high antioxidant capacity of phenols and flavonoids. Additionally, it has been reported that the presence of vitamins C and E, Zinc and Selenium, and oleic acid present in the avocado oil make it a powerful supplement for fertility enhancement (Abd El-Rahman and Omar, 2022; Almujaydil, 2023).

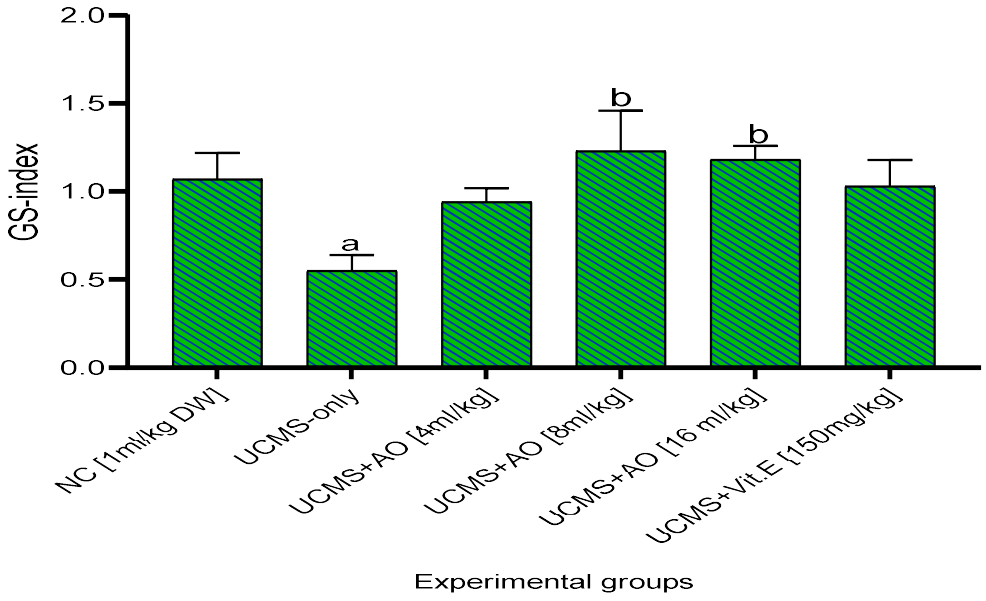

Figure I: Gonadosomatic Index (GSI) In UCMS Adult Male Wistar Rats Treated with Avocado Oil

NC - Normal control; DW - distilled water; UCMS - unpredictable chronic mild stress; AO - Avocado oil; Vit. E – Vitamin E. Superscripts a and b represent significant difference ($p < 0.04$) compared to NC and UCMS only group respectively.

Table III: Testicular Oxidative Stress Markers following Unpredictable Chronic Mild Stress in Adult Male Wistar Rats Treated with Avocado Oil

Experimental groups	ROS (IU/ml)	GSH (IU/mg Protein)	CAT (IU/mg Protein)
NC [1 ml/kg DW]	83.19±0.41	15.36±0.22	6.20±0.03
UCMS-only	106.94±0.74 ^{a}	9.26±0.31^{a}	4.18±0.04^{a}
UCMS + AO [4ml/kg]	100.95±0.70 ^{ab}	10.78±0.29^{a}	4.48±0.09^{a}
UCMS + AO [8ml/kg]	98.37±0.45 ^{abc}	14.58±0.55^{bc}	5.36±0.04^{abc}
UCMS + AO [16 ml/kg]	93.53±1.09 ^{abc}	14.72±0.63^{bc}	6.76±0.05^{abcd}
UCMS + Vit.E [150mg/kg]	87.62±0.95 ^{bcde}	16.68±0.43^{bcde}	5.58±0.21^{abce}

NC - Normal control; DW - distilled water; UCMS - unpredictable chronic mild stress; AO - Avocado oil; Vit. E – Vitamin E. Different superscripts represent significant difference ($p<0.001$): a= $p< 0.05$ compared NC; b= $p< 0.05$ compared to UCMS only; c= $p< 0.05$ compared to UCMS + AO 4ml/kg; d= $p< 0.05$ compared to UCMS + AO 8ml/kg; e= $p< 0.05$ compared to UCMS + AO 16ml/kg.

Table IV: Sperm DNA Parameters following Unpredictable Chronic Mild Stress in Adult Male Wistar Rats Treated with Avocado Oil

Experimental Groups	Sperm DNA Concentration (ng/ul)	DNA Absorbance (260/280)
NC [1 ml/kg DW]	2129.80±377.70	1.84±0.06
UCMS-only	3859.25±920.29[a]	1.66±0.07[a]
UCMS + AO [4ml/kg]	3102.60±526.89	1.68±0.06
UCMS + AO [8ml/kg]	1629.83±333.15[b]	1.87±0.04[b]
UCMS + AO [16 ml/kg]	1511.23±137.11[b]	1.88±0.04[b]
UCMS + Vit.E[150mg/kg]	2344.03±612.06	1.72±001

The sperm DNA parameters were significantly improved in the groups treated with avocado oil 8ml, and 16ml respectively. NC - Normal control; DW - distilled water; UCMS - unpredictable chronic mild stress; AO - Avocado oil; Vit. E – Vitamin E. Superscripts a and b represent significant difference ($p < 0.05$) compared to NC and UCMS only group respectively.

This study illustrated the interplay between UCMS and oxidative stress generation. The testicular ROS was significantly elevated, with a corresponding significant reduction in the endogenous antioxidant enzymes CAT and reduced glutathione (GSH) level in the UCMS-only group compared to the normal control or treated groups ($p<0.001$) (Table III). This suggest that UCMS has the potential to alter male fertility via the generation of significant free radicals that cause sperm DNA damage with resultant alteration in the fertilizing ability of the spermatozoa. This result agrees with previous studies that reported significant decrease in the activities of catalase and glutathione, and increased oxidative products following exposure to UCMS (Kolbashi *et al*., 2021; Meng *et al*., 2022). The administration of avocado oil before the onset of the various stress factors mitigated significantly the biomarkers of oxidative stress in the rat's testes by preventing the UCMS-induced testicular oxidative damage via enhancing the activities of endogenous antioxidant enzymes and GSH concentrations, and decreasing the total ROS levels. The protective role of avocado oil could be attributed to the modulatory effects of the monounsaturated and polyunsaturated fatty acids, vitamins, and phytochemicals present in the oil (Almujaydil, 2023). It has been reported that flavonoids inhibit oxidant enzymes such as NADPH oxidase, lipoxygenase and xanthine oxidase, in addition to enhancing the expression of c-glutamylcysteine synthetase, responsible for GSH production thus acting as a strong antioxidant that prevent free radical-induced cellular damage, and lowers oxidative stress in general (Mustafa *et al*., 2023).

The sperm DNA concentration in our study showed significant decrease in the UCMS + AO 16ml/kg group indicating the ability of avocado oil to prevent DNA damage, and restore normal sperm parameters and male fertility (Table IV). The sperm DNA concentration might be a proxy of sperm DNA integrity (Wu *et al*., 2019). In addition, research has shown that sperm mitochondrial DNA depletion occurs during spermatogenesis; therefore, higher sperm DNA concentration in the UCMS-only rats indicates abnormalities and impaired spermatogenesis from oxidative stress and

aberrant gene expression of mitochondrial transcription factor A (TFAM) and nuclear-encoded polymerase gamma (POLG), both of which regulate sperm DNA concentration (Lee *et al.,* 2019). Therefore, sperm DNA concentration has predictive value of male infertility and as such could be used as a diagnostic test for clinical infertility (Wu *et al*., 2019). The higher DNA purity seen in avocado oil groups at 8ml/kg and 16ml/kg exceeding 1.8 signifies improved DNA quality in these groups further depicting the ability of avocado oil to protect and/or maintain sperm DNA with excellent quality which can be used for quantitative polymerase chain reaction (qPCR) assay in genetic and epigenetic applications (Darbandi *et al*., 2018; Mothe *et al*., 2023). Research has shown that flavonoids which are abundant in avocado oil increase the sperm quality in infertile men, and reduce oxidative stress, apoptosis and sperm DNA fragmentation index (Mousavi *et al*., 2022).

CONCLUSION

Avocado oil at 16.0 ml/kg exacted significant protective effects against UCMS-induced testicular oxidative injury, sperm DNA damages and decreased gonadosomatic index via the reduction in total free radical enhancement of the endogenous antioxidant enzymes and improved sperm DNA quality, thus can be used for the management of male infertility, especially the unexplained type.

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Conflict of Interest

All the Authors declared no conflict of interest.

References

Aitken, R. J., Drevet, J. R., Moazamian, A. and Gharagozloo, P. (2022). Male Infertility and Oxidative Stress: A Focus on the Underlying Mechanisms. *Antioxidant*. 11, 306-327.

Akusu, O. M., Obinna-Echem, P. C., Opurum, P. C. and Chibor, B. S. (2021). Comparative Analysis of the Physicochemical Characteristics, Phytochemical Components and Fatty Acid Profile of Avocado Pear (Persea Americana L) Pulp and Seed Oil. *Europ J Agri Food Sci*, 3 (1),11-17.

Abd El-Rahman, H. A. and Omar, A. R. (2022). Ameliorative effect of avocado oil against lufenuron induced testicular damage and infertility in male rats. *Andrologia*, 14580, 1-14.

Almujaydil, M.S. (2023). The Role of Dietary Nutrients in Male Infertility: A Review. *Life*, 13, 519-534.

Arancibia, C., Riquelme, N., Zúñiga, R. and Matiacevicha, S. (2017). Comparing the effectiveness of natural and synthetic emulsifiers on oxidative and physical stability of avocado oil-based nanoemulsions. *Innovat Food Sci. Emerg Technol*, 44, 159-166.

Bardhi, E and Drakopoulos, P (2021). Update on Male Infertility. *J Clin Med*, 4771 (10), 1-3.

Benatta, M., Kettache, R., Buchholz, N. and Trinchieri, A. (2020). The impact of nutrition and lifestyle on male fertility. *Archive of Italian Urology Andrology*, 92(2), 122-130.

Choowong-in, P., Sattayasai, J., Boonchoong, P., Poodendaen, C., Wu, A.T.H., Tangsrisakda, N., Sawatpanich, T., Arun, S., Uabundit, N. and Iamsaard, N. (2021). Protective effects of Thai *Mucuna pruriens* (L.) DC. var. pruriens seeds on sexual behaviors and essential reproductive markers in chronic unpredictable mild stress mice. *J Trad Complement Med,* 12, 402-413.

Costagli, G, and Betti M (2015). Avocado oil extraction processes: method for cold pressed high quality edible oil production versus traditional production. *J Agri Eng,* 46 (467), 115-122.

Darbandi, M., Darbandi, S., Khorshid,H. R. K., Akhondi, M. M., Mokarram, P. and Sadeghi, M. R. (2018). A simple, rapid and economic manual method for human sperm DNA extraction in genetic and epigenetic studies. *Midd East Fert Soc J,* 23, 216-219.

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N. and Suleiman, S. (2023). Phytochemical screening and Antimicrobial Activity Evaluation of selected Medicinal plants in Ethiopia. *J Exp Pharmacol*, 15, 51-62.

Kolbasi, B., Bulbul, M. V., Karabulut, S., Altun, C. E., Cakici, C., Ulfer, G., Mudok, T. and Keskin, I. (2021). Chronic unpredictable stress disturbs the blood–testis barrier affecting sperm parameters in mice. Reprod Biomed Online, 1(1), 1-17.

Lee, S. H., Choi, K. H., Cha, K. M., Hwang, S. Y., Park, U. K and Jeong, M. S. (2019). Protective effects of Korean Red Ginseng against sub-acute immobilization stress-induced testicular damage in experimental rats. *J Ginseng Res*, 43 (1),125-134.

Mahmud MA., Shaba P., Yisa H Y., Gana J., Ndagimba R. and Ndagi S (2014). Comparative efficacy of Diazepam, Ketamine, and Diazepam-Ketamine combination for sedation or anaesthesia in cockerel chickens. *J Advanced Veteri Animal Res,* 1(3), 107-113.

Malmir, M., Mehranjani, M. S., Faraji, T. and Noreini S.N. (2021). Antioxidant effect of Vitamin E on the male rat reproductive system by a high oral dose of Bisphenol-A. *Toxicol Res Application*, 5,1-11.

Meng, X., Peng, L., Xu,J., Guo, D., Cao,W., Xu, Y. and Li. S. (2022). Betaine attenuate chronic restraint stress-induced changes in testicular damage and oxidative stress in male mice. *Reprod Biol Endocrinol,* 20(80),1-13.

Mothe, G. B., Scott, C., Malossi, C. D., Araujo-Junior, J. P. and Ferreira de Souza, F. (2023). DNA extraction methods of canine sperm cells to sexing by quantitative PCR. *Res Soci Develop,* 12(4), 1-8.

Mousavi, S.N., Dorraji, M.S., Pourmansouri, Z., Mohammadian, M., Chiti, H., Moghaddam, N. and Hosseini, E. (2022). Quercetin-loaded on whey protein improves male fertility parameters and atherogenic indices of rats on a western-style diet. *J Funct Foods,* 88, 104-114.

Mustafa, H. N. (2023). Ameliorative potential of the quercetin on lead-induced testicular damage: morphohistometric and biochemical analysis. *Afri J Urol*, 29(36), 1-8.

Olamitibo, D. J., Dayo, O. O., Oladimeji, A. M., Mathew, A., Olajide, O., Emmanuel, Oluwafemi, A. E., Amidu, S. T., & Ayobami, I. O. (2016). Effects of avocado seed extract in different trisextenders on sperm and oxidative stress indices of vitrified goat spermatozoa. *J Agri Sci Belgrade*, 61(4), 359-374. https://doi.org/ 10.2298/jas1604359o

Oyeyemi, M. O. and Ajuwan, C. T. (2020). Gonadosomatic index, epididymalmass index and haematology of male wistar rat treated with carrot juice. *Afri J Online,* 38(2), 83-84.

Sofowora, A. (1993). Phytochemical Screening of Medicinal Plants and Traditional Medicine in Africa. *Spectrum Books Ltd*, Ibadan, Nigeria, Pg 100-108.

Willner, P. (1997) Validity, reliability and utility of the chronic mild stress model of depression: A 10-year review and evaluation. *Psychopharmacology*, 134, 319-329.

World Health Organization (2018). International Classification of Disease, 11th Revision (ICD-11) Geneva: *WHO 2018*.

Wu, H., Huffman, A. M., Whitcom, B. W, Josyula, S., Labrie, S., Tougias, E., Rahil, T., Sites, C. K, and Pilsner, J. R. (2019). Sperm mitochondrial DNA measures and semen parameters among men undergoing fertility treatment. *Reprod Biomed Online*, 38(1), 66-75. doi: 10.1016/j.rbmo.2018.10.004.

Zou P, Xiaogang, W., Wang, Y., Chang, L., Qing, C., Huan, Y., Niya, Z., Yingfei, Z., Hongqiang, C., Guowei, Z., Jinyi, L., Jia, C., Lin, A. and Lei, S (2019). Mechanisms of Stress-Induced Spermatogenesis Impairment in Male Rats Following Unpredictable Chronic Mild Stress (UCMS). *Intl J Mol Sci,* (20) 4470,1-17.