Journal of Advances in Medical and Pharmaceutical Sciences

23(10): 35-47, 2021; Article no.JAMPS.77151
ISSN: 2394-1111

Fleurya Aestuans Leaves and Tetrahydroxyflavone Mitigate Lead Induced Testicular Toxicity in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2021/v23i1030265
Editor(s):
(1) Prof. ZoranTodorovic, University of Belgrade and University Medical Center "Bezanijskakosa", Serbia.
(2) Dr. Erich Cosmi, University of Padua, Italy.
Reviewers:
(1) Tamara Jovanovic, University of Belgrade, Serbia.
(2) Farah Saeed, Dow University of Health Sciences, Pakistan.
Complete Peer review History, details of the editor(s), Reviewers and additional Reviewers are available here: https://www.sdiarticle5.com/review-history/77151

Original Research Article

Received 08 October 2021
Accepted 17 December 2021
Published 20 December 2021

ABSTRACT

The effects of *Fleurya aestuans* leaves and tetrahydroxyflavone on the reproductive functions of lead acetate induced testicular toxicity in Wistar rats was investigated in this study. The study animals were divided into control group (A) and toxicity-induced groups (B, C, D, E, F, and G). To determine all particulate parameters under inquiry, the study used established procedures. Histological and tissue biochemical studies were performed on the testes and caudal epididymis. The most active compound in the extract was found to be tetrahydroxyflavone, and treatment of the extract resulted in a significant ($P<0.05$) improvements in male mating behaviors, conceptive hormones, sperm parameters, and testicular biochemical parameters. The frequency of mount,

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intromission and ejaculation rose significantly (P<0.05), but the latencies of mount, intromission, and ejaculation, decreased. Similarly, the treatment of the extract with tetrahydroxyflavone reversed the harmful effects of lead acetic acid on various organ systems in the research animals, such as the epididymis and testes. As a result, the study recommends that *Fleurya aestuans* leaves be used to treat reproductive problems. The dose in human being experiment may be beneficial to patients with reproductive problems.

Keywords: Fleurya aestuans; tetrahydroxyflavone; testicular toxicity; reproductive dysfunctions.

1. INTRODUCTION

Male infertility and reproductive dysfunctions are widespread health challenges and almost half of the human infertility is considered to be male etiology, [1]. It has also been observed that oligospermia is the most common reason for the expressed male infertility [2]. Recently, the generation of reactive oxygen species (ROS) in testicular tissues has been reported to be the reason of male infertility [3]. Nevertheless, diminishing the excessive reactive oxygen species (ROS) levels or protective agents against reactive oxygen species (ROS), such as antioxidants, may be useful therapeutic agents for male infertility [4].

In this regard, plants have a long folklore of use in aiding fertility, including fertility-enhancing properties and aphrodisiacal qualities.

Fleurya aestuans is an annual herbaceous plant of the Urticaceae family. It is most commonly found in agriculture, along roads, partially shaded areas in the wild, and other disturbed forest settings. The leaves are commonly used in soups and as a vegetable. In Nigeria, *Fleurya aestuans* is a common plant that is often considered a weed [5].

According to Oluwaseyi *et al.,* [6], the plant is known in Igbo as "*Ile-nkita (Ohuahihara*), in Yoruba as "*Ofuefue (Fiyafiya)*," in Hausa as "*Bulsum fage*", Spanish as *"Picapica*,"and Chinese as "*Huo yan sung ye ma*." In Guinea-Bissau, it's called *manding-mandinka nhafitiram,* in Sierra Leone, "*koranko mambuwure,*" and in Ivory Coast, "*akan-brong hom-hom*" [7].

The utilization of *Fleurya aestuans* leaves has amazing logical verification in the treatment of female hormonal disequilibrium [8] Different reports have uncovered that the leaves of *Fleurya aestuans* is utilized to treat migraine, cough, syphilitic yaws, gonorrhea, rheumatism menopausal problems and pyrexia [9].

In any case, the current study is an attempt to examine the viability of *Fleurya aestuans* leaves against lead acetate induced testicular toxicity in humans utilizing animal's models.

2. MATERIALS AND METHOD

2.1 Collection and Identification of Plant Material

Fresh *Fleurya aestuans* leaves were collected at the Madonna University and its surroundings and certified a plant taxonomist at the University of Port Harcourt's Department of Plant Science and Biotechnology. UPH/P/263 was the herbarium number assigned to the plant.

2.2 Preparation of Extract

Fleurya aestuans leaves were gathered, extraneous elements were removed, the leaves were dried at room temperature for one week, and mechanically pulverised. Using a sohxlet device, 690g of dry powdered leaves were defatted and successively extracted in 400ml of water-ethanol combination (25:70) for 72 hours in an extraction jar. To get the crude extract, the extract was concentrated using a rotary evaporator. The extracted yield was kept at 4°C in a home refrigerator until it was needed.

2.3 Quantitative and Qualitative Phytochemical Analysis

The quantitative and qualitative phytochemical analysis of the extract was performed according to the methods of Sofowara [10] with slight modifications.

2.4 Lethal Dose (LD_{50}) of the Extract

Using the technique of Lork [11] the lethal dose (LD_{50}) of *Fleurya aestuans* extract (FAE) did not produce any signs of toxicity in rats even up to the dose of 4600 mg/kg.

2.5 Experimental Design

A total of 70 Wistar rats weighing between 155.9 - 240.3g obtained from the animal house, Madonna University Elele, Nigeria were used for the study. The rats were housed in wire meshed cage under standard conditions (temperature 25-29°C and natural dark/light cycle and fed with a standard rat pelleted diet and tap water ad libitum. The animals were given a period of two week for acclimatization.

2.6 Animal Placement/Inducement

After acclimatization, the rats were weighed and randomly assigned into seven groups (n=10 in each group). Using the method of Falana and Oyeyipo, [12] testicular toxicity was induced in all test groups (Groups 2-7) by oral admnstraton of lead acetate at 2.25mg/kg. The rat groups were treated as follows:

Group A (Control group) were only allowed rat feed and tap water ad libitum for 30 days.

Group B (LA only group) were only treated with lead acetate.

Group C (Low dose extract group) were administered 50mg/body weight of *Fleurya aestuans* leaf extract for 30 days.

Group D (Medium dose extract group) were administered 75mg/body weight of *Fleurya aestuans* leaf extract for 30 days.

Group E (High dose extract group) were administered 200mg/body weight of *Fleurya aestuans* leaf extract for 30 days.

Group F (LATHF group) were co-administered LA + THF for 30 days.

Group G (THF only group) were administered 100mg/body weight of THF for 30 days.

2.7 Determination of Mating Parameters

The Yakubu and Akanji [13] technique was used to conduct the mating behaviour test. For the time being, healthy and sexually experienced male rats with active sexual motions have been chosen for the study. The male animals were brought to the study laboratory after receiving the extract and were exposed to red dim light (in a 1 w fluorescent tube in a 14' 14' laboratory) at the designated testing period for a few days (3-6 d) before the experiment. The female animals were falsely brought into estrus (heat) phase (as female rodents only allow mating during the estrus phase) by giving them a suspension of ethinyl estradiol orally at a dose of 100 mg/kg 48 hours before the pairing and subcutaneous progesterone at a dose of 1 mg/kg 6 hours before the pairing. Male animals different than the control and test animals were used to confirm the female animals' receptivity before the test. The research only included the most responsive females. The experiment was carried out at 20:00 h in the same laboratory and with the same level of light. The responsive female animals were placed in male animal cages at a ratio of one female to one male. The observation of mating behaviour began right away and lasted for the first two mating series. If the male failed to express sexual desire, the test was stopped. Any female animals who did not exhibit signs of receptivity were replaced with another artificially ‘warmed' female.

The following aspects of male sexual behaviour were observed:

Mount latency (ML) is the interval between placing a female animal near a male animal and the first copulation attempt. They were registered by real-time monitoring. The copulation is the behavior in which the male animal mounts on the back of the female.

The mount frequency (MF) is the number of times that the male animal copulates with the female animal without intromissions. MF is calculated in the copulation series until the first ejaculation.

The intromission latency (IL) is the interval between placing a female animal near a male animal and the first male intromission. Intromission is a copulatory behavior in which the male animal genital organ enters the female vagina.

The intromission frequency (IF) refers to the number of times the male copulates with intromissions and performs this action in a copulation series until the first ejaculation.

The ejaculatory latency (EL) is the interval between placing a female animal near a male and the first ejaculation.

2.7.1 Ejaculation frequency

The number of times the male rat ejaculates during the copulation series.

2.7.2 Postejaculatory interval

Time from ejaculation until the next intromission. Exceptionally, the first mount following ejaculation is taken as the endpoint of the postejaculatory interval.

Copulatory efficacy (CE) is measured by the formula CE=IF/(MF + IF). The IF/EL ratio was also measured. It shows to what extent the copulation was successful. The IL, IF, and CE of the groups were not counted and compared when at least one of their animals did not have intromissions and/or ejaculations.

The Inter-Copulatory Interval (ICI) is the mean time that elapses between a mount or intromission. The Hit Rate (HR) is obtained by dividing the number of intromissions by the sum of intromissions and mounts. ICI= EL/IF

The copulatory efficiency (CE): Proportion of mounts resulting in vaginal penetration relative to the total number of mounts. CE=IF/MF× 100

2.8 Collection of Blood Samples

After 30 days, the rats were anaesthetized with chloroform and slaughtered by cutting through the jugular vein. To extract the plasma, whole blood was collected into heparinized tubes and centrifuged at 3,000 rpm for 15 minutes at 4°C.

2.9 Determination of Sperm Parameters

Hemo cytometer and eosin stain were used to assess sperm parameters (count, motility, viability, and morphology).

2.10 Determination of Serum Hormone Assays

An enzyme linked immune sorbent assay was used to examine serum levels of LH, FSH, testosterone, and prolactin (ELISA).

2.11 Statistical Analysis

All values were represented as mean ± S.E.M and subjected to statistical analysis. Comparison was done using one – way analysis of variance (ANOVA). Values were considered significant when $P < 0.05$.

3. RESULTS

All Results Obtained from the study were presented in tables and expressed as mean plus/minus standard error of mean (M±S.E.M) as below.

3.1 Histology

Fig. 1 & 2 illustrates the photomicrographs of transverse sections of the rat's testes and epididymis in the control group and test groups respectively.

Table 1. Values of extract and tetrahydroxyflavone on mount and intromission parameters of study animals

Groups	ML	MF	IL	IF
A (CG)	76.00±8.59	50.80±0.80	47.00±0.02	21.20±0.49
B (LAOG)	53.40±2.66[a]	26.84±5.99[a]	49.60±1.08	09.00±0.32[a]
C (LDEG)	30.20±2.06[ab]	31.20±0.48[a]	22.33±1.24[ab]	18.40±0.40[b]
D (MDEG)	39.00±1.30[ab]	35.00±5.26[ab]	36.00±1.90[ab]	14.80±0.20[a]
E (HDEG)	48.40±0.40[a]	42.40±7.09[ab]	43.30±0.02	20.80±0.80[b]
F (LATHF)	29.11±4.03[b]	46.20±0.50[b]	38.01±1.00[ab]	25.50±0.60[b]
G (THFOG)	22.08±1.02[ab]	51.00±0.07[ab]	27.50.±0.01[ab]	19.00±2.00[ab]

KEY: ML=Mount Latency, IL=Intromission Latency, MF=Mount Frequency, IF=Intromission Frequency. Values are presented as mean ± sem. a = mean values are statistically significant compared to Group A rats, b = mean values are statistically significant compared to Group B rats.

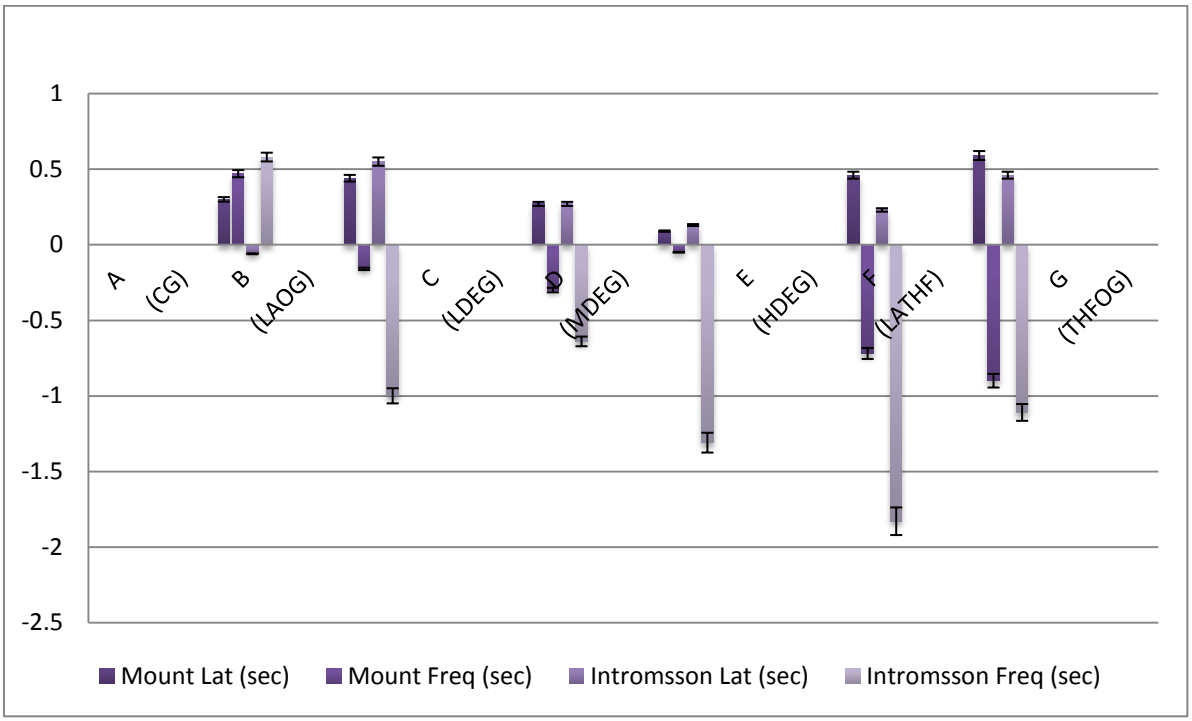


Fig. 1. % diff of mount and intromission parameters of study animals

Table 2. Values of extract and tetrahydroxyflavone on ejaculatory parameters of study animals

Groups	EL	EF	PEI
A (CG)	109.40±3.85	12.6±0.68	124.8±0.49
B (LAOG)	86.20±4.10[a]	3.20±0.86[a]	166.00±5.10[a]
C (LDEG)	92.60±2.18	10.00±0.00[ab]	111.80±3.37[ab]
D (MDEG)	80.20±2.06	8.20±0.20[ab]	98.00±7.50[ab]
E (HDEG)	67.80±3.06[ab]	5.60±0.24[ab]	82.80±2.18[ab]
F (LATHF)	75.50±0.03[ab]	19.00±0.33[b]	153.62±7.20[ab]
G (THFOG)	52.20±1.02[ab]	28.00±4.00[ab]	157.30.±2.01[ab]

KEY: *EL=Ejaculation Latency, EF=Ejaculation Frequency, PEI= Post Ejaculation Interval. Values are presented as mean ± sem.* [a] *= mean values are statistically significant compared to Group A rats,* [b] *= mean values are statistically significant compared to Group B rats*

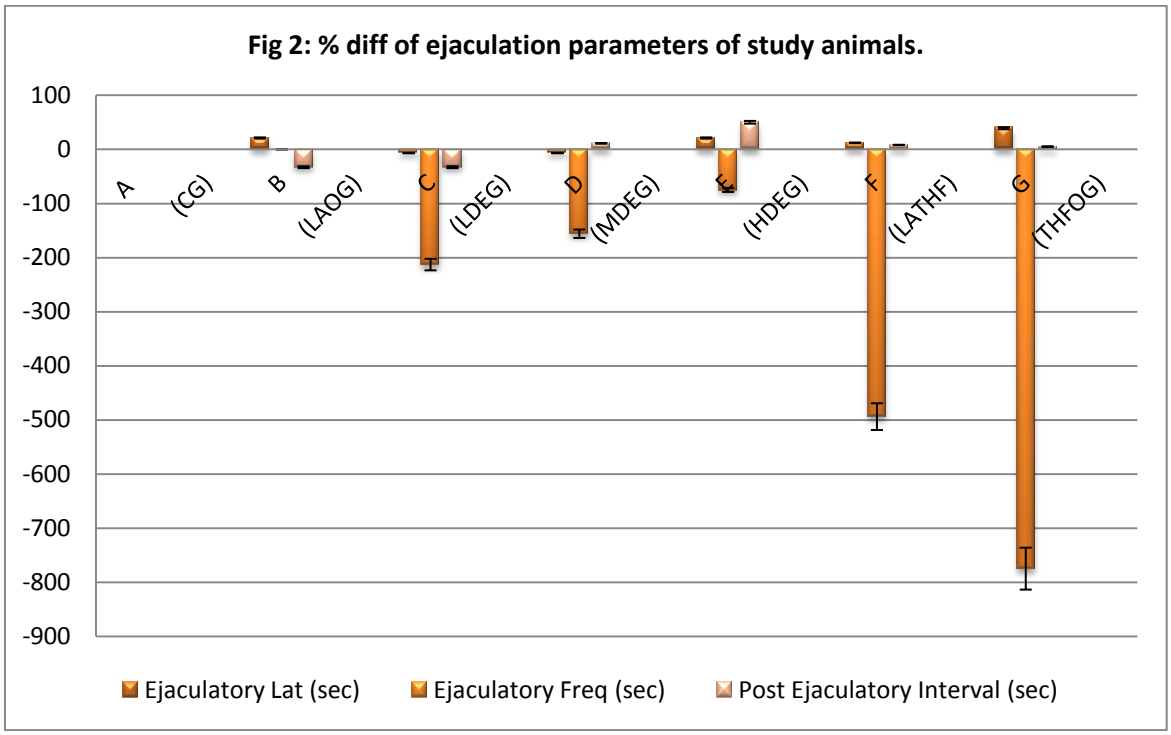


Fig. 2. % diff of ejaculation parameters of study animals

Table 3. Values of extract and tetrahydroxyflavone on computed copulatory parameters of study animals

Groups	ICI	CE	CE%
A (CG)	5.16±7.90	29.44±0.37	41.73±0.61
B (LAOG)	9.60±12.81[a]	25.11±0.05	33.53±5.34[a]
C (LDEG)	5.03±5.45	37.10±0.46[ab]	58.97±0.83[ab]
D (MDEG)	5.42±10.3	29.30±0.04	41.43±3.80 [b]
E (HDEG)	3.25±3.83[b]	32.91±0.10[b]	49.05±0.11[ab]
F (LATHF)	2.96±0.05[b]	35.60±0.55[b]	55.20±1.20[ab]
G (THFOG)	2.75±0.51[ab]	27.14±1.00	37.25.±2.85

KEY: *ICI=Intercopulatory Interval, CE= Copulatory Efficacy, CE= Copulatory Efficiency.Values are presented as mean ± sem.* [a] *= mean values are statistically significant compared to Group A rats,* [b] *= mean values are statistically significant compared to Group B rats.*

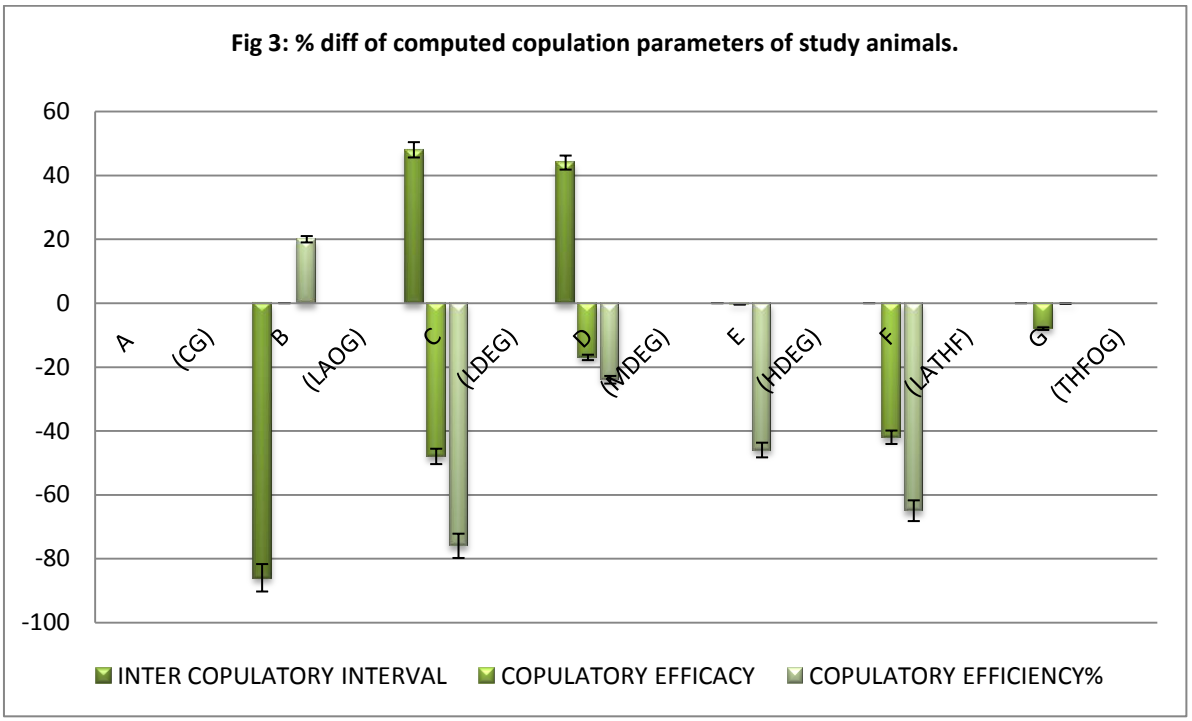


Fig 3. % diff of computed copulation parameters of study animals

Table 4. Values of extract and tetrahydroxyflavone on reproductive hormone of study animals

Groups	LH	FSH	TEST	PRL
A (CG)	1.38±0.00	0.22±0.00	1.59±0.00	6.83±0.45
B (LAOG)	0.29±0.00[a]	0.12±0.02[a]	0.77±0.00[a]	10.70±0.46[a]
C (LDEG)	0.48±0.00	0.48±0.01[b]	0.95±0.00[b]	9.82±0.01
D (MDEG)	0.95±0.01[b]	0.42±0.01[b]	0.82±0.01	7.72±0.24
E (HDEG)	1.40±0.02[b]	0.38±0.01[b]	0.90±0.02[b]	7.73±0.46
F (LATHF)	0.57±0.20[b]	0.40±0.50[b]	0.88±0.00	6.85±0.60[b]
G (THFOG)	1.38±0.12[b]	0.20±0.07	1.22±0.00[b]	6.92±0.00[b]

KEY:*Values are presented as mean ± sem.* [a] *= mean values are statistically significant compared to Group A rats,* [b] *= mean values are statistically significant compared to Group B rats.*

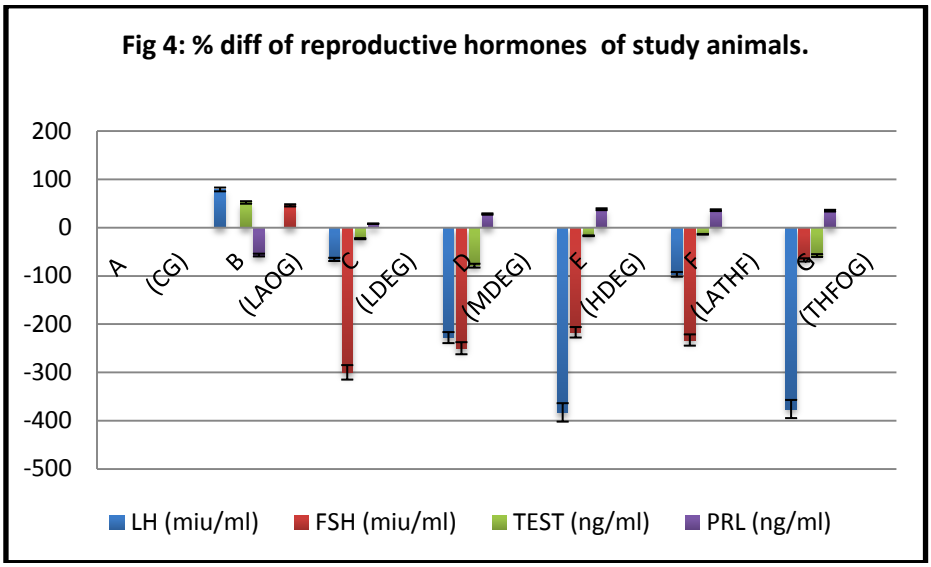


Fig 4. % diff of reproductive hormones of study animals

Table 5. Values of extract and tetrahydroxyflavone on sperm parameters of study animals

Groups	A (CG)	B (LAOG)	C (LDEG)	D (MDEG)	E (HDEG)	F (LATHF)	G (THFOG)
Appearance	**Milky**	**Brownish**	**Milky**	**Milky**	**Milky**	**Milky**	**Milky**
Volume (ul)	0.2	0.1	0.1	0.2	0.4[b]	0.2	0.3[b]
pH	8.0	6.0	7.0	8.0[b]	7.0	7.0	7.0
Viability (%)	75	60	70	80[b]	90[b]	70	75[b]
Viscosity	Normal	High	Normal	Normal	Normal	Normal	Normal
Sperm Count	400	100	400[b]	300	700[b]	500[b]	600[b]
Normal (%)	70	55[a]	65	80[b]	80[b]	75[b]	70
Abnormal (%)	25	45[a]	25[b]	30	20[b]	20[b]	30
Active (%)	85	45[a]	70[b]	65[b]	65[b]	70[b]	70[b]
Sluggish (%)	10	12	10	10	5[b]	10	10
Dead	20	45[a]	25[b]	20[b]	15[b]	20[b]	20[b]

KEY: *Values are presented as mean ± sem.* [a] *= mean values are statistically significant compared to Group A rats,* [b] *= mean values are statistically significant compared to Group B rats.*

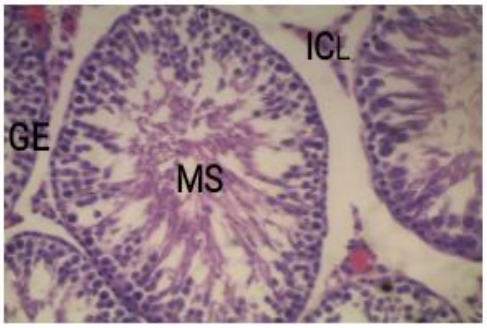
Table 6. Values of extract and tetrahydroxyflavone on oxidative stress markers of study animals

Groups	MDA	SOD	CAT	GSH
A (CG)	0.50±0.02	0.42±0.00	3.88±0.05	1.22±0.00
B (LAOG)	0.60±0.00[a]	0.27±0.04 [a]	2.62±0.00	0.92±2.00 [a]
C (LDEG)	0.55±0.00	0.30±0.10	2.88±0.03	1.15±0.00[b]
D (MDEG)	0.49±0.08[b]	0.40±0.00[b]	2.75±0.00	1.04±0.02[b]
E (HDEG)	0.41±0.00[b]	0.32±2.00	3.15±0.01	0.97±0.08
F (LATHF)	0.52±0.10	0.32±0.00	2.75±0.00	1.20±0.00 [b]
G (THFOG)	0.47±0.00[b]	0.45±3.00[b]	3.72±0.06	1.17±0.04[b]

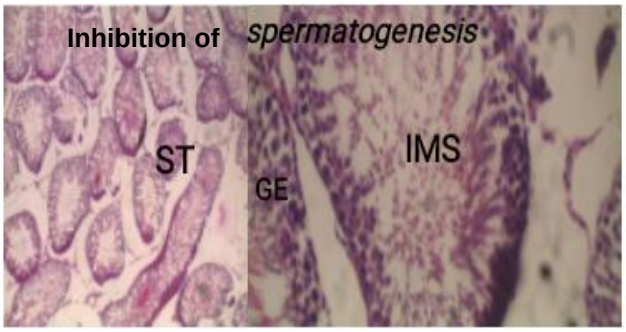
KEY: *Values are presented as mean ± sem.* [a] *= mean values are statistically significant compared to Group A rats,* [b] *= mean values are statistically significant compared to Group B rats.*

3.1.1 Histological examination of testes

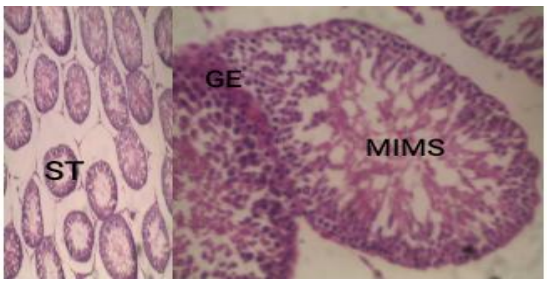
PLT. 1a shows that the tubular lumen is filled with mature spermatozoa. PLT. 1b (LAOG) shows that the tubular lumen contains numerous immature spermatozoa & scanty deflagellated structures of MS. This implies inhibition of sperm motility & spermatogenesis. PLT. 1c Tubular lumen contains mainly immature spermatozoa. Interstitial cells of Leydig are seen. PLT. 1d Tubular lumen contains few matured spermatozoa. Interstitial cells of Leydig are seen. PLT. 1e Tubular lumen contains few mature spermatozoa. PLT. 1f shows distorted germinal epithelium. Tubular lumen contains mainly immature spermatozoa. PLT. 1g Tubular lumen contains mature spermatozoa.



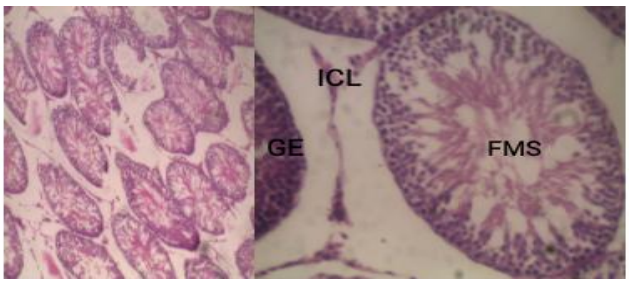
PLT**. 1a. (CG) x 600**



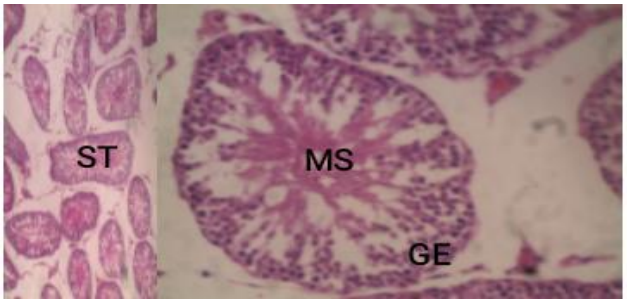
PLT**. 1b. (LAOG) x 125 & x 600**



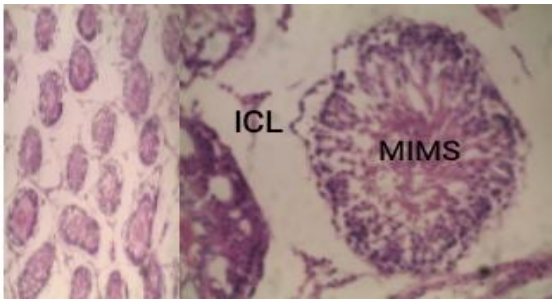
PLT**. 1c. (LDEG) x 125 & x 600**



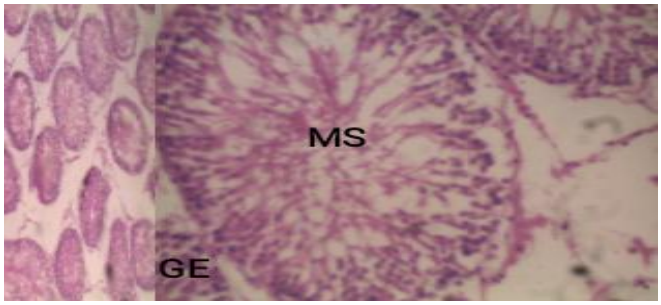
PLT**. 1d. (MDEG) x 125 & x 600**



PLT**. 1e. (HDEG) x 125 & x 600**



PLT**. 1f. (LATHF) x 125 & x 600**

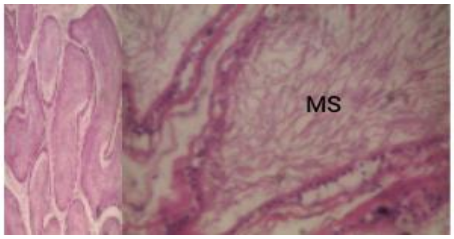


PLT**. 1g. (THFOG) x 125 & x 600**

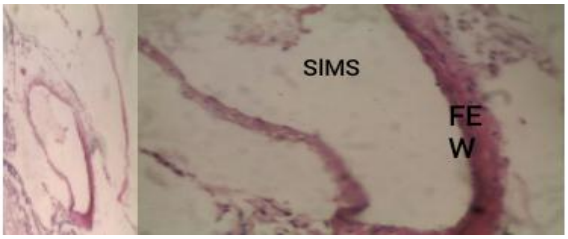
*Key**: FMS**= Few matured spermatozoa, **MS**= mature spermatozoa, **MIMS**= Mainly immature spermatozoa*

3.1.2 Histology of the epididymis of extract and LA groups

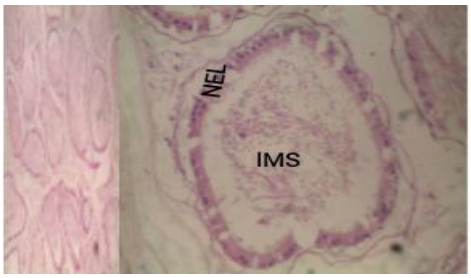
The results of photomicrographs of the epididymis are displayed in PLT. 2a to g respectively. PLT. 2a shows normal epithelial lining. Contain mature spermatozoa. PLT. 2b (LAOG) shows fibrosis of epidydimal wall. Epidydimis contains little or no spermatozoa which were underdeveloped implying inhibition of spermatogenesis. PLT. 2c-d shows a normal epithelial lining. Epidydimis contains immature spermatozoa. PLT. 2e shows normal epithelial lining. Contains mainly mature spermatozoa. Few immature spermatozoa are seen. PLT. 2f-g shows normal epithelial lining. Contains mature spermatozoa (flagellated structures)



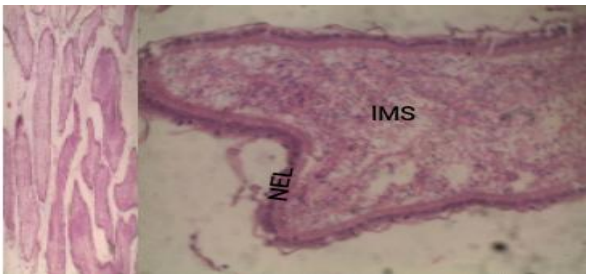
PLT**. 2a. (CG) x125 & x 600**



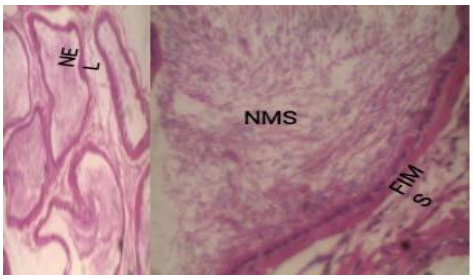
PLT**. 2b. (LAOG) x125 & x 600**



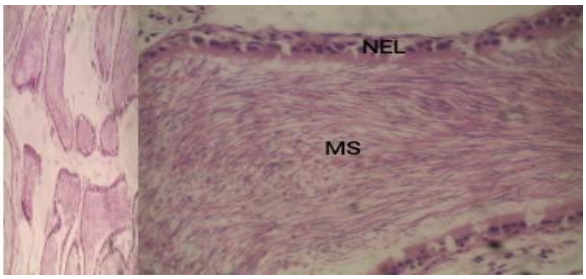
PLT**. 2c. (LDEG) x125 & x 600**



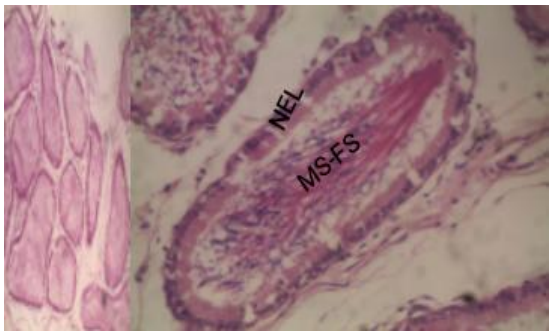
PLT**. 2d. (MDEG) x125 & x 600**



PLT**. 2e. (HDEG) x125 & x 600**



PLT**. 2f. (LATHF) x125 & x 600**



PLT**. 2g. (THFOG) x125 & x 600**

*Key: **SIMS**= Scanty immature spermatocytes, **FEW**= Fibrosis of epithelia wall, **NEL**= normal epithelial lining, IMS= immature spermatozoa **: NMS**= Numerous mature spermatocytes, **MS-FS**= mature spermatozoa (flagellated structures), **NEL**= normal epithelial lining, **FIMS**= Few immature spermatozoa*

4. DISCUSSION

Through production of gonadotropins and testosterone in systemic circulation, the hypothalamic gonadotropin releasing hormone (GnRH) guarantees proper physiology of the hypothalamo-hypophysio-gonadal axis, which is required for spermatogenesis, spermatozoa maturation, and reproductive behaviour [14, 15].

LH is also known as interstitial cell-stimulating hormone (ICSH) in men, and it activates the interstitial cells of Leydig in the testes. It is required for enhanced steriodogenesis and testosterone production from Leydig cells.

In males, follicle-stimulating hormone (FSH) works in tandem with testosterone to speed up the sperm production process [16]. The main male sex hormone and anabolic steroid, testosterone is predominantly released by the interstitial cells of Leydig in the testes. It works on the seminiferous tubules to begin and sustain spermatogenesis, and it plays a key role in male androgenicity. Testosterone is also required for the development and maturity of men's reproductive organs (Biney *et al.,* 2020).

The hormone prolactin, which is produced by the adenohypophyseal lactotrophs, has no recognised target organ or function in male reproduction. However, the presence of prolactin receptors in the choroid plexuses and brain suggests that this hormone has a hidden role in male fertility control. Progress in understanding of prolactin signaling cascades across species, in particular, shows that the physiological role of prolactin is conserved from rodents to humans. Although the hormone's physiological significance to male reproduction has not been definitively proven, it has been linked largely to male infertility. Acute hyperprolactinemia is known to decrease testosterone production and male fertility by blocking GnRH secretion via prolactin receptors on hypothalamic dopaminergic neurons or by inducing hypersecretion of adrenal corticoids in response to prolactin. Dopamine is thought to be involved in the release of endorphins from opiatergic neurons, which suppress GnRH production. Men's spermatogenesis and steriodogenesis are harmed as a result of this (Marinaki *et al.,* 2016).

In the current study, a reduction in serum reproductive hormones in male rats treated with lead acetate indicates that lead acetate has a suppressive impact on the hypothalamic-pituitary-testcular-pathway, hence, a repression of both steriodogenesis and spermatogenesis in group b rats (LAOG) of the current study. In addition, sperm count, sperm viability, and sperm morphology are all useful markers of male fertility. Lead acetate produced a substantial decrease in sperm count and viability in the current study, as well as an increase in aberrant sperm characteristics. The unfavorable and repressive impact of lead acetate on testosterone in group b (LAOG) might be related to the negative effect of lead acetate on sperm physiognomies. Because testosterone is known to play an important role in spermatogenesis, a drop in plasma testosterone levels can lead to Leydig cell dysfunction and testicular androgenic disorders [17].

The generation of reactive oxygen species (ROS) in testicular tissues suppressed male reproductive functions, according to a study by Agarwal *et al.* [3]. This might explain why lead acetate decreased male reproductive hormones and semen parameters in group b rats (LAOG) of the current investigation. Lead is one of the heavy metals that have been linked to increased lipid peroxidation, decreased saturated fatty acid content, and increased unsaturated fatty acid content in membranes. The results of this study are congruent with those of Akhigbe & Ajayi [18], Yakubu & Atoyebi [19], who found that treatment with toxic substances reduced reproductive hormones, steriodogenesis, and spermatogenesis in male wistar rats.

In contrast, co-administration of lead acetate with a hydro-ethanol extract of *Fleurya aestuans* leaves resulted in a dose-dependent increase in blood hormone concentrations and semen characteristics throughout the whole test group. This implies that the leaf extract may have nullified the fatal effect of lead acetate on the parameters evaluated, bringing them closer to the values found in group A (control group rats) of the current investigation. Following phytochemical and GC-MS analysis of the extract, some compounds such as tetrahydroxyflavone, sapogenin, catechin, and phenolic constituents' were present in the leaf extract of *Fleurya aestuans*.

At 100ug tetrahydroxyflavone treated rats, significant increases in conceptive hormones and semen parameters such as sperm counts and viability were found. In vitro and in vivo studies

have shown that tetrahydroxyflavone has a variety of antioxidant properties. It functions as a superoxide scavenger at low concentrations, especially against the highly reactive hydroxyl radical and peroxynitrite species. It enhances the activity or expression of antioxidant enzymes including superoxide dismutase, catalase, and heme oxygenase-1 at high doses. The strong antioxidant potentials of tetrahydroxyflavone may explain its effect on copulatory hormones and semen parameters possibly due to the capacity of tetrahydroxyflavone and other phenolic compounds to scavenge the oxidative free radicals produced by lead acetate in the hypothalamus, pituitary, and testes [20].

The extract's influence on sperm qualities may be attributed to its zinc concentration also. Poor zinc levels have been linked to low sperm counts, an increased defective sperm, decreased sperm viability and spermatocyte agglutination. Zinc supplementation has been shown to increase sperm quality [21]. Zinc also contains antioxidant capabilities to eliminate free radicals. Thus, the rise in plasma testosterone levels in this study might be owing to zinc's ability to enhance leydig cell and testosterone production. This might be because zinc is a neurotransmitter that stimulates the production of gonadotropins from the adenohypophysis, as shown by the results of Mahdavi *et al.,* [22].

Furthermore, sapogenin is a phytosteriod involved in the synthesis of pregnenolone, androgen, and other hormones. The action of the leaves of *Fleurya aestuans* extract on reproductive hormones is most likely owing to a stimulatory effect on the hypothalamic-pituitary-gonadal circuit by the phytosteroid component of the extract, which is important for steroidogenesis.

The current findings are similar to those of Haghmorad *et al.* [23] and Amah-Tariah *et al.* [8], who found that treatment with some therapeutic plant extracts augments reproductive functions in animal models.

5. CONCLUSION

The study therefore suggests the use of the plant resources in the correction of reproductive dysfunctions in Wistar rats. The dose in human trial may be beneficial for the patients who are having procreative disorders.

6. RECOMMENDATIONS

Molecular basis or molecular mechanisms of *Fleurya aestuans* should be demonstrated.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The research was approved by our institutional ethical committee with the reference number: UPH/CEREMAD/REC/MM78/052. International standard, rules and guidelines for use of animal for research was adhered to as approved by the committee [24].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cooper TG, Noonan E, vonEckardstein S, Auger J, Baker HW, Behre HM. World health organization reference values for human semen characteristics. Human Reproduction Update; 2010.
2. Biswas TK, Pandit S, Mondal S, Biswas SK, Jana U, Ghosh T. Clinical evaluation of spermatogenic activity of processed shilajit in oligospermia. Journal of Andrology; 2010.
3. Agarwal A, Virk G, Ong C, Plessis SS. Effect of oxidative stress on male reproduction. World Journal of Mens Health; 2014.
4. Gunes S, Hekim GN, Arslan MA. Asci R Effects of aging on the male reproductive system. Journal of Assisted Reproduction and Genetics; 2016.
5. Agbodjento E, Klotoé JR, Sacramento TI, Dougnon V, Tchabi FL, Déguénon E, Atègbo JM. Ethnobotanical knowledge of medicinal plants used in the treatment of male infertility in southern Benin. Advances in Traditional Medicine; 2020a.
6. Oluwaseyi Adegoke Adetunji, Jeremiah Olorunjuwon Olugbami, Ayodeji Mathias Adegoke, Michael Adedapo Gbadegesin, Oyeronke Adunni Odunola. Reno-hepatoprotective and antidiabetic properties of methanol leaf extract of *Fleurya aestuans* in wistar rats. Journal of Evidence-Based Integrative Medicine; 2021.
7. Soladoye MO, Itokun T, Chukwuma EC, Ariwaodo JO, Ibhanesebor GA, Agbo-Adediran OA, Owolabi SM. Our plants our

heritage: Annals of Biological Research; 2013.
8. Amah-Taria FS, Nwafor CC, Ajah AA. Bekinbo MT. Changes in some female reproductive parameters of Albino wistar rats by hydroethanol leaf extract of *Fleurya aestuans*. International Journal of Reproduction, Contraception, Obstetrics and Gynecology; 2016.
9. Essiett UA, Edet NI, Bala DN. Phytochemical and physiochemical analysis of the leaves of *Laportea-aestuan* (Linn) chew and laporteaaestuan (schumach)chew (male and female); Asian Journal of plant science and Research; 2011.
10. Sofowora A. Medicinal plants and traditional medicine in Africa. Ibadan, Nigeria: Spectrum Books Limited; 2008.
11. Lorke D. A new approach to practical acute toxicity testing. Archieve Toxicology; 1983.
12. Falana BA, Oyeyipo IP. Selenium and zinc attenuate lead-induced reproductive toxicity in male sprague-dawley rats. Research Journal of Medical Science; 2012.
13. Yakubu MT, Akanji MA. Effect of aqueous extract of *Massularia acuminata* stem on sexual behaviour of male wistar rats. Evidence-Based Complementary and Alternative Medicine; 2011b.
14. Asuquo IE, Edagha IA, Ekandem GJ, Peter AI. Carica papaya attenuates testicular histomorphological and hormonal alterations following alcohol-induced gonado toxicity in male rats. Toxicological Research; 2020.
15. Sower SA, Freamat M, Kavanaugh SI. The origins of the vertebrate hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-thyroid (HPT) endocrine systems: new insights from lampreys. Gen. Comp. Endocrinol; 2009.
16. Ulloa-Aguirre A, Reiter E, Crépieux P. FSH receptor signaling: Complexity of interactions and signal diversity. Endocrinology; 2018.
17. Zhang H, Liu Y, Zhang R, Liu R. Chen Y. Binding mode investigations on the interaction of lead (II) acetate with human chorionic gonadotropin. J Phys Chem B; 2014.
18. Akhigbe R, Ajayi A. Testicular toxicity following chronic codeine administration is via oxidative DNA damage and up-regulation of NO/TNF-α and caspase 3 activities. PLoS; 2020.
19. Yakubu MT, Atoyebi AR. *Brysocarpus coccineus* (Schum & Thonn) root reinstates sexual competence and testicular function in paroxetine-induced sexual dysfunction in male Wistar rats. Andrologia; 2018.
20. Klotoé J, Agbodjento E, Dougnon V, Yovo M, Sacramento T, Deguenon EL, Dougnon J, Atègbo JM. Exploration of the chemical potential and antioxidant activity of some plants used in the treatment of male infertility in Southern Benin. Journal of Pharmaceutical Research International; 2020.
21. Falana BA, Oyeyipo IP. Selenium and zinc attenuate lead-induced reproductive toxicity in male sprague-dawley rats. Research Journal of Medical Science; 2012.
22. Mahdavi B, Saneei S, Qorbani M. *Ziziphora clinopodioides* Lam leaves aqueous extract mediated synthesis of zinc nanoparticles and their antibacterial, antifungal, cytotoxicity, antioxidant, and cutaneous wound healing properties under in vitro and in vivo conditions. Appl Organometal Chem; 2019.
23. Haghmorad D, Mahmoudi MB, Haghighi P, Alidadiani P, Shahvazian E, Tavasolian P, Hosseini, M, Mahmoudi M. Improvement of fertility parameters with *Tribulus terrestris* and *Anacyclus pyrethrum* treatment in male rats. International Brazilian Journal of Urology; 2019.
24. National Institute of Health. Laboratory Animal Welfare. United State Department of Health and Human Services. 1985;14: 8.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/77151