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Original Research Article

Hydroethanolic Leaf Extract of *Erythrina senegalensis* Attenuates Diclofenac Sodium-Induced Testicular and Epididymal Perturbation in Male Wistar Rats

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Abstract

Clinical and experimental studies have demonstrated that diclofenac sodium (DFS) administration could result in male reproductive dysfunction. *Erythrina senegalensis* (ES) is a potent medicinal plant with antioxidants that may significantly impact cellular redox homeostasis, including male reproduction. Therefore, the study investigated the attenuative effects of hydroethanolic leaf extract of *Erythrina senegalensis* (HLEES) in DFS-induced testicular and epididymal toxicity in male albino rats. Thirty male Wistar rats of 5 rats per group were used in this study and were randomly divided into 6 groups (A–F) and treated for 21 days. Rats in group A (control group) received distilled water orally; Group B was given DFS at 10 mg/kg body weight intraperitoneally (IP). Group C and D were given HLEES at 200 and 400 mg/kg body weight orally respectively. Groups E and F were given DFS at 10 mg/kg + HLEES at 200 and 400 mg/kg respectively. Spermatological profiles and testicular histopathology were assessed. DFS-treated rats showed significant ($p<0.05$) reduction in relative organ weight and sperm parameters. Also, DFS treatment increased the percentage of sperm abnormalities relative to the control, and the groups administered HLEES only. Co-administration of HLEES with DFS improved the spermatological parameters and ease some effects of DFS on the reproductive and accessory organs mentioned above. HLEES was also seen to play a beneficial role in the histopathological effects of DFS on the testicular tissues. The current findings have shown that this evaluated plant extract may have ameliorative effects on DFS-induced testicular and epididymal injuries in rats.

Keywords: Diclofenac sodium, *Erythrina senegalensis*, spermatozoa, testes, albino rats.

INTRODUCTION

Diclofenac Sodium (DFS) is a medication that has been proven to have damaging consequences on certain human and animal organs and tissues (Owumi & Dim, 2019). It is a phenylacetic acid derivative with significant biological activity that functions as a non-steroidal anti-inflammatory drug (NSAID), an analgesic, and an effective inhibitor of pain and rheumatic inflammation (El-Megharbel *et al*., 2015), and musculoskeletal disorders in humans and animals (Adeyemi & Olayaki, 2018). Prior studies have revealed that DFS treatment could result in detrimental effects on the reproductive system (Owumi *et al*., 2020). Several investigations have highlighted the placental translocation of DFS (Shintaku *et al*., 2009). The use of DFS has been linked to significantly lower testicular weight and sperm counts, with the testes' histological structure showing the most indications of degeneration (Vyas *et al*., 2019). The metabolism of DFS has been associated with excessive ROS production, which induces oxidative stress and death by progressive apoptosis (Inoue *et al*., 2004). Hence, this study was conducted with the aim of minimizing the harmful effects of DFS on the male reproductive system. The toxic effects of DF were attributed to the production of reactive metabolites, 5-hydroxydiclofenac and N, 5-dihydroxydiclofenac, which could disrupt the natural balance and promote excess reactive oxygen and nitrogen species (RONS) in the tissues. This may then induce oxidative stress, organ, and tissue damage in rats (Bort *et al*., 1999).

Plants have been used for a wide range of human needs since the dawn of time, including food, nutrition-related medicines, and the treatment of diseases in both humans and animals (Jothy *et al*.,

2012). Plants are considered to be safer than conventional allopathic drugs. It has been observed that complaints of the adverse effects of conventional drugs are considerably more prevalent than reports of herbal toxicity (Pal and Shukla, 2003). Extracts from many plants' leaves, stems, roots, and fruits have been used to produce pharmaceuticals (Al-Attar *et al*., 2017; Imo *et al*., 2019). They are employed as remedies to prevent, treat, or manage a plethora of medical disorders, including relatively recent ones like HIV/AIDS (Langlois-Klassen *et al*., 2007). As a consequence, there is growing interest in assessing various plant extracts for their pharmacological efficacy in the treatment of organ disorders (Konda *et al*., 2016; Iseghohi & Orhue, 2017).

Erythrina senegalensis (ES), also known coral tree, is a member of the Fabaceae family.

Traditional healers use the leaves, stem, and root bark to manage various illnesses, including malaria, gastrointestinal disorder, fever, dizziness, jaundice, diarrhea, nose bleeding, and pain (Kone *et al.*, 2011). It has been demonstrated that the stem bark contains antibacterial properties, hepatoprotective effects, and to a significant extent antioxidant activities (Togola *et al*., 2008). Nene-Bi *et al*. (2013), also reported that this plant extract has antifungal, and antiplasmodial activities, as well as anti-inflammatory properties (Koné *et al*., 2011). However, despite the studies on the traditional uses of this plant, there is a dearth of scientific information on the therapeutic effects of ES on some male reproductive functions. Therefore, this study was initiated to evaluate the therapeutic effects of HLEES against DF-induced testicular and epididymal perturbation in male rats.

MATERIALS AND METHOD

Chemicals and kits

DFS is an injectable liquid purchased from the North China Pharmaceutical Co. Ltd, 115 Hainan Road, Shijiazhuang, Hebei, China. . Each 3 ml ampoule contains 75 mg of DFS. All the reagents used are of analytical grade.

Plant Material

ES leaves were harvested from the premises of the College of Agriculture Garkawa, Plateau state. The plant was identified by a taxonomist; a voucher specimen number UAM/FH/242/21 already exists in the College of Forestry Herbarium, Federal University of Agriculture, Makurdi, Benue State.

Preparation of HEESL

The leaves were washed under a running tap and air dried for a period of one month in the laboratory at room temperature, the leaves were pulverized using an electric blender and then sieved using a locally made mesh. Briefly, the solvent mixture was prepared by adding 800 ml of distilled water to 200 ml of absolute ethanol and mixed. Then, 100 g of the pulverized sample was macerated in 1000 ml of aqueous ethanol mixture and allowed to stand for 72 hrs. The mixture was sieved with a white piece of cloth and the liquid obtained was filtered with Whatman no. 1 filter papers. The filtrate was concentrated in a water bath at 45 °C, and the extract obtained was dried to a constant weight in a desiccator. The concentrated HLEES were weighed, and every 100g of the powdered leaves yielded 7g of extracts.

Experimental animals and management

Thirty adult male Wistar rats (*Rattus norvegicus*) having body weights of 200–250 g, were used for this study. They were purchased as litters at the age of 6 weeks from National Veterinary Research Institute (NVRI), Vom, Plateau State. Then they were kept in plastic cages to grow unto maturity and acclimatized for about 4 weeks in the Department of Veterinary Physiology and Biochemistry research laboratory, Federal University of Agriculture, Makurdi, Nigeria. The rats were kept under normal environmental conditions of 12 h dark and 12 h light cycle, with an average temperature of 29°C. They were fed with standard animal feeds, produced by Grand Cereal and Oil Mills Ltd, Jos, Nigeria, and clean water ad libitum. The rats were handled with care according to International guidelines for the use of laboratory animals (NIH, 1978).

Preparation of DFS

The method of Hassan *et al.*, (2021) was adopted for the preparation of DFS to be administered with some modification. The dose to be administered in volume to groups B, E, and F rats were calculated using the 10mg/kg body weight dosage, and a single dose of the drug was suspended in 0.2 ml of normal saline (0.9 g/dL NaCl) solution.

Experimental Procedure

Thirty (30) male albino rats weighing (100-118g) were used. The rats were assigned to six experimental groups of five rats each.
Group A received 0.2 ml distilled water intraperitoneally (ip)
Group B received 10mg/kg of DFS ip.
Group C received 200mg/kg of HLEES orally.
Group D received 400mg/kg of HLEES orally.
Group E received orally 10mg/kg of DFS ip and 200mg/kg of HLEES.
Group F received 10mg/kg of DFS ip and 400mg/kg of HLEES orally.

All treatments with DS and HLEES were done concurrently for 21 days. During this treatment, the rats were observed for signs of toxicity and death daily.

Animal Sacrifice and Organ Collection

On day 20, the rats were fasted overnight, individually weighed, and their ultimate weights were recorded. On day 21, the rats were sacrificed after sodium pentobarbital anaesthesia (100 mg/kg i.p.) and sacrificed by cervical dislocation.

Organ weights

The testes, epididymides, and accessory sex organs (seminal vesicles, vas deference, and prostate glands) were removed and dissected, and the index weight (I.W.) of the excised organs was computed as follows: I.W. = organ weight (g) / body weight (g) ×100%, and the average value obtained for each paired organ was considered one observation, with values, reported as g/100 g body weight. Each animal's testis was fixed in Bouin's fluid for histological examination.

Testis Index, Testicular Coefficient, and Gonadosomatic Index

The testis index was calculated by dividing the left testis weight by the total b.w. and multiplied by 100 (Dikhil *et al*., 2013) Testicular coefficient was calculated by dividing the total organ weight by b.w. and then multiplying it by 100 (Yu *et al*., 2014). Gonadosomatic index (GSI) was calculated by dividing gonad weight with total b.w. and then multiplied by 100, where gonad weight = (weight of the right testis + weight of the left testis)/2 (Latif *et al*., 2008).

Semen Analysis

Sperm motility

Determination of cauda epididymal sperm motility was done using the method described by Abu *et al*. (2016). The individual motility was determined by the formula;

$$\text{Motility (individual) (\%)} = \frac{\text{Number of motile sperm}}{\text{Total number . of sperm (motile+immotile)}} \times 100$$

Sperm Concentration

Sperm count was determined using an improved Neubauer hemocytometer by the method described by (WHO, 1999; Oyeyemi and Ajani, 2015. Epididymal spermatozoa were obtained by the invasive opening of the cauda epididymis and released into a sterile universal specimen bottle, containing 1 ml of normal saline. Briefly, 5 µl of epididymal fluid was delivered onto a glass slide covered with a 22×22 mm coverslip and examined under the light microscope at a magnification of ×400. The microscopic field was scanned systematically and each spermatozoon encountered was assessed.

Sperm Viability Test

The viability (percentage of live spermatozoa was determined using an eosin nigrosin stain as described by (WHO, 1999; Gupta, 2014).

$$\text{Viability (\%)} = \frac{\text{Number of viable sperm}}{\text{Total number of sperm (viable + non - viable)}} \times 100$$

Sperm Morphologies

Sperm morphology was determined by examining air-dried slides under oil immersion as described by WHO (1999). The sperm cells were scored as follows:

Normal Morphology: Sperms with normal head and tail. Abnormal morphology: sperm cells with isolated heads – misshapen head or not; head misshapen head with abnormal tail and fused sperm. The percentage of abnormal forms was evaluated; Normal semen has fewer than 30% of abnormal forms (Sood, 2016).

Determination of Acrosome Integrity

The sperm acrosome integrity was determined by the method described by Dott and Foster. (1972). Acrosome integrity was determined by placing a drop (100 µL) of sperm sample on a clean, grease-free slide and mixed with a single drop of Giemsa stain. The spermatozoa were allowed to interact with the stain for at least 2 min and then a smear was prepared. The prepared smear was air-dried and examined under an oil immersion objective (100× magnification) to determine the percentage of spermatozoa with intact acrosomes. The spermatozoa that pick the Eosin-Nigrosine stain means acrosome integrity is compromised or dead. The spermatozoa with intact acrosome integrity do not pick the stain. The mean results were expressed as percent intact acrosomes.

Histopathological Investigations

For the histopathological studies, the testicular tissues were fixed in Bouin's fluid for 24 h and fixed tissues proceeded for paraffin embedding, staining with hematoxylin–eosin (H&E), and sectioned at 5µ mM thick sections. Microscopic observations were made at 200× magnifications with proper resolution and subsequently made microphotography (Obeys *et al.*, 2013, Vyas *et al*., 2016)

Statistical Analysis

The data were computed using Prism Graph Pad software (Graph Pad Company, USA) program version 8.01 and presented as Mean ± SEM. Statistical analysis was performed using the one-way analysis of variance (ANOVA) method, followed by the Tuckey

post hoc test. The significance of differences was set at $P < 0.05$.

RESULTS

Table 1: Effect of HLEES and DFS on the body weight, relative organ weights, and the organosomatic index of male Wistar rats

Weights (g)	Treatment Groups					
	Control	DFS	HLEES 200 mg/kg	HLEES 400 mg/kg	DFS+ HLEES 200 mg/kg	DFS+ HLEES 400 mg/kg
Initial	175± 11.5	183± 14.1 [a]	173± 11.9	189± 17.9	186±1.45	185±1.15
Final	221±16.2	164± 8.72 [a]	220± 12.0 [c]	131± 16.6 [d]	179±0.89	178±0.91
Left Testes	1.50±0.1	0.93±0.02 [a]	1.53± 0.06 [b]	1.55±0.4 [b]	1.45±0.01 [b]	1.44±0.00 [b]
Right Testes	1.57±0.7	0.9± 0.02 [a]	1.3± 0.06 [b]	1.19±0.0 [b]	1.13±0.05 [b]	1.27±0.01 [b]
Epid*.	0.57±0.01	0.24±0.02 [a]	0.32±0.00 [b]	0.34±0.01 [c]	0.21±0.02	0.25±0.03
Vas d**	0.08± 0.02	0.06±0.01 [a]	0.07± 0.01 [b]	0.06± 0.02 [c]	0.06±0.02	0.05±0.01
S. V*.**	1.71 ±0.08	0.08±0.01 [a]	1.42±0.09 [b]	1.37±0.05 [c]	1.16±0.01	1.35±0.08
Pros**.**	0.17± 0.08	0.08± 0.01 [a]	1.42± 0.09 [b]	1.37± 0.01 [b]	0.16±0.01 [b]	0.35±0.03 [b]
Testis Index (TI)	0.68±0.01	0.56±0.01 [a]	0.68±0.01	0.86±0.01	0.81±0.01 [b]	0.79±0.01 [b]
Test Coeff (TC)	1.37±0.01	1.11±0.01	2.81±0.01	2.07±0.01	1.45±0.01	1.44±0.01
Gonado Ind (GI)	1.55±0.02	0.90±0.01 [a]	1.44±0.01	1.35±0.01	1.27±0.02 [b]	1.35±0.01 [b]

Values are mean ± SEM. n=5. Values with different alphabet superscripts on the same column are significantly ($p< 0.05$) different.

Key: Epid* = Epididymis, Vas d ** =Vas deferens, S.V*** Seminal Vesicles' Pros**** =Prostate

In contrast with the control group and the groups that received only HLEES, DFS administration caused a significant ($p< 0.05$) decrease in Body weight, relative organ weights, TI, TC, and GI in the rats administered DFS. However, when the rats received dose-dependent co-treatments of HLEES and DFS, there was a significant (p 0.05) reversal effect.

Table 2: HLEES assuages sperm motility, viability, concentration, morphology, and acrosome integrity in DFS-treated male Wistar rats

Dose (mg/kg)	Sperm Motility (%)	Viability (%)		Sperm Conc*. ($x10^6$/ml)	Morphology (%)		Acrosome Integrity (%)	
		Live Sperm	Dead Sperm		Normal	Abnormal	Intact	Not Intact
Control	36.67±0.82	120±6.10	3.10±0.00	34.57±0.72	133.7±0.9	18.0±1.00	121±2.11	68.8±2.11
DFS	18.23±0.5	65.7±5.47	8.3±1.10	20.32±1.1	66.33±1.20	126.0±1.73	110±2.06	82.00±1.17
HLEES 200 mg/kg	33.0± 0.8	119±5.30	4.2±0.07	33.0± 0.8	132.7±5.24	14.67±0.33	122±4.68	68.7±4.68
HLEES 400 mg/kg	35.33±0.33	123±5.32	4.3±0.07	37.30±0.23	141.3±4.4	19.67±1.06	120±4.41	61.3±4.11
HELEE 200mg/kg + DFS	20.0±1.20	74.5±2.51	3.4±0.02	24.56±1.70	94.33±2.33	28.7±0.80	113.7±1.00	65.6±3.56
HELEE 400mg/kg + DFS	22.00±2.08	77.3±3.53[a]	3.2 ±0.12	25.67±1.73	115.3±3.71	25.7±0.33	113.3±1.35	65.6±3.56

Values are mean ± SEM. n=5. Values with different alphabet superscripts on the same column are significantly ($p<0.05$) different.Key: * = Sperm Conc= Sperm concentration, % = Percentage

Treatment of rats with HLEES alone at graded doses did not affect the parameters studied as compared to the control group. Rats administered DFS alone showed a significant decrease in sperm motility, and sperm concentration, and a significant increase in the percentage of abnormal sperm and acrosomal integrity (dead) relative to the control group. But interestingly, co-treatment of the rats with HLEES with DFS significantly showed an increase in these aforementioned parameters (Table 2).

HLEES treatment modulated the testicular injury induced by DFS in male Wistar rats

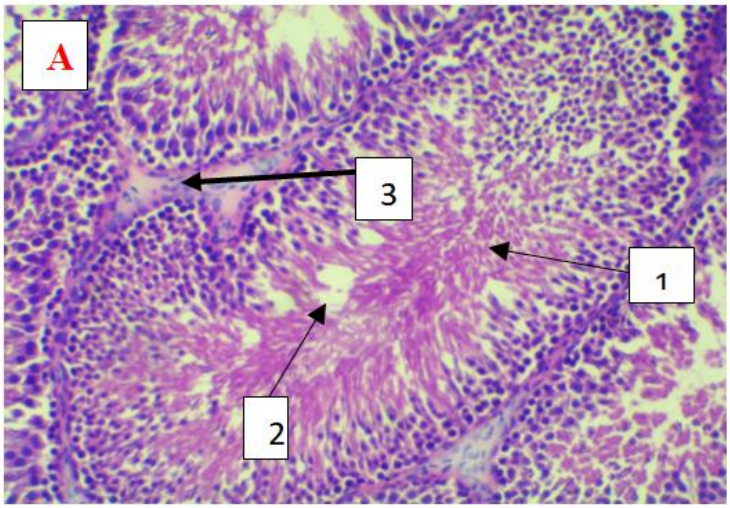

Fig. 1 (A): Section through the Testis of control rat showing normal Seminiferous tubules with copious spermatozoa (1) normal lumen of Seminiferous tubules & germinal layers, (2) normal Interstitium (3) H&E, 40X

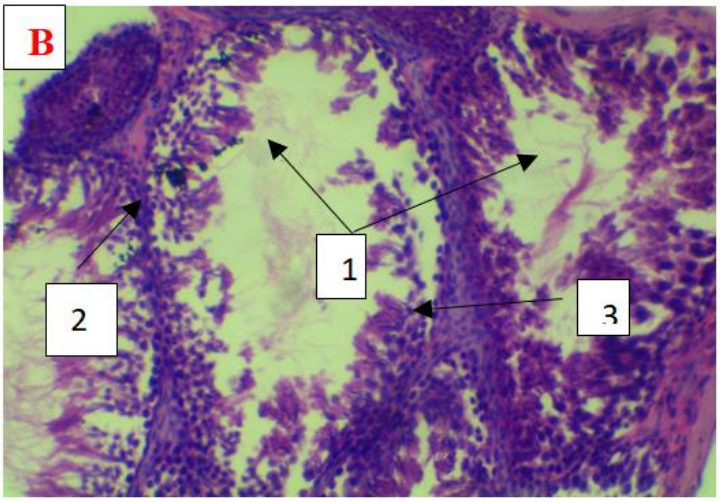

Fig. 1 (B): Section through the testis of the therapeutic (DFS) group showing, sloughing off of the seminiferous tubules with vacuolation and degeneration with the depletion in the germinal layer resulting in increased space between it (1) Increased thickens the wall of Seminiferous tubules (2) Degeneration of Leydig cells & the interstitium (3) & (4) H&E, 40X.

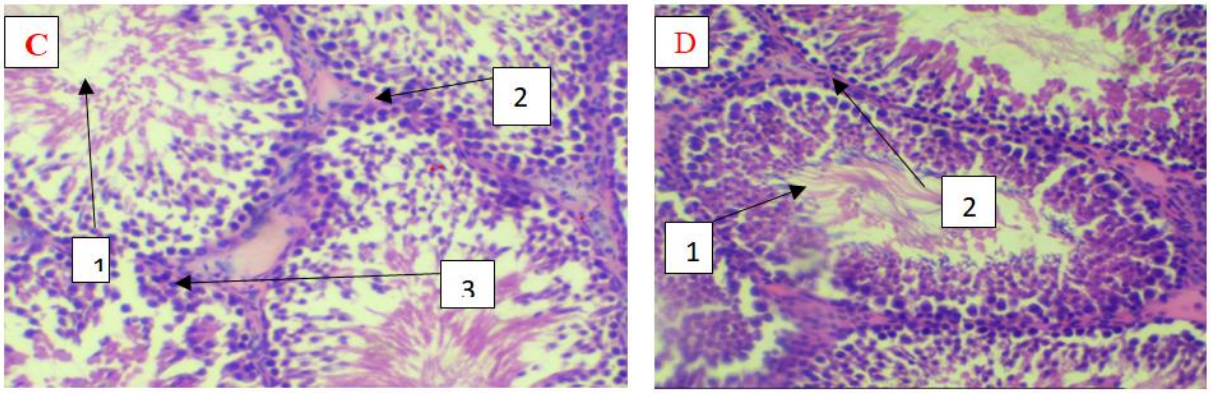

Figure 1 (C and D): Histo-architecture of testis of groups C and D (HLEES 200 and 400 mg/kg) (1) the seminiferous tubules are covered by basal lamina which was lined with stratified germinal epithelium, involved Sertoli and germ cells in various stages of spermatogenesis. Leydig cells or interstitial cells exhibit in between inter seminiferous tubules. Spermatogonia (2) Thickened interstitial tissues (3) the lumen of the seminiferous tubules showed vacuolation in some regions.

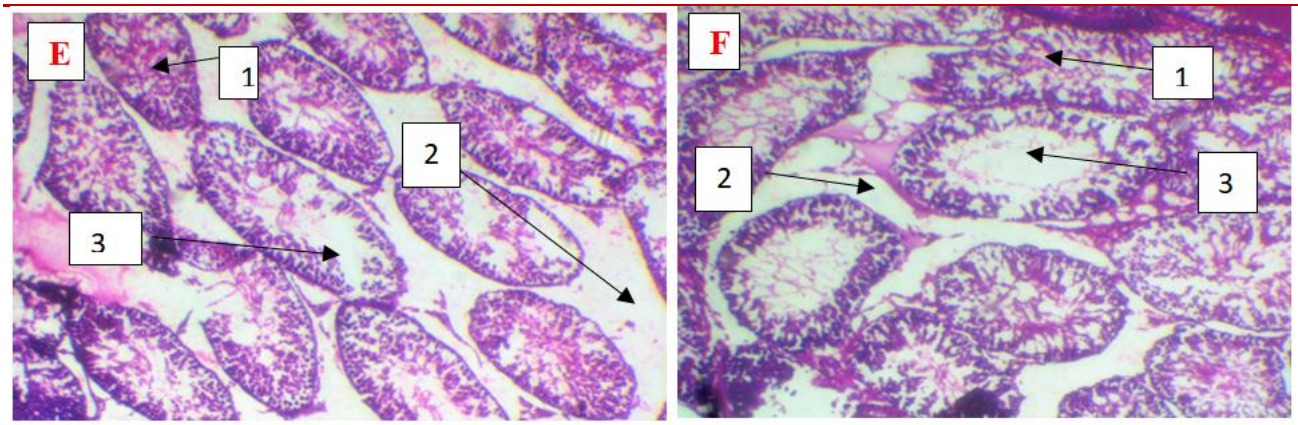

Fig. 1 (E and F): Shows the histo-architecture of DFS plus HLEES HLEES 200 and 400 mg/kg respectively this group showed some amelioration in the effects of DFS with resolved lesions

DISCUSSIONS

Diverse types of toxic elements, drugs, endocrine disruptors, and intensified physical shocks have been shown to have negative effects on male fertility, and some of these toxicants are known to target testicular activities causing a diminution in male reproduction capacity (Gan, 2010; Campion *et al*. 2012; Jana and Sen, 2012; Jeng, 2014; Sweeney *et al*., 2015). Organ toxicity associated with nonsteroidal anti-inflammatory drugs (NSAIDs) was previously recognized as a prevalent feature and related to adverse effects (Subramanian, 2009; Gan, 2010; Vohra and Raut, 2016). The current study also aimed to investigate the reversal effects of *Erythrina senegalensis* hydroethanolic leaf extract on DFS-induced testicular and epididymal perturbation in male Wistar strain albino rats.

In this study, rats treated with DFS only at the dose of 10 mg/kg have significantly lower body weights when compared with rats in the control and the groups that were administered HLEES only. The reduction in body weight may have been caused by the reported study-related decrease in food intake, intestinal nutrient malabsorption, and impaired feeding conversion. This may be linked to DFS's significant effect on the hypothalamus endocrine function, which controls appetite and water intake. This finding is in line with what Agu *et al.* (2020), reported in an experiment with animals exposed to administered an allopathic drug. However, the groups co-administered DFS and HLEES at varying doses showed a significant increase in body weights. The increase in body weight of rats concurrently treated with HLEES and DF shows that HLEES treatment prevents reduction in body mass and improves the rats' fitness. These attributes showed the beneficial effects of HLEES during DF treatment in rats.

Treatment of rats with DFS only showed a significant reduction in the testicular and accessory organ weights and Agu *et al*. (2020) have previously stated that reduced testicular and accessory organ weights are two credible predictors of gonadal toxicity. It is also acknowledged that the weight of the reproductive organs is a reliable indicator of the level of spermatogenesis in rats (Raji *et al*., 2005). Correspondingly, it is asserted that a significant change in the absolute or relative weight of an organ following the administration of a drug is a marker of the drug's toxic effects (Maina *et al*., 2008). Thus, the decrease in testicular weight following daily DFS treatment demonstrates the drug's deleterious effects on rat testes. This may be the typically influenced of abnormal reactive oxygen species (ROS) generation in the testicular tissues (Azu *et al*., 2010). The co-treatment of DFS with HLEES at 200 mg/kg and 400 mg/kg in groups 5 and 6, respectively, alleviated the loss in the weight of the accessory reproductive organs and the testes in the rats. Inhibited steroidogenesis and spermatogenesis may have contributed to the considerable weight loss of the reproductive organs in the current investigation. Since the amount of differentiated spermatogenic cells constitute a significant component of the testicular weight, a decrease in testicular weight would appear to imply a disruption to the testicular germ cells (Aly and Hassan, 2018). There was a significant decrease in the testis index (TI), gonadosomatic index (GSI), and testicular coefficient (TC) in the group that was administered DFS only relative to the control and the groups that were administered HLEES only (Table 1). However, the co-administration of DFS and HLEES caused a significant increase in the GSI, TC, and TI in the experimental rats. GSI is inversely proportional to the reproductive efficiency of the rats (Silva *et al*., 2014), and the testicular coefficient (TCT) value, which correlates with testicular reproductive toxicity (Yuan *et al*., 2012).

One important biomarker of chemical toxicity in male reproduction in animals is spermiotoxicity (Reddy *et al*., 2011), and it is established that the spermatozoa produced by the testes are transported, concentrated, stored, matured, and preserved by the epididymis (Adedara *et al*., 2017). This study

demonstrates that HLEES co-administration protected against DFS-induced epididymal toxicity. DFS's noxious effects on the spermatozoa and epididymal internal milieu are indicated by the significant diminution in the functional sperm dynamics, more specifically, declines in sperm motility, concentration, and count when compared with those of the control group and this result agrees with the earlier reported by other authors (Fausto *et al.* 2001, Winnall *et al*. 2007, Brunetti *et al*. 2010; Jana and Sen 2012). The lipid peroxidation of unsaturated fatty acids in the sperm plasma membrane, which results in a loss of fluidity and function, could be the cause of the lower sperm concentration, motility, and typical sperm morphology in DFS-treated rats (Aitken *et al*., 1993). The significant decrease in sperm capacitation may be due to the harmful effects of DFS on the flagellum, a vital part of the propulsion mechanism of the sperm cells. Sperm that are of low counts, slow or immobile have a lower chance of passing through the cervical mucosa for fertilization, and limited oval fertilization results in sterility (Gupta *et al*., 2006).

Acute drug exposure has also been shown to cause an increase in germ-cell apoptosis in laboratory animals. Additionally, it may result in spermatogenic cell degeneration, azoospermia, and decreased weights of the reproductive organs (Amin *et al*., 2008; Turk *et al*., 2008). In the current investigation, the DFS-treated group's (group 2) ability to fertilize was severely diminished. High percentages of abnormal/deformed sperm cells compared to the control could be the cause of the rats' decreased fertility. Excitingly, HLEES appears to have therapeutic potential against DFS-mediated spermatotoxicity in treated rats as evidenced by a significant increase in the functional sperm parameters (sperm count, concentration, motility, and acrosomal integrity (live sperm cells) in co-administered DFS and HLEES rats. Given the dose-dependent improvement in sperm parameters observed, it is plausible that HLEES also possesses spermatogenic potentials. This is because the antioxidant found in ES may be responsible for this effect. However, this study's drawback is the dearth of a comprehensive analysis of the impact of several epididymal areas, including the caput, and corpus, on sperm functionality.

The histological analysis of the control rats' testes shows normal seminiferous tubules, the normal lumen of seminiferous tubules with copious spermatozoa at different stages of development, germinal layers, and normal interstitium. However, treatments with DFS alone show perniciousness as evidenced by diminished organosomatic index, testicular cell population dynamics and aberrant testis histology. Furthermore, there is degeneration, vacuolation, and apoptosis in spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids rigorously affected by high doses of DFS causing the disruption of the Sertoli cells' microenvironment and this may affect the machinery for protein synthesis required for germ cell differentiation. The normal testis secretes most of the specific protein throughout the spermatids' elongation and sperm maturation (Monsees *et al*., 2000; Karaguzel *et al*., 2014). The groups that received HLEES only 200 and 400 mg/kg (Fig1 C and D) showed seminiferous tubules covered by a basal lamina and involved Sertoli and germ cells in various stages of spermatogenesis. Leydig cells or interstitial cells exhibit in between inter seminiferous tubules. Spermatogonia are oval in shape and rest on the basal lamina of seminiferous tubules, directly above them are spherical primary spermatocytes, recognized by copious cytoplasm and large nuclei. The group that received HLEES at 400 mg/kg show normal testicular architecture but has more space between the seminiferous tubules.

It is intriguing to observe how the toxic effects of DFS on the testicular tissues were substantially lowered when HLEES and DFS were administered together, as shown in Fig. 1 (E and F). This finding suggests that HLEES has therapeutic potential for improving the morphology of the testicular cells and maintaining physically and functionally active seminiferous tubules.

CONCLUSION

By reducing the spermiotoxic effects and histological alterations caused by DFS, the current study's findings imply that HLEES has therapeutic benefits against DFS-induced testicular and epididymal pertubation. ES may be a viable treatment option that might provide defence against the testicular and epididymal damage caused by DFS and other drugs. The molecular mechanism behind its inhibitory action requires further investigation.

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Statement of Ethical Approval

All procedures performed in experiments involving experimental animals were approved by the Ethics Committee, College of Veterinary Medicine, Federal University of Agriculture, Makurdi, Benue State, Nigeria.

Competing Interest: The authors declare no competing interests.

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