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Ameliorative effect of aqueous *Cissus populnea* suspension on cotton seed-induced testicular damage in male Wistar rats

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Abstract

Background: Testicular damage is an important etiological factor in male infertility. Despite reported decline in global incidence of infertility over the past years, pockets of cases are still ironically noticed to occur in developing countries due to limitation of accessibility to advanced management methods, hence their resort to alternative herbal therapy.

Methods: Testicular damage was induced using cotton seed. *Cissus populnea* was cut into chunks, air-dried, pulverized, powdered and suspended in water. Thirty-two (32) matured male Wistar rats were randomly divided into 4 groups (Group 1–4) designated as control, 100 mg/kg *C. populnea* (CP), cotton seed meal (CSM) and CSM + CP groups, each consisting of 8 rats. Group 1 was fed with normal rat chow, Group 2 was fed with 100 mg/kg CP, and Group 3 was fed with CSM for 8 weeks. Rats in Group 4 were fed with CSM for 8 weeks and dosed with 100 mg/kg aqueous *C. populnea* suspension for another 8 weeks. At the expiration of test period, the rats were sacrificed, blood sample collected, and plasma obtained for luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, estrogen, catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) measurements. Semen was collected for analysis and testes harvested for histological studies.

Result: There is a significant decrease ($p < 0.05$) in plasma FSH, LH, testosterone, estrogen, GSH, catalase, SOD, and GPx in rats fed on CSM when compared with values obtained in the control and aqueous *C. populnea* suspension-fed rats. Seminal fluid analysis showed a significant reduction ($p < 0.05$) in the sperm count, motility, morphology, vitality, and non-vitality among rats fed with CSM when compared to control rats. The histologic features of the testes showed abnormal interstitial appearances and absent Leydig cells in many areas among cotton seed-fed rats. Improvements in reproductive hormones, sperm qualities, and histological features were observed to occur in CSM group following administration of aqueous *C. populnea* suspension.

Conclusion: Based on the findings from this study, it can be concluded that aqueous *C. populnea* suspension ameliorates cotton seed-induced hypothalamo-pituitary–testicular axis functional disruption and testicular damage.

Keywords: Cotton seed, *Cissus populnea*, Testicular damage, Reproductive hormones, Antioxidant

1 Background

Generally, testicular damage as a contributor to male infertility manifests as alteration in sperm quality (concentration, morphology and motility) in at least one of two seminal fluid analyses, collected between 1 and 4 weeks apart [1]. The importance attached to

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child-bearing ranges from its natural or religious values to traditional or sociocultural values; varies among tribes, ethnic groups and races; and determines the extent to which solutions are sourced for. Globally, the prevalence of infertility varies widely, being less in developed countries and more in developing countries, where limited resources for its management (investigation, diagnosis, and treatment) exists [2]. Risk factors associated with male infertility include age > 30 years, obesity, occupational exposure to harmful physical and chemical agents (benzene, carbon disulfide, ethers, glycol toluene, and xylene), pesticide (Dibromochloropropane and Ethylenedibromide), occupational stress, long-term strenuous exercise, diet (alcohol, caffeinated drinks) and long-term testicular exposure to heat and electronic devices (laptops, cell phones) [3–9]. Drugs such as antineoplastic agents (busulfan, cyclophosphamide, chlorambucil, and methotrexate), glucocorticosteroids, hormonal steroids (diethylstilbestrol, estrogen, medroxyprogesterone acetate), antibiotics (cotrimoxazole and sulfasalazine), thyroid supplements, spironolactone, cimetidine, colchicine, marijuana, neuroleptic agents and opiates are also implicated [3]. The male is solely responsible for about 20% of all cases of infertility and contributory in about 50% of all infertility cases [10]. Identifiable causes of male infertility could either be hormonal or damage at the hypothalamus, pituitary or testicular level, which in some instances translates to features suggestive of hypogonadotrophism or hypogonadism as underlying factors.

Advancements in technology has made problems initially believed to be unsurmountable, a simple exercise to proffer solutions to. Infertility treatments range from simple medication therapy to very expensive and invasive induction and manipulation of eggs and sperm [11]. Despite global improvements in infertility management, there are still rising cases of male infertility in Nigeria. This could be ascribed to inaccessibility to modern diagnostic equipment and prevailing economic hardships with consequent constraints in the affordability of its management cost. Thus, people who cannot bear the cost but believe in alternative medicine and those with previous failed infertility treatments are inclined to searching for readily available and cheaper alternative herbal remedies in order to restore their child-bearing potentials. *Cissus populnea,* commonly known as 'Okoho' by the Idoma, Igbo and Igala tribes; 'Dafara' or 'Latutuwa' by the Hausas; 'Orogboro,' 'Ajara,' or 'Afato' by the Yoruba tribes of Nigeria, is one of such herbs generally considered to enhance fertility and reproductive capability in man, though base mostly on anecdotal belief with little or no scientific basis. While some researchers reported that the plant has fertility enhancement potentials [12], others reported that the plant has no effect on fertility [13].

Before clinical trials of any kind in human, similitudes of human disease conditions are usually induced in animal models for scientific understanding of the pathological basis of the condition and identification of the point of intervention that will ameliorate the condition. This study thus created an artificial testicular structural dysfunction using cotton seed meal and determined the ameliorating effect of aqueous *Cissus populnea* suspension in male Wistar rats.

2 Methods

2.1 Preparation of cotton seed meal

The cotton seed meal (CSM) was prepared using Ground corn (329 g/kg), Soybean meal (234 g/kg), Whole cotton seed (390 g/kg), Vitamins and Mineral salt (47 g/kg) [14].

2.2 Care of animals

The care of rats was done in accordance with the US Public Health Service Guidelines [15]. Cages were made of non-toxic plastic materials spacious enough to prevent rat escape; allows for the rigors of regular cleaning, disinfection and rat handlings; prevents accidental entrapment of rats; and free of sharp edges or projections that may injure the rats. Animal house is well ventilated, relatively silent from noise, maintained at a room temperature of 22–28 °C and under the natural cycle of daylight and night darkness.

2.3 Experimental design

The research was a longitudinal study involving thirty-two (32) adults male Wistar rats which were grouped as follows:

Group 1 (n = 8): Fed on normal rat chow and water ad libitum for 8 weeks.

Group 2 (n = 8): Fed on normal rat chow and 100 mg/kg aqueous *Cissus populnea* suspension for 8 weeks.

Group 3 (n = 8): Fed on cotton seed diet for 8 weeks.

Group 4 (n = 8): Fed on cotton seed diet for 8 weeks after which 100 mg/kg aqueous *Cissus populnea* suspension were administered for another 8 weeks.

The various doses of aqueous *Cissus populnea* suspension were freshly constituted shortly before dosing.

2.4 Specimen collection, storage and processing

After the completion of the experiment, all rats were anesthetized (using diethyl ether as anesthetic agent) and blood was collected via ocular puncture and cardiac puncture into heparinized and EDTA bottles. The collected blood samples were centrifuged at 5000 rpm for 5 min to obtain the plasma which was stored at − 20 °C till assayed. The epididymis was harvested for semen collection and estimation of sperm parameters. Also, the

testes were harvested and kept in Bouin solution until fixed for histological studies.

2.5 Determination of epidemical sperm parameters

Sperm density was determined by method described by Anthony et al. [16]. Sperm count was done using the improved Neubauer hemocytometer, and sperm morphology was assessed under dark field microscopy to detect any abnormalities in head, neck, mid-piece or tail regions.

2.6 Hormonal and biochemical assay

Plasma follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and estrogen were assayed using Accu bind ELISA kits as described by the manufacturer. Calibration curves were generated using the absorbance obtained for the calibrators against their concentrations, and concentrations of the hormone were generated from their absorbance read-off of the calibration curves [17]. Plasma glutathione (GSH), glutathione peroxidase (GPx), catalase, and superoxide dismutase (SOD) activities were assayed using Randox commercial kits (Randox Laboratories Ltd UK) [18–21].

2.7 Histological studies

The testes were cut into 0.5 cm thick slabs and fixed in Bouin solution for 72 h. They were then passed through graded alcohol, cleared in xylene, embedded in molten paraffin, and blocked out. Serial sections of 5 µm thick were cut from these blocks, stained with hematoxylin and eosin stains, and examined under light microscopes (CETI, UK).

3 Data analysis

The statistical analysis was done using SPSS package for windows version 25.0. Descriptive statistics was used to describe and represent variables. Differences in mean between the groups were determined using one-way ANOVA. The level of significance was set at $p < 0.05$.

4 Results

4.1 Serum hormones

Result of reproductive hormone showed a significant decrease ($p < 0.05$) in plasma FSH, LH, testosterone and estrogen in rats fed on CSM when compared with values obtained in the control and aqueous *Cissus populnea* suspension-fed rats (Table 1).

4.2 Antioxidant status in male Wistar rats

Result of plasma antioxidant concentrations revealed a significant decrease ($p < 0.005$) in plasma concentrations of GSH, catalase, SOD, and GPx in rats fed on CSM when compared to values obtained from those administered with CP (Table 2).

4.3 Sperm qualities in male Wistar rats

Result of seminal fluid analysis showed a significant reduction ($p < 0.05$) in the sperm count, motility, morphology, vitality, and non-vitality among rats fed with CSM when compared to control rats (Table 3).

4.4 Photomicrograph of the testes

The histologic features of the testes showed normal seminiferous tubules, interstitium and Leydig cells appearances; and adluminal area packed with spermatozoa

Table 1 Effect of aqueous *Cissus populnea* suspension on serum hormones in male Wistar rats

Parameters	Control	100 mg/kg CP	CSM	CSM + 100 mg/kg CP	*F* value	*p* value
FSH (mIU/mL)	3.80 ± 0.25	4.47 ± 0.36	2.64 ± 0.63	3.32 ± 0.44	19.214	0.000
H (mIU/mL)	2.60 ± 0.16	3.01 ± 0.10	1.90 ± 0.82	2.41 ± 0.61	14.226	0.000
EST (ng/mL)	3.80 ± 0.28	3.94 ± 0.16	2.33 ± 0.24	2.50 ± 0.43	61.178	0.000
Estrogen (ng/mL)	25.11 ± 0.78	27.72 ± 1.16	13.29 ± 1.80	17.91 ± 1.51	72.665	0.000

Values are mean standard deviation (mean ± standard deviation) and level of statistical significance considered at $p < 0.05$. Where *FSH* follicle stimulating hormones, *LH* luteinizing hormones, *TEST* testosterone, *CP Cissus populnea, CSM* cotton seed meal

Table 2 Effect of aqueous *Cissus populnea* suspension on antioxidant status in male Wistar rats

Parameters	Control	100 mg/kg CP	CSM	CSM + 100 mg/kg CP	*F* value	*p* value
Cat (mmol/L)	69.71 ± 4.63	79.18 ± 2.81	45.00 ± 2.77	52.18 ± 6.88	94.604	0.000
SOD (U/mg prt)	2.99 ± 0.20	4.29 ± 0.11	2.02 ± 0.10	2.63 ± 0.24	6.920	0.000
GPx (ng/g)	26.76 ± 0.56	27.66 ± 1.67	21.53 ± 6.10	22.20 ± 4.68	25.445	0.001
GSH (ng/g)	4.24 ± 0.22	5.36 ± 0.34	3.00 ± 0.41	4.30 ± 0.22	4.360	0.000

Values are mean standard deviation (mean ± standard deviation) and level of statistical significance considered at $p < 0.05$. Where *Cat* catalase, *SOD* superoxide dismutase, *GPX* glutathione peroxidase, *GSH* glutathione, *CP Cissus populnea*, *CSM* cotton seed meal

Table 3 Effect of aqueous *Cissus populnea* suspension on sperm qualities in male Wistar rats

Parameters	Control	100 mg/kg CP	CSM	CSM + 100 mg/kg CP	*F* value	*p* value
Count ($\times 10^6$)	21.00 ± 3.43	36.83 ± 5.39	7.33 ± 2.15	14.30 ± 1.50	72.705	0.000
Motility (%)	91.00 ± 2.93	96.86 ± 1.35	45.50 ± 5.73	50.00 ± 23.91	47.905	0.000
Morphology (%)	3.75 ± 0.46	3.74 ± 0.49	3.25 ± 0.46	3.40 ± 0.00	2.605	0.039
Vitality (%)	87.50 ± 2.67	94.00 ± 3.83	84.25 ± 4.05	85.50 ± 7.03	6.256	0.000
Non-vitality (%)	1.125 ± 0.230	1.125 ± 0.232	1.000 ± 0.000	1.121 ± 0.233	1.803	0.134

Values are mean standard deviation (mean ± standard deviation) and level of statistical significance considered at $p < 0.05$. Where *CP Cissus populnea, CSM* cotton seed meal

among the control; and abnormal interstitial appearances and absent Leydig cells were observed in many areas among cotton seed-fed rats (Fig. 1).

5 Discussion

Male reproductive functions are controlled by different hormones notably LH, FSH, estrogen and testosterone [22]. The plasma activities of these hormones are a function of the hypothalamo-pituitary–testicular axis which is initiated by the pulsatile release of gonadotropin releasing hormone from the hypothalamus [22]. This, in turn, stimulates the secretion of anterior pituitary trophic hormones, with LH acting on the testicular and Leydig cells for steroidogenesis and FSH acting on Sertoli cells for spermatogenesis [23]. Result obtained from this study showed a significant decrease ($p < 0.05$) in plasma concentrations of FSH, LH, testosterone and estrogen (2.64 ± 0.63, 1.90 ± 0.82, 2.33 ± 0.24, and 13.29 ± 1.80, respectively) in rats fed on cotton seed when compared with values obtained in the control rats (3.80 ± 0.25, 2.60 ± 0.16, 3.80 ± 0.28, 25.11 ± 0.78) and aqueous *Cissus populnea* suspension-fed rats (4.47 ± 0.33, 3.01 ± 0.10,

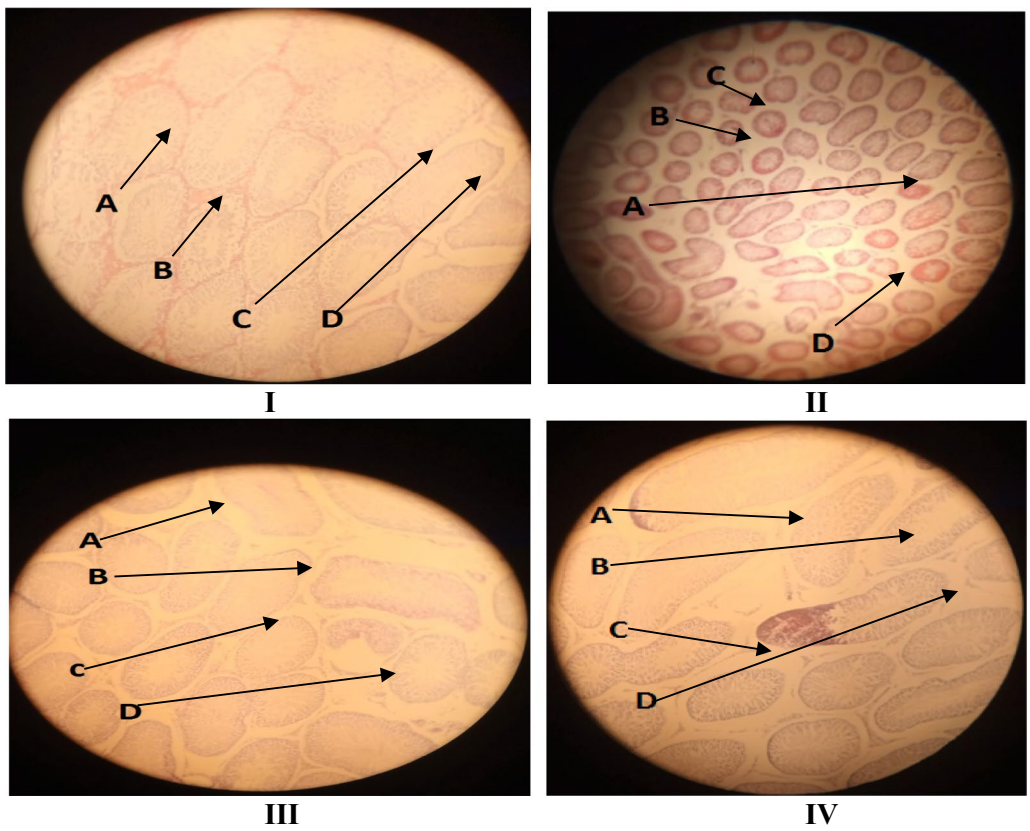

Fig. 1 Photomicrograph of the testes (Mag × 100). Where **A**: Seminiferous tubules, **B**: Lumen, **C**: Leydig cells, **D**: interstitium; I = Control, II = 100 mg/kg *Cissus populnea* suspension, III = Cotton seed, IV = Cotton seed + 100 mg/kg *Cissus populnea* suspension

3.94 ± 0.16, and 27.72 ± 1.16, respectively) (Table 1). These findings corroborate report from similar studies where animals fed on cotton seed showed a significant reduction in serum level of estrogen, FSH and LH [24, 25]. Meanwhile, improvements were observed in the concentrations of these hormones following administration of aqueous *Cissus populnea* suspension after initial cotton seed meal with values of 3.32 ± 0.44, 2.41 ± 0.61, 2.50 ± 0.43, and 17.91 ± 1.51, respectively, for FSH, LH, testosterone and estrogen as compared to 2.64 ± 0.63, 1.90 ± 0.82, 2.33 ± 0.24, and 13.29 ± 1.80, respectively, observed in rats fed on cotton seed only (Table 1). This corroborates reports from similar studies involving aqueous *Cissus populnea* root [26, 27] but is in contrast to others [13]. Cotton seed oil consumption has been implicated in the induction of infertility in human as it is rich in gossypol which have contraceptive effects and by decreasing sperm concentration, motility, and viability [28–30]. The observed ameliorative effect of aqueous *Cissus populnea* suspension on hormonal parameters may be due to exhibition of gonadotrophic activities by certain phytochemicals therein present on the pituitary gland thereby enhancing spermatogenesis and reproductive processes [31]. The significant decrease in plasma FSH, LH, and testosterone concentrations among cotton seed-fed rats is an indication that the damage done by the CSM also involves higher brain (hypothalamus) level.

Oxidative stress has been implicated in infertility. The extent of oxidative stress in a living system can be inferred from the concentration of antioxidants which is inversely proportional to its rate of usage. Result obtained from this study showed a significant decrease ($p < 0.05$) in plasma concentrations of catalase, SOD, and GPx, and GSH (45.00 ± 2.77, 2.02 ± 0.10, 21.53 ± 6.10, and 3.00 ± 0.41, respectively) in cotton seed-fed rats when compared to values obtained from control (69.71 ± 4.63, 2.99 ± 0.20, 26.76 ± 0.56 and 4.24 ± 0.22) and rats administered with *aqueous Cissus populnea* suspension (79.18 ± 2.81, 4.29 ± 0.11, 27.66 ± 1.67 and 5.36 ± 0.34, respectively) (Table 2). This indicates that the administered cotton seed increases systemic ROS generation which caused some degree of hypothalamic damage and affectation of the hypothalamo-pituitary–testicular axis. The administered aqueous *Cissus populnea* suspension however ameliorates the existing oxidative stress as indicated by improvements in the observed decrease in plasma catalase, SOD, GPx, and GSH concentrations to 52.18 ± 6.88, 2.63 ± 0.24, 22.20 ± 4.68, and 4.30 ± 0.22 respectively (Table 2). In this situation, the non-enzymatic (GSH) and enzymatic (catalase, SOD, and GPx) antioxidants act synergistically to scavenge the cotton seed-induced generated ROS and thus annul or minimize their damaging effects.

Seminal fluid analysis remains an important non-invasive investigative means of diagnosing male infertility. The generally acceptable parameters for assessing male fertility potentials include ejaculation volume ≥ 1.5 mL, sperm concentration ≥ 15 million spermatozoa/mL, and total sperm count ≥ 39 million spermatozoa per ejaculate [32–34]. Normal morphologic and vitality feature requires that at least 4% of spermatozoan are of normal form, 58% living, having 40% of total spermatozoan motile (progressive + nonprogressive) and 32% of this showing progressive movement [32–34]. Result from this study showed a significant reduction ($p < 0.05$) in the sperm parameters (Count 7.33 ± 2.15; motility 45.50 ± 5.73; morphology 3.25 ± 0.46; vitality 84.25 ± 4.05; and non-vitality 1.000 ± 0.000) among rats fed with cotton seed meal when compared to control rats (Count 21.00 ± 3.43, Motility 91.00 ± 2.93; morphology 3.75 ± 0.46; vitality 87.50 ± 2.67; and non-vitality 1.125 ± 0.230) and aqueous *Cissus populnea* suspension-fed rats (Count 36.83 ± 5.39, Motility 96.86 ± 1.35; morphology 3.74 ± 0.49; vitality 94.00 ± 3.83; and non-vitality 1.125 ± 0.232) (Table 3). Similar alterations in sperm characteristics had earlier been reported among Wistar rats fed with cotton seed by researchers [35, 36]. The observed low sperm count, morphologic alterations and reduction in sperm motility among rats fed with cotton seed meal are attributable to gossypol present in the administered CSM which has immobilizing effect on spermatozoa and generate large amounts of ROS that oxidatively damage the germinal epithelium and spermatozoan plasma membrane causing reduction in the spermatozoan qualities (counts, morphology, motility, viability), sperm dysfunction or even sperm death. Meanwhile, improvements in these parameters (Count 14.30 ± 1.50; motility 50.00 ± 23.91; morphology 4.00 ± 0.00; vitality 85.50 ± 7.03; and non-vitality 1.121 ± 0.233) were observed after the administration of aqueous *Cissus populnea* suspension. This is suggestive of spermatogenic effect of aqueous *Cissus populnea* suspension as against the earlier reported no effect on fertility [13]. The observed spermatogenic effect of aqueous *Cissus populnea* suspension is ascribed to the presence of phytochemicals with antioxidative activities which mop-up the gossypol-induced generated ROS thereby annulling the damaging effects on the testes and sperm cells. However, in vitro studies using Sertoli cell lines have been reported to increase cell proliferation which plays critical roles in spermatogenesis [31, 37].

The photomicrograph of the testes showed normal seminiferous tubules, interstitial and Leydig cells appearances; and adluminal area packed with spermatozoa among the control rats (Fig. 1). On the other hand, abnormal interstitial appearances and absent Leydig cells were observed in many areas among cotton seed-fed rats

(Fig. 1). Improvements in the histologic appearances were however observed to occur among rats administered with aqueous *Cissus populnea* suspension as evident by having few germinal cell loss, partial abnormal interstitial appearances, absent Leydig cells in few areas and adluminal areas sparingly packed with spermatozoa (Fig. 1). These findings agree with report from similar studies [31, 37] and confirm the observed improvements in serum hormones and other biochemical parameters by the administered aqueous *Cissus populnea* suspension. The observed testicular damage among rats fed with cotton seed reflects existing oxidative stress caused by the associated imbalance between systemic ROS generation and antioxidants activities. The observed improvements occur as a result of some phytochemicals present in the aqueous suspension which stimulate the hypothalamo-pituitary–testicular axis and regenerate the hypothalamus and germ cells in the testes.

6 Conclusion

It can be inferred from the results obtained in this study that aqueous *Cissus populnea* suspension ameliorates cotton seed-induced hypothalamo-pituitary–testicular axis functional disruption and testicular damage. The use of aqueous *Cissus populnea* suspension by the indigenous males in some parts of Nigeria to improve their reproductive capacity is thus justifiable. The fertility improvements identified with the extract could be ascribed to the antioxidant properties of certain phytochemicals therein present which act to mop-up ROS and stimulate intrinsic enzymatic and non-enzymatic antioxidant activities, as oxidative stress is known to be of etiological importance in infertility. For equivalent dose in human base on surface area, 16.2 mg/kg of aqueous *Cissus populnea* suspension is expected to have similar therapeutic effect in human as the 100 mg/kg of the extract administered to the rats.

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Authors' contributions
O.E.W. conceptualize the research, contributed to laboratory work, data collection and manuscript writing; F.A.O. contributed to purchase of rats recruitment, daily cleaning cages, dosing of rats, laboratory work, data collection contributed and manuscript revision; A.O.O. contributed to laboratory work and data collection and manuscript writing; M.A.A. contributed to recruitment of participants, data collection and manuscript writing; while A.A.A. contributed to laboratory work, data collection, data analysis, and manuscript editing. All authors read and approved the manuscript.

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Availability of data and materials
The data collected, analyzed and used for this study are available from the corresponding author specifically on reasonable request.

Declarations

Ethics approval and consent to participate
This research was carried out with strict compliance with the US Public Health Service Guidelines on animal handling. The approval for the research was given by the research ethics committee of the Faculty of Basic Medical Sciences on the 4th day of November 2020, with the reference number: FBMS/R/11/202. The rights of the animals were respected.

Consent for publication
Not applicable.

Competing interests
The authors declared that there is no competing interest.

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