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Research Article

Effects of Seed Fractions of *Buchholzia coriacea* on Reproductive Functions of Male Wistar Rats

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ABSTRACT

Buchholzia coriacea seeds have been reported to induce reproductive toxicity. In this study, the possible mechanism(s) by which these seeds induce male reproductive toxicity were examined. Methanol fraction (MFBC-50 and 100 mg/kg), hexane fraction (HFBC-50 mg/kg) or ethylacetate fraction (EFBC-50 mg/kg) of *B. coriacea* seeds were administered daily (p.o) for 6 weeks to rats and thereafter sacrificed. Sperm profile was examined microscopically while sex hormones were assayed using ELISA technique. Oxidative stress biomarkers were assayed spectrophotometrically in serum and testis. All treatment significantly decreased sperm motility and sperm count while sperm with defective morphology increased when compared with control. The MFBC (50 mg/kg) decreased luteinizing hormone (LH) and follicle stimulating hormone (FSH). The HFBC decreased testosterone, LH and FSH while EFBC decreased testosterone and FSH. Serum and testicular malondialdehyde (MDA) increased while serum and testicular superoxide (SOD), and testicular catalase decreased in HFBC and EFBC groups. MFBC (100 mg/kg) decreased serum SOD, testicular SOD and testicular catalase. Marked derangement of the testicular epithelium was observed in treated rats. *Buchholzia coriacea* seeds mediate male reproductive toxicity by precipitation of oxidative stress and suppression of the pituitary-testicular axis

Keywords: *Buchholzia coriacea, sperm profile, sex hormones, oxidative stress, toxicity*

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INTRODUCTION

Africa is richly endowed with diversity of plant resources and is estimated to contain about 45,000 different plant species with potentials for development. Out of these, about 5,000 species are used for medicinal purposes (Mahomoodally, 2013). Sometimes, a single medicinal plant is used in the treatment of various diseases and illnesses (Adjanohoun *et al*., 1996). One of such plants is *Buchholzia coriacea*. *Buchholzia coriacea* is a perennial plant with large glossy leaves and conspicuous cream white flowers in racemes at the end of the branches (Mbata *et al*., 2009). It is one of the 36 species of the *Capparidaceae* family and commonly found in Tropical African countries such as Nigeria, Gabon, Guinea and Cameroon. The seed is also known as wonderful kola, due to its supposed potency against a wide range of human diseases.

The use of medicinal plants is poorly regulated in Africa. It is therefore imperative for researchers to continually examine the effects of these healing plants on various systems of the body so as to avoid serious deleterious effects due to abuse of these medicinal plants (Helwig, 2005). The seeds of *B. coriacea* have mostly been reported to be beneficial to health. Some of the medicinal properties includes as antimicrobial (Ezekiel and Onyeoziri, 2009), anti-malarial (Okoli *et al*., 2010), anti-diabetic (Adisa *et al*., 2011), antihelmintic (Fred-Jaiyesimi, 2011), antinociceptive (Onasanwo *et al*., 2013) and antidepressant (Onasanwo *et al*., 2016).

However, a preliminary study using crude methanol *B. coriacea* seed extract suggests possible detrimental effect of on male reproductive physiology (Obembe *et al*., 2012). The authors documented a decrease in epididymal weight with autolysis of the constituent epididymal spermatozoa in treated rats. The mechanism of action was however not conclusive. This study aimed to evaluate the effects of methanol, hexane, and ethylacetate fractions of *B. coriacea* seeds on male reproductive parameters so as to determine the most potent fraction and the possible mechanism(s) of action.

MATERIALS AND METHODS

Plant Sample Collection: Fresh seeds of *B. coriacea* were obtained from Oje Market, Ibadan and were authenticated at

the herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where voucher number FHI 109920 was earlier assigned to a similar specimen. The seeds were washed thoroughly with distilled water to remove adhering particles, after which they were sliced, properly shade- dried and pulverized.

Extraction and isolation of *B. coriacea* fractions: Crude methanol extract of *B. coriacea* seeds was prepared as earlier described (Obembe *et al*., 2012)**.** Briefly, 3.7 kg of the dried macerated seeds was soaked in pure methanol for 72 hours and filtered using Whatman filter paper (1 mm). The filtrate was concentrated with rotary evaporator (Heidolphlaborota 400 Effiecient, Germany) at 40 ^{0}C. The crude extract was further concentrated using a vacuum oven at 40^{0}C and a pressure of 700 mmHg. The crude extract weighed 202.22 g and percentage yield was 5.47 %. Concentrated crude methanolic extract (150 g) was turned into a clean beaker and 100 ml of methanol was added and made into solution after which 100 ml of distilled water was added and stirred. The mixture was poured into a separating funnel and 200 ml of pure hexane was added and carefully shaken. This mixture was allowed to stand for 10 minutes in order to ensure proper partitioning of the two phases after which the hexane layer (top layer) was collected after releasing the methanol/water layer. This process was repeated until a clear layer was obtained for the hexane portion. The procedure was repeated using ethyl acetate to obtain the ethyl acetate fraction leaving behind the methanol fraction.

The various isolated fractions that were obtained were concentrated using the rotary evaporator and vacuum oven respectively at 40^{0}C. Hexane fraction (HFBC) obtained weighed 11.106 g and percentage yield was 0.07 %. Ethylacetate fraction (EFBC) obtained was 3.726 g (percentage yield 0.02 %) and methanol fraction (MFBC) was 101.2 g (percentage yield 0.63 %).

Animal grouping: All rats were housed in the Central Animal House of Osun State University, and were fed with standard rat pellets and clean water *ad libitum.* All procedures conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and use of animals as amended (2002) and all experiment was carried out in accordance with the National Institutes of Health Guide for Care and Use of Laboratory animals (2011). Twenty five male Wistar rats (180-200g) were used for this study. Rats were randomly assigned into 5 groups of 5 rats each. Group 1 served as the control and received the vehicle (Tween 80) only, Group 2 and 3 were treated with MFBC (50 and 100 mg/kg) respectively while Group 4 received HFBC (50 mg/kg) and Group 5 received EFBC (50 mg/kg). All treatment was administered by oral gavage once daily for duration of 6 weeks. The rats were then anaesthetized using sodium pentobarbital (30 mg/kg, i.p) and sacrificed by cervical dislocation. The testis, epididymis and hypothalamus were excised and preserved. Prior to sacrifice, serum was obtained for assay of sex hormones and oxidative stress biomarkers.

Sperm analysis: The excised caudal epididymis (left) was lacerated and sperm obtained was analyzed microscopically using Olympus research microscope (Olympus, Japan). The sperm was categorized as belonging to one of three motility categories - progressive, non-progressive and immotile, according to WHO guidelines (2010). Sperm viability was assessed by using the improved one step eosin-nigrosin staining technique. A fraction of each suspension of the sperm samples was mixed with equal volume of eosin-nigrosin stain and air dried smears were prepared on glass slides for each samples according to Bjorndal *et al* (2003). Normal live sperm cells exuded the eosin-nigrosin while dead sperm cells took up the stain. Sperm count was done according to Shi and Haug (1990), by counting in five Thoma chambers of improved Neubauer hemocytometer.

Hormonal assay: Serum levels of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) were assayed using the ELISA technique by commercially available kits. The kits were obtained from Calbiotech Inc. (California, USA) and contained the respective enzyme label, substrate reagent and quality control sample. The quality control was carried out at the beginning and end of the assay in other to ascertain acceptability with respect to bias and within variations. Testosterone kit used had a sensitivity of 0.075 ng/ml with intra and inter assay variations of 3.9 and 4.3 % respectively. Luteinizing hormone kit had a sensitivity of 0.12 mIU/ml with intra and inter variation of 7.6 and 10.83 % while FSH kit had a sensitivity 0.353 mIU/ml with intra and inter assay variation of 5.6 and 6.4 %.

Testicular homogenate: One of the each excised testis (left) were washed in 1.15 % KCl solution, blotted with filter paper and weighed. They were then homogenized on ice pack in four volumes of homogenizing fluid (phosphate buffered saline, pH 7.4) using Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 rpm for 10 mins in a cold centrifuge (4 ^{0}C) to obtain post mitochondria fraction. Biomarkers of oxidative stress were estimated from the supernatant obtained using bioassay kit.

Assay of oxidative stress biomarkers: Biomarkers of oxidative stress namely malondialdehyde (MDA), superoxide dismutase (SOD) and catalase were assessed spectrophotometrically from serum and testicular homogenate obtained using respective commercially available kits obtained from BioAssay systems (San Francisco, USA). The MDA assay was measured based on reaction of MDA with thiobarbituric acid (TBA), forming a MDA-TBA adduct that is quantified colometrically (Uchiyama and Mihara, 1978). Superoxide dismutase activity was determined by measuring the inhibition in photo-reduction of nitroblue tetrazolium (NBT) by SOD enzyme (Alici and Arabaci, 2016) and catalase was measured by monitoring decrease in absorbance resulting from decomposition of H_2O_2 (Aebi, 1984).

Histopathology: Excised right testis and right epididymis were immediately preserved in 4% paraformaldehyde for 6 hours. This was then replaced with Bouins' fluid. Tissues were fixed with 10% formalin, embedded in paraffin wax, and then

sectioned with a microtome to obtain 4-5 μm thick paraffin sections. De-waxed sections are then stained with hematoxylin and eosin and thereafter examined microscopically.

Statistical analysis: Data were expressed as mean ± standard error of mean (SEM). Comparison of means were made by one way analysis of variance (ANOVA) using SPSS version 16 (SPSS Inc., Chicago USA). P<0.05 was considered significant

RESULTS

Visceral and sex organ weights: Administration of various fractions of *B. coriacea* seeds had no significant effect on the weights of liver, kidney, spleen, lungs and brain of rats. Also, *B. coriacea* seeds had no effect on the weights of sex organs - testis, epididymis, seminal vesicle and prostate gland. However, HFBC significantly reduced the heart weight of treated rats when compared with the control (Table 1).

Sperm profile: Administration of MFBC (50 and 100 mg/kg), HFBC (50 mg/kg) and EFBC (50mg/kg) caused significant decrease in sperm motility and sperm count of treated rats when compared with the control (Table 2). Also, a significant increase in sperm cells with abnormal morphology was observed in rats treated with MFBC (100 mg/kg), HFBC (50 mg/kg) and EFBC (50 mg/kg), but not MFBC (50 mg/kg). However, sperm viability and semen volume were not affected by any of *B. coriacea* fractions.

Serum and testicular antioxidants: A significant reduction in serum SOD was observed in all treated rats when compared with the control while testicular SOD was lowered by MFBC (100 mg/kg), HFBC and EFBC. Also, the levels of MDA were significantly elevated by HFBC and EFBC in both the serum and testicular tissue, but MFBC at both doses administered had no significant effect on serum and testicular MDA. Administration of MFBC (100 mg/kg), HFBC and EFBC caused a significant decrease in testicular catalase levels. However, no significant effect was observed on catalase levels in the serum of all *B. coriacea* treated rat groups (Table 3).

Table 1:
Relative organ weights of *B. coriacea* treated rats

	Control	MFBC (50 mg/kg)	MFBC (100 mg/kg)	HFBC (50 mg/kg)	EFBC (50mg/kg)
Testis	1.27 ± 0.04	1.29 ± 0.04	1.29 ± 0.06	1.27 ± 0.09	1.35 ± 0.05
Epididymis	0.67 ± 0.05	0.68 ± 0.04	0.65 ± 0.04	0.60 ± 0.03	0.66 ± 0.03
Seminal Vesicle	1.24 ± 0.16	1.16 ± 0.10	1.04 ± 0.12	0.91 ± 0.19	0.91 ± 0.08
Prostate	0.36 ± 0.04	0.41 ± 0.05	0.36 ± 0.04	0.28 ± 0.04	0.37 ± 0.04
Liver	7.65 ± 0.05	8.05 ± 0.51	6.80 ± 0.36	6.74 ± 0.37	7.71 ± 0.45
Kidney	0.70 ± 0.05	0.70 ± 0.04	0.66 ± 0.03	0.63 ± 0.02	0.73 ± 0.04
Heart	0.78 ± 0.05	0.83 ± 0.08	0.70 ± 0.03	0.65 ± 0.03*	0.81 ± 0.04
Spleen	0.67 ± 0.06	0.69 ± 0.03	0.70 ± 0.03	0.64 ± 0.04	0.77 ± 0.04
Lungs	1.76 ± 0.20	1.92 ± 0.10	1.72 ± 0.12	1.71 ± 0.10	1.85 ± 0.11
Brain	1.54 ± 0.09	1.68 ± 0.05	1.61 ± 0.05	1.52 ± 0.06	1.63 ± 0.15

*Values are expressed as mean ± S.E.M, n=5, *(P<0.05) indicates significant difference from control.*

Table 2:
Effect of *B. coriacea* on sperm profile

	Control	MFBC (50 mg/kg)	MFBC (100 mg/kg)	HFBC (50 mg/kg)	EFBC (50mg/kg)
Sperm motility (%)	93 ± 1.22	78 ± 2.0*	76 ± 2.45*	76 ± 2.45*	72 ± 2*
Sperm viability (%)	97.4 ± 0.6	96.8 ± 0.73	96.8 ± 0.73	96.8 ± 0.73	96.8 ± 0.73
Sperm volume (ml)	5.18 ± 0.02	5.18 ± 0.02	5.18 ± 0.02	5.18 ± 0.02	5.18 ± 0.02
Sperm count (million/ml)	145.8 ± 2.22	127.6 ± 5.83*	114.0 ± 5.47*	116.2 ± 3.06*	112.0 ± 4.11*
Abnormal morphology (%)	11.47 ± 0.38	12.72 ± 0.51	12.75 ± 0.60*	12.73 ± 0.68	13.95 ± 0.53*

Values are Mean ±SEM, n=5, *p<0.05 indicate significant difference compared to control

Table 3:
Effect of *B. coriacea* on serum and testicular oxidative stress

	Control	MFBC (50 mg/kg)	MFBC (100 mg/kg)	HFBC (50 mg/kg)	EFBC (50mg/kg)
Serum SOD (iμ/ml)	2.46 ± 0.15	1.80 ± 0.07*	1.84 ± 0.14*	1.6 ± 0.07*	1.74 ± 0.07*
Serum Catalase(iμ/ml)	25.86 ± 1.52	25.14 ± 1.05	21.44 ± 1.09	20.76 ± 1.68	24.90 ± 0.94
Serum MDA (nmol/L)	27.80 ± 2.50	33.94 ± 1.27	37. 98 ± 2.11	47.62 ± 2.63*	46.28 ± 1.62*
Testicular SOD (iμ/ml)	8.08 ± 0.19	7.7 ± 0.37	6.14 ± 0.23*	6.04 ± 0.25*	5.84 ± 0.29*
Testicular Catalase(iμ/ml)	223.12 ± 5.60	214.46 ± 3.09	186.64 ± 5.31*	192.36 ± 3.40*	202.68 ± 6.40*
Testicular MDA (nmol/L)	1.66 ± 0.09	1.64 ± 0.07	1.72 ± 0.06	2.28 ± 0.11*	2.36 ± 0.14*

Values are Mean ±SEM, n=5, *p<0.05 indicate significant difference compared to control

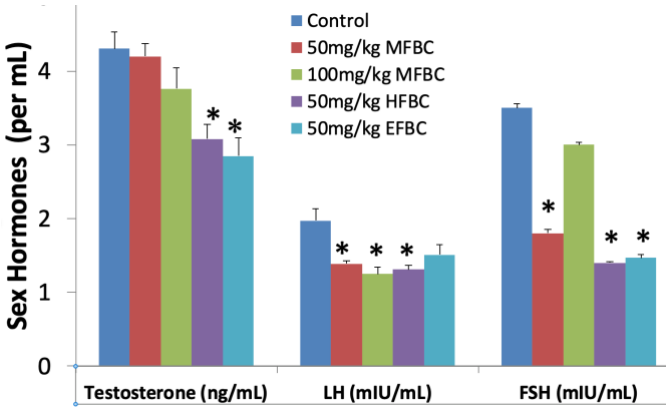

Figure 1:
Sex hormones of *B. coriacea* treated rats. Values are expressed as Mean ± SEM, n=5. *p<0.05 indicates significant difference from control

Sex hormones: Figure 1 showed that MFBC (50 mg/kg) significantly decreased serum LH and FSH while MFBC (100 mg/kg) significantly decreased LH, but had no significant effect on FSH. Treatment with MFBC at both doses administered had no effect on testosterone. The HFBC significantly lowered the serum levels of testosterone, LH and FSH while EFBC caused a significant decrease in testosterone and FSH.

Histopathology: Plates 1 and 2 showed that various fractions of *B. coriacea* seeds had varied effects on the cytoarchitecture of testis and epididymis. Control rats had normal testis as evidenced by the copious spermatogenic cells and well outlined germinal epithelium. However, MFBC (50 and 100 mg/kg) caused necrosis of spermatogenic cells. Loss of spermatogenic cells from the basal compartment of the testis into the lumen was also observed coupled with a marked reduction in the height of the germinal epithelium. The germinal epithelium was also observed to have irregular outline (Fig 2). The epididymis of the control rats was densely populated by mature spermatids. The epididymis of MFBC treated rats had a marked decrease in the density of constituent spermatids when compared with the control (Fig 3). The lower dose of MFBC (50 mg/kg) obviously had a greater effect on the histomorphology of testis and epididymis than the higher dose (100 mg/kg). Hexane fraction had no visible effect on the histomorphology of testis and epididymis but EFBC decreased the height of the testicular germinal epithelium and decreased the density of the epididymal spermatozoa.

DISCUSSION

The medicinal value of *B. coriacea* plant against wide range of diseases and illnesses has been reported in literature (Adisa *et al*., 2011; Onasanwo *et al*., 2016; Walker, 1953; Adjounahum and Ake, 1972). However, a potential detrimental effect of the plant seeds on male fertility in experimental rats has been documented (Obembe *et al*., 2012). Evidence abounds of several other beneficial medicinal plants, but with documented deleterious effects. For example, even though they have proven antibiotic and antimalarial properties, the antisteroidogenic and anti-fertility effects of quinine (Sairam, 1978), chloroquine (Adeeko and Dada, 1998), *Quassia amara* (Raji and Bolarinwa, 1997; Obembe and Raji, 2012; Obembe *et al*., 2014) and *Alstonia boonei* (Raji *et al*., 2005) have been reported.

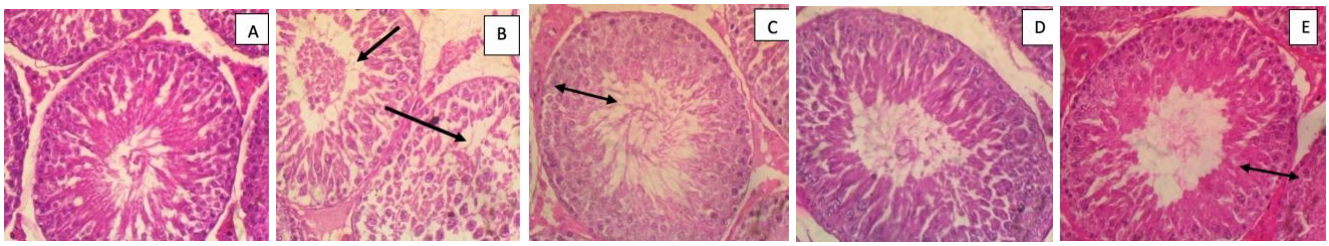

Plate 1:
Transverse sections through the testis of rats treated with *B. coriacea* seed fractions (X400). A– Control, B- MFBC (50 mg/kg), C- MFBC (100 mg/kg), D- HFBC (50 mg/kg) and E- EFBC (50 mg/kg). Arrow indicates necrosis of spermatogenic cells and loss these cells from basal to luminal compartment. A marked decrease in height of the testicular germinal epithelium of treated rats was also observed when compared with the control.

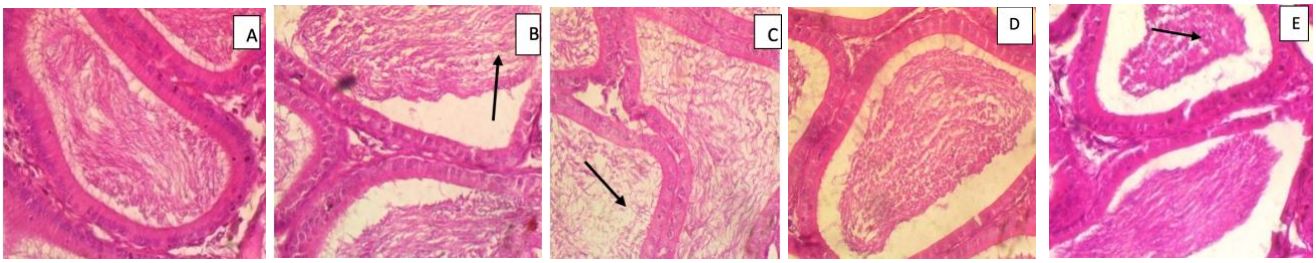

Plate 2:
Transverse sections through the epididymis of rats treated with *B. coriacea* seed fractions (X400). A– Control, B- MFBC (50 mg/kg), C- MFBC (100 mg/kg), D- HFBC (50 mg/kg) and E EFBC (50 mg/kg). Arrow indicates decrease in the density of spermatids when compared with the control.

The analysis of basic sperm parameters serves as diagnostic indices for male fertility (Bieniek *et al*., 2016). Results from this study showed that *B. coriacea* seeds exhibited significant anti-steroidogenic and anti-fertility effects in male rats. The MFBC, HFBC and EFBC significantly decreased sperm motility and sperm count and significantly increased the number of sperm cells with abnormal morphology when the sperm profile of treated rats was compared with the control (Table II). This indicates that this plant seeds interferes with the functional competence of the sperm *in vivo.* All fractions however had similar outcome on the sperm motility and sperm count as there was no significant difference between the extents of the decrease in these sperm parameters by administration of the various fractions. The observed decrease in sperm motility in this study is consistent with earlier reports on the crude extract of these seeds (Obembe *et al*., 2012).

The observed decrease in sperm motility and sperm count in this study may be attributed to the debilitating effects of MFBC, HFBC and EEBC on antioxidant levels, resulting in concurrent over production of reactive oxygen species (ROS) and induction of oxidative stress (Aitken, 2004). Oxidative stress is a cellular condition associated with an imbalance between the production of free radicals, mainly ROS, and the scavenging capacity of antioxidants. Normally, there is a balance between ROS production and antioxidant scavenging activities in the male reproductive tract. This balance culminates into just about the minimal amounts of ROS essential for the regulation of normal sperm functions, such as sperm capacitation, acrosome reactions, sperm motility, and fusion of sperm membrane with the oolemma (Saalu, 2010).

Malondialdehyde (MDA) is the terminal product of lipid peroxidation and serves as its index (Dinvoko-Kotsova, 2002). The observed increase in MDA in the serum and testis of HFBC and EFBC treated rats reflects the status of the metabolism of free radicals, the degree to which the cells are attacked by free radicals and the degree to which lipids were peroxidated (Mirela *et al*., 2012). Catalase acts as a preventive antioxidant and SOD is a chain breaking antioxidant and they both play key role in the protection against the injurious effects of lipid peroxidation (Dinvoko-Kotsova, 2002). Where SOD stops its function, catalase exerts its function. The observed decline in serum and testicular SOD of MFBC, HFBC and EFBC treated rats indicates the treatment probably caused down-regulation of expression of the genes coding for SOD (Kamalakannan *et al*., 2005). Similarly, testicular catalase was significantly decreased by MFBC, HFBC and EFBC, further emphasizing the deleterious effects of treatment on the antioxidant level in the testis. The combination of increased ROS generation and increased lipid peroxidation, as evidenced by increased MDA, and decreased antioxidant level, as evidenced by decrease in catalase and SOD of the testicular tissues, indicates the generation of oxidative stress in the testis.

Gonadotropins (LH and FSH) and testosterone are the main regulators of sperm cell development. Reactive oxygen species and the induction of oxidative stress have been indicated to negatively influence steroidogenesis and gametogenesis (Pandey and Jain, 2013). A positive correlation between generation of oxidative stress in the male reproductive system and fertility disorders has been documented (Resim *et al*., 2015; Saalu, 2010). The induction of oxidative stress as evidenced by increased MDA, decreased SOD and decreased catalase in the testis of HFBC and EFBC treated rats was likely responsible for the reduced testosterone levels (Figure 1) and poor sperm quality seen in these rats. The decline in testosterone of HFBC treated rats may also be due to reduced LH level in these rats. LH stimulates testicular Leydig cells to secrete testosterone and decline in LH is known to reduce testosterone level (Ramaswamy and Weinbauer, 2014). Serum FSH was also significantly reduced by HFBC, EFBC and MFBC (50 mg/kg). These implies that the reproductive toxicity action of the *B. coriacea* seeds may not be limited to the testis, but also affect the anterior pituitary gland, where gonadotropes secreting these gonadotropins are located. Follicle stimulating hormone is well documented to play critical role in the initiation and maintenance of spermatogenesis (Ramaswamy and Weinbauer, 2014). Observed derangement of the testicular germinal epithelium, necrosis and loss of spermatogenic cells coupled with marked decrease in the density of epididymal spermatids of MEBC (50 mg/kg) and EFBC treated rats as revealed by histopathological examination can be adduced to the observed decrease in serum FSH. Interestingly, the lower dose of MFBC (50 mg/kg) administered caused a greater decline in serum FSH level and consequently a greater debilitating effect on the cytoarchitecture of testis than the higher (100 mg/kg) dose (Figures 2 and 3). The significant reduction in the heart weight of HFBC treated rats call for attention and investigation on the effect of this seed on cardiac cells in future research. In a separate study (unpublished), we also observed a significant reduction in heart weight of rats treated with crude methanol extract (50 mg/kg) of this seed.

From the foregoing, it has been demonstrated that *Buchholzia coriacea* seeds exhibits toxic effects on the male reproductive system by impairment of pro-oxidant-antioxidant balance in the testicular tissues and reduction serum levels of the sex hormones by suppressing the pituitary-testicular axis. The consequence of these was reduction of sperm motility and sperm count and derangement of the cytoarchitecture of testis and epididymis. The methanol fraction mediated its action by decreasing testicular antioxidants and decreasing gonadotropic hormones. In addition to these, hexane and ethylacetate fractions of this seeds induced oxidative stress by increasing lipid peroxdation. Hexane fraction appeared to have the most potent effect of all *Buchholzia coriacea* seed fractions used in this study as it affected all estimated indices of the pituitary-gonadal axis. Since male reproductive toxicology may be a prelude to a form of male contraception, the reproductive toxicity effect of *Buchholzia coriacea* seeds may be taken to advantage for further study of its efficacy in male contraception.

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Declaration of interest *– None*

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