

Preliminary study on the effects of *Buchholzia Coriacea* seed extract on male reproductive parameters in rats

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Summary: The effects of methanol extract of *Buchholzia coriacea* seed was studied on male reproductive system of albino rats. Administration of 200mg/kg *b.w.*(p.o.) of the extract for 6 weeks resulted in significant reduction ($P<0.05$) in the weight of the epididymis and seminal vesicle, but not the testes and prostate gland. Also the weight of the visceral organs- lungs, liver, heart and kidney were unaffected. A marked decrease ($P<0.05$) in sperm motility and volume was also observed in sperm collected from the caudal epididymis of the treated animals. Sperm count and morphology were not significantly affected ($P>0.05$). Total tissue protein of the epididymis and testes of the treated rats was significantly increased ($P<0.05$) and fertility was zero in the treated rats. Histological section showed that the epididymal ducts were mostly empty, though the epithelial lining appeared normal. There were fewer spermatozoa and late stage spermatids in the testes, with normal testicular epithelium. The results suggest that the extract of *Buchholzia coriacea* may have antifertility effects in male rats, the site of action most probably the epididymis.

Keywords: *Buchholzia coriacea*, antifertility, epididymis, testes, sperm.

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INTRODUCTION

Buchholzia coriacea belongs to the family *Capparaceae* and is widely distributed in several tropical countries. Its leaves and seeds are reputed scientifically to have good antihelminthic (Ajaiyeoba *et al.*, 2001), antibacterial (Mbata *et al.*, 2009), antimicrobial (Ezekiel and Onyeoziri, 2009), hypoglycemic (Adisa *et al.*, 2011), antimalarial (Okoli *et al.*, 2010), abortifacient and cytotoxicity effects (Adjanooun *et al.*, 1996).

Many antibiotic and antimalarial agents are known to have antifertility actions. For instance, the antisteroidogenic and anti-fertility actions of quinine and chloroquine have been well reported (Sairam, 1978; Adeeko and Dada, 1998). Also, the induction of male infertility in experimental animals resulting from treatment with medicinal plants and their products has drawn attention of researchers. The antisteroidogenic and anti-fertility actions of extracts of *Quassia amara* and *Alstonia Boonei* stem bark, both of which have documented antimalarial properties have also been reported (Raji and Bolarinwa, 1997; Raji *et al.*, 2005).

Although the beneficial effects of *Buchholzia coriacea* seed extract have been exploited, its use as an antibacterial and antimalarial agent has made it imperative to evaluate its effects on reproductive functions. This is in view of the fact that both malaria and infertility are worldwide phenomena and the need to avoid risk of infertility resulting from malarial chemotherapy.

This study was therefore designed to investigate the effects of methanol extract of *B. coriacea* seed extract on male reproductive functions in experimental rats.

MATERIALS AND METHODS

Buchholzia coriacea

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. Voucher number FHI 109920 was assigned to the specimen. The seeds were washed thoroughly with distilled water to remove adhering particles after which they were sliced and properly shade-dried, and pulverized. The

powdered seeds (2.36kg) were macerated in methanol (80% v/v) for 15days with daily shaking. The solvent was decanted every five days and each time replaced with fresh solvent. The extract obtained was concentrated to a dark-brown residue on a rotary evaporator at 40°C and weighed. The Methanol Extract of Seeds of *B. Coriacea* (MEBC) obtained was concentrated to dryness (93.02 g) by lyophilization. Percentage yield was 3.94 %.

Experimental Animals

Adult male Wistar strain albino rats (180-200g) were housed in well ventilated wire mesh cages in the Animal House of the College of Health Sciences, Osun State University with constant 12-h light 12-h dark cycle. They were fed standard rat feed and clean water *ad libitum* and were allowed two weeks of acclimatization.

All procedures in this study 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 (World Medical Association, American Physiological Society, 2002).

Experimental Design

Buchholzia coriacea seed extract (200mg/kg *b.w.*) was administered daily (p.o.) for 6 weeks to an experimental group while a control group concurrently receive vehicle (distilled water) for same duration. Each group was made up of 5 male rats of Wistar strain each. One rat was kept as a backup for each group in case of unexpected mortality. At the end of week 6, all male rats were sacrificed by decapitation. Reproductive and visceral organs were excised, cleared of fat and connective tissue and weighed to the nearest milligram. Tissues and visceral organs for histological studies were preserved in Bouin's solutions while reproductive organs were stored at -20°C for estimation of total protein.

Fertility test

After 5 weeks of treatment with *B. coriacea* seed extract, male rats were introduced to matured fertile female rats in the ratio 1:1 for a period of seven days and the number of litters resulting from cohabitation was recorded.

Sperm Analysis

Sperm characteristics analysis was performed on spermatozoa samples collected from the caudal epididymis using Olympus research microscope (Olympus, Japan) under x40 microscope objectives. Progressive motility was assessed immediately. Five-microlitre drop of diluted sperm suspension was placed on a pre-warmed slide and two drops of warm 2.9% sodium citrate was added and covered

with cover slip. Progressive forward motility was examined and scored to the nearest 10 (Morrissey *et al.*, 1988). Viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain. The motile (live) sperm cells were unstained while the non-motile (dead) sperms absorbed the stain. The stained and unstained sperm cells were counted and an average value for each was recorded from which percentage viability was calculated. Sperm count was done under the microscope with the aid of the improved Neubauer hemocytometer. Counting was done in five Thoma chambers (Shi and Haug, 1990). The epididymis was immersed in 5 ml normal saline in a measuring cylinder and the volume displaced was taken as the volume of the epididymis (Freund and Carol, 1964).

Sperm morphology was evaluated by staining the sperm smears on microscope slides with two drops of Walls and Ewa stain after they were air-dried. The slides were examined under the microscope under oil immersion with x 100 objectives. The abnormal sperm cells were counted and the percentage calculated according to the method described by Wyrobek and Bruce (1980).

Estimation of Tissue total protein

Epididymis and testes were washed in ice cold 1.15%KCl solution, blotted with filter paper and weighed. They were then chopped into bits and homogenized in four volumes of the homogenizing buffer (pH 7.4) using Teflon homogenizer. The resulting homogenate was centrifuged at 10,000rpm for 10mins in a cold centrifuge (4°C) to obtain post mitochondrion fraction. The supernatant was collected and total protein estimated using Randox total protein kit (Randox laboratories Ltd, United Kingdom).

Statistical Analysis

Data were expressed as mean \pm standard error of mean (SEM). Comparisons were made by using Analysis Of Variance (ANOVA) followed by post hoc Newman-Keul's test. P<0.05 was considered significant.

RESULTS

There were no changes in animal behavior or body weight during treatment with *B. Coriacea*. In *B. Coriacea* treated rats, caudal epididymal weight was significantly lower (P<0.05) when compared with the control (Table 1). There was no significant change in testicular weight of the extract treated rats when compared with the control. Also the weight of seminal vesicle was significantly lower (P<0.05) while no significant effect was recorded on the weight of prostate gland. However, there were no

Table 1: Effect of *B. coriacea* on weight (g) of sex and accessory sex organs

	Control	Treated
Testes (g)	1.18± 0.02	1.02 ±0.08
Caudal Epididymis (g)	0.30 ± 0	0.1±0.03 *
Seminal Vesicle	1.6 ± 0	0.31 ± 0.14 *
Prostate	0.26 ± 0.07	0.14± 0.04

Values are Mean ±SEM, n=5, * P< 0.05 indicates significant difference from control

Table 2: Effect of *B. coriacea* on weight (g) of visceral organs

	Control	Treated
Lungs	1.62±0.17	1.34 ± 0.15
Liver	6.54 ±1.31	6.72 ± 1.06
Heart	0.6 ± 0.09	0.6 ± 0.06
Kidney	0.68 ± 0.07	0.58 ± 0.05

Values are Mean±SEM, n=5

Table 3: Effect of *B. coriacea* on Sperm Motility, Count, Volume and Morphology

	Control	Treated
Motility (%)	93.75 ± 1.25	62.5 ± 17.08 **
Count (million/ml)	120.49 ± 5.40	113.5 ± 18.88
Volume(ml)	5.20 ± 0	5.17 ± 0.03*
Abnormal Morphology (%)	10.49 ± 0.34	12.09 ± 1.85

Values are Mean ±SEM, n=5, * P< 0.05, ** P< 0.01 indicates significant difference from control

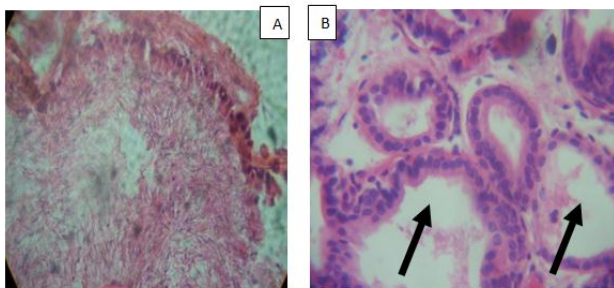


Figure 1: Transverse sections through the Epididymis of control (A) and rats treated with 200mg/kg *B. Coriacea* seed extract (B). Arrow indicates loosely packed epididymal ducts in the treated group when compared with control (× 100 Magnification).

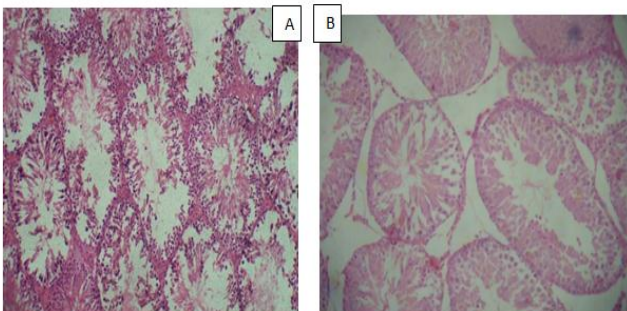


Figure 2: Transverse sections through the Testes of control (A) and rats treated with 200mg/kg *B. Coriacea* seed extract. No visible lesion is observed when compared with the control (× 100 Magnification).

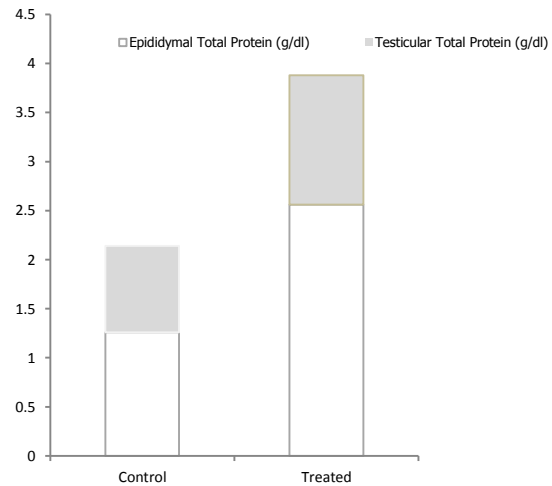


Figure 3: Effect of *B. coriacea* on Epididymal and Testicular Total Tissue Protein

significant changes ($P>0.05$) in the weight of the visceral organs of *B. Coriacea* treated rats when compared with the control (Table 2).

Most of the sperm of the control rats had normal counts, motility and morphology. In *B. Coriacea* treated rats, caudal epididymal sperm showed evidence of toxicity. Sperm motility was significantly lower in the treated group when compared with the control ($P<0.01$). Also semen volume was significantly lower ($P<0.05$). However, there was no significant difference in sperm count and percentage of sperm with abnormal morphology (Table 3). Histopathological examination of the treated rats revealed that the epididymal ducts are mostly empty, some containing deeply eosinophilic material likely to be cytoplasmic fragments of spermatids (Fig 1). The lining cuboidal epithelium appear however normal. The testes appeared to have fewer spermatozoa and late stage spermatids, but no visible lesion were observed (Fig 2).

Figure 3 showed that *B. Coriacea* seed extract significantly increased the testicular and epididymal total tissue protein ($P< 0.05$). Cohabitation of *B. Coriacea* treated rats with fertile female rats produced no offspring even though vaginal smear examination of all female rats revealed presence of sperm cells, a confirmation that there was mating. However, cohabitation of the control group produced nine offspring per rat.

DISCUSSION

Several beneficial effects of extract of seeds of *B. coriacea* have been described in literature and it has earned the name wonderful kola in folkloric use (Mbata et al, 2009; Adisa et al, 2011). It has been reported to have medicinal use in the treatment of malarial and other fever and a potent effect when mixed with palm oil and taken orally (Adjanohoun et al., 1996). More recently, its antimalarial property was scientifically proven (Okoli et al, 2010). The

impact of the extract on reproductive system, particularly in males has not been investigated. The data generated in this study demonstrate that the extract interferes with functional competence of sperm *in vivo* and thus suggest a possible exploitation of this plant seed as an antifertility agent.

B. Coriacea was observed in this study to exert its reproductive toxic effect mainly on the epididymis, decreasing its weight and causing autolysis of its constituent spermatozoa. Its adverse or beneficial effect on the testes was insignificant. Sperm acquire the capability for motility during their epididymal transit and in the normal course; all caudal epididymal sperm are motile (Zhou *et al*, 2008). The decline in sperm motility in the treated group further suggests that *B. coriacea* may have an epididymis specific toxic effect. The lack of effect on weight of various visceral organs indicates that this extract is well tolerated by these organs (Table 2). No sign of acute toxicity was observed at any dose given orally to albino rats up to a maximum dose of 2000mg/kg (Adisa *et al*, 2011; Nweze and Azuzu 2009).

The contributory factors to the initiation of spermatozoa motility, mainly in the form of proteins and small molecular weight glycoproteins emanate from the epididymal epithelia cells (Zhou *et al*, 2008). Derangement of the epididymal epithelium and impairment of motility of caudal epididymal sperm of *B. Coriacea* treated rats is a reflection of the effect of the seed extract on the physiologic anatomy of the epididymis (Faisal *et al*, 2006). The increase in epididymal and testicular tissue total protein observed after *B. Coriacea* treatment may be an ameliorative or defensive response to the debilitating effect of this extract on the reproductive tissues.

The lack of effect on sperm count and morphology showed that there was probably no interference with testicular spermatogenesis. Results from testicular histopathology further corroborate this assumption (Fig 2). The seminal vesicles contributes the bulk (about 60%) of the seminal fluid (Gustavo, 2001). The effect of treatment in decreasing this accessory organ weight may decrease its secretory capability and may be responsible for the decrease in semen volume recorded.

Lack of offspring after mating affirms the outcome of this study, which suggests that *B. coriacea* seed extract may have antifertility potentials, with the probable site of action as epididymis. Since male reproductive toxicology may be a prelude to a form of male contraception (Faisal *et al*, 2006), the negative consequence of *B. Coriacea* on sperm may be taken to advantage for further study on this seed for efficacy in male contraception.

Though a direct effect of *B. coriacea* on cellular mechanisms of spermatogenesis cannot be totally ignored, impairment of hormonal mechanisms concerned with the regulation of spermatogenesis can

also not be ruled out. Further ongoing study in our laboratory is expected to show the effects of *B. coriacea* on serum levels of reproductive hormones and to determine whether these effects are reversible. Serum parameters to affirm non reproductive organ toxicity will also be examined. Also graded doses of treatment will be administered to determine at what dose does *B. Coriacea* becomes toxic to the reproductive function and if its effect is reversible.

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