

Full Length Research Paper

Effect of *Securidaca longepedunculata* root-bark methanol extract on testicular morphometry of New Zealand rabbits

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The aim of this work was to determine the effect of the root-bark extract of *Securidaca longepedunculata* in the improvement of fertility in buck rabbits as envisioned by morphometric indices of the testes. Testicular morphometry of New Zealand rabbits was studied following treatment with different doses of the extract. The extract was administered *per os* in three doses of 0, 50 and 100 mg/kg body weight to three treatment groups of rabbits A, B, and C respectively for 29 days in a completely randomized designed (CRD). Eighteen mature buck rabbits between the ages of 18 and 24 months and with initial body weight (1.1 to 1.9 kg) were used. Each group comprised of three rabbits replicated twice. After the period of treatment, rabbits were weighed and sacrificed. Mean absolute paired testes weight, testes length and testes width were obtained and expressed relative to body weight at sacrifice. The results showed a significant difference in the mean relative paired testes weight, testes length and testes width between groups ($p < 0.05$). The treated group given 50 mg/kg body weight of the extract had the highest mean absolute and relative paired testes weight of 3.325 ± 0.1349^a g and 0.2098 ± 0.0139^a % with mean absolute and relative testes length of 2.4522 ± 0.0250^a cm and 0.1538 ± 0.0040^a %. While the group given 100 mg/kg body weight of the extract had the lowest values of 2.634 ± 0.2762^b g and 0.1565 ± 0.0124^b % with mean absolute and relative paired testes length of 2.1600 ± 0.0807^b cm and 0.1299 ± 0.0039^b % respectively. It was concluded that administration of the extract at 50 mg/kg body weight yielded higher testicular parameters of buck rabbits. It was recommended that the use of the extract in bucks should be done with caution in relation to dose and length of treatment and that a detailed research should be carried out to evaluate the semen quality of bucks treated with 50 mg/kg the extract.

Key words: *Securidaca longepedunculata*, root bark, testes, morphometry, buck, rabbit.

INTRODUCTION

The increasing population in the developing countries has led to increase in demand for animal protein. Rabbit meat

presents the most affordable source of animal protein to mitigate the problem of protein malnutrition in Nigeria.

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Rabbit production also provides high returns on investment, high quality meat products with high protein level of about 20.8%, low sodium, low fat and cholesterol levels which compares favorably with the local bush meat (Shinkut et al., 2016). The presence of caecal microbes enables the rabbit to digest large amounts of fibrous feed better than most non ruminant species (Taiwo et al., 1999). It is for this reason that the costs of beef, chevon, mutton, chicken and frozen fish are higher compared to rabbit meat (Shinkut et al., 2016). For efficient and maximum production of rabbits for meat, a thorough understanding of the reproductive potential of the rabbit is invaluable. The importance of the breeder male spermatozoa for fertilizing eggs is rivalled only by his genetic influence on the progeny performance. The basic knowledge of the morphometric characteristics of the reproductive organs is essential for reproductive assessment and prediction of sperm production, storage potential and fertilizing ability of the breeder male (Gage and Freckleton, 2003). Testicular morphometry and histological changes in rabbit bucks have been used to access the male reproductive status (Abadjieva, 2016).

The plant *Securidaca longepedunculata* is a plant commonly known as Violet tree in English and Krinkhout in Afrikaans. In Swahili it is known as Chipvufanaor mufufu. In Nigeria, the Hausas call it Uwarmaganigunar while the Ibos call it Ezeogwu, Fulani name is 'aalali' is a medium size tree measuring 8 to 9 m height with visible violet (or white) flowers, pale smooth bark, common in North-Central Nigeria, and is generally widespread in hot temperate part of Africa. When in flower the plant is distinctly ornamental. The fruits are round, with a characteristic membranous wing up to 45 mm, purplish green when young, becoming pale straw colored between April and August (Alqasim, 2013). The genus *Securidaca* comprises about 80 species, characterized by papilionaceous purplish flowers and mostly scandent shrubs and lianas, which produce compounds known as securixanthenes with antimicrobial and antioxidant properties (Da Costa et al., 2013). *S. longepedunculata* stem bark and roots are still found amongst the most traded medicinal plants in Africa (Tabuti et al., 2012).

The root extracts are used for treating venereal diseases, skin cancer, skin infections, flu; they are also used for contraceptive purposes, abortion, constipation, coughs and fever. Other uses of the root extracts are sexual boost, toothache, tuberculosis, rheumatism, pneumonia, and as blood purifier and it is also used as an aphrodisiac for men (Mongalo et al., 2015). It is used in treating infections related to nervous and circulatory system, dysentery, malaria, typhoid and frequent stomach ache (Maroyi, 2013; Mustapha, 2013a, b).

Traditionally, the root and bark are taken orally either powdered or as infusion for abortion, infertility, venereal diseases, headache among other diseases (Nordeng et al., 2013). In Limpopo, the Venda people mix the powdered root with maize and sorghum beverages for

sexually weak men (Togun and Egbunike, 2006). A root decoction may be drunk in beer as an aphrodisiac, for sexual impotence, toothache, fungal infections and malaria among other diseases (Maroyi, 2013; Ogunmefun and Gbile, 2012; Mongalo et al., 2015; Motlhanka and Nthoiwa, 2013). Moreover, the dried root is ground into powder, along with that of *Parkia biglobosa* and then taken with cow's milk as a sexual boost. The pounded root may be mixed with that of *Zanthoxylum humile* and taken with soft porridge to treat erectile dysfunction (Semenya and Potgieter, 2013). *S. longepedunculata* Fresen (Polygalaceae) is a multi-purpose plant with a long history of use in African traditional medicine to treat various sexually transmitted infections and other health conditions (Mongalo et al., 2015). Phytochemically, extracts from various parts of *S. longepedunculata*, especially the root bark, contain numerous valuable compounds including xanthenes, some benzyl benzoates and triterpene saponins amongst others.

Toxicity studies, both *in vivo* and *in vitro*, revealed that extracts are only toxic at relatively high concentrations. Furthermore, extracts have antimicrobial, antioxidant, antiparasitic, anti-diabetic, anti-inflammatory, antimalarial, insecticidal, pesticidal, and anticonvulsant properties. Some African medicinal plants have been ethnobotanically and scientifically implicated in the treatment of a variety of human infections (Mongalo and Mafoko, 2013; Mongalo, 2013; Zongo et al., 2013). The pharmacology of these plants may be attributed to various classes of compounds occurring within these plants. In general, these medicinal plants may have relatively low toxicity (Belayachi et al., 2013). Fresh leaves are made into paste with little or no water along with the bark of *Gardenia erubescens* and *Jussiaea suffruticosa* with shea butter and applied externally to treat skin cancer and skin infections respectively (Mustapha, 2013a). Smoke from dry leaves is inhaled to treat headaches while the boiled leaves are taken orally for contraceptive purposes (Mustapha, 2013b). The leaves are either chewed fresh or both orally and nasally administered to treat infertility and to expel the placenta among other uses (Augustino et al., 2011). A decoction from the stem bark may be taken orally for abortion, infertility problems, venereal diseases and some other diseases (Kadiri et al., 2013). The powdered stem bark has antimicrobial activity against a variety of organisms including *Neisseria gonorrhoea*, *Candida albicans*, *Trichomonas vaginalis* and the agent for syphilis (Hedimbi and Chinsebu, 2012). However there is a need to explore the biological activity of various extracts from the species against microorganisms such as, *Klebsiella granulomatis*, *Mycoplasma hominis*, *Mobiluncus* spp. and *Mycoplasma genitalium* as the most common causative agents of gonorrhoea, bacterial vaginitis, donovanosis and other urogenital infections (Mongalo et al., 2015).

Plant part used for abortion is dried root boiled into

distilled water along with Lállè (Hausa name)/nalli (Fulani name) (leave of *Lawsonia inermis*) and a pap is made from the juice (Alqasim, 2013). Dried root, ground into powdered form along with Dòòráwà (Hausa name)/nareehi (Fulani name) (root of *Parkia biglobosa*) when mixed with cow's milk is also used as a sexual boost (Alqasim, 2013). The aqueous root and ethanol extracts yielded alkaloids, cardiac glycosides, flavonoids, saponins, tannins, volatile oils, terpenoids and some steroids (Haruna et al., 2013a; Auwal et al., 2012; Gbadamosi, 2012) while chloroform and ethanol extracts indicated flavonoids, saponins, coumarins, tannins and alkaloids (Adebayo and Osman, 2012). The aqueous root bark extract was slightly toxic to albino rats with an LD₅₀ of 0.771 g/kg (Auwal et al., 2012), while Agbaje and Adekoya (2012) reported an LD₅₀ of 3.16 g/kg when administered orally to rats. Moreover, acute toxicity studies of the aqueous whole root extract on mice revealed LD₅₀ values of 1.740 and 0.020 g/kg for the oral and intraperitoneal application routes respectively (Adeyemi et al., 2010). Elsewhere, the 80% ethanol extract of the root bark exhibited an LD₅₀ of 0.547 g/kg against albino mice (Keshebo et al., 2014). These findings may well suggest that the root bark extract has greater acute toxicity than the whole root extract following oral administration. Antifungal activity has been reported (Karou et al., 2012; Alitonou et al., 2012). Hyperglycemic activity has also been reported (Keshebo et al., 2014).

The inclusion effects of 0.5 ml/kg *C. populnea* extract and inclusion of *S. longepedunculata* showed that *C. populnea* plant extract enhanced the reproductive profiles of male and female *C. gariepinus* brood stocks and brought about a significant increase in egg weight but on the other hand the inclusion of *S. longepedunculata* caused a significant reduction in egg weight at the two different concentrations of the plant extract while fish on diet 4 (0.5ml/kg SL) showed the lowest fecundity count. The reduction could be attributed to the concentration of toxic substances in the leaves of the plant (Ademola et al., 2017). *S. longepedunculata* inclusion also improves spermatogenesis in low concentration but at high dose, there was low sperm count and low motility which could be as a result of toxicity of the extract (Akah and Nwambie, 1994). The testosterone, progesterone and estrogen values as well as the milt volume, sperm motility and milt count were significantly reduced ($p < 0.05$) in fish fed with diet inclusion of 1.0 ml *S. Longepedunculata* (Ademola et al., 2017).

This also agreed with the finding of Dandekar et al. (2002) that *S. longepedunculata* contains some compounds that have negative effect on animal reproductive parameters. The possible mechanisms for the anti-gonad action of *S. longepedunculata* extract could be by exerting a direct inhibitory action on the testis which affects androgen biosynthesis pathways and the pituitary gland, thereby causing changes in Gonadotrophin concentrations and subsequent

spermatogenic impairment or changing the concentration of neurotransmitters (Sarkar et al., 2000). A total of 61 plants species from 36 families were found to be used traditionally to treat male sexual disorders, of the 61 plant species, only *S. longepedunculata* is also traditionally used as a contraceptive. The common methods of application are decoctions and/or infusions in water, beer or milk taken orally (Abdillahi and Van Staden, 2012).

Erectile dysfunction (ED) is a neurovascular event and entails the inability to sustain an erection during coitus as well as a decreased libido. Findings indicated the use of 12 species, 10 of them with new documentations. Only *Osyris lanceolata* and *S. longepedunculata* were previously recorded in the treatment of ED (Lourens et al., 2015). *S. longepedunculata* is believed to have an aphrodisiac property, may improve sperm quality and enhance fertility (Bahmanpour et al., 2006). Plants with aphrodisiac property may be useful in solving fertility problems (Bahmanpour et al., 2006).

The aim of this work was to determine the effect of the root-bark extract of *S. longepedunculata* in the improvement of fertility in buck rabbits as envisioned by morphometric indices of the testes.

MATERIALS AND METHODS

Study location

This study was conducted at the Rabbitry Unit of the University of Abuja Research farm, Abuja Nigeria. University of Abuja Nigeria is geographically located on latitude 8.941°N and longitude 7.092°E at an altitude of 300 m above sea level.

Experimental animals and management

The animal experiments followed the principles of the Laboratory animal care (Canadian Council on Animal Care Guide, 1993). Eighteen (18) mature buck rabbits between the ages of 18 to 24 months and with initial weight (1.1 to 1.9 kg) were obtained from a rabbit farmer in Jos, Plateau state, middle belt of Nigeria. Rabbits were housed in battery cages and acclimatized for one month. During the acclimatization period all the rabbits in this study were fed standard commercial feed containing 18% crude protein twice daily and clean tap water was provided *ad-libitum*.

Collection and identification of plant materials

S. longepedunculata was collected within the premises of the University of Abuja permanent site by the help of the local people. The plant was subsequently identified and confirmed by the Herbarium and Ethno-Botany Unit of National Institute for Pharmaceutical Research and Development Idu- Abuja (NIPRD), where a voucher specimen (NIPRD/H/6576) was deposited.

Processing of *S. longepedunculata* root-bark

Some roots from each *S. longepedunculata* plant were removed in such a way that the tree still remained alive. The roots were dusted

and peeled to obtain the bark. The obtained root bark was cut into pieces, dried in the shade to minimize loss of volatile constituents and reduced to size with pestle in a mortar.

Extraction

The plant material (500 g) was extracted by cold maceration in methanol for 48 h and concentrated in a rotary evaporator at reduced pressure to obtain a dark brown mass of the crude methanol extract.

Experimental design

Eighteen (18) mature buck rabbits between the ages of 18 and 24 months and with initial weight (1.1 to 1.9 kg) were randomly allocated into three experimental groups (A, B and C) with three rabbits per group replicated twice.

Rabbits in group A (control), B and C were administered 0, 50, and 100 mg/kg body weight of *S. longepedunculata* root bark extract respectively *per os* with the aid of an improvised oral catheter for 29 days. At the end of the treatment, rabbits were weighed and sacrificed. Both testes in each buck were dissected out, freed of all connective tissues and blotted on paper to remove blood. Both testes from each buck were weighed to get the paired testes weight using a sensitive weighing balance (Ohaus SP-602 Scout Pro Digital Balance, USA) while testes length and width were measured using a vernier caliper (Series 530 - Standard Model, Mitutoyo, USA). Mean absolute paired testes weight, testes length and testes width were obtained and expressed relative to body weight at sacrifice.

Statistical analysis

Data collected was subjected to a one-way analysis of variance (ANOVA) using SPSS, Version 17.0. Mean differences with values of $P < 0.05$ was considered statistically significant and was separated using Tukey's HSD test.

RESULTS

Extraction yield

The plant material (500 g) yielded 54.51 g (10.9%) of the crude methanol extract.

Effect of extract on testes morphometry

The absolute paired testes weight showed significant difference between treatments ($P < 0.05$). The highest absolute paired testes weight occurred in the 50 mg/kg body weight group, while control group had the lowest (Table 1). Mean absolute paired testes weight in control group was statistically similar to that of rabbits in 100 mg/kg body weight group ($p > 0.05$). The mean paired relative testes weight was significantly different between treatments ($P < 0.05$). Rabbits in 50 mg/kg body weight group had the highest relative paired testes weight. The lowest relative paired testes weight was seen in 100

mg/kg body weight group. The absolute testes length was significantly different between treatments ($P < 0.05$). Rabbits in group B had the highest paired absolute testes length while rabbits in the group C had the lowest absolute paired testes length. However, the absolute paired testes length of control rabbits was similar to those of rabbits in the group C.

The relative paired testes length was significantly different between treatments ($P < 0.05$). Rabbits in the group B had the highest relative paired testes length but were statistically similar to the control group. The lowest relative paired testes length was seen in group C. There was no significant difference in the absolute paired testes width when rabbits in each group were compared ($P > 0.05$). Significant differences were observed when the relative paired testes width between each group was compared ($P < 0.05$). Relative paired testes width was highest in the control group followed by group B. Whereas the relative paired testes width of group C was the lowest.

DISCUSSION

The crude methanol extract yield obtained in this study agreed with the crude methanol extract value of (10.9%) reported by Okoli et al. (2006). The extract seems to have some anabolic effects considering the body weight of the control and the treated groups. The treated groups have significantly higher body weight than the control. This effect may be due to steroids present as part of the constituents of the extract. It has been reported that the aqueous root and ethanol extracts yielded alkaloids, cardiac glycosides, flavonoids, saponins, tannins, volatile oils, terpenoids and some steroids (Haruna et al., 2013a; Auwal et al., 2012; Gbadamosi, 2012). The knowledge of the ability of the testes to produce spermatozoa is of immense importance in rabbit breeding program. The higher values in relative paired testes weight, length and width observed in the present study with the introduction of *S. longepedunculata* extract at 50 mg/kg body weight is a pointer that *S. longepedunculata* root-bark extract promotes testicular growth. This indicates that administration of *S. longepedunculata* root-bark extract at 50 mg/kg body weight for 29 days may be good for the development of spermatogenic potentials of the buck as reflected on the paired testes weight observed in this study. This agrees with Akah and Nwambie (1994) who reported that *S. longepedunculata* inclusion also improves spermatogenesis in low concentration. Testes size is a good indicator of the present and future spermatozoa production of an animal (Morris et al., 1979; Perry and Petterson, 2001; Gupta and Mohanty, 2003; Togun and Egbunike, 2006). The knowledge of basic morphometric characteristics of the reproductive organs have been found to provide valuable information on the evaluation of breeding and fertility potential of animals.

Table 1. Mean±SEM testicular parameters of buck rabbits treated with *Securidaca longepedunculata* root bark methanol extract at sacrifice.

Testicular parameter	Doses of extract (mg/kg body weight of rabbit)		
	0	50	100
	Mean±SEM	Mean±SEM	Mean±SEM
Body weight (g)	1450 ± 50.00 ^b	1600 ± 44.72 ^{ab}	1666.7 ± 61.46 ^a
Absolute paired testes weight (g)	2.5855 ± 0.0981 ^b	3.325 ± 0.1349 ^a	2.634 ± 0.2762 ^b
Relative paired testes weight (%)	0.1783 ± 0.0026 ^{ab}	0.2098 ± 0.0139 ^a	0.1565 ± 0.0124 ^b
Absolute paired testes length (cm)	2.2108 ± 0.0468 ^b	2.4522 ± 0.0250 ^a	2.1600 ± 0.0807 ^b
Relative paired testes length (%)	0.1537 ± 0.0073 ^a	0.1538 ± 0.0040 ^a	0.1299 ± 0.0039 ^b
Absolute paired testes width (cm)	0.9472 ± 0.0073 ^a	1.0155 ± 0.0304 ^a	0.9347 ± 0.0368 ^a
Relative Paired testes width (%)	0.0657 ± 0.0020 ^a	0.0637 ± 0.0030 ^{ab}	0.0561 ± 0.0012 ^b

a-c = Means in the same row with different superscripts are significantly different (p<0.05).

The fact that the group that received 50 mg/kg body weight of the *S. longepedunculata* root-bark extract resulted in absolute and relative paired testes weight which were greater than the control shows that the reproductive potential of these testes were higher compared to those that did not receive the *S. longepedunculata* root-bark extract. Larger testes (without any abnormality) have been reported to produce more spermatozoa than smaller testes (Oyeyemi et al., 2002; Galmessa et al., 2003; Britto et al., 2004; de Soya, 2007). Testes weight had been reported to be positively correlated with sperm volume, mass motility, sperm concentration, and testosterone but negatively correlated with dead sperm cell and primary abnormality in male goat (Johnson et al., 1984; Rahja et al., 1995; Daramola et al., 2007).

The positive effect of 50 mg/kg *S. longepedunculata* on the testes morphometry may have been contributed by its antimicrobial, antioxidant and aphrodisiac properties. Rabbits are known to suffer venereal diseases which could influence the testes parameters. Sub clinical infections may not be noticed. It has been reported that the root extracts of *S. longepedunculata* are used for treating venereal diseases, syphilis, skin cancer, skin infections and serves as a blood purifier (Maroyi, 2013; Mustapha, 2013a; Mustapha, 2013b). A decoction from the stem bark may be taken orally to treat infertility problems, epilepsy and venereal diseases (Kadiri et al., 2013). The powdered stem bark is also mixed with hot water and taken orally to treat syphilis and gonorrhoea (Hedimbi and Chinsebu, 2012). In another perspective the aphrodisiac properties may be playing a role on the positive testicular parameters observed at 50 mg/kg. A root decoction may be drunk in beer as an aphrodisiac (Motlhanka and Nthoiwa, 2013).

The lower values in relative paired testes weight, length and width of the group given 100 mg/kg body weight could be due to testicular aplasia or atrophy. Morton (1988) reported that in sacrificed animals, a decreased weight of the testes indicates wide spread or diffuse loss

of seminiferous epithelial cells. 100 mg/kg body weight of the extract for 29 days yielded lower testicular parameters. The result of 100 mg/kg of the extract for 29 days having lower values of testicular parameters measured, may be due to some level of toxicity at that dose and this is in agreement with Mongalo and Mafoko (2013), Mongalo (2013) and Zongo et al. (2013). In these reports, toxicity studies, both *in vivo* and *in vitro*, revealed that extracts are only toxic at relatively high concentrations. The findings of the present studies also concur with the report that the pharmacology of these plants may be attributed to various classes of compounds occurring within these plants. In general, these medicinal plants may have relatively low toxicity (Belayachi et al., 2013). The findings in this study is supported by that of Auwal et al. (2012) who reported that the aqueous root bark extract was slightly toxic to albino rats with an LD₅₀ of 0.771 g/kg, Agbaje and Adekoya (2012) reported an LD₅₀ of 3.16 g/kg when administered orally to rats. Moreover, acute toxicity studies of the aqueous whole root extract on mice revealed LD₅₀ values of 1.740 and 0.020 g/kg for the oral and intraperitoneal application routes respectively (Adeyemi et al., 2010). Elsewhere, the 80% ethanol extract of the root bark exhibited an LD₅₀ of 0.547 g/kg against albino mice (Keshebo et al., 2014). These findings may well suggest that the root bark extract has greater acute toxicity than the whole root extract following oral Administration (Mongalo et al., 2015). Out of the 61 plant species, used traditionally to treat male sexual disorders, only *S. longepedunculata* is also traditionally used as a contraceptive (Abdillahi and Van Staden, 2012), they are also used for contraceptive purposes and abortion (Maroyi, 2013; Mustapha, 2013a; Mustapha, 2013b). These reports are pointers that this plant has toxic potentials. The inclusion of *S. longepedunculata* in a dietary supplement of *Clarias gariepinus* caused a significant reduction in egg weight and fecundity count at the two different concentrations of the plant extract. The testosterone, progesterone and estrogen values as well as the milt volume, sperm motility

and milt count were significantly reduced ($p < 0.05$) in fish fed with diet inclusion of 1.0 ml *S. longepedunculata* (Ademola et al., 2017; Ajiboye et al., 2012; Akah and Nwambie, 1994). This also agrees with the finding of Dandekar et al. (2002) that *S. longepedunculata* contains some compounds that have negative effect on animal reproductive parameters. The possible mechanisms for the anti-gonad action of *S. longepedunculata* extract could be by exerting a direct inhibitory action on the testis which affects androgen biosynthesis pathways and the pituitary gland, thereby causing changes in gonadotrophin concentrations and subsequent spermatogenic impairment or changing the concentration of neurotransmitters (Sarkar et al., 2000). 100 mg/kg body weight of the extract for 29 days of the experiment could be toxic enough to elicit the changes observed. The investigation of other parameters of fertilization is necessary for a complete understanding of the action of this extract in this dose and period of treatment.

Conclusion

The administration of 50 mg/kg body weight of methanol extract of *S. longepedunculata* root-bark extract for 29 days yielded higher values of some of the testicular parameters such as relative paired testis weight and relative paired testis length and may be applied to improve the reproductive capacity of rabbit bucks at that dose. It is recommended that the use of the extract in bucks should be done with caution in relation to dose and length of treatment and that a detailed research should be carried out to evaluate the semen quality of bucks treated with 50 mg/kg the extract to ascertain its effect on semen quality.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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