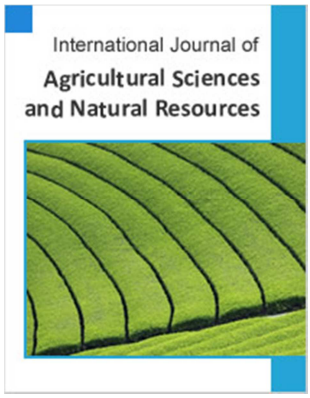
International Journal of Agricultural Sciences and Natural Resources
2018; 5(1): 1-5
http://www.aascit.org/journal/ijasnr
ISSN: 2375-3773

AASCIT American Association for Science and Technology



Keywords
Securidaca longepedunculata,
Root-Bark,
Fertility,
Testis Morphometry,
Semen Quality

Received: October 31, 2017
Accepted: November 23, 2017
Published: January 11, 2018

Semen Quality and Testis Morphometry Effects of *Securidaca longepedunculata* (Fresen) Root Bark Extract in Wistar Rats

Chibuogwu Ijeoma Chika*, Oluwafemi Rufus Adebisi, Momoh Ibrahim

Animal Science Department, University of Abuja, Abuja, Nigeria

Email address
ijeoma.chibuogwu@uniabuja.edu.ng (C. I. Chika),
adebisi.oluwafemi@uniabuja.edu.ng (O. R. Adebisi), aromealfa76@gmail.com (M. Ibrahim)

*Corresponding author

Citation
Chibuogwu Ijeoma Chika, Oluwafemi Rufus Adebisi, Momoh Ibrahim. Semen Quality and Testis Morphometry Effects of *Securidaca longepedunculata* (Fresen) Root Bark Extract in Wistar Rats. *International Journal of Agricultural Sciences and Natural Resources.* Vol. 5, No. 1, 2018, pp. 1-5.

Abstract
A decoction of the root bark of *Securidaca longepedunculata* is frequently taken traditionally by men as an aphrodisiac and may have effect on fertility. A total of twenty-four male Wistar rats (*Rattus norvegicus*) within the age of two to three months old were used to study the effect of *Securidaca longepedunculata* root bark methanol extract on male fertility using testes morphometry and epididymal semen quality as fertility indices in a Completely Randomized Designed experiment. *Securidaca longepedunculata* root bark methanol extract was administered *per os*, in four different doses of 0, 25, 50 and 75mg/kg body weight to four groups of rats comprised of six rats per group. Treatment spanned 59 days. All rats were weighed at the beginning and end of the treatment and reproductive organs were harvested intact. Paired relative testis weight, relative testis length and relative mean testis width were measured. Also, spermatozoa motility, semen concentration and percentage of live spermatozoa from the caudal epididymis were measured. Rats in the control group had the highest ($p<0.05$) mean body weight gain (81.00g) while rats in the treated group had low weight gains with the lowest (37.00g) recorded in the group given 75mg/kg body weight of the extract. The relative paired testes weight was highest ($p<0.05$) in the group given 75mg/kg body weight of the extract (0.0111) followed by the group given 50mg/kg body weight of the extract (0.0109) and least in the control group (0.0094). Sperm motility percentage was highest in the group given 50mg/kg body weight of the extract (70.57%) followed by the group given 75mg/kg body weight (64.77%). While percentage of live spermatozoa was highest in the group given 75mg/kg body weight of the extract (91.43%) followed by the group given 50mg/kg body weight of the extract (88.90%) and least in the control group (83.67%). *Securidaca longepedunculata* root bark extract may have the potential to increase the fertility of male animals. However, *Securidaca longepedunculata* root bark extract at doses above 50mg/kg body weight may not be recommended for animals bred for meat because it may reduce weight gain. Certain phytochemicals or anti-nutritive factors present in the root bark may be responsible for the negative effect.

1. Introduction

In a developing country like Nigeria, the daily protein intake has been so far below the requirement as recommended by world health organization [1]

Protein contains the essential amino acid needed by man as precursors to nucleic acid, co-enzyme, hormone, immune response and repairing of worn-out tissues [2].

Poor intake of protein is known to cause malnutrition diseases like decrease in birth weight and gestation duration, increased stress sensitivity, decreased sperm quality, increased obesity, decreased in brain size and altered fat distribution among others [3] [4] [5] [6].

To mitigate this problem, there is need to device a means to increase maximum production of animals to make it cheaply available for the common masses to afford. To this end, the laboratory rat is being used as an ideal model from which laboratory result can be applied to higher animals like farm animals to improve their reproductive performance

Several plants known to have aphrodisiac properties have also been found to be remedies for impotence and infertility [7]. The plant *Securidaca longepedunculata* (Fresen) is believed to have an aphrodisiac property and seems to improve male potency. However, there is a need to determine the effect of the root bark on male fertility [8]

The aim of this work was to determine if the methanolic root-bark extract of *Securidaca longepedunculata* Fresen can be used to improve reproductive capacity of male Wistar rat (*Rattus norvegicus*) using semen quality, body weight gain and testis morphometry as indices.

2. Materials and Methods

2.1. Experimental Site

The experiment was carried out at the Animal Unit of Animal Science Department, University of Abuja, Nigeria. University of Abuja is located between latitude 8°57' and 8°55'N and longitude 7°05' and 7°06'E. Temperature ranges from 28°C-33°C in the day time and 22°C-5°C in the night.

2.2. Eperimental Animals

A total of twenty-four male rats (*Rattus norvegicus)* within the range of two to three months of age were obtained from Nigerian Veterinary Research Institute (NVRI) Jos, Plateau state. The rats were housed in battery cages and acclimatization for a period of two weeks. Rats were fed with standard growers' mash and clean water was given *ad-libitum*. Feed was introduced to the animals twice daily both in the morning and evening and a good hygienic environment was maintained throughout the experiment.

2.3. Collection and Identification of Plant Material

The leaves of *S. longepedunculata* was collected and identified at the Herbarium and Ethno Botany Unit of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD) Idu-Abuja, where a voucher specimen (NIPRD/H/6576) was deposited.

The root bark was obtained, rinsed gently with water to remove dirt particles, cut into smaller pieces and shade dried. It was then reduced into smaller sizes with the aid of pestle and mortal.

About 2.0kg of the plant material was macerated in cold methanol for 48 hours and filtered. The filtrate was concentrated in a rotary evaporator at reduced pressure to obtain a dark brown semi-solid mass.

2.4. Experimental Design and Treatment

A total of 24 male Wistar rats aged two to three months with initial body weights of 145±8.25g between groups were randomly allocated into four experimental groups (A, B, C and D) in a Complete Randomized Design. Each group comprised of six animals in each battery cage.

Group A, B, C and D was given 0, 25.0, 50.0 and 75.0g/kg body weight of the extract respectively *per os* using an oral canula. Treatment was given daily for 59 days. Thereafter, the animals were weighed, humanely killed and the reproductive organ harvested for morphometric study and epididymal semen evaluation.

2.4.1. Epididymal Semen Collection

The scrotum was excised to remove the testes. Each cauda epididymis was dissected free of fatty tissue, and minced in 2.0mls of normal saline. This was centrifuged or allowed to stand for 5mins. Thereafter, the decanted supernatant was used as the semen sample.

2.4.2. Evaluation of Semen Quality

Sperm motility

Semen sample, that is, an aliquot of this solution was observed under light microscope. The mean motility estimation was taken as final motility scored for each sample, and presented in percentage.

Determination of sperm concentration

Caudal epididymal semen was drawn up to the '0.5' mark of a WBC pipette. Semen dilution fluid (1.0% formol saline) was drawn into the pipette while rotating the pipette to mix contents until volume reached the '11' mark. This gave a dilution factor of 1:20. The tip of pipette was placed at the junction between the cover glass and hemocytometer chamber to allow diluted semen to flow into the chamber. Both chambers of the hemocytometer were filled, allowed to settle in a humid chamber for about five minutes and viewed under the microscope at a magnification of X40. Spermatozoa within four $1mm^2$ area at corners of the chamber were counted to get the number of spermatozoa in 0.4ul of diluted semen (0.1mm X 1.0 mm X 1.0mm X 4 squares = 0.4ul). The number of spermatozoa in 1ml of diluted semen was calculated. The number of spermatozoa in 1.0 ml of concentrated epididymal semen was then calculated

by multiplying the number of spermatozoa in diluted ependymal semen by the dilution factor of 1:20. This gave a standard of 5 X 10^4 of the number of spermatozoa in 0.4ul of diluted semen. The average for both chambers was used.

Spermatozoa life percentage

Semen sample was mixed with eosin/nigrosin stain at the ratio of 2:1. A drop of the mixture was put on a microscope slide and examined at 400X using a light microscope. About 200 spermatozoa was counted. Unstained spermatozoa were considered to have its plasma membrane and acrosome intact and was alive. While stained cells were considered membrane compromised and therefore, dead [9]. Life/death percentage was taken as, number of spermatozoa alive divided by total number of spermatozoa multiplied by 100 i.e. $\left(\frac{No.of\ sperm\ alive}{Total\ No.of\ spermatoza}\right)$ X 100.

2.4.3. Testis Morphometric Study

Both testes from each experimental animal was removed intact and blotted on tissue paper to remove all the blood stain and weighed on a Mettler Toledo scientific weighing balance. Testes length and width was also measured using Vernier caliper (Unique Wink Vernier caliper made in Thailand).

2.5. Data Analysis

One Way Analysis of Variance was used to analyze all data collected from experimental animals. When there were significant differences at $P < 0.05$ between means, Duncan Multiple Range Test was used to separate the means.

3. Result and Discussion

About 2.0kg of the plant material yielded a dark brown semi-solid mass that weighed 218.04g and had a percentage yield of 10.9%. The yield agrees with Okoli *et al*. [10] who had a yield of 10.9% from the methanol extraction of *S. longepedunculata* root bark.

The effect of the extract on the body weight of treated rats is seen in Table 1 below.

Table 1. *Effects of different doses of Securidaca longepedunculata root bark extract on body weight gain.*

	Doses of *Securida longepedunculata* (mg/kg body weight)					
Weight parameters	**0**	**25**	**50**	**75**	**SEM**	**Sig**
Mean Initial Weight	153.25[a]	145[d]	148.25[c]	149.25[b]	0.904	*
Mean final live weight	234.25.00[a]	190.25[c]	215[b]	186.25[d]	96.65	*
Mean weight gain	81.00[a]	45.25[c]	66.75[b]	37.00[d]	5.25	*

SEM= Standard Error of Mean

a-d= means in the same row having different superscripts are significantly different at P<0.05

The mean weight gained values were statistically significant (p<0.05) between treatments. Rats in the control group had the greatest weight gain while rats given 75mg/kg body weight of the extract had the lowest weight gain. Similar findings were observed by Luka [11] when doses above 50mg/kg body weight of the extract were given to rabbits. This finding could be due to phytochemicals in the root bark that have anti nutritive values. Junaidu *et al* [12] demonstrated the presence of flavonoid, saponin, alkaloids, cardiac glycoside and saponins glycosides in *S. longepedunculata* root bark. While, Auwal *et al* [13] demonstrated the presence of alkaloids, cardiac glycosides, flavonoids, saponins and tannins with saponins and tannins in larger quantities. As reported by Gemede and Ratta [14] certain anti-nutrients in plant foods such as phytic acid, lectins, tannins, saponins, amylase inhibitors and protease inhibitor are responsible for deleterious effects related to the absorption of nutrients and micronutrients. They reduce the availability of nutrients, inhibit growth and reduce body weight.

Table 2. *Effect of different doses of Securidaca longependunculata root bark methanol extract on testis morphometry.*

	Levels of *Securidaca longependunculata* (mg/kg body weight)				
Testis parameters	**0**	**25**	**50**	**75**	**SEM**
Body weight at slaughter (g)	234	190.25	215	209	5.85
Relative mean paired testis weight (g/g)	0.0094[b]	0.0099[b]	0.0109[a]	0.011[a]	0.0023
Relative mean paired testis length (mm/g)	0.0060 [a]	0.0070 [a]	0.0064 [a]	0.0067 [a]	0.0002
Relative mean paired testis width (mm/g)	0.0020 [a]	0.0020 [a]	0.0024 [a]	0.0021 [a]	0.0018

SEM= Standard Error of Mean

a-d= means in the same row having different superscripts are significantly different at P<0.05

The relative paired testis weight was significantly different (p<0.05) between groups. While the mean relative testis length and width were not significantly different (p>0.05) between groups. The relative testis weight was highest in the group given 75mg/kg body weight of extract but was statistically similar (p>0.05) to the group given 50mg/kg body weight of the extract. The least value was seen in the control group. The basic knowledge of morphometric characteristics of reproductive organs in an animal give valuable information for the evaluations of its breeding ability and potential fertility. Testis size is a good indication of present and future sperm production as well as the breeding potential of any male animal [15] [16]. Melon [17] found a strong association between testicular weight and total daily sperm production in Wistar rats. It has been discovered that larger testes without any abnormality produce higher or

more spermatozoa than the smaller ones [18]. Morton [19] reported that in sacrificed animals, testes with decreased weight show wide spread diffused loss of seminiferous epithelia cells. Thus, a high testis weight may be an indicator of good quality testes which is synonymous to better spermatogenesis.

The result for semen evaluation is presented in Table 3 below.

Table 3. *Effects of different doses of Securidaca longepedunculata root bark extract on semen parameters.*

	Levels of *Securidaca longependunculata* (mg/kg body weight)				
Semen parameter	**0 (Control)**	**25**	**50**	**75**	**SEM**
Motility (%)	63.20^{c}	55.20^{d}	70.57^{a}	64.77^{b}	1.66
Concentration	44^{c}	46.67^{b}	42.67^{d}	65.33^{a}	2.77
Live (%)	83.67^{d}	88.17^{c}	88.90^{b}	91.43^{a}	0.87

SEM= Standard Error of Mean

a-d= means in the same row having different superscripts are significantly different at $P<0.05$

There was a significant difference ($P<0.05$) in the percentage of linear progressive motile spermatozoa between treatments. Rats treated with 50mg/kg body weight of extract had the highest value of 70.6% followed by the group treated with 75mg/kg body weight of the extract which had 64.77%. It has been reported that there is a significant correlation between motility and fertility [20] [21]. The extract of the root bark of *Securidaca longepedunculata* improved the percentage motility of rat spermatozoa especially when given the moderate dose of 50mg/kg body weight of the extract. Thus, may enhance the capacity of rat spermatozoa to successively fertilize an ovum.

The sperm concentration was significantly different ($P<0.05$) between treatments. However, the difference was not dose dependent as it was highest in the group given 75mg/kg body weight of the extract but lowest in the group given 50mg/kg body weight. Sperm concentration is a component of total sperm output and is a variable related to fertility.

The percentage of life spermatozoa showed significant differences ($P<0.05$) between treatments. Rats administered 75mg/kg body weight had the highest percentage of life spermatozoa while control rats had the least percentage of life spermatozoa but rats given 50mg/kg body weight of extract had a value that was statistically similar ($p>0.05$) to the group administered 25mg/kg body weight of extract. This may suggest that the extract has a booster effect on spermatozoa survival. Bitter leaf (*Vernonia amygdalina*) was found to have a similar booster effect on spermatozoa survival and it was attributed to its antioxidants, the flavonoids and vitamin content that could maintain sperm morphology, sperm survival and sperm function [22]. *Securidaca longepedunculata* root bark is known to contain flavonoids and this may be responsible for its booster effect in this study.

4. Conclusion and Recommendation

The result of this study shows that the administration of methanol extract of the root bark of

Securidaca longepedunculata root bark extract at doses 50mg/kg body weight and 75mg/kg body weight caused increases in relative testis weight of Wistar rats and may improve spermatogenesis and fertility capacity of Wistar rats

Securidaca longepedunculata root bark extract at doses 50mg/kg body weight and 75mg/kg body weight improves the percentage motility and percentage liveability of rat spermatozoa and may have higher capacity to successively fertilize an ovum.

Only the concentration of rat spermatozoa in the group given 25mg/kg body weight and 75mg/kg body weight of the extract was improved when compared to the control

Securidaca longepednculata beyond 50mg/kg body weight of the extract may be harmful to rats as it reduced body weight gain.

Although 75mg/kg body weight of the extract had better effect on more semen and testes parameters in rat than the 50mg/kg body weight dose, its negative effect on body weight gain rules it out as the dose of choice. Thus, 50mg/kg body weight of *Securidaca longepedunculata* root bark extract may be recommended to be administered to rats in a breeding program.

It is also recommended that *Securidaca longepedunculata* root bark be analyzed for anti-nutritive factors or toxic phytochemicals that may be responsible for drastic weight loss in the rats given 75mg/kg body weight of the extract.

Furthermore, there is a need to investigate the hematological, serum biochemical and histopathological changes in rats treated with the root bark to fully understand the possible toxic effects of the plant.

References

[1] World Health Organization, Food and Agriculture Organization of the United Nations, United Nations University: Protein and amino acid requirements in human nutrition. Report of a joint FAO/WHO/UNU expert consultation (WHO Technical Report Series 935) 2007; 265 pages. ISBN: 92 4 120935 6.

[2] "Front Matter." Institute of Medicine: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, DC: The National Academies Press, 2005. doi: 10.17226/10490.

[3] Agbon, C. A. (2007). Nutritional Status of Rural Preschool Children in the University of Agriculture Abeokuta Extension Villages. Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka [PhD dissertation].

[4] Muzi-Filho, H., Bezerra, C. G. P., Souza, A. M., Boldrini, L. C., Takiya, C. M., Olivera, F. L., Nesi, R. T., ……Cunha, V. M. (2013). Undernutrition affects cell survival, oxidative stress, Ca^{2+} handling and signaling pathways in vas deferens, crippling reproductive capacity. *PLoS ONE, 8* (7), e69682. doi: 10.1371/journal.pone.0069682.

[5] Melo, M. C., Almeida, F. R., Caldeira-Brant, A. L., Parreira, G. G., Chiarini-Garcia, H. (2013) Spermatogenesis recovery in protein-restricted rats subjected to a normal protein diet after weaning. *Reproduction, Fertility and Development 26*, 787-796. https://doi.org/10.1071/RD13032

[6] Haque, M., Starr, L. M., Koski, K. G., Scott, M. E. (2017). Differential expression of genes in fetal brain as a consequence of maternal protein deficiency and nematode infection. International Journal for Parasitology http://www.sciencedirect.com/science/article/pii/S002075197302540 https://doi.org/10.1016/j.ijpara.2017.07.005

[7] Andel, T. N., Boer, H. and Towns, A. (2015). Gynaecological, andrological and urological problems: an ethnophamacological perspective. *In* Ethnopharmacology (first edition) Heinrich, M and Jager, A. K (eds). John Wiley & Sons, West Sussex, UK. 464 pages.

[8] Mongalo, N. I., McGaw, L. J., Finnie, J. F., Staden, J. V. (2015). *Securidaca longepedunculata* Fresen (Polygalaceae): a review of its ethnomedicinal uses, phytochemistry, pharmacological properties and toxicology. *Journal of Ethnopharmacology*, 165: 215-26 doi: 10.1016/j.jep.2015.02.041.

[9] Aalseth, E. P. and Saacke, R. G. (1986). Vital staining and acrosomal evaluation of bovine sperm. *Gamete Research*, 15 (1): 73-81.

[10] Okoli, C. O., Akah, P. A., and Ezugworie, U. (2005). Anti-inflammatory activity of extracts of root bark of *Securidaca longepedunculata* Fres (Polygalaceae). *African Journal of Traditional, Complementary and Alternative Medicines 2* (3): 54-63.

[11] Luka, M. (2013). The Effect of the Root-Bark Methanol Extract *of Securidaca Longepedunculata* on Libido, Body Weight and Paired Testis Weight. A project submitted for the award of the Bachelor of Agriculture, Faculty of Agriculture, University of Abuja, Nigeria.

[12] Junaidu, S., Shehu K., Aliero, A. A., Bawa, J. A. and Suleiman, I. (2014). Evaluation of antifungal and phytochemical properties of Violet Tree (*Securideca longepedunculata* Fres). *Global Journal of Science Frontier Research*: *C Biological Science*, 14 (5): Version 1.0 page 1-6.

[13] Auwal, S. M., Atiku, M. K., Wudil, A. M. and Sule, M. S. (2012). Phytochemical composition and acute toxicity evaluation of aqueous root bark extract of *Securidaca longepedunculata* (linn). *Bayero Journal of Pure and Applied Sciences*, 5 (2): 67-72.

[14] Gemede, H. F. and Ratta, N. (2014). Anti-nutritional factors in plant foods: Potential health benefits and adverse effects. *International Journal of Nutrition and Food Sciences, 3* (4), 284-289. (http://www.sciencepublishinggroup.com/j/ijnfs) doi: 10.11648/j.ijnfs.20140304.18.

[15] NseAbasi N. E. (2015). Testicular and epididymal morphometric characteristics: viable indicators of reproductive ability of farm animals. *American Journal of Biomedical Science and Engineering, 1* (4): 39-44.

[16] Galmessa, U., Raina, V. S., Gupta, A. K. and Mohanty, T. K. (2003). Testicular biometry and semen quality in Karan Fries bulls. *Indian Journal of Diary Science*, *56*: 317-319.

[17] Melon, F. C. A., Matta, S. L. P., Paula, T. A. R., Gomes, M. L. M., and Oliveira, L. C. (2010). The effects of *Tynnanthus fasciculatus* (Bignoniaceae) infusion on testicular parenchyma of adult Wistar rats. *Biological Research 43:* 445-450. http://dx.doi.org/10.4067/S0716-97602010000400009

[18] Oyeyemi, M. O., Oke, A., Olusola, C., Ajala, O., Oluwatoyin, O. and Idehem, C. O. (2002). Differences in testicular parameters and morphological characteristics of spermatozoa as related to age of West African Dwarf bucks. *Tropical Journal of Animal Science*, *5*: 99-107.

[19] Mortons, D. (1988). The use of rabbits in male reproduction toxicology. *Environmental Health Perspective*, *77*, 5-9.

[20] Love C. C. (2011). Relationship between sperm motility, morphology and the fertility of stallions. *Theriogenology, 76* (3), 547-57. doi: 10.1016/j.theriogenology.2011.03.007. Epub 2011 Apr 16.

[21] Ingrid, D., Philippa, K., Gilles, L., Olivier, P., Franck, P., Pierre, D., Xavier, D. (2015). Mass sperm motility is associated with fertility in sheep. *Animal Reproduction Science*, *161*, 75-81.

[22] Saalu L. C., Akunna, G. G., and Ajayi, J. O. (2013). Modulating role of Bitter Leaf on spermatogenic and steroidogenesis functions of the rat testis. *American Journal of Biochemistry and Molecular Biology, 3*: 314-321. doi: 10.3923/ajbmb.2013.314.321.