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Research Article

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Evaluation of the Fertility Activity of Aqueous Leaf Extract of *Desmodium velutinum* in Male Wistar Rats

ABANIWO Rose Mafo[1], ONIWON Wisdom Otaru[1], SULE Fatima Ajuma[1], MOMOH Theophilus Boniface[2], IDAKWOJI Precious Adejoh[1]

[1] Department of Biochemistry, Faculty of Natural Sciences,
Kogi State University, Anyigba, Kogi State, Nigeria
[2] Department of Plant Science and Biotechnology, Faculty of Natural Sciences,
Kogi State University, Anyigba, Kogi State, Nigeria
Corresponding author: MOMOH Theophilus Boniface
(theophilusmomoh@rocketmail.com)

Abstract

This study evaluated the fertility potentials of the aqueous leaf extract of *Desmodium velutinum* (DVAE). Acute toxicity study was carried out on the extract using standard method of Lorke. The pro-fertility experiment was carried out using 20 adult male and 8 female Wistar rats. The male rats were randomized into 4 groups of 5 rats each. Group 1 served as normal control and received 20 ml/kg of Dist. H_2O; groups 2 and 3 received 200 and 400 mg/kg DVAE respectively while group 4 served as standard control and received 5 mg/kg sildenafil citrate. All treatments were done orally for 28 days within which the mounting and mating frequencies were observed in male rats paired with oestrous female rats. 24h after the last treatment, the rats were euthanized and the epididymis of rats was resected and the effects of the extract on sperm count/motility/morphology and epididymal weight were evaluated. The extract produced a significantly ($P< 0.05$) and dose- dependent increase in the mounting and mating frequencies compared to control. There was similarly a significant ($P< 0.05$) increase in the mean sperm count/ motility compared to the normal control. The mean epididymal weight of the treatment groups were also significantly ($P< 0.05$) increased by the extract compared to the normal control. The extract also dose- dependently produced a significant ($P< 0.05$) increase in the percentage of normal sperm cells compared to the normal control. With the observed effects of the extract on mounting and mating frequencies, sperm count, sperm motility, percentage of normal sperm cells and epididymal weight, it was concluded that the extract of *Desmodium velutinum* possesses pro-fertility effects.

Keywords: *Desmodium velutinum*, Fertility, Sperm motility, Sperm count, Wistar rats

Introduction

Fertility is the latent ability of an organism to reproduce itself while infertility is the incapability to conceive and carry a pregnancy to live birth. It is a worldwide medical and social problem that affects above 10-15% of married couples (Bonanomi *et al.*, 2002). The vertebrate reproductive cycle depends upon delicate interrelationships between the sex hormones and the pituitary gonadotropic hormones (Nagabhashanam *et al.*, 2005). Male infertility is the male's incapability to cause pregnancy in a fertile female. It occurs due to some disorders such as hormonal disturbances, low sperm production, poor sperm quality and abnormal sperm function and others. Semen quality is used as an alternative measure of male fecundity (Cooper *et al.*, 2009).

Several potential approaches for infertility have been investigated for a long period of time, including hormonal, immunological and chemical approaches. The extremely high cost of imported drugs coupled with the inadequacy of modern health care personal and infrastructure excluded a very large majority of the third world population from any modern health care programme. Today the concerted ill effects with the continuing economic crisis have aggrieved the abject poverty affecting the majority of Africans. Consequently traditional medicine remains and will remain for a long time the main source and method of health for most of the developing countries. Although studies have reported the pro- fertility effect of many medicinal plants, to the best of our knowledge, no such study has been carried out on *Desmodium velutinum.*

Desmodium velutinum Dc (Fabaceae) is a perennial semi-erect shrub of tropical and sub-tropical regions. It grows up to 3 m high. The plant is used traditionally for treatment of a number of diseases such as Jaundice, rheumatism, puerperal fever, paralysis, oedema, filarial and post-natal care to avoid secondary complications. It also provides general support to the body during periods of influenza, cough, cold, neuralgia and headache; it is also used as a dietary supplement (Guyton *et al.*, 2008). The flavonoid and alkaloid fraction of *Desmodium velutinum* have been known to possess antioxidant and anti-inflammatory activities. Whereas its water decoction extract possesses anti-nociceptive activity, ethanol extract of the plant stem has also been shown to have hypocholesterolemic and antioxidant effect in isoproterenol induced myocardial infarction (Habig *et al.*, 2014). The leaves of *Desmodium velutinum* may be a source of pharmacological active agents useful in enhancing fertility. Thus this study evaluated its fertility effects in male Wistar rats.

Materials and Methods

Animals

Wistar rats of both sexes weighing 150–220g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed throughout the experiment.

Chemicals and Drugs

All the chemical used were of analytical grade and purchased from Sigma-Aldrich, U.S.A and Sildenafil citrate (Viagra®) was purchased from a pharmacy shop in Nsukka, Enugu state.

Plant Collection and Identification

The leaves of *Desmodium velutinum* were collected from a natural habitat in Nsukka Area of Enugu State, Nigeria. The plants were identified and authenticated at Pharmacognosy Department, University of Nigeria, Nsukka.

Figure 1: *Desmodium velutinum* in its natural habitat

Preparation of Extracts

The leaves of *Desmodium velutinum* were rinsed with distilled water to remove all debris. The leaves were then shade- dried for seven (7) days and pulverized using an electric blender. A known quantity (2000 g) of the pulverized leaves was cold -macerated in distilled water for 48 h after which it was filtered using Whatmann filter paper (Size No1). The filterate was concentrated using free- dryer to afford the extract. For the purpose of this study, the aqueous leaf extract of *Desmodium velutinum* shall henceforth be reffered to as DVAE.

Acute Toxicity Study

The acute toxicity study was carried out on the extract according to the method of Lorke (1983).

Experimental Design

Twenty (20) Male Wistar rats and eight (8) female rats were used for the experiment. The male rats were randomly divided into 4 groups of 5 animals each and treated daily for 28 days as follows:
Group I: Control (20ml/kg Dist. H_2O)
Group II: 200 mg/kg DVAE
Group III: 400 mg/kg DVAE
Group IV: 5 mg/kg Sildenafil

Mounting frequency test

On the 24th day of the treatment each male rat in each group was put in a cage with two estrous female rats. Oestrous was induced in the female rats using 1mg progesterone and 100µg ethinylestradiol 6 and 48 hours respectively before the pairing (Varsha *et al.*, 2013). The rats were observed for mounting behavior. The number of times the male rat mounts the female within five minutes time frame was counted and recorded.

Mating frequency test

On the 26th day of treatment the sexual episode/intromission is usually established when a male rat mount a female rat and lick its penis. The number of times each male rat in all the groups mounted a female and licked its penis was recorded for a period of five minutes (Varsha *et al.*, 2013).

Sperm analysis

At the end of the 28- day treatment, the animals were anaesthetized under chloroform vapour and sacrificed. The epididymis was exposed by scrotal incisions and transferred into petri-dish. The weight of the epididymis was recorded for each rat. The epididymis was crushed using a blunt forceps in a petri-dish and 1 ml of normal saline was added to semen and mixed thoroughly using a syringe to draw and release the mixture continuously (Verma *et al.*, 2002). The semen mixture was then sucked into a red blood cell pipette to the 0.5 mark, then normal saline was sucked up to the 101 mark. The normal saline in the stem of the pipette was discarded and the content of the bulb of the pipette was mixed thoroughly. A drop of the mixture was placed on the counting chamber which then spreads under the cover slip by capillary action. The counting chamber was then mounted on the slide stage of the microscope and viewed under x40 magnification. A grid system divides the counting chamber into five major squares each containing 16 smaller boxes. The count included all the sperm cells within the five major squares using the top and right or left and bottom system of counting as described by Verma *et al*. (2002) and Zaveneid and Polakoski (1977). The sperm count for a rat was calculated as = n x $1x10^{-6}$/ml of semen.

Sperm motility

A drop of the semen mixture was placed on a glass slide using 2 ml syringe, the preparation was placed on a microscope. Sperm motility was assessed as described by Sonmez *et al*. (2007). The motility of epididymal sperm was evaluated microscopically within 2–4 min of their isolation from the caudal epididymis and data were expressed as percentages of fast motile, slow motile and non-motile spermatozoa. The percentage of motility was evaluated visually at x40 magnification.

Statistical Analysis

Data were expressed as mean standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA, followed by Duncan's multiple comparisons test. Mean values were considered statistically significant when p-value is less than 0.05.

Results

Acute Toxicity Study

The extract up to a dose of 5000 mg/kg did not produce any sign of toxicity nor mortality. The oral LD_{50} the extract was then taken to be > 5000 mg/kg (Lorke's method).

Table 1: Observations from the Acute Toxicity Study of the Aqueous Extract of *Desmodium velutinum* in Wistar Rats

Phase	Group	Treatment (mg/kg)	D/T	Observed Sign of Toxicity
I	1	DVAE (10)	0/3	-
	2	DVAE (100)	0/3	-
	3	DVAE (1000)	0/3	-
II	1	DVAE (1600)	0/1	-
	2	DVAE (2900)	0/1	-
	3	DVAE (5000)	0/1	-

D= Death, T=Total number of animals treated

Mounting Frequency

The administration of the extract produced a significant (P≤0.05) and dose- dependent increase in mounting frequency compared to the control. At 400 mg/kg, the effect of the extract on mounting frequency was higher compared to the standard drug- sildenafil (Figure 2).

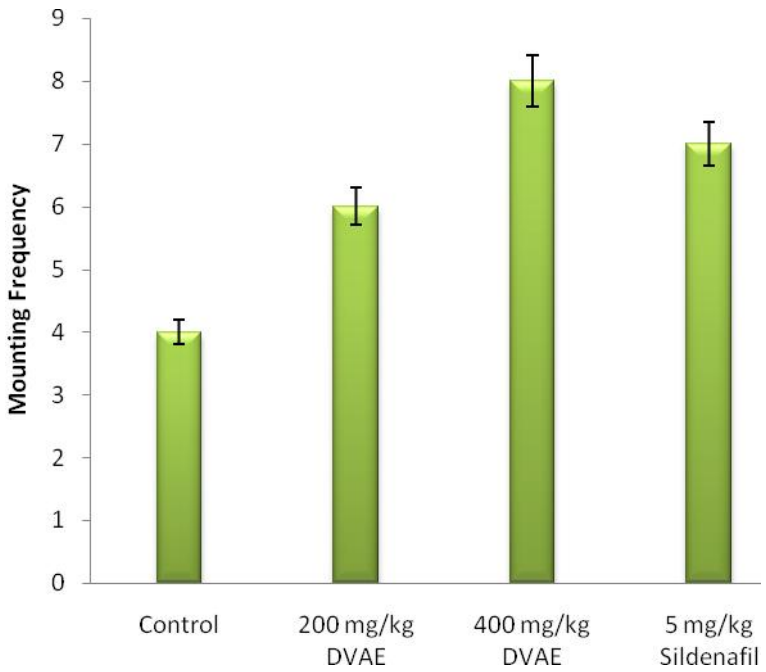

Figure 2: Effect of the Aqueous Extract of *Desmodium velutinum* on male mounting frequency in male rats

Mating Frequency (sexual episode)

There was a significant (P≤0.05) increase in mating frequency in rats treated with DVAE compared to the control. At both doses (200 and 400 mg/kg) of DVAE, the effect was not comparable to that of Sildenafil (Figure 3).

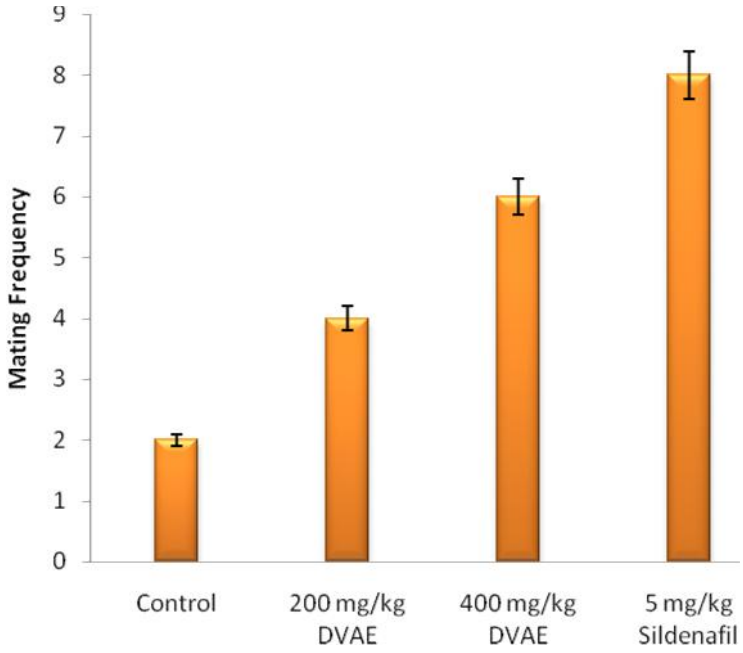

Figure 3: Effect of the Aqueous Extract of *Desmodium velutinum* on mating frequency in male rats

Effect of Aqueous Extract of *Desmodium velutinum* Leaves on the Sperm Count and Epididymal Weight of Wistar rats

The mean sperm counts of the DVAE- treated groups were significantly (P < 0.05) higher compared to the control group. The mean epididymal weight of same groups were also significantly (P < 0.05) higher than the control group but lower than that of the standard drug- sildenafil (Table 2).

Table 2: Effects of Aqueous Extract of *Desmodium velutinum* Leaves on Sperm count and Epididymal Weight in Adult Male Wistar rats

Groups	Sperm Count (million cells/ml)	Epididymal Weight (g)
Dist. H_2O (20ml/kg)	36.14 ± 2.26^{a}	0.13 ± 0.01^{a}
DVAE (200mg/kg)	40.21 ± 2.48^{a}	0.15 ± 0.05^{a}
DVAE (400mg/kg)	50.32 ± 2.78^{b}	0.25 ± 0.03^{b}
5 mg/kg Sildenafil	54.56 ± 2.38^{b}	0.27 ± 0.03^{b}

Data are presented as mean ± SD. (n=5). Means with different alphabets as superscript are significantly (P < 0.05) different

Effect of Aqueous Extract of *Desmodium velutinum* Leaves on Sperm motility of Wistar Rats

As presented in Figure 4, there was a significant ($P < 0.05$) and dose- dependent increase in actively motile sperm compared to control. The effect of the extract at 400 mg/kg was comparable to that of the standard drug- sildenafil.

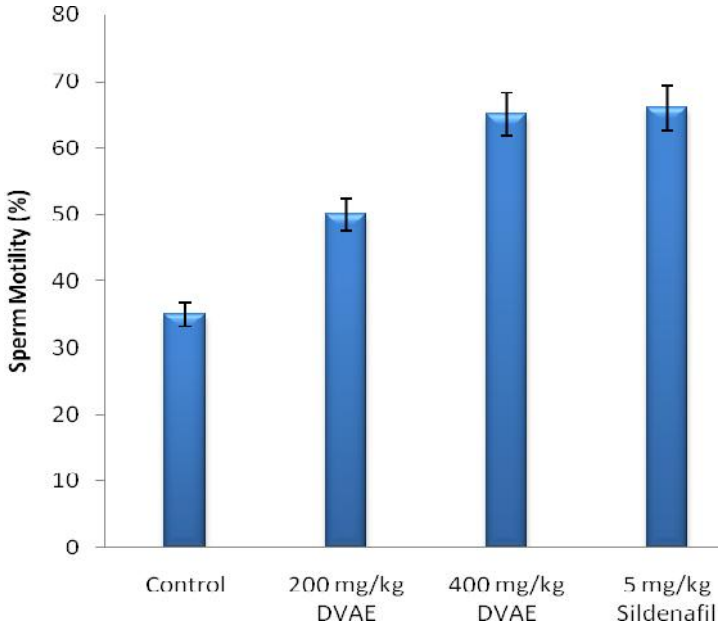

Figure 4: Effect of Aqueous Extract of *Desmodium velutinum* Leaves on Sperm motility of Wistar Rats

Effect of Aqueous Extract of *Desmodium velutinum* Leaves on Sperm Morphology of Wistar Rats

Figure 5 shows the effect of treatments on the morphology of sperm of rats. The extract produced significant ($P < 0.05$) increase in normal sperm with a corresponding decrease in abnormal sperm compared to control. The increase in normal sperm and decrease in abnormal sperm produced by the extract at 400 mg/kg was higher compared to that of Sildenafil.

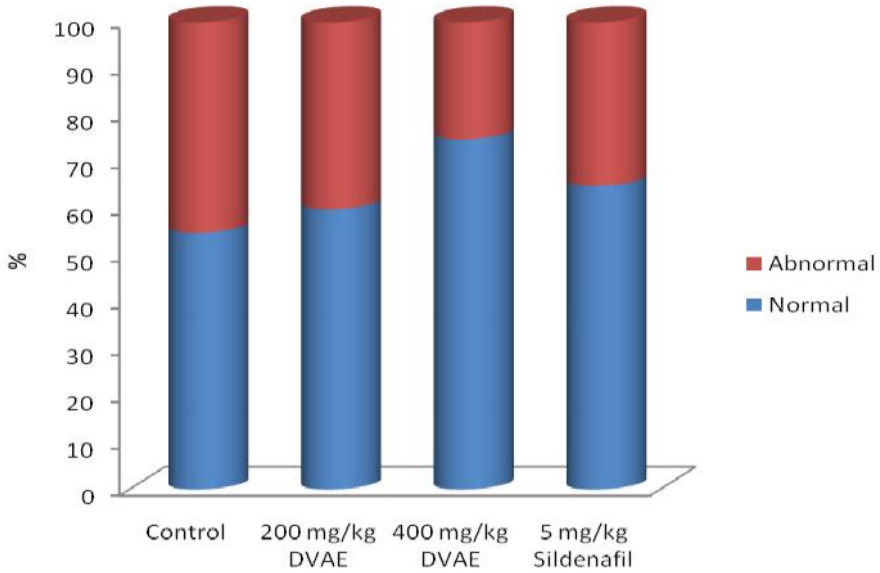

Figure 5: Effect of Aqueous Extract of *Desmodium velutinum* Leaves on Sperm Morphology of Wistar Rats

Discussion

On an average, about 10% of all couples face difficulty in starting a family and this creates a feeling of great personal failure, particularly in regions where religious and socio-economic traditions have made it almost imperative for everyone to have children. A significant association had been found between impaired semen quality including sperm count, motility and morphology. Herbal medicine remains a major solution to the problem of infertility for most of the developing countries. Hence a need exists for scientific validation of these plants with folkloric use. This study evaluated the fertility- enhancing effect of aqueous leaf extract of *Desmodium velutinum* on male wistar rats.

We first carried out an acute toxicity study on the extract to establish its safety. Results revealed that up to a dose of 5000 mg/kg, the extract did not produce mortality or any sign of toxicity. That is to say the extract is relatively safe when administered acutely. This is an advantage over the orthodox fertility drug which usually comes with side effects especially when administered in high doses. Further studies are however recommended to ascertain the safety of the extract when used for a longer period of time.

In this study, we observed the mounting and mating frequencies which are usually considered as indications of sexual arousal and desire (Neil *et al.*, 1990). Disorder of sexual desire (libido) can involve either a deficient or compulsive desire for sexual activity and may include hypoactive sexual desire (HSD), a persistent or recurrent deficient or absence of sexual fantasy and desire for sexual activity (APA, 1994). The significant increase in mounting frequency observed with the extract- treated rats compared to the control indicates an increase in sexual desire thus aphrodisiac activity. There was also an increase in mating frequency as observed across the groups. The significant increase in mating frequency produced by the extracts implies aphrodisiac properties specifically, arousal, motivation and vigor which enable penetration and consequently sexual intercourse (Yakubu *et al.*, 2007).

In this study, we also observed an increase in sperm count and motility. This was produced by the extract at both 200 and 400mg/kg doses. Thus, *Desmodium velutinum* have beneficial effects on male reproductive functions in rats. The epididymis weight of the rats also increased significantly with administeration of the extract. The significant increase in the epididymis could be due to increased androgen biosynthesis. Androgens have been shown to be necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands and studies have shown that the level is positively correlated with the weight of testis, epididymis, seminal vesicle and prostate glands (Setty *et al*, 1997). It is known that a major function of the epididymis is sperm maturation which leads to the acquisition of fertilizing ability and viability of spermatozoa. Therefore, improvement in the activities of the epididymis could have led to an increase in progressive motility of sperm in the experimental rats.

High percentage abnormal spermatozoa levels alongside low sperm count and motility have been associated with reduced fertility (Raji *et al* 2003). In this study, there was an observed increase in the normal sperm cells levels compared to the control. This increase in the normal cells enhances the fertilizing capacity of the Semen.

Conclusion

Conclusively, with the observed increase in mounting and mating frequencies in male rats and also the positive effects on the sperm count, sperm motility and epididymal weight of the rats treated with the extract, it is safe to say that the extract of *Desmodium velutinum* possesses aphrodisiac and pro-fertility effects.

References

American Psychiatric Association (APA) (1994). DSM-IV: Diagnostic and statistical manual of mental disorders. 4th edition. Washington, DC: American Psychiatric Press.

Bonanomi M, Lucente G. and Selvstrini B. (2002). Male fertility: core chemical structure in pharmacological research. Contraception. 2002;65(4):317-20

Cooper T. G, Noonan E, Von Eckardstein S, Auger J, Baker H. W, Behre H. M, Haugen T. B, Kruger T, Wang C. (2009). World Health Organization reference values for human semen characteristics. *Hum Reprod*; 16(3):231–45

Gyton, E., Araya, H., Horie, T., Hayashi, M. and Awazu, S. (2008). An alteration in the liver microsomal membrane of the rat following paracetamol overdose. *J. Pharm. Pharmacol.*, 39(2): 1047-1049.

Habig, W.H., Pabst, M. J. and Jakoby, W.B. (2014). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.*, 24(2): 130 - 139.

Lorke, D. (1983). "A new Approach to Practical Acute Toxicity Testing." *Archives of Toxicology* 54: 275-287.

Nagabhashanam R, Kodarkar M. S. and Sarojini R. (2005). Animal Physiology. 2nd ed. New Delhi: Oxford & IBH Publishing Company Pvt. Limited; 2005

Neil D, Vogel G, Hagler M, Kors D, Hennessey A. (1990). Diminished sexual activity in a new animal model of depression. *Neurosci and Biobehavioural Rev*; 14: 73-76.

Raji Y, Udoh U. S, Mewoyaka O. O, Ononye F. C. and Bolarinwa A. F. (2003) Implication of reproductive endocrine malfunction in male antifertility efficacy of *Azadirachta indica* extract in rats. *Afri. J. Med.Med.Sci* 32:159-165.

Serry B. S, Riar S. S. and Kar AB. (1997). Androgenic control of epididymal function in rhesus monkey and rabbit. *Fert Steril*. 22:674–81.

Sonmez M., A. Yuce, and Turk, G. (2007). The protecttice effect of melatonin and Vitamin E on anti-oxidant enzyme system activities and epididymal sperm characteristics of homocycysteine treated male rats. *Reproductive Toxicology*, 23, 226–231.

Varsha Z, Dinesh D, Vaibhao T. and Shital, P. (2013). Evaluation of potential aphrodisiac activity of *Moringa oleifera* seed in male albino rats. *Int J of Pharmacy and Pharmaceutical Sci*; 5(4): 683-689.

Verma RJ and N. J. Chinoy (2002). Effect of papaya seed on contractile response of cauda epididymal tubules. *Asian Journal of Andrology*, 4(1), 77-78.

Yakubu M. T, Akanji M. A, Oladeji AT. (2007). Sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. *PHCOG Rev*.; 1(1): 49-52.

Zaneveld, L. J. D. and Polakoski, K. L. (1977). Collection and physical examination of the ejaculate. In E. S. E. Hafez (Ed.), Techniques of human andrology. Armsterdam: North Holland Biomedical Press, 147-156.

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