www.ajbrui.org

Afr. J. Biomed. Res. Vol. 20 (January, 2017); 65- 73

Full Length Research Paper

Aphrodisiac Effects of Methanol Extract of *Smilax Kraussiana* Root in Experimental Rats

***Nwafor P.A and Oniyide V.K**

Department of Pharmacology & Toxicology, Faculty of Pharmacy University of Uyo, P.M.B 1017, Uyo, Nigeria

ABSTRACT

The aphrodisiac effect of methanol extract of *Smilax kraussiana* was investigated in adult albino male rats The extract was investigated on sexual behaviour based on the parameters of sexual indices such as mount and intromission latencies and ejaculation latency. Others include mount and intromission frequencies, erection frequency, post ejaculation interval as well penile erection in adult albino male rats. The extract caused increase in mount frequency, intromission frequency and erection frequency. Others were penile erection and ejaculation latency. These increases were statistically ($p < 0.001$) significant. Similarly, the extract also caused decrease in both mount and intromission latencies and in post ejaculation interval. These effects were observed in both sexually - active and in - active rats. There was an increase in body weights of all the animals treated with the extract coupled with those of sexual organs such as testis, epididymis and vas deference, these increases were statistically significant ($p < 0.001$) relative to control. The methanol extract of *Smilax kraussiana* caused a significant decrease in serum cholesterol level while the serum concentrations of aspartate and alanine aminotransferases as well as the alkaline phosphatase were elevated. These effects were statistically significant ($p < 0.001$) relative to control. The free radical scavenging ability of the extract against DPPH showed that the extract possessed some antioxidant properties. The photomicrograph of the sexual organs showed no histological abnormalities, rather there was an increase in seminiferous tubules proliferation. . Phytochemical screening of the extract showed that it contained alkaloids, saponin, cardiac glycoside, tannins and phlobatannins. The median lethal dose was calculated as 243.86mg/kg. The observed effects may in part be due to the secondary metabolites of the extract. These effects of the extract justified the folkloric use of the plant.

Key Words: Aphrodisiac, *Smilax kraussiana*, extract, male rats

*Author for correspondence: *paulnwafor@yahoo.com;* Tel: +234 803 677 8861

Received: August, 2016; Accepted: October, 2016

Abstracted by:
Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

In Homo sapiens, reproduction is initiated by the mating of a male and a female in sexual intercourse which facilitates the coming together of a sperm and egg for the purpose of fertilization. For there to be a normal sexual intercourse and sexual fulfillment in males, the male sexual organs (the copulatory organ, the penis) and factor relating to erection must function normally. Inability to perform this function effectively is a major problem facing reproductive process. This is known as sexual dysfunction. Sexual dysfunction can be managed by the use of aphrodisiac (Yakubu *et al*., 2007). An aphrodisiac can therefore, be defined as any food or drug that arouses the sexual instinct, induces veneral desire, and increase pleasure and performance (Neelesh *et al*., 2011). Aphrodisiacs are substances able to excite libido or arouse sexual instinct (Sandroni, 2001). These substances can be categorized according to their mode of action into three groups: by increasing libido (i.e. sexual desire), by increasing potency (i.e. effectiveness of erection) and by increasing sexual pleasure (Sandroni, 2001). Sexual dysfunction is a serious medical and social symptom that occur in 10-52 % of men and 25-63 % of women. Of men aged 40-70 years, an estimated 34.8 % have moderate to complete erectile dysfunction. It has been estimated that more than 152 million men worldwide experienced erectile dysfunction in 1995, and that the number will rise by 170 million, to approximately 322 million by the year 2025 (Hosseinzadeh *et al*., 2008). Enhanced sexual performance may provide increased relationship, satisfaction

and self-esteemed in human. Therefore, the study of aphrodisiac is important because they may provide a means to treat the psychological component of sexual dysfunction as opposed to the current treatments, which only treat the mechanical component (Singh *et al*., 2010).

A medicinal plant can be described as any plant in which one or more of its organs contain substance that can be used for therapeutic purpose, investigative or which are precausor for the synthesis of newer or useful drug (Sofowora 1982 modified). Many plants have been investigated for aphrodisiac properties and many of these plant extracts are traditionally employed among different cultures to enhance sexual performance. Among these are: Myristical fragrans (Hout) - Nutmeg, Allium sativa L (Garlic), Cannabis indica L (Indian hemp) among others (Noumi *et al*., 1998; Drewes *et al* 2003).One of the plants used locally among the Akai Nsit Ubium community in Nsit Ubium Local Government Area of Akwa Ibom State is Smilax kraussiana. (Okon Etefia, personal communications 2011) Previous work on the plant indicated, acute toxicity (Nwafor *et al*; 2006), phytochemical and antimicrobial studies (Hamid *et al*., 2011), anti-inflammatory property, hepatotoxic effect (Iwu & Anyanwu, 2006), analgesic and anti-inflammatory activities (Nwafor *et al*., 2010) and in-vivo antiplasmodial and antipyretic activities (Okokon et. al., 2012) A search in literature revealed no documented scientific literature on its use on sexual dysfunction hence this work is intended therefore, to determine the rationale for its ethno-botanical use and the mechanism for its effectiveness as an aphrodisiac.

MATERIALS AND METHODS

Collection and identification of plant material: The root of Smilax kraussiana was obtained from Akai Nsit Ubium in Nsit Ubium Local Government Area of Akwa Ibom State. It was identified and authenticated by Dr. (Mrs.) Margaret Bassey in the Department of Botany and Ecological Studies, University of Uyo. A voucher specimen of the plant with voucher number: Reg. No.UU/HER.No40e was deposited in the herbarium of Department of Botany and Ecological Studies, University of Uyo.

Plant extraction: The root was chopped to small pieces, air-dried for two weeks. The dried root was pulverized (reduced to coarse powder) using pestle and mortar. The powered root sample was macerated for 72 h with 250 ml of methanol. The extract was concentrated to dryness in a water bath and gradually reduced as the volume of the extract decreases at 40 ^{o}C to give dark brown viscous extract. Extract was stored at -4 ^{o}C until used.

Phytochemical screening: The phytochemical screening of the extract was performed according to the methods of Clarke (1975), Odebiyi and Sofowora (1978), Trease and Evans (1989) and Harbourne (1984). Tests for alkaloids, saponins, flavonoids, tannins, terpenes, anthraquinones, simple sugars and cardiac glycosides.

Animal preparation: Adult and mature albino rats of both sexes weighing 150-250 g were obtained from the Animal House of University of Calabar, Calabar. The male and female rats were kept separately and quarantined for 2 weeks for acclimatization and maintained under standard conditions (12 h light/12 h dark cycle) at the animal house of the Department of Pharmacology and Toxicology, University of Uyo, Uyo. The animals were fed with grower marsh (Grand Bendel Feed, Ltd., Edo State, Nigeria) and water was given ad-Libitum. Female receptivity was induced using the method of Padashettey & Maishra (2007) with a little modification. 48 h prior to the pairing in the cage, 17- β- estradiol was administered to the female subcutaneously (sc) to induce the estrous cycle, making the females receptive to the males. 40 h later, progesterone (500 µg/kg) dissolved in corn oil was administered (sc) to the female rats to enhance the effect of the 17- β- estradiol.

The male rats were characterized into sexually-active, sluggish and impotent. This was done by pairing the male rats with a sexually mature receptive female in a transparent plexiglass cage (46 cm x 41cm x 41 cm) at 19.00 h, using a red bulb and observed the following parameters:

Mount latency (ML): Time taken for the first mount to occur following the introduction of female.

Intromission latency (IL): Time taken from the introduction of female to first intromission (vaginal penetration).

Ejaculation latency (EL): This is the interval between first intromission and first ejaculation.

Mount frequency (MF): This is the number of mounts observed in 30min.

Intromission frequency (IL): This is the number of intromissions observed in 30min.

Erection frequency (EF): This is the number of erections observed in 30min.

Post ejaculation interval (PEI) or Post Ejaculation latency: This is the time from ejaculation to the subsequent intromission.

Penile erection: This is the number of times the rat bent down to lick the penis in 30min (characterized by penile body elongation and dorsiflexion reddening and engorgement of the glans)

Only the results obtained from the last three pre-experimental test were used. Rat achieving ejaculation in all the three tests were defined as sexually active. One or two of the three pre-experimental tests were considered as sexually sluggish. Those that failed to achieve ejaculation in all three tests were considered sexually impotent. The sexually impotent males were discarded and the sexually active and sluggish males were used for the experiment (Zaloni *et al*., 2009).

Effect of extract on male sexual indices: Sexually, mature, active male rats were used for this experiment. Thirty-six male rats were used to determine the aphrodisiac effect in sexually-active males. They were divided into six (6) groups of six rats each. Group 1 was given normal saline (10 ml/kg, i.p) and served as the negative control. Groups II, III and IV were given 24, 48, 72 mg/kg bw., (i.p.) of extract of respectively. Group V was administered with testosterone (1 mg/kg bw.) and served as the reference group. Group VI was given testosterone (1 mg/kg bw., sc) and 10 minutes later, extract (48 mg/kg i.p.) was administered. All agents were administered for 5 days. On the sixth day, food was withdrawn 3 h prior to the experiment. Each male rat was introduced into the plexiglass cage 10 min prior to the introduction of the female rat, for acclimatization. The rats were observed for 30 min. However, experiment was

terminated if any of the following conditions occur before 30 min.: immediately after the post ejaculation intromission, if the intromission does not occur within 30 min, if in any case the post-ejaculation interval exceed 30 min, if the ejaculation latency exceeds 30 min. The above experiment was repeated using sexually sluggish male rats.

Effect of extract on body weight and sexual organs.: After 5 days of treatment, all the treated and the control rats were weighed, and the changes in their body weight were recorded. The animals were sacrificed by cervical dislocation, the sexual organs were carefully removed and their weights were determined. The weight of the liver and kidney were also determined. (Padshetty *et al*., 2007).

Effect of extract on biochemical parameters.: Biochemical analyses were carried out in adult albino rats. After five (5) days of treatment of all rats, the animals were anaesthetized with ether, sacrificed and the blood samples were collected centrifuged at 5000 rpm for 15 min and the clear sera was separated, collected and submitted to the Department of Chemical Pathology, University of Uyo Teaching Hospital, for the following investigations: cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

Effect of extract on 2,2-diphenyl -1-picrylhydrazyl (DPPH) - induced scavenging activity: The free radical scavenging ability of the extract against DPPH (1,1,-diphenyl-2picryl-hydrazyl) free radical was evaluated (Ursini,1994). Briefly 1 ml aliquot (0.05 g of the extract was dissolved in 20 ml of methanol) was mixed with 1 ml of 0.4 mM methanolic solution consisting of DPPH radicals, the mixture was then left in the dark for 30 min before measuring the absorbance at 516 nm.

Histological studies: After five (5) days treatment of all the respective groups, the testis and the seminal vesicle from each group were excised during the dissection and the following tissues: testis and seminal vesicle were fixed in 10 % buffered neutral formalin. The tissues were then transferred to Department of Histopathology, University of Uyo Teaching Hospital for slide preparations, photomicrograph evaluations and interpretations.

Statistical analysis: Multiple comparisons of Mean±S.E.M were carried out by one way analysis of variance (ANOVA) followed by Tukey-Krammar multiple comparisons post tests. A probability level of less than 5% was considered significant.

RESULTS

Phytochemical screening : Phytochemical screening of the extract revealed the presence of alkaloids, saponins, cardiac glycosides, tannins and phlobatannins. Flavonoids were however, equivocal.

Acute Toxicity Studies: (median lethal dose): The median lethal dose (LD_{50}) was determined to be 243.86mg/kg body weight. The physical signs of toxicity were writhing, gasping, palpitation, decreased respiratory rate and death.

Effect of the extract in sexually - active male rat: The effect of extract in sexually-active male rats is as shown in Table 1. The extract decreased mount latency, intromission latency and post ejaculation interval. The extract however increased ejaculation latency, mount frequency, intromission frequency, erection frequency and penile erection. These effects were statistically significant ($p<0.01$ - 0.001) relative to control.

Effect of extract on sexually - inactive male rat: The effect of extract in sexually-inactive male rats is as shown in Table 2. The extract increased ejaculation latency, mount frequency, intromission frequency, erection frequency and penile erection . The extract, however, decreased mount latency, intromission latency and post ejaculation interval. These effects were statistically significant ($p <0.001$) relative to control.

Effect of extract on body weight and sexual organs: Measurement of the body weight of the animals throughout the experimental period showed significant differences between the treated and control groups. All the animals gained weight. Increase in weight observed in testis, epididymis, vas deference were statistically significant ($p < 0.01$) while liver though, increased, was not statistically significant. Kidney and seminal vesicle remain equivocal (Table 3).

Effect of extract on cholesterol and liver function enzymes: The extract decreased cholesterol and AST levels in the serum. However, there were increased ALT and ALP levels in the serum. These effects were statistically significant ($p <0.05$ - 0.001) relative to control (Table 4).

Effect of extract on DPPH: The effect of the extract on free radical scavenging activity is as shown in Fig 1. The extract showed a dose - dependent free radical scavenging property.

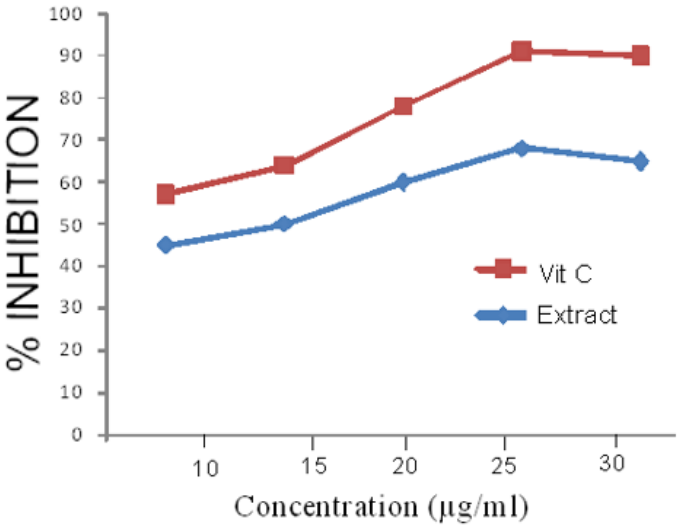

Fig. 1:
The effect of extract on DPPH scavenging radical

Table 1:
Effect of Methanolic Root Extract of *Smilax kraussiana* on Sexual Behaviour of Sexually-Active Male Rat

Control	Mount Latency (Seconds)	Intromission Latency (Time In Seconds)	Ejaculation Latency (Time In Seconds)	Post Ejaculation Intromission	Mount Frequency	Intromission Frequency	Erection Frequency	Penile Erection
Control	17.50±1.04	20.60±0.45	5.70±0.02	6.05±0.12	17.20±0.64	11.20±0.24	8.30±0.05	6.00±0.37
24mg/kg	3.80±0.12[b]	4.20±0.70[b]	11.10±0.70[b]	5.10±0.57[a]	33.50±0.22[b]	29.70±5.82[b]	36.30±1.20[b]	29.70±0.48[b]
48mg/kg	4.20±0.40[b]	6.30±0.84[b]	9.06±0.31[b]	3.66±0.12[b]	55.50±0.42[b]	42.80±1.05[b]	39.70±1.40[b]	36.30±0.11[b]
72mg/kg	5.30±0.47[b]	7.60+1.63[b]	13.87±0.11[b]	4.82±0.55[b]	16.70±1.35[ns]	11.20±2.44[ns]	12.20±0.21[b]	9.80±0.21[b]
Testosterone	5.20±0.79[b]	6.00±1.06[b]	6.98±0.13[b]	3.90±0.06[b]	36.00±1.04[b]	24.80±0.45[b]	25.50±0.45[b]	21.50±0.50[b]
Testosterone +48mg/kg	6.80±1.04[b]	9.00±1.32[b]	7.60±0.07[b]	6.00±0.61[ns]	35.80±0.50[b]	28.00±0.24[b]	31.00±0.12[b]	26.70±0.30[b]

Values represent Mean ± S.E.M ; Significance related to control: [a]p<0.01; [b]p<0.001; (n=6); ns=not significant

Table 2:
Effect of Methanolic Root Extract of *Smilax kraussiana* on Sexual Behaviour of Sexually-Inactive Male Rat

	Mount Latency (Time In Seconds)	Intromission Latency (Seconds)	Ejaculation Latency (Time In Seconds)	Post Ejaculation Intromission	Mount Frequency	Intromission Frequency	Erection Frequency	Penile Erection
Control	35.60±0.85	40.60±0.31	7.70±0.12	11.85±0.27	14.00±0.12	9.50±0.44	7.20±0.30	7.00±0.11
24mg/kg	10.00±0.58[b]	13.20±0.21[b]	13.20±0.21[b]	7.50±0.14[b]	26.00±0.02[b]	20.20±1.05[b]	22.20±0.11[b]	18.20±0.10[b]
48mg/kg	7.80±0.10[b]	12.00±0.25[b]	12.80±0.10[b]	6.80±0.12[b]	34.20±0.67[b]	28.50±0.45[b]	31.70±0.08[b]	30.00±0.12[b]
72mg/kg	16.00±0.57[b]	17.20±0.14[b]	13.60±0.21[b]	8.00±0.10[b]	18.30±0.04[b]	14.80±0.50[b]	20.80±0.16[b]	16.20±0.50[b]
Testosterone	7.60±0.49[b]	11.60±0.80[b]	8.50±0.17[b]	5.06±0.14[b]	31.00±0.21[b]	25.30±0.18[b]	28.50±0.15[b]	23.30.54[b]
Testosterone +48mg/kg	11.30±0.49[b]	13.00±0.31[b]	8.96±0.14[b]	10.00±0.10[b]	23.00±0.31[b]	19.00±0.14[b]	27.20±0.65[b]	21.50±0.40[b]

Values represent Mean ± S.E.M; Signifance relative to control: [b]p<0.001; (n=6)

Table 3:
Effect of Methanol Root Extract of *Smilax kraussiana* on Body Weight and Weight of Organs(mg)

	Initial Weight	Final Weight	Testis	Epididymes	Vas Deferens	Seminal Vesicle	Liver	Kidney
Control	195.20± 0.11	198.00±0.56.	1.67±0.10	0.59±0.06	0.16±0.02	0.75±0.09	6.15±0.31	1.27±0.056
24mg/kg	196.30±0.4	208.00± 0.98 [c]	1.99±0.07[b]	0.705±0.01[a]	0.17±0.01	0.38±0.56 [c]	5.17±0.19	1.02±0.28
48 mg/kg	200.30±2.08	213.30±0.83 [c]	2.07±0.04 [b]	0.76±0.03[c]	0.17±0.02	0.90±0.08	7.30±0.26	1.36±0.03
72mg/kg	194.33±11.36	210.00±0.93 [c]	2.13±0.01 [b]	0.81±0.13 [c]	0.52±0.14 [c]	0.197±0.01 [c]	7.67±0.56	0.52±0.13
Testosterone	197.00±7.7	212.20±0.72 [c]	2.17±0.07[c]	0.80±0.03 [c]	0.19±0.03	0.97±0.09 [c]	6.50±0.26	1.29±0.09
Testosterone + 48 mg/Kg	198.10±8.12	205.3±4.81 [c]	2.09±0.05 [b]	0.77±0.02 [c]	0.18±0.03	0.85±0.42	6.68±063	1.31±0.76

Values represent Mean ± S.E.M; Significance related to control: [a]p<0.05; [b]p<0.01,[c]p<0.001; (n=6); weights are in grammes (g)

Table 4:
Effect of Extract on Some Biochemical Parameters.

Group	Dose (mg/kg)	Cholesterol	AST	ALT	ALP
1.	ControL	4.75± 0.02	95.96±0.11	16.88±0.15	136.13±0.16
2.	24	2.06± 0.10[b]	76.63±0.10 [b]	33.9±0.07 [b]	191.38±0.70 [b]
3.	48	2.34±0.08 [b]	88.13±0.15 [b]	25.88±0.15 [b]	148.38±0.11 [b]
4.	72	2.74±0.04 [b]	100± 0.05 [b]	38.63±0.05 [b]	191.39± 0.16 [b]
5.	Testosterone	4.68±0.32[ns]	110.38±0.01 [b]	33.5±0.81 [b]	123.13±0.15 [b]
6.	Testosterone+48	4.03±0.12[a]	73.0±0.14 [b]	27.88±0.45[b]	157.63±0.14 [b]

Values represent Mean ± S.E.M; Significance related to control: [a]p>0.05; [b]p>0.001; (n=6); ns=not significant

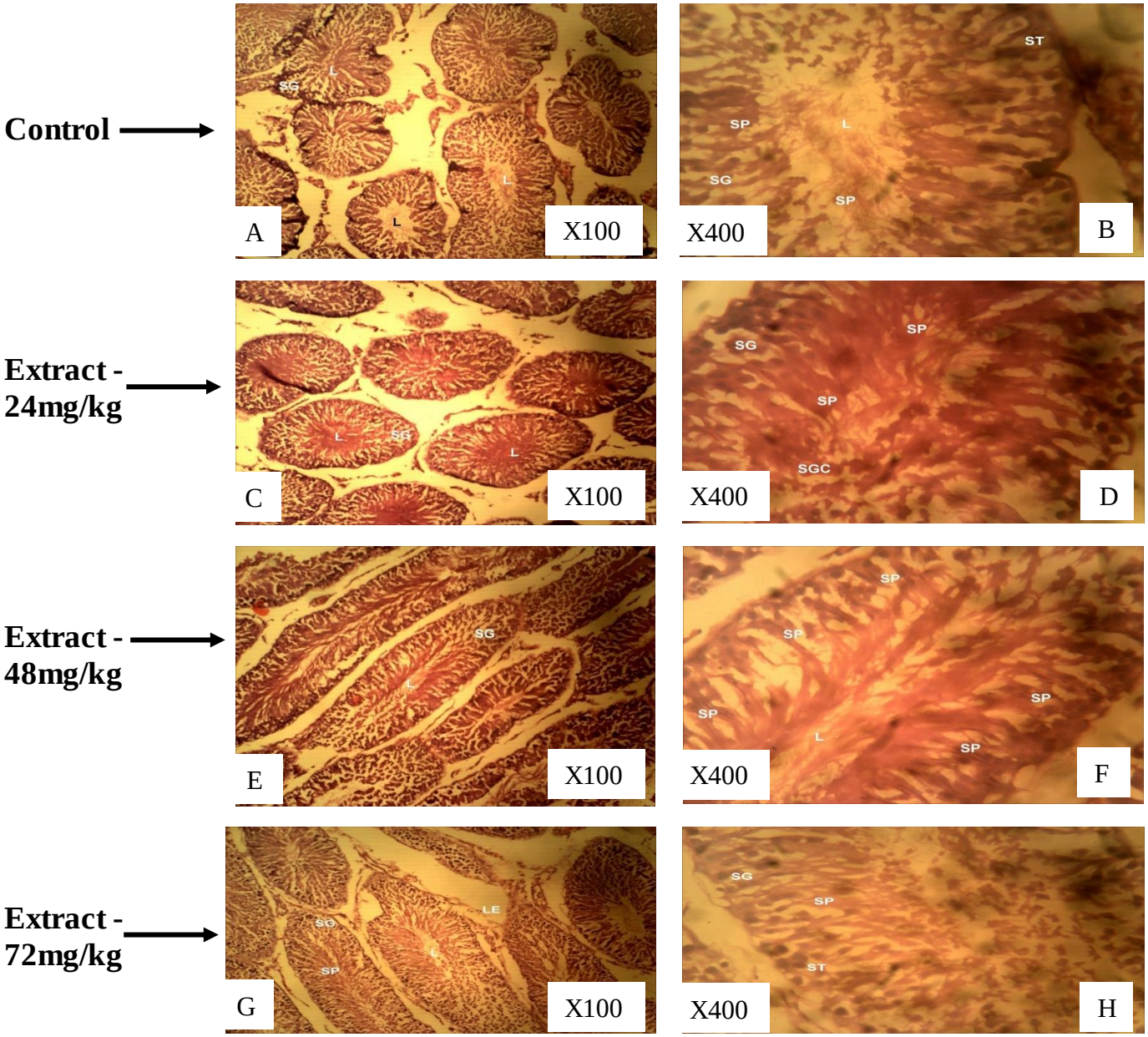

Plate 1
Cross section of testis of rat administered with normal saline (A, B) and methanolic Root Extract of *Smilax kraussiana* at 24mg/kg (C, D) with Lumen containing artificially sloughed-off germ cells (SGC), 48mg/kg (E,F) and 72mg/kg (G,H) with reduced sperm cells. [spermatogonia (SG), sperm cell (SP) and lumen (L).]Sertol, cell (ST)

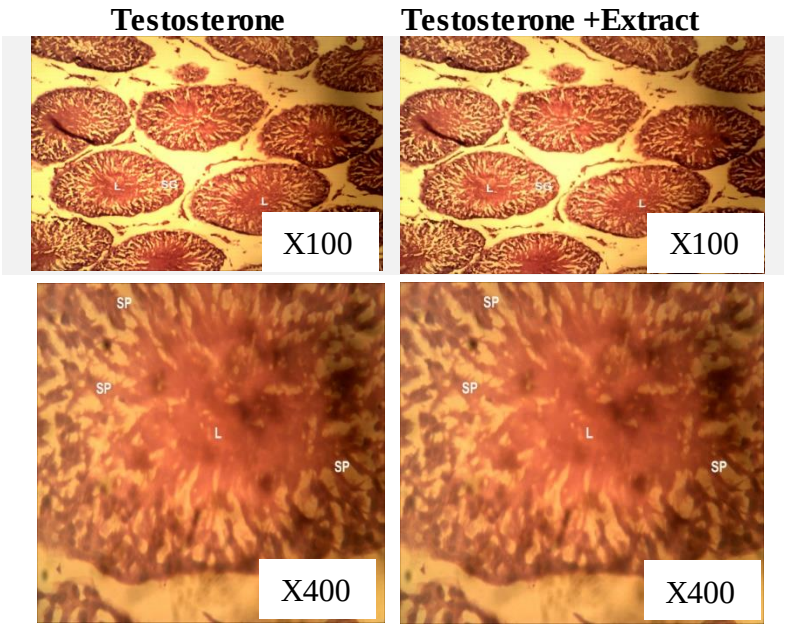

Plate 2
Cross section of testis of rat administered with testosterone with or without the extract at 48mg/kg. [spermatogonia (SG), sperm cell (SP) and lumen (L).]. Sertol, cell (ST)

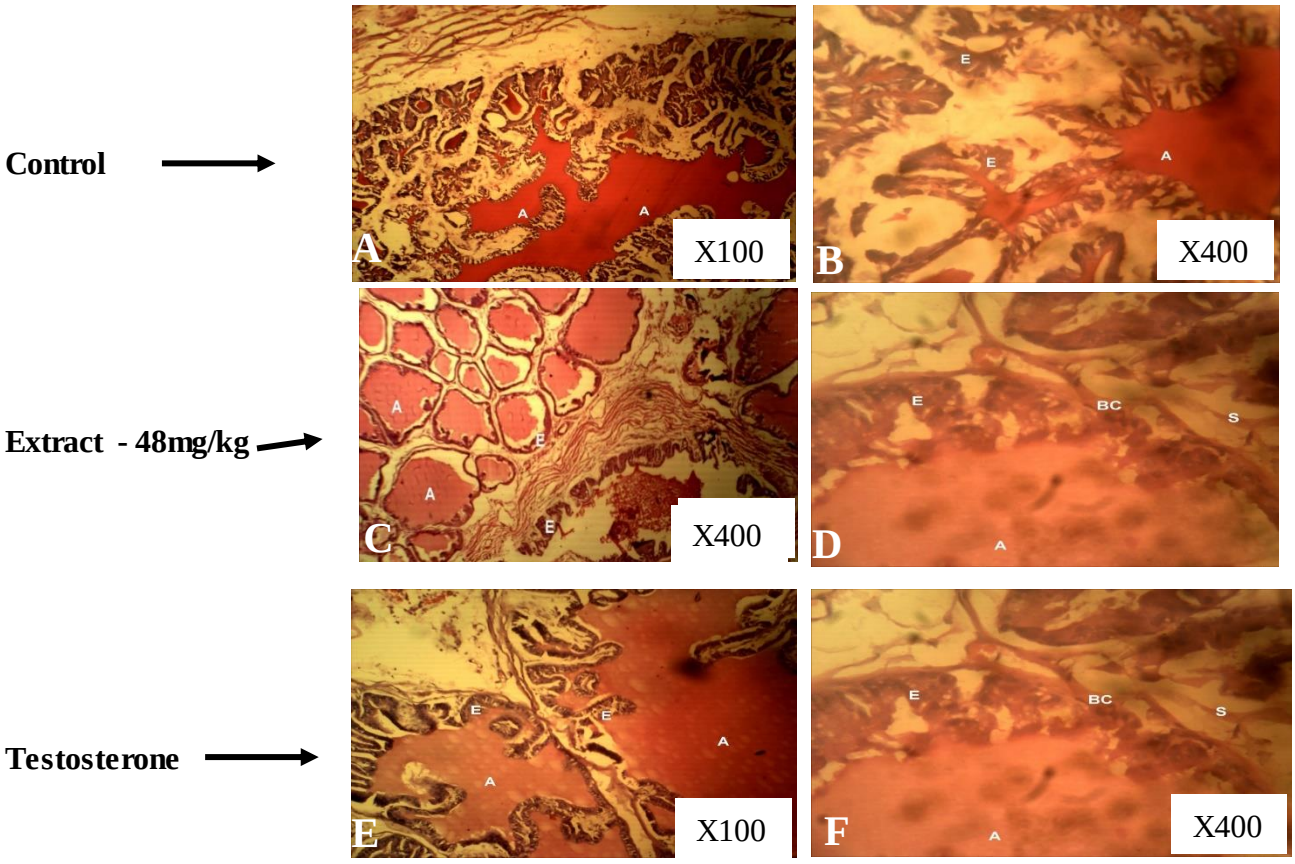

Plate 3:
showing the cross-section of the seminal vesicle of rat administered with normal saline (A, B), methanolic Root Extract of *Smilax kraussiana* (C and D) or testosterone (E and F)

Effect of the extract on organs of localization: Histological sections showed the testis and seminal vesicles with testicular and numerous seminiferous tubules containing germ cells at various stages of development. The layer of spermatogonia and sperm cells are normal in density in the extract - treated rats (Plates 1-8)

DISCUSSION

The indices of sexual dysfunction occur in both male and female. Sexual dysfunction affects women (43%) and men (31%). (Hosseinzadah *et al*.,2008). There are several approaches to restore sexual function in both males and females (Montorsi *et al*.,1993). These ranged from the use of synthetic drugs, mechanical maneuvers to natural products among others. Many plant extracts are traditionally employed among different cultures in order to manage sexual inadequacies (sexual dysfunction) (Yakubu *et al*.,2007). One of the plants whose extract is employed is *Smilax kraussiana*. It is used in Tanzania for the treatment of sexual dysfunction in males (Hamid and Aiyelaagbe.,2011). It is also utilized in the treatment of inflammation and sexual dysfunction among the Ibibios of South Eastern Nigeria (Okon Etefia, personal Communications, 2011).

The results of the present study suggest that the extract of *Smilax kraussiana* caused an anabolic effect which is comparable to the testosterone treated rats. Administration of the extract resulted in weight gain in treated animals. Testes and epididymal weights were also increased significantly, a moderate but significant increase in seminal vesicle weight was observed. Steroid is one of the substances/drugs that has been known to cause increased body and sexual organ weights, and increase in these parameters could be regarded as a biological indicator for effectiveness of the herbal drug in improving genesis of steroidal hormone. (Mayank and Dixit ,2007). Since androgenic effect is attributable to testosterone level in the blood, it is likely that the plant extract may have a role in testosterone secretion allowing better availability of the hormone to the gonads.

Methanol extract of *Smilax kraussiana* root produced statistically significant increase in the indices of sexual vigour of mount and intromission frequencies as compared to the control group. However, the extract did not produce any significant increase in the rat treated with the highest dose. Mount and intromission frequencies are considered the indices of both libido and potency. Thus, the increase in both indices indicated that *Smilax kraussiana* enhanced both the libido and potency (Tajuddin *et al*., 2005)

The extract reduced mount latencies, intromission latencies and post ejaculation intromission. Reduced mount latencies, intromission latencies and post ejaculation intromission have been correlated with invigoration of endocrine system thereby, resulting in enhanced sexual motivation, arousal and performance Abdulwaheb *et al*., 2006; Mayank and Dixit, 2007). Mount and intromission frequencies are maintained and sustained by penile erection and its frequencies. These parameters are also indications of aphrodisiac properties inherent in the plant extract (Ratnasooriya and Dharmasin.,2000; Yakubu *et al*.,2005). Post erection index (PEI) also known as post ejaculation interval is important for evaluating the effect of the extract on erectile function (Vikas *et al*.,2008). The shorter it takes for erection to be initiated after ejaculation the more powerful is the aphrodisiac effect of the extract/drug. The extract increased penile erection of the male rats. An increase in PEI was observed in treated groups, indicated the involvement of nitrous oxide (NO) based intervention (Mayank and Dixit, 2007). The effect on penile erection is more pronounced when compared to the administration of testosterone.

Cholesterol is the precursor in the synthesis of many physiologically important steroids such as bile acids, steroid hormones and vitamin D, its requirement for normal testicular activity has been well established (Bedwell *et al*.,1994; Watcho *et al*.,2004). A medicinal plant with potential for aphrodisiac activity should result in statistically significant increase in testicular cholesterol and/or decrease in serum cholesterol concentration (Yakubu *et al*., 2005). Such increase may imply stimulation in steroidogenesis, which may lead to increased testosterone concentration. The increase in testosterone concentration normally reflect a corresponding increase in libido (Yakubu *et al*., 2005). The extract decreased cholesterol in the serum relative to control. This decrease in cholesterol level helped to authenticate the result obtained from the sexual behavior which indicated that extract caused an increase in libido.

The enzyme alkaline phosphatase was found to increase the prostratic secretion and help to maintain the appropriate pH of seminal fluid, which in turn is necessary for retaining the sperm motility (Padashetty and Maishra,2007). A significant increase in alkaline phosphatase level was observed in all the animals treated with the extract indicating that the extract enhances sperm motility in the animals. AST is an enzyme localized in the liver, kidney, heart, muscle and other organs (Yakubu *et al*., 2005). while ALT is a specific enzyme in the liver (Nwafor *et al*.,2006), both enzymes can be used to evaluate liver damage (cytolysis). Excessive as well as insufficient liver enzymes indicate dysfunction of the liver. *Smilax kraussiana* extract produced a significant decrease on AST in low dose and middle dose but a significant increase in high dose. This suggests that the extract is safe at low and moderate doses but toxic at high doses. The increase in ALT is a sign of dysfunctional liver.

Reactive oxygen metabolites, such as hydrogen peroxide, superoxide radical, nitric oxide radical, among others, appear to play many diverse roles in the maintenance and disruption of cell physiology. On one hand, they have been found to perform cell signaling functions in different cell types, including spermatozoa, they have also been linked to ageing, disease, and apoptosis (Aitken.,1995). The role of oxidative stress in the aetiology of male infertility has been clearly established by a series of studies (Aitken *et al*.,1998; Twigg *et al* .,1998). High concentration of these reactive oxygen species (ROS) is associated with loss of sperm motility and decrease in sperm oocyte fusion (Aitken., *et al*, 1998), thus making the health of the sperm to depend upon antioxidant. 2,2-diphenyl-1-picylhydrazyl (DPPH) is a stable radical that has been used widely to evaluate the antioxidant activity of various natural products (Singh *et al*.,2010). In this study, the ability of DPPH scavenging activities has been evaluated in the extract. The effect of DPPH was assayed in the presence of the extract and

was observed that the antioxidant property of the extract was dose - dependent. Antioxidants help to protect tissues and organs such as testis, epididymis and seminal vesicles from oxidative stress (Joseph *et al* 2007) Therefore, observed enhanced libido in animals treated with extract might in part be due to the presence of antioxidant property inherent in the extract.

The phytochemical screening of the plant revealed the presence of alkaloid, saponins, cardiac glycoside, tannins and phlobatannins with the exclusion of flavonoids. The presence of indole alkaloid and saponin in the extract may also contribute to its sex-stimulating activity as they are known to enhance the androgen level (Ageel *et al*.,1994). The photomicrographs indicated that the extract is non- toxic to the organs of reproduction in male rats rather, it enhanced the proliferation of the cells of seminiferous tubules which is responsible for spermatogenesis.

In conclusion therefore, though the work is not conclusive as cellular mechanism and the structural elucidation of the compound responsible for these effects are not elaborated, however, the results obtained from this investigation confirmed that the indices of libido (sexual desire), potency (effectiveness of erection) and sexual pleasure validate the folkloric use of the plant among the indigenes (Ibibios) of South Eastern part of Nigeria in the treatment of sexual dysfunction. These effects may in part be due to the secondary metabolites of the plant.

Conflict of interest declaration

The Authors declare no conflict of interest.

Acknowledgements

The authors gratefully acknowledge Mr. Nsikan M. Udo, Department of Pharmacology & Toxicology for technical assistance, Departments of Chemical Pathology and Histopathology for their analysis and interpretations of the specimens, and Mr Ikechukwu Ezeocha of CHI Pharmaceuticals Ltd; Nigeria, for the gift of 17ß –estradiol.

REFRENCES

Abdulwaheb, M., Makonnen, E., Debella, A., Abebe, D (2007) Effect of Catha edulis foresk (Khat) extracts on male rat sexual behavior. Journal of Ethnopharmacology 110: 2(21), 250 – 256

Ageel, A. M, Islam, M. W., Ginawi, O. T and AL-Yahya, M. A. (1994). Evaluation of the aphrodisiac activity of Listea chinensis (laureaceae) and orchis malaculata (orcidaceae) extract in rats. *Phytotheraphy Research* 8:103-105.

Aitken, R. J., Paterson, M., Fisher, H., Buckingham, D. W. and Duin, M. V. (1995). Redox regulation of tyrosine phosphorylation in human spermatozoa and its role in the control of human sperm function. *Journal of Cell Science* 108:2017-2025.

Aitken, R. J., Gordon, E., Harkiss, D., Twigg, J. P., Milne, P., Jenning, Z. and Irvine, D. S. (1998). Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biology of Reproduction* 59:1037-1046

Bedwell, R. S, Edwards, M. S., Katoch, M., Bahuguna, A., and Dewan, R. (1994). Histological and Biochemical changes in Testes of Zinc deficient BALB/C strain of Mice. *Indian Journal of Experimental Biology*, 32: 243-247.

Clarke, E.G.C.(Ed.) (1975) Isolation and identification of drugs. Vol. 2 Pharmaceutical Press, London pp .905

Drewes, S.E., George, J. and Khan, F. (2003) Recent findings on natural products with erectile dysfunction activity. Phytochemistry 62: 1019 – 1025

Hamid, A.A. and Aiyelaagbe, O. O. (2011). Phytochemical investigations and antimicrobial Studies of *Smilax kraussiana* Twig (Smilacaceae). International Journal of Academics Research 3(2): 824 – 828

Harborne, J.B.C.(1984) Phytochemical methods: a guide to modern technique of plant analysis. 2nd edition. Chapman and Hall Ltd., London p283

Hosseinzadeh, H., Zlae T. and Sadeghi, A.I. (2008). The Effect of Saffron, Crocus Sativus Stigma, Extract and its Contituence safronal and crocin on Sexual Behavior in Normal Male Rats. *Phytomedicine* 15:491-495.

Iwu, M.M. and Anyanwu, B.N. (1982) Phytotherapeutic profile of Nigerian herbs. 1. Anti- inflammatory and anti – arthritic agents. Journal of Ethnopharmacology 63: 263 – 272 Joseph, S., Sabulal, B., **George, V., Sauna, T.P., Janardhaanan, K.K.,(2007)** Antioxidative and anti-inflammatory activities of chloroform extract on *Ganoderma lucidum* found in South India. Scientia Pharmaceutica. 77: 111 – 121

Mayank, T. and Dixit, V.K. (2007). Aphrodisiac Activity of *Dactylorhiza hata girea* (D. Don) SOO in Male Albino Rat. Evidence-Based Complementary and Alternative Medicine 4:29-31.

Montorsi, F., Briganti, A., Salonia, A., Rigatti, P. and Burnette, A. L. (2006). Can Phosphodiesterase Type Inhibitor cure erectile Dysfunction European Urology, 49; 979-986

Neelesh, M., Sanjay, J., Vipin, B. G. and Savita, V. (2011). Recent Studies on Aphrodisiac Herbs for the Management of Male Sexual Dysfunction - a Review. *Acta Poloniae Pharmaceutica -Drug Research*, 68(1):3-8.

Noumi, E., Amivam – Zollo, P.H. and Lontsi, D (1998) Aphrodisiac plants used in Cameroon. Fitoterapia 2: 125 – 134

Nwafor, P.A., Ekpo, M., Udezi., T.W., Okokon, J.E, Bassey, A.I. (2006) Acute toxicity potential of methanolic extract of Smilax kraussiana leaves in rats. International Journal of Pharmacology 2(4): 463 - 466

Nwafor, P.A., Nwajiobi, N., Uko E. and Obot, J.S. (2010). Analgesic and anti- inflammatory Activities of Ethanol Extract of *Smilax kraussiana* Leaf in Mice. *African Journal of Biomedical Research*, 13:141-148.

Odebiyi, O.O., Sofowora, E.A. (1978) Phytochemical screening of Nigerian medicinal plants. Lloydia 41: 234 - 235

Okokon, J.E., Ndehekedehe, I., Akpan, E.J. (2012) In- vivo antiplasmodial and antipyretic activities of *Smilax krausiana*. Phytopharmacology 3(2): 376 – 385

Padeshetty, S.A. and Maishra, S.T. (2007). Aphrodisiac Studies of *Tricholepis glaberrima* with supportive action from Antioxidant Enzyme. *Pharmaceutical Biology*,45 570-586.

Ratnasooriya, W. D. and Dharmasiri, M. G. (2000). Effects of *Terminalia catappa* Seeds on sexual Behaviour and Fertility of Male **Rats. Asian Journal of Andrology, 2: 213-219.**

Sandroni, P. (2001) Aphrodisiac past and present: ahistorical review.

Clinical Autonomic Research 11: 303 – 307 Singh, B., Vikas, G., Parvenu, B. and Dhamendra, K. (2010). Pharmacological Potentials of Plant used as Aphrodisiac. *International Journal of Pharmaceutical Science Review5:16-17*.

Sofowora, A.(1982) Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd Ibadan, Nigeria p.1 – 25.

Tajuddin A., Shamshad, A. Abdul L., Iqbal A. Q. and Kunwar Y. A. (2005). An experimental study of sexual Function improving Effect of *myristica fragan* Hout (nutmeg). BMC Complementary and Alternative Medicine 5:16-20.

Trease, G.E., Evans, W.C. (1989) Pharmacognosy. English Language Book Society. 13th Edition,Bailliere Tindall, London. Pp. 683 – 684

Twig, J. P., Irvine D. S. and Aitken, K. J. (1998). Oxidative Damage of DNA in Human Spermatozoa does not precute Pronucleus Formation at Intracytoplasmic Sperm Injection: *Human Reproduction*. 13:1861 - 1871. Singh, B., Vikas, G., Parvenu, B. and

Ursini, F. M. (1994). A Novel Antioxidant (1dB1031) Affecting Molecular Mechanism with Cell Free Radial. *Biomedicine*. 16:547-553.30.)

Vikas, S., Mayank, T., Nagendra, S. C. and Vinod, K. D. (2008). Evaluation of Anabolic, Aphrodisiac and Reproductive Activity of *Anacyclus Pyrethrum* D. C. in Male Rat. *Scientia Pharmaceutica* 77:97-110.

Watcho P, Kamtchouing P, Sokeng, S. D., Moundipa, P. F., Tantchou, J., Essame, J. L. and Koueta, N. (2004). Androgenic Effect of *Mondia whitei* Roots in Male Rats. *Asian Journal of Andrology, 6: 269-272*.

Yakubu, M. T, Akanji, M. A. and Oladiji, A. T. (2005). Aprodisiac Potentials of Aqueous Extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in Male Albino Rats. *Asian Journal of Andrology*, 7: 399-404.

Yakubu, M.T., Akanji, M. and Oladiji, A.T. (2007). Male sexual Dysfunction and Method used in assessing Medical Plant with Aphrodisiac Potential. *Pharmacognosy Reviews*, 1:49-56.

Zaloni P., Zavatti M., Montanari C. and Baraldi M. (2009). Influence of *Eurycoma longifolia* on the Copulatory Activity of sexually Sluggish and Impotent Male Rats. Journal of Ethnopharmacology, 126:308-313.