African Journal of Pharmacology and Therapeutics Vol. 2 No. 3 Pages 94-100, 2013
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Research Article

Contraceptive and sexual behavioural effects of methanol extract of *Smilax kraussiana* root in rodents

Paul A. Nwafor [a,*], Ofonimeh J. Idiong [a], and Kufreh Davies [b]

[a] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria*
[b] *Department of Human Physiology, Faculty of Basic Medical Sciences, University of Uyo, Nigeria*

* **Corresponding author:** Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, P.M.B. 1017 Uyo, Nigeria; **Tel**: +234-803-6778861; **Email**: paulnwafor2013@yahoo.com

Background: *Smilax kraussiana* root has an age-long historical use for family planning among the Uruan Local Government Area of Akwa Ibom State, Nigeria. Traditionally, a decoction of the root is made either with boiling water or local gin and administered.

Objective: To investigate its contraceptive potential with a view of ascertaining the scientific basis for its use in family planning, and establish if any, its mechanism for action.

Method: The pulverized root was macerated in methanol for 72h and filtered with Whatman filter paper No.4. It was stored – 4^0C until when required. Randomized female rats having regular estrus cycle were divided into groups and were administered with various doses of extract. They were observed for estrus, ovulation and contraception as well as progestational and sexual behaviour.

Result: The result showed that it has anticonceptive effect, altered estrus, and ovulation cycle and possessed estrogenic effect which resulted in contraception. It also increases lordosis frequency, lordosis quotient as well as intromission frequency.

Conclusion: The anticonceptive, estrogenic and progestational properties as well as its copulatory behaviours are predicated on the properties of its phytochemical constituents which includes alkaloids, saponins and flavonoids. This therefore, justifies its folkloric use in family planning.

Keywords: *Smilax kraussiana*, contraceptive extract, rodents, sexual behaviour.

Received: July, 2013
Published: October, 2013

1. Introduction

A large number of medicinal plants have been screened for contraceptive activity in an attempt to replace hormonal contraceptives. Some of them have shown promising activities (Farnsworth et al, 1980; Nwafor et al, 2012). One of the most popular plants used for contraceptive activity among the Uruan Local Government Area of Akwa Ibom State, Nigeria, is *Smilax kraussiana.* It is commonly known as West African Sarsaparilla. Traditionally known as *Odufat* by the Ibibios, *Uruk – ekwong* by the Efiks, *Jiabanammuo* by the Ibos and *Kurangawofi* by the Hausas of Nigeria respectively. Earlier work on the root of the plant revealed that it contained saponins, tannins, simple sugar, cardiac glycosides and flavonoids (Nwafor et al, 2006).

The chief components are sarsaparilloside along with parillin as a breakdown product, including desglucorhamno parillin and aglycone sarsapogenin (Fukunaga et al, 1997). The plant *Smilax kraussiana* is a widely used shrub in traditional medicine. The leaf is an excellent antidote in the treatment of poison, decoction

ISSN 2303-9841

of the twig is used to hasten delivery while the root is used for rheumatism, gout, kidney problems, gonorrhea and syphilis, febrifuge and malaria (Odugbemi and Akinsulire, 2008). The acute toxicity potential of the leaves, the anti-inflammatory as well as its analgesic activities have been reported (Nwafor et al, 2006; Nwafor et al, 2010). Okokon et al, (2012) showed the antiplasmodial and antipyretic activities of the plant. The root is used as a contraceptive. The root of *Smilax kraussiana* was investigated for its contraceptive activity in rodents with the aim of ascertaining the scientific basis for the use of this plant for family planning and to establish if any, it mechanism of action.

2. Materials and Methods

2.1 Collection of Plant Materials

The fresh root was collected from Uruan Local Government Area of Akwa Ibom State, Nigeria. The plant was identified at the Department of Botany and Ecological Studies, University of Uyo, Nigeria. A voucher specimen number **Ref. No. UU/HER. No40e** was assigned to it and was deposited at Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.

2.2 Extraction and Phytochemical Analysis

The fresh root of the plant was dried at room temperature (25±2 °C), pulverized by grinding using pestle and mortar. Then, 60 g of the ground root were macerated in methanol (250 ml) for 72 h, filtered with Whatman filter paper No. 4 and dried in water bath at 45 °C. This gave a mean yield of 14.65±0.34 g w/w of extract. The extract was stored in –4 °C from where it was used when required.

The phytochemical screening of the extract was performed according to the methods of Clarke (1975), Odebiyi and Sofowora (1978), Trease and Evans (1989) and Harborne (1998). Tests for alkaloids, saponins, tannins, terpenes, simple sugars, cardiac glycosides were carried out.

2.3 Animals

Adults and young immature albino female mice (weighing 25-30 g and 13-18 g, respectively) and albino female rats (weighing 165-200 g) were used in the study. All the animals were housed in a cross-ventilated room (temperature 22±2.5 °C, 12 hr light/dark cycle) and were fed with standard growers mash (Bendel Feeds, Edo State, Nigeria) and water *ad libitum*. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care (Based on Helsinki convention) and guidelines and rules of Faculty of Pharmacy, University of Uyo, for animal experimentation.

2.4 Effect of extract on conception

Adult female mice having regular estrus cycle confirmed by daily vaginal smear analysis were used. The selection of animals for use was determined by the presence of at least two consecutive 4 day estrus cycles. The animals were randomized and divided into four groups of six animals each. The first group was administered with normal saline (5 ml/kg) intraperitoneally (IP) in divided doses for 4 days. Groups 2-4 were administered with different doses (24-72 mg/kg) of extract for 4 days. On the fifth day, fertile males were introduced using 3:1 (F/M) ratio and allowed to remain with females until the experiment was terminated due to pregnancy.

2.5 Effect of extract on estrogenic and antiestrogenic activities

The estrogenic and antiestrogenic activities of the methanol extract were assessed in bilaterally ovariectomized immature rats using the method of Edgren and Calhoun (1957). The end points used to determine the estrogenic effect of the extract include: uterine wet weight, degree of vaginal cornification and quantal vaginal opening. Exactly 1 week after bilateral overiectomy, the rats were randomized into the various experimental groups. Then, 17-β-estradiol was dissolved in corn oil and administered subcutaneously (SC) at a dose of 0.1 μg/rat per day for 4 consecutive days as a reference standard. For evaluating estrogenic activity, different groups of animals received only the extract (IP) at various doses whereas for the antiestrogenic activity, various doses of the extract were administered conjointly with 17-β-estradiol (0.1 ug/rat per day) for 4 consecutive days. Controls were simultaneously maintained and received vehicle only. Animals were sacrificed 24 h after the last treatment.

2.6 Effect of extract on estrus cycle

For estrus cycle determination, eighteen cycling female rats were randomized and divided into three (3) groups of six rats each. Group 1 received normal saline (5 ml/kg IP) and served as the control group. Groups 2 and 3 received extract in divided doses 24 mg/kg and 48mg/kg intraperitoneally (IP) respectively. All injections were administered at late stage of proestrus. The animals were examined every morning to observe any changes in the estrus cycle for eight days (Telleria et al, 1997).

2.7 Effect of extract on ovulation

To establish the effect of the extract on ovulation, eighteen (18) cycling female rats were randomized and divided into three groups of six (6) each. Groups 1 received normal saline (5 ml/kg IP). Group 2 and 3 received the extract in divided doses 24 mg/kg and 48 mg/kg (IP), respectively. All injections were administered at late stages of proestrus. At the end of the estrus, the animals were laparotomized, and the ovaries examined with hand lens, to see if there was interference with ovulation or not.

2.8 Progestational and antiprogestational effect of extract

Progestational and antiprogestational effects were evaluated in mature female rats by the traumatization method of Ohta (1982) with a slight modification taking the decidual response as end point. Rats at estrus were ovariectomized and the day designated day 1 (D_1). Then, 17-β- estradiol was injected subcutaneously at a dose of 1μg/day for 3 consecutive days (D_1 – D_3), thereafter, the rats received a subcutaneous injection of

progesterone (3 mg) daily for 7 consecutive days (D_4-D_{10}). On the 6th day, all rats received a single subcutaneous injection of 0.1µg 17-β-estradiol, the animals were laparotomized under light ether anaesthesia and the decidual stimulus applied to the right horn of the uterus by traumatizing it with needle. Animals were killed on the day following the last progesterone injection and the uteri were removed and inspected for the presence of deciduoma.

For evaluating the progestational effect of the extract, different doses were administered for 7 days intraperitoneally instead of standard progesterone. For antiprogestational effect, different doses of the extract were administered conjointly with progesterone for the same period.

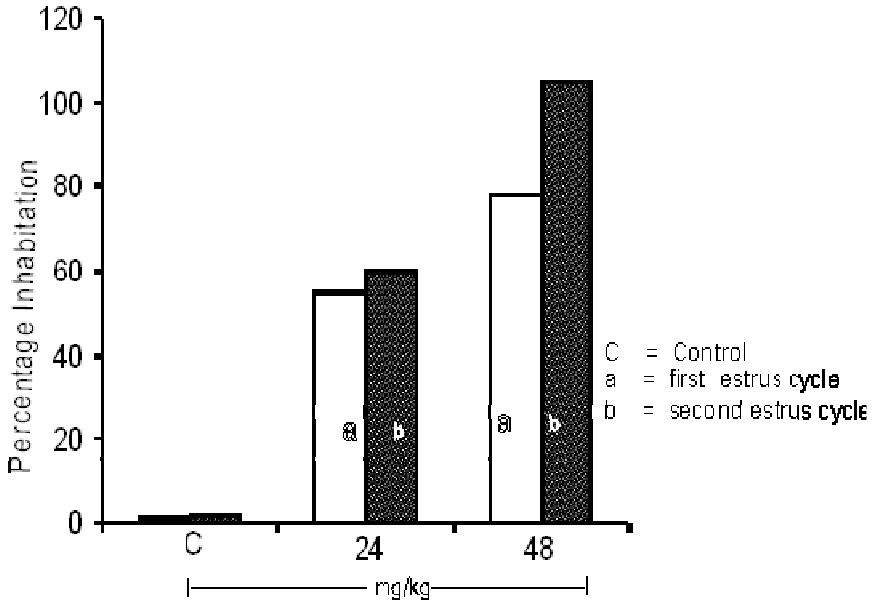

Figure 1: Effect of extract on estrus cycle in rats

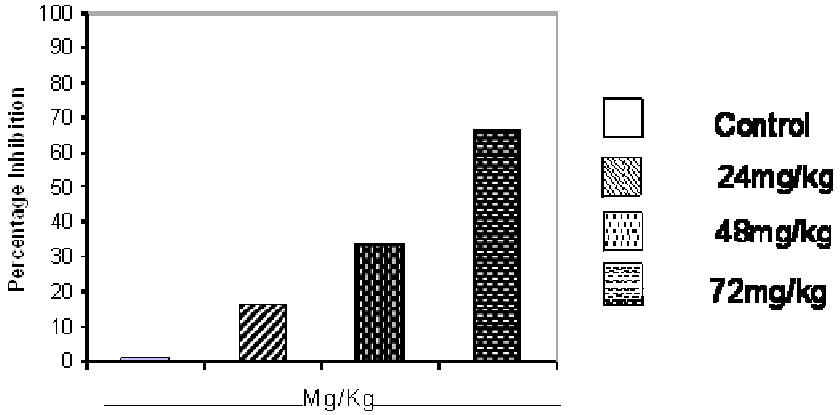

Figure 2: Effect of extract on ovulation in rats

2.9 Test on sexual behaviour

The sexual behaviour of the female rats were tested during the period of darkness (21:00 GMT) in a quiet room for a duration of 30mins for each parameter. Some of the parameters of the male sexual behaviour were adopted as a means of achieving degree of compliance of the female rats.

After 10min adaptation period in the transparent plexiglass copulation cage (46 cm x 41 cm x 41 cm) a female rat was presented to each male by dropping gently into the cage. The following parameters were recorded or calculated according to standard method (Ageel et al, 1994; Carro-Juarez et al, 2004): Mount latency (ML), the time taken from the introduction of the female to the occurrence of the first mount; Mount frequency (MF), number of mounts preceding ejaculation; Intromission Latency (IL), the time from introduction of the female to the occurrence of first intromission; Intromission frequency (IF), the number of intromissions preceding ejaculation; Lordosis frequency (LF), the number of lordosis within 30min. (Lordosis or its response is a display of a rigid posture with arching of the back, elevation of the hind quarters, and deviation of the tail to facilitate male mounting and intromission). Female sexual behaviour was expressed as the ratio of the number of lordosis responses to mounts, i.e. mounts alone, mounts with intromissions, and mounts with intromissions and ejaculations) by the male sexual partner. The Lordosis Quotient (LQ) was calculated as a percentage of the total number of

lordosis responses divided by the total number of mounts.

2.10 Statistical analysis

Results were expressed as multiple comparisons of Mean ± S.E.M. Significance was determined using one-way ANOVA followed by Turkey-Kramer multiple comparison post test. A probability level of less than 5% was considered significant.

2.11 Ethical approval

Permission and approval for animal studies were obtained from the Faculty of Pharmacy Animal Ethics Committee, University of Uyo.

3. Results

3.1 Phytochemical screening

The result of the phytochemical screening showed that it contained alkaloids, saponins, and flavoniods. Others are terpenes, cardiac glycosides and anthraquione. Tannins were however absent.

3.2 Effect on conception

The extract protected 50% of the rats administered with 24 mg/kg from conception for over two gestational periods while the remaining 50% that littered were protected for one gestational period. Animals pretreated with 48 mg/kg and 72 mg/kg respectively, offered 67% protection from conception for over three gestational periods while 33% that littered were protected for two gestational periods. There was no abnormalities observed the pups (**Table 1** and **2**)

3.3 Estrogenic and antiestrogenic effects

On the estrogenic and antiesstogenic effects of the extract, it caused increase in uterine wet weight, vaginal opening and cornification. This increase was statistically ($p < 0.01$) significant. Similar results were obtained in mice. In the presence of 17-β-estradiol, both animals (mice and rat) showed increase in estrogenicity (**Table 3** and **4**).

3.4 Effect on estrus and ovulation

The effect of extract on estrus is as shown in **Figure 1**. Rats that received extract at late proestrus phase (D1.) of the cycle, were prevented from proceeding into estrous phase the next morning. Animals pretreated with 24 mg/kg altered 83% of the estrous cycle while higher doses (48 and 72 mg/kg), achieved 100% alteration of estrous cycle. Similarly, the interference of the extract with ovulation was dose-dependent, with the highest dose 72 mg/kg exhibiting 67% inhibition (**Figure 2**)

3.5 Progestational and antiprogestational effects

The extract exhibited progestational effect in all the rats pretreated with different doses of it. However, in the presence of a reference hormone (I7-β-estradiol), it showed antiprogestational effect (**Table 5**)

3.6 Effect of extract on sexual behaviour

The effect of the extract on sexual behaviour in female rat is as shown in **Table 6**. The extract increases the lordosis frequency in a dose-related manner. These increases were statistically significant ($p < 0.001$). Similar effects were shown in lordosis quotient and intromission frequency respectively.

4 Discussion

The results of this study showed that *Smilax kraussiana* root possessed anticonceptive, estrogenic and progestational effects in rodents. It also enhanced sexual behaviour in female rats. The extract interfered with ovulation and altered the estrus cycle. This conclusion is supported by the following observations: (i) Pretreated animals that were kept with fertile males were protected from conception over some varied gestational periods. (ii) An increase in uterine weight, premature vaginal opening and cornification were observed in young ovariectomized rats. (iii) There was positive deciduoma formation confirming it progestational effects. (iv) Pretreated animals failed to ovulate the day following their proestrus phase. (v) The estrus cycle of pretreated animals were altered. (vi) Mount, intromission and Lordosis frequencies were increased in pretreated females while intromission and lordosis latencies decreased.

Estrogenicity in premature animals is characterized by premature opening of vagina and its cornification (Turner and Bagnara, 1971). It is known that the administration of estrogen produces uterotrophic effects in several animal species including rats and mice (Edgren and Calhoun, 1957, Jacob and Morris, 1969). These effects are associated with growth and proliferation of the endometrial cells number, vaginal opening and cornification (Ljungkrist, 1971). Due to their uterotrophic effects, estrogens have been reported to accelerate the passage of ova through the uterine tubes and the uterus, the premature expulsion of eggs is presumed to be the basis of their anticonceptive activity. Furthermore, the disruption of the estrogen/progesterone balance may result in an unfavourable endometrial environment, since degeneration at the implantation site has been demonstrated in animals (Kincl and Dorfman, 1965). These observations taken together showed that the extract possessed estrogenic activity.

The interference in estrus cycle, ovulation and estrogenicity observed with the administration of the extract indicated its potential in interfering with fertilization and implantation of ovum. Steroidal saponins have been found to inhibit estrus cycle and reduce fertility in animals upon continuous administration (Tamura etal, 1997). Progestational effects in animals administered with different doses of extract indicated positive decidual response. Progesterone is known to alter cervical secretion making it viscid, scanty and hostile for sperm penetration. Large doses of progesterone also bring about complete abortion of pregnancy of up to seven weeks in 60-85% cases (Smith and Aronson, 2002). These effects of progesterone may in part contribute to its anticonceptive activities.

Table 1: Anticonceptive effect of methanol extract of *Smilax kraussiana* root on adult female rats

Dose(mg/kg)	Mean no of pups	Duration of protection (no. of gestational periods)*	Percentage of animals protected (%)
Control (normal saline 5ml/kg)	5.8 ± 0.33	nil (0/6)	0
24	5.30 ± 0.13	2(3/6)	50
48	5.50 ± 0.14	3(4/6)	67
72	5.00± 0.00	3(4/6)	67

** Numerator indicates number of rats protected; denominator indicates total number of rats in the group*

Table 2: The effect of methanol extract of *Similax kraussiana* root on weight and length of pups

Dose (mg/kg)	D_1		D_{10}		D_{21}	
	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)
Control (normal saline 5ml/kg)	5.70±0.06	6.42±0.07	6.87±0.08	7.80±0.06	15.61±0.21	14.07±0.22
24	5.77±0.07	6.50±0.13	7.70±0.32	7.53±0.18	15.30±0.40	13.97±0.18
48	5.76±0.07	5.28±0.04	6.61±0.02	7.20±0.03	14.80±0.21	13.62±0.07
72	5.27±0.03	5.56±0.13	6.93±0.09	6.69±0.18	14.51±0.04	13.20±0.06

D_1 = Day one of delivery
D_{10} = Day ten after delivery
D_{21} = Day twenty one after delivery

Table 3: Determination of estrogenic and antiestrogenic effect of *Smilax kraussiana extract* in young immature female rats

Dose (mg/Kg)	Relative uterine wet Weight(mg/100g b.wt)	Quantal Vaginal opening	Vaginal cornification
Normal Saline (5ml/kg)	0.21± 0.006	-	-
24	0.25 ± 0.006[a]	±	±
48	0.31 ± 0.009[b]	+	+
72	0.31± 0.003[b]	+	+
17 – β estradiol	0.93 ± 0.010[b]	+	+
48 + 17–β-estradiol	0.86± 0.006[b]	+	+

Values represent mean ± SEM
Significance relative to control: [a]p< 0.01; [b]p< 0.001
(n = 6)

 ISSN 2303-9841

Table 4: Determination of estrogenic and antiestrogenic effect of *Smilax kraussiana extract* in young immature female mice

Dose (mg/Kg)	Relative uterine wet Weight (mg/100 g b.wt)	Quantal Vaginal opening	Vaginal cornification
Normal Saline (5ml/kg)	0.10± 0.003	-	-
24	0.12 ± 0.003^{ns}	±	±
48	0.17 ± 0.002^{b}	+	+
72	0.14± 0.007^{a}	+	+
17 – β estradiol	0.59 ± 0.019^{b}	+	+
48 + 17 – β estradiol	0.42± 0.005^{b}	+	+

Values represent mean ± SEM
Significance relative to control: $^{a}p< 0.01$; $^{b}p< 0.001$
(n = 6)

Table 5: Progestational and antiprogrestational effects of *Smilax kraussiana* root extract in rats

Extract (mg/kg)	Drug Progesterone (3mg)	Initial Body Weight (kg)	Final Body Weight (kg)	Quantal Rats Showing Positive Decidual Response
Normal Saline (5ml/kg)	3	114.27±5.17	115.00±4.84	6/6
24	-	119.0±6.96	122.70 ±7.71	6/6
48	-	131.40±9.56	132.50 ±9.91	6/6
72	-	135.70±5.62	137.30 ±5.56	6/6
48	3	145.10±14.19	133.30 ±8.35	0/6

Table 6: Effect of methanolic extract of *Smilax kraussiana* root on sexual behaviour in female rats

Drug extract	Mount frequency	Mount latency (sec)	Intromission frequency	Intromission latency (sec)	Lordosis frequency	Lordosis Quotient (sec)
Normal Saline (5ml/kg)	11.67±1.04	23.67±9.11	6.83±0.50	44.83±0.05	3.83±0.45	32.82
24mg/kg	13.67±1.30	15.67±1.20	11.67±0.09^{b}	15.67±0.11^{b}	13.30±0.22^{b}	97.29
48mg/kg	15.00±0.50^{a}	20.00±0.3	12.67±0.06^{ns}	12.50±0.13^{b}	14.67±0.40^{b}	97.80
72mg/kg	14.83±0.11^{a}	48.83±0.31^{b}	12.50±0.40^{b}	24.00±0.50^{b}	16.00 ±0.50^{b}	107.88
17-β-estradiol	17.00±0.40^{b}	35.33±0.15^{ns}	13.83±0.07^{b}	33.55±0.41^{b}	17.83±0.60^{b}	104.88
17-β-estradiol + 48mg/kg	17.83±0.41^{b}	44.83±0.15^{a}	13.33±0.40^{b}	29.67±0.40^{b}	18.33±0.60^{b}	102.80

Values represent mean ± SEM
Significance relative to control: $^{a}p< 0.01$; $^{b}p< 0.001$
Ns = not significant
(n = 6)

The extract facilitated copulatory behaviour in the female rats. This was indicated by increases in mount, lordosis and intromission frequencies which portrayed sexual receptivity and compliance. This is further supported by a dose-dependent increase in lordosis frequency and its quotient. Estrogen is known to increase sexual desire (Thornton and Finn, 1999), Uphouse and Maswood, 1999). The observed effects may in part be due to estrogenic property.

Phytochemical screening of the extract revealed that it contained flavonoids and alkaloids, bioactive compounds that possess effects on reproductive system. These two compounds (flavonoids and alkaloids) were reported to possess antifertility activities (Gupta et al, 2003, Nataraj et al, 2007). These further lent credence to the antifertility effects of the extract.

 ISSN 2303-9841

In conclusion therefore, the anticonceptive, estrogenic and progestational properties as well as its copulatory behaviour are predicated on the properties of its phytochemical constituents. This therefore justifies its folkoric use in family planning. However, further work is in progress to elucidate the structure of the active ingredients and their activities.

Conflict of Interest declaration

The authors declare no conflict of interest

Acknowledgements

The authors gratefully acknowledge Mr. Nsikan M. Udo and Miss Sifonobong J. Akpan, Department of Pharmacology & Toxicology for their technical assistance, and Mr. Ikechukwu Ezeocha of CHI Pharmaceuticals Ltd, Nigeria, for the gift of 17-β-estradiol.

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 ISSN 2303-9841