Original Article

Effects of Post-coital Administration of Alkaloids from *Senna alata* (Linn. Roxb) Leaves on some Fetal and Maternal Outcomes of Pregnant Rats

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Abstract

Background: The abortifacient claim of *Senna alata* (*S. alata*) was scientifically validated recently with alkaloids speculated to be the bioactive agent. This speculation is yet to be substantiated or refuted by scientific evidence. The present study was aimed to investigate the pregnancy terminating effects of the alkaloids from *S. alata* leaves.

Methods: Twenty four Pregnant rats (143.99±1.21 *g*) allocated randomly to four groups: A, B, C and D respectively received, 0.5 *ml* of distilled water, 250, 500 and 1000 *mg/kg* body weight of the *S. alata* extracted alkaloids orally, once daily from day 10 until day 18 post-coitum. The indices of abortifacient were evaluated at the end of the exposure period. The results were analyzed by both the analysis of variance and Duncan's multiple range test and $p<0.05$ was considered as statistically significant.

Results: Thin-layer chromatographic separation produced five spots with R_f values of 0.28, 0.33, 0.39, 0.47 and 0.55 which gave positive reaction with Meyer's and Wagner's reagents, respectively. The number of implantation sites and corpora lutea, as well as the concentrations of FSH, LH, progesterone, weight of uterus, uterine/body weight ratio, glucose and cholesterol decreased significantly ($p<0.05$) whereas the resorption index, pre- and post-implantation losses, uterine protein content and alkaline phosphatase activity increased significantly. None of the alkaloid treated animals presented with provoked vaginal opening or bleeding except fetal deaths. The alkaloid decreased the maternal weight gain, as well as feed and water intake.

Conclusion: Overall, the alkaloids from *S. alata* leaves exhibited anti-implantation, anti-gonadotropic, anti-progesteronic, embryonic resorptive, feto-maternal toxic activities but not complete abortifacient. The alkaloids alone may not be the sole abortifacient bioactive agent in the leaf extract.

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Introduction

Since the dawn of history, nature and natural sources, such as plants, animals, microbes, and minerals have remained a veritable source of bioactive compounds with medicinal values. Among these sources, plants have been the most explored and exploited for their bioactive medicinal components. Their constituents that have always served as "lead compounds" or templates for the rational development of drugs are of more specific efficacies and fewer side effects (1). One of the botanicals of interest in which the abortifacient claims in the folk medicine have

been substantiated by scientific evidence (2) but with no documentation in the open scientific literature on its bioactive abortifacient agent(s) is *Senna alata*.

Senna alata (Linn.) Roxb (Leguminosae), also known as Craw-Craw plant or Ringworm plant (English), asunwon oyinbo (Yoruba-Western Nigeria), Nelkhi (Igbo-Eastern Nigeria), Filisko or Hantsi (Hausa-Northern Nigeria) is an erect tropical, annual herb which grows up to 0.15 *m* high. The large, leathery compound leaves are bilateral and fold together at night. The fruit is a pod, while the seeds are small and square in shape. The plant has been claimed to be used in the management of several diseased conditions such as hepatitis, dermatitis, jaundice, gastroenteritis, eczema, constipation and diarrhoea (3, 4). Our ethnobotanical studies have also revealed that the leaves are used to "wash the uterus" (2).

Previous studies have shown that *S. alata* have antifungal, antibacterial and antioxidant activities (3, 5–8). Furthermore, Yakubu et al. (2) reported that the aqueous leaf extract of the plant contained saponins (1.22%), flavonoids (1.06%), cardiac glycosides (0.20%), phenolics (0.44%), alkaloids (0.52%), cardenolides and dienolides (0.18%). Aqueous leaf extract of *S. alata* has also been scientifically validated for its acclaimed use as an abortifacient (2). However, there is no study in the open scientific literature that has reported on the exact bioactive abortifacient agent(s) in *S. alata* leaves. Therefore, the present study aimed to validate the speculation that the alkaloids in aqueous extract of *S. alata* leaves are responsible for its abortifacient activity. The focus on alkaloid was a follow-up from our previous study that speculated on alkaloids to be responsible for the abortifacient activity of the crude extract of *S. alata* leaves and from several studies that implicated the phytochemical property of colchicine, quinazoline alkaloids, *e.g.* vasicine and vasicinone in several botanicals such as *Xylopia aethiopica*, *Peganum harmala* epigeal parts, *Areca catchu* nuts and *Gloriosa superba* roots as abortifacient bioactive agents and/or their role in the contraction and relaxation of uterine muscles (9–12).

Methods

Plant material and authentication: The plant leaves, obtained from herb sellers at Oja Tuntun, (New Market) in Ilorin, Nigeria, was authenticated at the Herbarium Unit of the Forestry Research Institute of Nigeria (FRIN) in Ibadan, Nigeria. A voucher specimen (FHI 10845) was deposited at the Herbarium of the Institute.

Chemicals and reagents: Assay kits for both glucose and cholesterol were products of Randox Laboratories, Ltd, United Kingdom, while those of progesterone, follicle stimulating and luteinizing hormones were products of Inteco UK LTD, United Kingdom. Thin Layer Chromatographic (T LC) plates and silica gel were products of Merck (Darmstadt, Germany). Para-nitrophenyl phosphate and other reagents were products of Sigma-Aldrich Inc., St. Louis, USA.

Animals: Male and female Wistar rats (*Rattus norvegicus*) weighing 178.91±3.07 and 143.99± 1.21 *g*, respectively, were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals which were housed individually in plastic cages and placed in a well-ventilated room (temperature: 28-31 ℃; photoperiod: 12 *hr* natural light and 12 *hr* darkness; humidity: 50–55%) were provided with unrestricted access to rat pellets (Bendel Feeds and Flour Mills, Ewu, Nigeria) and water. The animals were also handled according to the guidelines of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes–ETS 123 (13).

Extraction of alkaloids: The leaves of *Senna alata* which were oven-dried at 40 ℃ for 48 *hr* were pulverised using a Mikachi Blender (MK-1830, China). Alkaloids were extracted from the powder according to the procedure described by Manske (14). A known amount (500 *g*) of the powder was extracted in 1.2 *L* of hexane for 72 *hr* and filtered with Whatman No. 1 filter paper. The hexane extract (containing fats, oils, terpenes, waxes) were discarded and the resulting residue extracted again in 1.2 *L* of methanol for a week and subsequently filtered. The filtrate was evaporated using a rotavapor (R110, Gallenkamp, England, UK) and the process was repeated two more times. The three filtrates were combined, concentrated to give a methanolic, green slime (90 *g*) which was treated with 1 *M* HCl and then basified by adding 5 *M* NaOH with continuous stirring until a cloudy precipitate appeared. A known volume (500 *ml*) of chloroform and 200 *ml* of 1 *M* NaCl were added and the process was repeated three more times. Equal volumes (150 *ml*) of 1 *M* NaCl and 5 *M* NaOH were added to the organic layer in a sepa-

rating funnel after which the mixture was evaporated to yield brownish-black slurry (18 *g*) of alkaloids that corresponded to a yield of 3.60% that was used for subsequent experiments.

Analysis of the alkaloids: The procedure described by Singh and Sahu (15) was adopted for the preparation of thin layer chromatography (TLC) plates. Furthermore, about 10 *µl* of the test solution (extract) was spotted onto the thin layer plate using a micropipette after which the plates were developed in chloroform: methanol (10:2) in which 0.01 *g/ml* of butylated hydroxyl toluene and butylated hydroxyl anisole were added to prevent oxidation (which may lead to increase in the number of bands with time) (16). The plates were later dried at room temperature and exposed to iodine vapour. Chromatograms were visualized under ultra violet light (UV, 254 *nm*). The alkaloids were confirmed with Meyer's and Wagner's reagents. The relative values related to the solvent front (R_f) of the spots were also computed.

Analysis of abortifacient activity: The method described by Salhab et al. (17) was adopted. Twenty-four pregnant rats were allocated in a complete randomize design to four groups (A, B, C and D) consisting of six animals each. Animals in Group A (controls), orally received 0.5 ml of distilled water with the aid of an oropharyngeal cannula while those in Groups B, C and D orally received 0.5 *ml* of the alkaloids corresponding to 250, 500 and 1000 *mg/kg* body weight, respectively. The doses were as used in the previous study (2). The administration was done once daily from day 10 until day 18 of pregnancy (period of organogenesis in Wistar rats) (18). By the 19th day (24 *hr* after their last dose), the animals underwent a laparatomy under diethyl ether vapour shortly before sacrifice. The following parameters were recorded/computed: number of live fetuses; number of dead fetuses; average weight of live foetuses; survival ratio (%) =(number of live fetuses/ number of live+dead fetuses) ×100; number of rats that aborted; percentage of rats that aborted= (number of rats that aborted/number of rats assessed) ×100; number of rats with vaginal bleeding; number of implantation sites; number of corpora lutea; implantation index=(total number of implantation sites/number of corpora lutea) ×100; pre-implantation loss=(number of corpora lutea–number of implantation sites/number of corpora lutea) ×100; post-implantation loss= (number of implantation sites–number of live fetuses/number of implantation sites) ×100; number of resorption sites=number of implantation sites in the control animals–number of implantations in the test animals; resorption index=(total number of resorption sites/total number of implantation sites) ×100. The weights of the animals both before pairing and prior to sacrifice, as well as feed and water intake were also recorded.

Determination of estrogenic/anti-estrogenic activities: The *in vivo* estrogenic/anti-estrogenic response of the rats to the alkaloids was evaluated by adopting the procedure described by Kanno et al. (19). Twenty-four ovariectomized, female rats (159.67±7.12 *g*) were allocated to four groups (A, B, C and D) of six animals per group. Group A (controls), orally received 0.5 *ml* of distilled water while animals in Groups B, C and D orally received 0.5 *ml* of the alkaloids corresponding to 250, 500 and 1000 *mg/kg* body weight, respectively. The administration which commenced from the eighth day of ovariectomy, lasted for another seven days. On day 16, the weights of the animals were determined prior to sacrifice and uterine to body weight ratio was computed. The uterine protein, glucose, cholesterol, and alkaline phosphatase activity were determined using standard procedures (20–23). Percentage of animals with vaginal opening and cornification were also computed.

Preparation of serum and uterine homogenates: The serum and uterine homogenates were prepared according to the procedures described by Yakubu and Bukoye (24).

Determination of some reproductive hormones: The procedures outlined in the manufacturer's protocol were adopted for the quantitative determination of progesterone, follicle stimulating and luteinizing hormones in the serum of the animals.

Statistical analysis: Data which were expressed as the mean±SD of six independent replicates were statistically analyzed using one-way analysis of variance and Duncan Multiple Range Test. Data were considered statistically significant at $p<0.05$.

Results

The alkaloids yielded 0.30 *g* which corresponds to 1.50% of the starting material of 500 *g*. The five different spots gave R_f values of 0.28, 0.33, 0.39, 0.47 and 0.55. The spots gave positive reaction with both Meyer's and Wagner's reagents producing a creamy precipitate and reddish-brown spots on a grey background (TLC).

The alkaloid truncated the development of the fetuses as none of them survived in the experiment groups as against the average number of live fetuses of 10.26 in the distilled water-treated control animals (Table 1). The average weight of live fetuses in the controls was 4.92 *g* against none in all the extract-treated animals. The percentage of fetal death in the 250, 500 and 1000 *mg/kg* body weight of the alkaloid-treated animals was 6.03, 6.00 and 6.50, respectively. There was neither episode of abortion nor vaginal bleeding in all the alkaloid-treated animals. Both the number of implantation sites and corpora lutea decreased significantly ($p<0.05$). While the implantation index was similarly high in all the alkaloid-treated animals, the resorption index, as well as pre- and post- implantation losses were many fold higher than the controls (Table 1). Although, all the pregnant rats gained weight at the end of the experimental period (final maternal weight) compared with their weight prior to pairing with the males (initial maternal weight) (Table 1), the weight gained by the alkaloid-treated animals was about 50% less than the control animals. Furthermore, the feed and water intake by the animals treated by different doses of the alkaloid decreased significantly ($p<0.05$).

All different doses of the alkaloids significantly ($p<0.05$) decreased the serum concentrations of the follicle stimulating hormone, luteinizing hormone and progesterone in the pregnant animals (Table 2).

The alkaloids also decreased ($p<0.05$) the absolute weight of the uterus, computed uterine to body weight ratio, and concentrations of uterine glucose and cholesterol (Table 3). These decreases were, however, not dose-related. In contrast, the concentration of uterine protein and the activity of alkaline phosphatase increased significantly ($p<0.05$). Furthermore, the alkaloids did not provoke vaginal opening or cornification in any of the animals (Table 3).

Discussion

Analysis of the TL chromatogram indicated that the mixture consisted of five alkaloids as evidenced by the creamy precipitates and reddish-brown spots produced with Meyer's and Wagner's reagents, respectively. In the present study, alkaloids from *S. alata* leaves significantly affected the fetal and maternal parameters of the animals. For instance, the death of the fetuses in the alkaloid-treated pregnant rats may not only suggest inhibition of mitotic division of the fetuses (25), since the animals were exposed during the period of organogenesis, but may also show its relevance to the reduction in the concentrations of gonadotropins and progesterone in the present study. These are indications that the alkaloids are fetotoxic. The present study differs fro m that previously reported by Yakubu et al. (2) on the aqueous leaf extract of the plant where only the 500 and 1000 *mg/kg* body weight of the extract produced fetal death. Furthermore, the absence of abortion and vaginal bleeding in the alkaloid-treated animals which were hitherto observed with the crude extract in our previous study suggest zero abortifacient activity for the alkaloid. Therefore, the

Table 1. Effect of the alkaloids from *Senna alata* leaves on some abortifacient parameters of pregnant rats

Parameters	Doses (*mg/kg* body weight)			
	Controls	250	500	1000
Number of implantation sites	10.26±0.22 [a]	6.78±0.43 [b]	6.21±0.61 [b]	6.28±0.95 [b]
Number of corpora lutea	10.66±0.14 [a]	7.62±0.69 [b]	7.14±0.83 [b]	7.22±1.03 [b]
Implantation index (%)	91.74	88.98	86.97	86.98
Pre- implantation losses (%)	3.75	11.02	13.02	13.01
Post- implantation loss (%)	0	100	100	100
Number of resorption sites	0.00±0.00 [a]	3.48±0.08 [b]	4.05±0.09 [c]	3.98±0.05 [b]
Resorption index (%)	0	51.33	65.22	63.38
*Weight of animal before pregnancy (g)	144.00±3.28 [a]	144.00±2.19 [a]	142.50±3.83 [a]	145.46±0.41 [a]
*Weight of animal after pregnancy (g)	185.50±3.83 [b]	166.00±3.89 [c]	162.00±2.69 [c]	167.33±1.03 [c]
Maternal weight gain (%)	28.82	15.28	14.08	15.04
Feed intake (*g/day* per rat)	20.32±0.75 [a]	14.50±0.87 [b]	15.33±0.47 [b]	14.89±0.70 [b]
Water intake (*ml/day* per rat)	19.83±1.45 [a]	16.63±0.75 [b]	14.57±1.89 [b]	11.99±1.83 [c]

Values are expressed as mean±SD of six independent determinations; a-d: Test values carrying superscripts different from the control for each parameter in the same row are significantly different ($p<0.05$). * Maternal weights of the animals before pregnancy were compared with their corresponding weights after pregnancy for each treatment group ($p<0.05$)

Table 2. Effect of alkaloids from *Senna alata* leaves on some female reproductive hormones of pregnant Wistar rats

Parameters	Doses (*mg/kg* body weight)			
	Controls	250	500	1000
Serum follicle stimulating hormone (*mIU/ml*)	15.00±0.00 [a]	10.00±0.00 [b]	4.66±0.03 [c]	2.50±0.00 [d]
Serum luteinizing hormone (*mIU/ml*)	12.50±0.00 [a]	10.00±0.00 [b]	9.50±0.54 [b]	5.50±0.54 [c]
Serum progesterone (*ng/ml*)	30.00±0.00 [a]	4.50±0.54 [b]	5.00±0.00 [b]	10.00±0.00 [c]

Values are expressed as mean±SD of six independent determinations; a-d: Test values carrying superscripts different from the control for each parameter in the same row are significantly different ($p<0.05$)

abortifacient activity of the aqueous extract of the plant leaf reported earlier (2) is not due to the alkaloidal content alone, but to some other phytochemicals such as saponins and flavonoids which may act synergistically or additively to produce the desired result.

It is interesting to note that both the number of implantation sites and corpora lutea decreased in the alkaloid-treated animals when the process of implantation ought to have been completed (implantation takes place normally within 5–6 days post-coitus in rats) before the exposure of the animals to the alkaloid mixture (treatment commenced from day 10 of pregnancy). The reason for this decrease is not immediately known but may not be unconnected with the consequence of some general hormonal effect (reduced progesterone) and/or absence of conceptuses growth (26). It is also possible that blastocytes were not activated and well-positioned before implantation, probably due to impaired muscular activity of the uterus (27). This trend of reduction is similar to the previous findings of Yakubu et al. (2).

The implantation index and pre-implantation loss evaluates the number of blastocysts implanted in the uterus while the resorption index and post-implantation loss relate to the number of implanted blastocysts and those that have not developed (18, 28). Therefore, the high implantation index, and pre- and post- implantation losses suggest that pregnancy was interrupted by the alkaloid, probably creating an environment that was not conducive for the fertilized eggs.

Normally, an abortion in most cases is accompanied by vaginal bleeding and when this is absent, the resorption will increase. Therefore, the increase in the resorption index in the alkaloid-treated animals confers antifertility effects (such as anti-implantation, anti-blastocystic and anti-zygotic) of the alkaloid. The alkaloid did not exhibit complete abortifacient effect since the pregnant animals exposed to the alkaloid presented with closed vagina just like the controls. The findings in the present study is similar to the report by Elbetieha et al. (29) where the administration of 200, 400 and 800 *mg/kg* body weight of ethanolic extract of Salvia fruticosa did not cause pregnancy failure but increased the number of resorption in the pregnant rats.

The alkaloids reduced the sense of taste and appetite of the animals as evidenced by the decrease in feed and water intake. Such reduction may account for the decrease in the computed percentage gain of maternal weight and may also be a consequence of impaired growth and development of the uterine contents (30). All these findings except those of feed and water intake, as well as maternal weight gain, contrast the previous report by Yakubu et al. (2).

It is well-known that for the implantation of the

Table 3. Effect of alkaloids from *Senna alata* leaves on some indices of oestrogenicity in pregnant rats

Parameters	Doses (*mg/kg* body weight)			
	Controls	250	500	1000
Weight of uterus (*g*)	1.80±0.21 [a]	1.33±0.05 [b]	1.53±0.05 [c]	1.55±0.05 [d]
Uterine/body weight ratio (%)	3.56±0.01 [a]	1.44±0.29 [b]	0.87±0.05 [c]	0.99±0.02 [c]
Uterine alkaline phosphatase activity (*nM/min/mg* protein)	1.51±0.02 [a]	2.10±0.06 [b]	2.19±0.14 [b]	2.97±0.15 [c]
Uterine glucose concentration (*mg/100 mg* uterus)	89.13±2.37 [a]	65.89±4.02 [b]	56.52±0.00 [c]	41.30±2.38 [d]
Uterine cholesterol concentration (*mmol/L*)	6.99±0.41 [a]	4.36±0.70 [b]	3.19±0.26 [c]	2.20±0.00 [d]
Uterine protein content (*mg/ml*)	275.50±1.64 [a]	311.00±13.76 [b]	398.50±10.69 [c]	370.25±18.74 [d]

Values are expressed as mean±SD of six independent determinations; a-d: Test values carrying superscripts different from the control for each parameter in the same row are significantly different ($p<0.05$)

fetus and sustenance of pregnancy, an exact equilibrium must exist between the secretion of estrogen and progesterone; this regulation is controlled by luteinizing and follicle stimulating hormones (31). Thus, the reduction in the gonadotropins may equally be responsible for the reduced concentration of progesterone in the present study and this may not only account for the death of the fetuses, but also for the increase in the resorption sites. The reduction in progesterone may suggest impaired endometrium function, which will adversely affect the normal secretion of special proteins required to nourish an implanted fertilized egg, and consequently, pregnancy failure. Thus, it is possible that the alkaloids posses anti-gonadotropic and anti-progestogenic activities which are inimical to the continued development of the fetuses.

Many plant extracts with anti-fertility properties are known to exhibit estrogenic activity by increasing protein synthesis, uterine weight, water uptake and retention of fluid leading to ballooning of the uterus, uterine content of glucose, cholesterol, glycogen and alkaline phosphatase activity, thereby changing the uterine milieu and creating non-receptive conditions in the uterus (32). Therefore, the contrasting effects the alkaloids on some of these parameters in the present study suggest that estrogenic activity was not total but selective. The alkaloids exhibited more anti-oestrogenic activity (71.43%) than oestrogenic (28.57%). This is similar to our previous finding (2).

Conclusion

Overall, the alkaloid from *S. alata* leaves at the oral doses of 250, 500 and 1000 *mg/kg* body weight on daily basis from days 10 until day 18 post-coitum exhibited several potential effects on the maternal and fetal outcomes of pregnant rats (anti-implantation, anti-gonadotropic, anti-progesteronic, selective estrogenic, embryonic resorption and fetotoxic activities), but it could not induce abortion in the animals. Therefore, the alkaloids may not alone be responsible for the abortifacient effects of the crude extract of *S. alata* which had been reported earlier. Finally, work is in progress on isolating the other phytochemicals (saponins and flavonoids) and evaluating their synergistic effects in pregnant animals.

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Conflict of Interest

Authors declare no conflict of interest

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