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Research Article

Abortifacient Potential of *Piper guineense* Schum and Thonn. (Piperaceae) Seed Consumption in Wistar Albino Rats

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Abstract

Background and Objective: *Piper guineense* Schum and Thonn. has been known to be a very useful plant in folk medicine for a very long time. It is locally used in Nigeria as spice to increase lactation in breast feeding mothers. Although, there is a folklore belief that it is not safe in pregnancy, no scientific justification has been reported. This study investigated abortifacient potential of ethanol extract of *P. guineense* seeds in Wistar albino rats. **Materials and Methods:** Pregnant female rats, divided into 3 groups (n = 6/group) were intraperitoneally injected with extract (10 or 20 mg kg⁻¹) or vehicle (Tween 80) once daily between GD11 and GD15. The rats were anesthetized and laparotomized on GD20 and fetuses were delivered. Number of implantation sites and fetal characteristics (number, stillbirths and body weights) were noted, as well as maternal weights of the uterus and ovary. Maternal body weights were also recorded on GD0, GD10 and GD20. **Results:** Number of fetuses delivered were fewer in extract treated rats than control and implantations were also fewer in 20 mg kg⁻¹ extract treated rats. There were resorptions and dead fetuses in extract treated animals which were absent in control animals. These effects were observed to be dose-dependent; stillbirths occurred at a rate of 39 and 69%, respectively; whereas, the calculated abortifacient activity was about 66 and 79%, respectively. Maternal weight gain was prevented, while fetal birth weight was significantly (p<0.0001) decreased in extract treated rats compared to control. In addition, ovarian and uterine weights of extract treated rats were significantly (p<0.0001) reduced compared to control. **Conclusion:** From the obtained results, it is concluded that *P. guineense* seed possesses abortifacient property in rats and should be avoided in pregnancy.

Key words: Abortifacient, fetus, *Piper guineense*, resorption, stillbirth

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Piper guineense Schum and Thonn. is a perennial climbing plant, widely distributed throughout the tropical and subtropical regions. It is usually found in wet places in the evergreen rain-forests, forest edges, gallery forest along rocky rivers and grows up to 750-1650 m high. It belongs to the family Piperaceae and has over 700 species. Some common names of the plant include, Black pepper, Ashanti pepper, False cubeb and Guinea cubeb^{1,2}. It is equally identified by local names by different ethnic groups in Nigeria such as Uziza (Igbo), Iyere (Yoruba) and Oduša (Ibibio).

Piper guineense is employed for various ethno-medicinal purposes. It is used to treat gastrointestinal upsets, sickle cell anemia, dental caries and respiratory diseases like asthma³⁻⁶.

Piper guineense is also used as anti-hypertensive, anti-cancer, anti-allergic, anti-inflammatory and antimicrobial agent^{7,8}. Previous pharmacological studies on extracts of different parts of *P. guineense* have indicated that the plant possesses analgesic, antiplasmodial⁹, anti-cancer¹⁰, oxytocic^{11,12}, gastro-protective, anti-ulcer⁷ and antioxidant effects^{13,14}. Aqueous extract of the dried fruits of *P. guineense* had equally been reported to cause stimulation of testicular and epididymal function in rats¹⁵, while Uhegbu *et al.*¹⁶ had reported that it has beneficial effects on lipids and biochemical parameters. The plant has also been shown to contain alkaloids, cardiac glycosides, flavonoids, steroids and terpenes¹⁷.

Medicinal plants are accessible medicinal resources used frequently by a large population of people for the treatment and prevention of diseases. Ethno-medicinal practice either encourages or discourages the use of certain natural remedies by pregnant women and breast feeding mothers in many cultures. In Nigeria and some other cultures the seeds of *P. guineense* are used as spices in diets by breast feeding mothers to enhance lactation. The seeds of the plant are also used traditionally to expel the placenta after childbirth. The plant is however believed by some to be unsafe in pregnancy but there is no scientific evidence of this. The objective of this study was to investigate the safety (abortifacient potential) of ethanol *P. guineense* seed extract, when administered during pregnancy in Wistar albino rats.

MATERIALS AND METHODS

Preparation of plant material: *Piper guineense* seeds were obtained from a market (Ikwerre Road, Port Harcourt, Nigeria) in July, 2016. The plant was properly identified and sample was deposited in institution's herbarium with a reference number (UPH/P/042). The seeds were washed, air-dried for about 2 weeks and pulverized to fine powder using an electric

blender. The sample (1750 g) was macerated in 70% ethanol (3500 mL) for 72 h with continuous agitation. The macerated mixture was filtered with Whatman No. 541 filter paper and the filtrate was transferred into a rotary evaporator (Model: RE-52A, Lab Science, England) to evaporate the solvent. The extract obtained was transferred into a crucible and remaining solvent was evaporated in a steam water bath (II- G Technique and Techmel, USA) at a temperature of 40°C to obtain a viscous extract and yield of 8.6%. This was stored in a refrigerator until it was used for the experiments.

Phytochemical analysis of extract: Phytochemical screening was carried out on extract using standard procedures¹.

Experimental animals: Eighteen female (130-160 g) and 9 male Wistar albino rats (200-230 g) were obtained from the Department of Experimental and Pharmacology and Toxicology Animal House, University of Port Harcourt, Nigeria and used for the experimental study. They were kept in a well ventilated room and housed in standard animal cages. They were maintained under standard laboratory conditions at an ambient room temperature of 24-27°C and natural lighting conditions. They were fed with standard rat diet (Live Feeds Plc, Lagos, Nigeria) and clean tap water *ad libitum*. The animals were allowed to acclimatize for a period of 14 days before they were used for the experiment. Experimental procedures were in line with approval of Ethics Committee of our institution.

Experimental design: The female and male rats were mated (2:1) and the presence of copulatory plug in female rats confirms pregnancy and indicates gestation day (GD)0. Pregnant animals were separated and grouped into three (n = 6 animals each). Extract was dissolved in Tween 80 and two of the groups were administered either 10 or 20 mg kg⁻¹ ip of the extract, while the 3rd group received only vehicle daily from GD11 to GD15 in each group. The LD₅₀ of *Piper guineense*¹⁸ is 100 mg kg⁻¹ ip. So, the doses used corresponded to 10 and 20% of the LD₅₀, respectively.

Body weights of pregnant animals were recorded on GD0, GD10 and GD20. Animals were anaesthetized deeply with diethyl ether and fetuses were delivered following laparotomy on GD20. Fetal indices like live or dead number, birth weights, as well as implantations and resorptions were noted. The ovaries and uteri were harvested and weighed.

Statistical analysis: Data were expressed as Mean ± SEM. Data were analyzed by Student's t-test using GraphPad Prism 5 software where applicable and probability value was set at p < 0.05.

RESULTS

Phytochemical screening of extract: Phytochemical test of the extract indicated the presence of alkaloids, tannins, saponins, glycosides, flavonoids, carbohydrates and phenols as shown in Table 1. All the compounds were abundantly present except flavonoids, saponins and carbohydrates (Table 1).

Maternal body weight of Wistar albino rats after treatment with extract: Body weights of control rats increased over time during gestation period when compared to GD0 as shown in Fig. 1. Animals' body weights of extract treated rats at GD10 and GD20 were higher compared to the initial body weights (GD0), but the values were not statistically significant ($p < 0.05$) (Fig. 1).

Table 1: Phytochemical constituents of ethanol extract of *Piper guineense* seed

Chemical constituent	Inference
Alkaloids	+++
Saponins	++
Tannins	+++
Flavonoids	+
Carbohydrates	++
Glycosides	+++
Phenols	+++

+: Low, ++: Moderate, +++: High

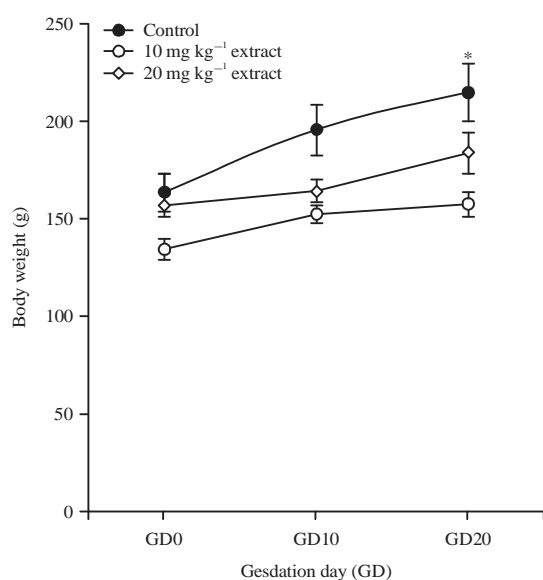


Fig. 1: Ethanol extract of *Piper guineense* seeds inhibits maternal weight gain in Wistar rats

Data expressed as Mean \pm SEM, n = 6 rats per group. * $p < 0.05$ compared to GD0

Pregnancy indices in Wistar albino rats treated with extract:

Number of implantations (39) observed in control rats were equal to the number of fetuses that were delivered (Table 2). In addition, there were no still births nor resorptions in control animals. On the contrary, although many implantations sites (41) were observed in rats that were given 10 mg kg⁻¹ extract, only 23 fetuses were delivered among which 9 were still births, 39% (Table 2). The resorptions that occurred in this group were equivalent to about 66% abortion. Furthermore, few implantation sites (24) were seen in rats that received 20 mg kg⁻¹ extract and 14 fetuses were delivered among which 9 were still births (64%). With 8 resorption occurring in this group, abortion rate was about 79% (Table 2).

Furthermore, there was reduction ($p < 0.0001$) in fetal birth weights in the two experimental groups when compared with birth weight of fetuses in control rats (Table 2).

Reproductive organ weights of rats after treatment with extract:

There were decreases ($p < 0.0001$) in the weights of ovary and uterus of all extract administered rats compared to control group (Fig. 2).

DISCUSSION

The findings of the abortifacient potential of *Piper guineense* seed extract (10 or 20 mg kg⁻¹) following its administration during second trimester of pregnancy in Wistar

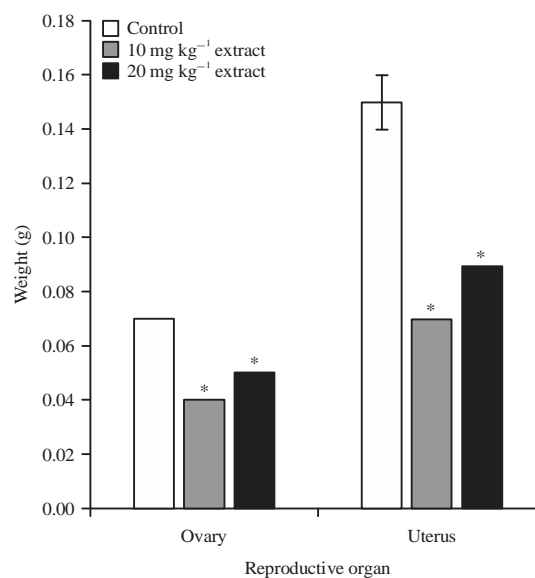


Fig. 2: Ethanol extract of *Piper guineense* seeds reduces ovarian and uterine weights of Wistar rats

Data expressed as Mean \pm SEM, n = 6 rats per group. * $p < 0.0001$ compared to control

Table 2: Effect of ethanol *Piper guineense* seed extract on pregnancy indices in Wistar rats

Dose (mg kg ⁻¹)	Total number of implantations	Total number of live pups delivered	Number of still births	Number of resorptions	Loss of pregnancy (abortifacient activity) (%)	Birth weight (g) [‡]
Control	39	39	0	0	0.00	5.53±0.07
10	41	14	9	18	65.85	3.64±0.14*
20	24	5	9	10	79.17	3.19±0.09*

n = 6 rats per group. [‡]Values expressed as Mean ± SEM, *p<0.0001 compared to control group

rats are herein presented. Successful pregnancy depends on implantation and subsequent growth of the fetus in utero through the period of gestation until delivery. Loss of pregnancy or abortion in animals can occur when there is premature termination of an implanted fetus. In the present study, number of implantation sites at the end of gestation period were normal in the rats that were treated with the lower dose of the extract (10 mg kg⁻¹), but this was reduced when the higher dose of the extract (20 mg kg⁻¹) was administered. At the two doses, the extract caused resorption of pregnancies as fewer number of the implanted embryos developed successfully at the end of gestation. This consequently resulted in few number of fetuses delivered in extract treated rats relative to control rats. Besides, stillbirths occurred following extract treatment (39 and 64%, respectively).

These results indicated that the extract potentially interfered with normal fetal development. At high concentrations, it appears that the extract possibly deteriorates or dissolves implantation after pregnancy, i.e., preventing implantation growth. Successful implantation after fertilization of the ovum depends on physiologically adapted uterus which is influenced by activities of ovarian hormones¹⁹. The reduction of uterine and ovarian weights by the extract may therefore contribute to some of the effects seen in this study. An earlier study had indicated that *P. guineense* induces luteolytic effect on corpus luteum²⁰ and corpus luteum plays essential role in the maintenance of early pregnancy. *Piper guineense* has equally been shown to possess oxytocic properties^{11,12}, so its uterine contractile ability is likely to affect retention of the fetus after implantation. Furthermore the resorptions observed may result from impairment of fetal growth. Intrauterine fetal growth inhibition may be partly due to toxicity to placenta function which can result to low birth weight of the fetus. So, it is not surprising that the birth weights of the fetuses of extract treated mothers were reduced. Reduction in birth weight increases the health risks of many adult diseases such as Type 2 diabetes, hypertension and respiratory tract infections²¹.

Furthermore, extract treatment did not reduce body weight but hindered maternal body weight gain. These results

strongly indicate that *P. guineense* seeds consumption during pregnancy can result in negative consequences. These negative effects may be due to the phytochemical constituents that are present in the plant extract which may be toxic to reproductive structures like tannins and alkaloids^{3,22,23}. In an earlier study, *Piper guineense* was reported to prevent pregnancy in mice completely²⁴. In that study, the extract was administered to both sexes of animals for 21 days while being mated and at the end of the study period no pregnancy occurred in all extract treated female mice. This supports the findings of this study, which indicate that the plant is capable of having negative influence on female reproductive activity in experimental animals.

CONCLUSION

Piper guineense seed extract treatment to pregnant rats altered maternal and fetal indices negatively which were characteristic of abortifacient activity. Number and birth weight of fetuses of extract administrated mothers were reduced and there was high percentage of still births which was absent in fetuses of the mothers that did not receive extract. Extract treatment equally induced resorption of pregnancy and inhibited body weight gain of mothers, suggesting that *Piper guineense* seed is capable of inducing abortion and affect maternal health in rats. The limitation of this study includes non-characterization of the active principles of the plant extract which can be considered in future studies. In addition, elucidating the mechanism of abortifacient activity of the plant is a possible concern of future studies.

SIGNIFICANCE STATEMENT

This study has demonstrated that treatment with ethanol extract of *Piper guineense* seeds to pregnant rats affects pregnancy outcome negatively in the animals. This study will provide baseline data to help the researcher effectively probe the active principles of the plant extract responsible for the activity. The study will also give a lead to the researcher to unravel the mechanism of action.

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