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*Full Length Research Paper*

# Effects of ethanol extract of *Piper guineense* seeds (Schum. and Thonn) on the conception of mice (*Mus Musculus*)

**A. P Ekanem[1]*, F. V. Udoh[2] and E. E. Oku[3]**

[1]Institute of Oceanography, University of Calabar, P. M. B 1115, Calabar, Nigeria.
[2]Department of Pharmacology, University of Calabar, P. M. B. 1115, Calabar, Nigeria.
[3]Department of Zoology and Environmental Biology, University of Calabar, Nigeria.

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**The effects of *Piper guineense* seeds (Piperaceae) on conception of mice (*Mus musculus*) were investigated. Thirty sexually mature mice (3 weeks old) were distributed in plastic cages with each cage containing two females and one male animal. Similar cages and same number of animals were set up as controls. Animals were fed a mixture of feed with extracts of *P. guineense* seed at various test concentrations (10, 20, 30 and 40 mg/kgBW). Controls were fed similar ration of normal diet without extract. The experiment lasted for 42 days, consisting of 21 days of feeding with extracts with males and females mice staying together in cages, and 21 days of feeding without extracts but male and female animals kept in separate cages to stop further sexual behavior. All the female mice in the control cages gave birth to young ones between the 28 and 35th day of the test periods but no animal in the test group showed signs of pregnancy and none had implantation of fetus in the womb. Histopathology sections of testis and ovary of test animals showed inflamed cells of the gonads compared to normal cells in the controls. Three alkaloidal amides (Piperanine, Δα,β-dihydrowasanine and isobutyl-(E,E)-2,4-decadienamide) were isolated by HPLC analysis of the extracts. On the bases of the results obtained, it was concluded that seeds of *P. guineense* contain substances that interferes with conception in mice.**

**Key words:** *Piper guineense*, conception, mice.

## INTRODUCTION

*Piper guineense,* popularly known as African black pepper or hot leave is widely consumed in some part of West Africa especially Nigeria and Ghana on account of its nutritional and medicinal properties (Negbenebor et al., 1999). It belongs to the family Piperaceae or Sapotaceae (Macmillian, 1984). In traditional herbal medicine, the seeds are put into a variety of uses, for instance, in some parts of Nigeria, the seeds are consumed by women after child birth, to enhance uterine contraction for the expulsion of placenta and other remains from the womb (Udoh et al., 1999), as an adjuvant in the treatment of rheumatic pains and as an antiasthmatics (Sofowora, 1982) and also for the control of weight (Mba, 1994).

The seed and leaf extracts are capable of exhibiting a depolarizing neuromuscular activity in a concentration related manners (Udoh et al., 1999). The antiparasitic, antimicrobial and antifungal activities of the leaf and seeds of *P. guineense* have also been reported (Ekanem and Obiekezie, 2000; Ngane et al., 2003; Ekanem et al., 2004). According to Noumi et al. (1998) in Mbongue et al. (2005), leaves of *P. guineense* have been used by

*Corresponding author. E-mail: albertekanem@yahoo.com

traditional medical practitioners for the treatment of respiratory diseases and correction of female infertility problems, and the seeds as an aphrodisiac. The objective of the study was to investigate the influence of the seed extracts of *P. guineense* on the fertility of female mice.

## MATERIALS AND METHODS

### Collection and preparation of samples

Seeds of *Piper guineense* were purchased from a local market in Calabar, Nigeria. The plant was authenticated by Dr. S. W. Hausaini, University of Jos, Nigeria. A voucher specimen deposited in the Department of Botany, University of Calabar, Nigeria. Seeds were washed in water to remove sand and debris, sun dried under low intensity (28°C) sunlight for 24 h and oven dried in a hot air oven at 60°C for 48 h. Dried seeds were crushed to powder with mortar and pestle and reduced to fine powder with an electric blender. The powder was extracted by Soxhlet method. A total of 500 g of the powder was extracted by wrapping 50 g per batch in a thimble and inserted into the extractor connected to 1l pyrex round bottom flask containing 500ml of absolute ethanol and extracted at 60°C for 48 h. The extract was evaporated to dryness in vacuo at 40°C using a rotary evaporator. A total yield of 20% (200 g) extract was recovered from the process.

### Preparation of working solutions

The extract was used for the preparation of test solution by dissolving a known weight in 5% diemethyl sulphoxide (DMSO) to ensure complete dissolution and then in distilled water. The stock solution was used in the preparation of test solutions by dilution in distilled water. A preliminary test was conducted to guide in the selection of concentration boundaries used for the final test. The working solutions selected after the preliminaries were 10, 20, 30, and 40 mg/kg. Control tests were also administered under the same experimental conditions except the absence of *P. guineense* extracts.

### Methods of survey

After obtaining an official permission from the Cross River State Ministry of Health's Ethical committee, thirty sexually matured laboratory mice (*Mus musculus*) of the same age and weight groups were obtained, randomly distributed into 10 cages with 3 animals (2 females to 1male) in each cage. Initial weights of the mice were taken with an electronic weighing balance before they were introduced into the cages. Animals were fed twice daily (for a period of twenty one days) by mixing extract with feed and water for the test and feed with DMSO and water for the controls. Daily observations were made for changes in behavior and indication of toxicity. Cages were kept clean daily throughout the duration of the experiment. The experiment was replicated three times.

After the first twenty one days of feeding with extract for the test and normal ration without extract for the control, mail mice were removed from their female counterparts and kept separately to avoid further sexual behavior. The weights were again taken for all the animals in different cages. Animals were left in the cages for another twenty one days feeding normal diets without extract for both test and the controls. Animals that showed signs of pregnancy (unusual weight increments and distended abdomen) were separated and kept in separate cages to reduce aggression and disturbance from cage mates.

### Bioassays

The date and time of littering and the weight after littering were noted. The difference in weights in the different groups between the initial and the final and between the end of feeding with the extract and the periods with no extract were also measured. Lapratomy was carried out on the female mice of all the test groups for examination of the uterus for possible implantation. Male animals from both test and control groups were also sacrificed and the testes removed and fixed in Bouins fluid for histopathological processes. Tissues were stained and counterstained with haematoxylin and eosin stains respectively. Toxicity studies of *P. guineense* seed extract on mice were carried out. 10 mice were put in each cage and treated with graded concentrations of the extracts (40, 60, 80 100 and 120 mg/kg). Male mice were treated separately from their females' counterparts in the toxicity experiment. The concentration at which 50% of the test animals died ($LC_{50}$) was noted and the experiment was replicated three times.

### HPLC and LC-MS analysis of *P. guineense* seeds

HPLC and LC-MS were conducted using an Agilent 1100 series LC/MSD system (Agilent Technologies) equipped with a quaternary pump, diode array and multiple wave length detector, thermostated column compartment, degasser, MSD trap with an electrospray source and HP Chemstation software, Bruker Daltonics 4.0 and data analysis 4.0. A pre-packed 150 x 4.6 mm (3um particle size) phenyl-hexyl column (Phenomenex, Torrance, CA) was selected for HPLC analysis. The absorption spectra were recorded from 200 to 400 nm for all peaks. The column temperature was set at 45°C and the mobile phase included water (containing 0.1% formic acid solvent A), acetonitrile (containing 0.1% formic acid, solvent B) in a gradient system, 0min 18% B linear gradient to 68 % B in 30 min, and the a linear gradient to 95% B in 25 min. The total running time was 55 min, and the post running time was 10 min. The flow rate was 1.0 ml/min and injection volume 10 µl. he electro spray mass spectrometer (ESI-MS) was operated under positive ion mode and optimized collision energy level of 100% scanned from m/z 100 to 1000. ESI was conducted using needle voltage of 3.5 KV. High purity nitrogen (99.99%) was used as nebulizers at 45 psi. The ESI interface and mass spectrometer parameters were optimized to obtain maximum sensitivity. The LC-MS was run under positive mode.

### Statistical analysis

The homogeneity of the replicates of the groups was checked by the Mann Whitney U-test before the data of the replicates were pooled together and treated as one group**.**

## RESULTS

### Effects of *P. guineense* in the fertility of *M. musculus*

At the end of the 21 days of administration of ethanol

**Table 1.** Effects of *Piper guineense* (Mg/Kg) on conception and littering of mice.

| Conc. (Mg/Kg) | Observations (littering) | | | | |
|---|---|---|---|---|---|
| | 21 days | 28th day | 30th day | 35th day | Remarks |
| 0 (Control) | All animals were heavy | 1 animal littered, producing 4 young mice | 2 animals littered, producing 7 young mice | All animals littered, producing a total of 65 mice | All animals littered |
| 10 | No animal was heavy | 0 | 0 | 0 | None littered |
| 20 | | 0 | 0 | 0 | None littered |
| 30 | | 0 | 0 | 0 | None littered |
| 40 | | 0 | 0 | 0 | None littered |

**Table 2.** Extract of *Piper guineense* (Mg/Kg) against mortalities (%) of mice.

| Conc. (Mg/Kg) | Observation (% Mortality) | | | | |
|---|---|---|---|---|---|
| | 24 | 48 | 72 | 96 | Remarks |
| 0 | 0 | 0 | 0 | 0 | No mortality |
| 40 | 0 | 0 | 0 | 0 | No mortality |
| 80 | 0 | 0 | 0 | 0 | No mortality |
| 100 | 0 | 0 | 10 | 0 | 10% |
| 120 | 0 | 10 | 0 | 10 | 20% |

extract of seeds of *P. guineense* at various concentrations to mice, there were no signs of pregnancy in any of the animals in the test groups, but a slice reduction in weight was observed in a concentration related manner. All the animals were agile and active. In the controls, there were reasonable increase in size and weight of mice and there were obvious signs of pregnancy in the females as indicated by abdominal distension, sluggishness and reduced appetite.

After discontinuation of administration of extract of *P. guineense* and the separation of males from female cages for another 21 day, one animal in the control littered on the 28th day giving birth to 4 young mice, two animals also littered two days after (30th day) in the control and by the 35th day all the female mice in the control had given birth to young ones. None of the animals in the test groups gave birth to young ones (Table 1). The females were sacrificed, dissected and abdominal contents examined but there was no young animal found in any of them.

## Toxicity of *P. guineense*

Results of the toxicity test did not indicate reasonable level of mortality in the highest concentration of 100 mg/kg tested. At the end of 96 h test period, one and two animals were confirmed death in 100 and 120 mg/kg respectively. There was no mortality of animals in the controls.

The results are as shown in Table 2. istopathological examinations showed inflammation of testesticular cells (Figure 1) in the animals fed with *P. guineense* extract. Similar changes were not observed in the control (Figure 2). The ova from the animal fed with the extracts also showed hyperplastic cells (Figure 3) that were not seen in the controls (Figure 4).

## Identification of active compounds in *P. guineense*

Results of the HPLC analysis (Figure 5) of extracts of *P. guineense* seeds yielded over 50 compounds. Most compounds were elicited after 28-26 min. After subjecting the extract to LC-MS analysis, most compounds were found to contain nitrogen in their structures. Three major compounds identified were (1) Piperanine which showed MS ions at 288 [M+]+135.1; Δα,β-dihydrowasanine (2) with MS ions at 318 [M+]+, 286, and 265 N-isobutyl-(E,E)-2, and (3) 4-decadienamide with MS ion at 224 [M+]+, and 168 respectively. The structures of these compounds are shown in Figure 6.

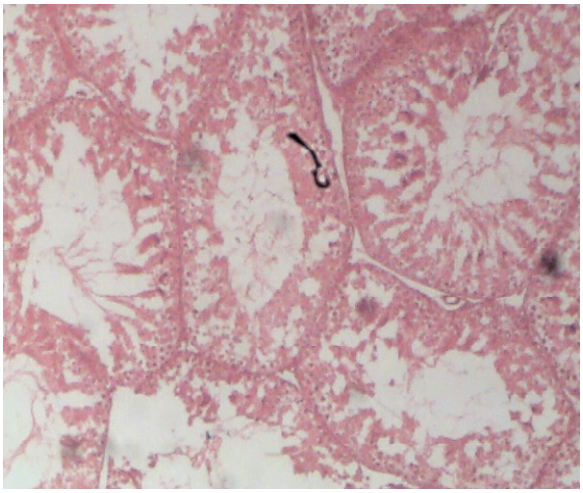

**Figure 1.** Control testis with normal distribution of cells (100X)(Photographed with Moticam 352).

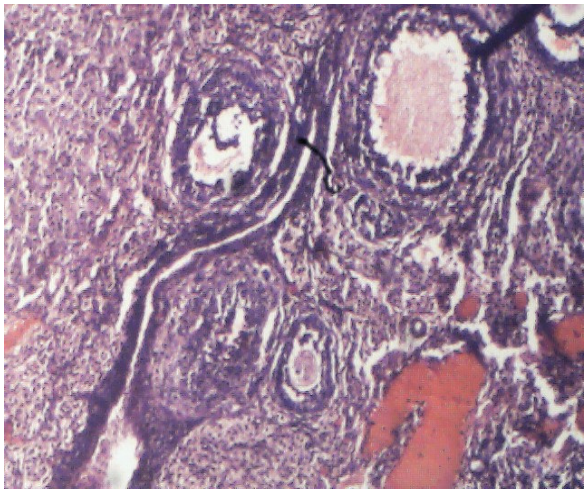

**Figure 3.** Control ovary with normal cells (100X) (Photographed with Moticam 352).

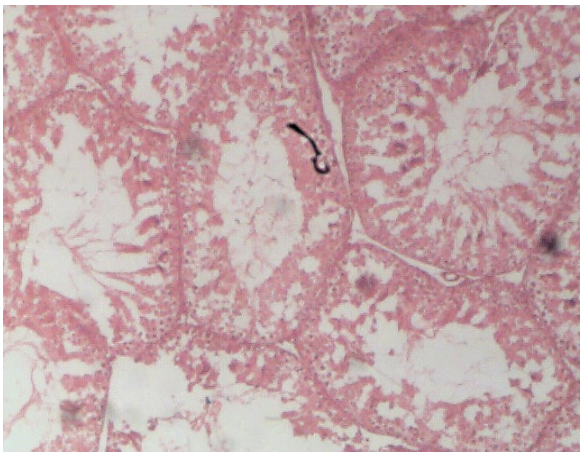

**Figure 2.** Treated testis with enlarged cells (100X) (Photographed with Moticam 352).

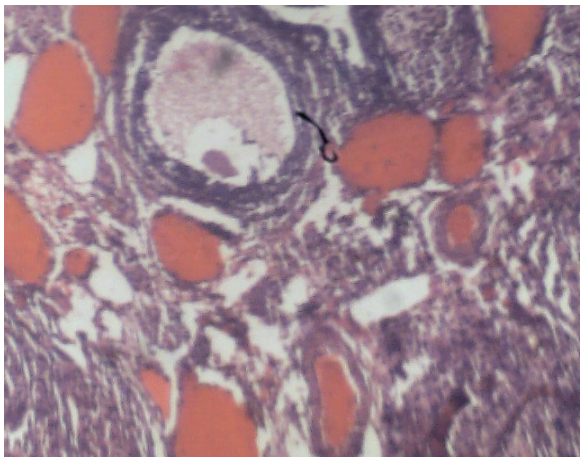

**Figure 4.** Treated Ovary with enlarged cells (100X) (Photograhed with Moticam 352).

## Statistical analysis

The test of significance difference between the test and control could not be conducted with any statistic because there was zero conception in all the treatments including the lowest concentration (10 mg) tested**,** whereas, all the animals matched against the test organisms gave birth to young mice in all the cages used as control.

## DISCUSSION

The in-vivo administration of ethanol extract of *P. guineense* to male and female mice resulted in interference with conception. Animals treated with the extracts neither showed obvious signs of neither pregnancy (heaviness, sluggishness and distended abdomen) nor any developing embryo when they were dissected at the end of the test periods. Whereas, all the animals in the control were pregnant (heavy, sluggish with distended abdomen) and gave birth to young animals at various time within the test periods. There have been reports (Mbongue et al., 2005) on the antifertility effects of aqueous extracts of seeds of *P. guineense* on male wistar rats but no report has been given its effects on female reproduction. Both seeds and leaves of *P. guineense* are widely consumed in the Eastern and Southern parts of Nigeria and other parts of

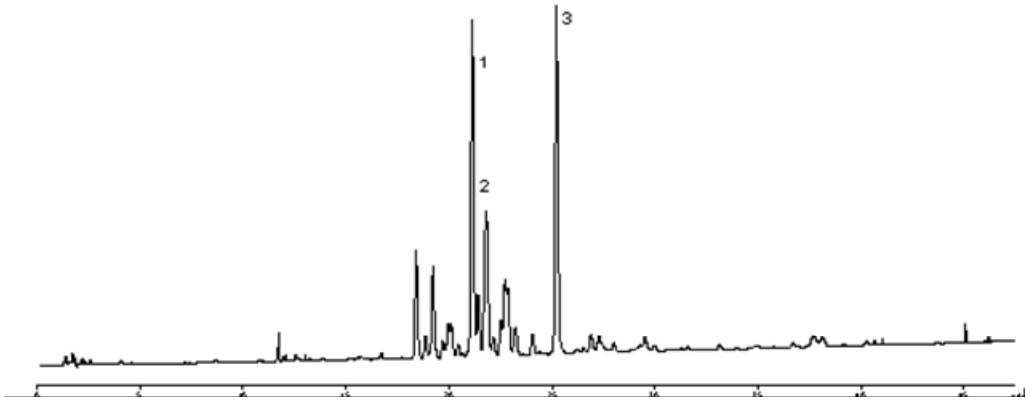


**Figure 5.** HPLC chromatogram of ethanol extract of *Piper guineense*.1 = (Piperanine, 2 = Δα, β-Dihydrowasanine, 3 = N- isobutyl- (E, E)-2, 4-decadienamide).

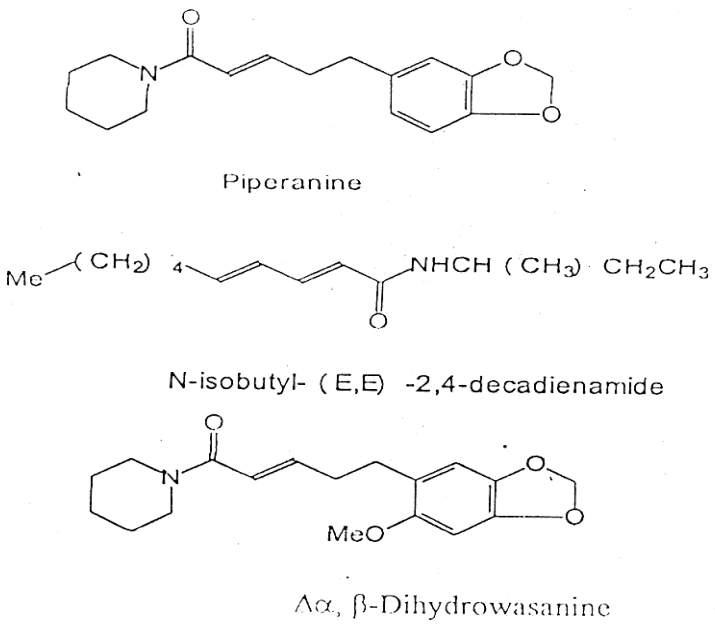


**Figure 6.** Structures of three compounds identified from the ethanol extract of *Piper guineense*.

West Africa as spice and additives to meals and for the treatment of various aliments (Mba, 1994; Negbenebor et al., 1999). Infertility is becoming a house hold problem in Nigeria and has severally been attributed to witchcrafts. It is likely that the consumption of some herbal products that has not been fully investigated may in addition to enhancing treatment of diseases but also interfere with normal functioning of certain organs of the body. For instance, if extracts of seeds of *P. guineense* contains substances capable of enhancing uterine muscle contraction for the expulsion of the placenta in women after birth as reported by Udoh et al., (1999), then it is possible that it may interfere with implantation of fetus in the womb because of the rigorous movements of the uterine muscle. This may explain the lack of conception in the animals fed a mixture of *P. guineense* extracts in diets as shown in the results of the present study. Moreover, the facts that female animals fed a mixture of

the extract in meal were not pregnant even after mating may be as a result of lack of viability of sperm cells produced by the males. Apart from the fact that histopathological analysis of testicular cells in this study showed slight deformities, a reduced fecundity of wisters male rats fed with extracts of *P.guineense* has been reported (Mbongue et al., 2005).The reduced fecundity was attributed to the reduction in the level of α-glucosidase and fructose which plays important role in sperm motility and supply of energy (Johnson and Everitt, 1988). Perhaps the presence of some alkaloidal amides (Piperanine, Δα,β-dihydrowasanine, isobutyl-(E,E)-2, 4-decadienamide) isolated from the extracts as shown by the HPLC analysis in this study may have contributed to the effects obtained but needs to be confirmed by further studies.

The study on the effects of *P. guineense* on the fertility of mice led to the conclusion that the ethanol extract has negative effects on mice reproduction. A mouse belongs to the same class (mammalia) as man and thus the likelihood that similar effects may occur in man is alleged. This is further confirmed by various studies on other members of the Class in which conceptions were prevented in animals that were treated with the plants.

The threshold of test concentration used in this study were all active against the conception of mice because even animals administered with the lowest concentration (10 mg/Kg) did not show any sign of fetal development nor gave birth to any young one. This may be as a result of high concentration of extract used in this study. Further study is recommended with lower concentrations of extract of *P. guineense* to establish threshold concentrations at which conception can be achieved in mice for the purposes of statistical comparisons. Furthermore, since *P. guineense* is a well cherished food spice among some West Africans and for its medicinal values, there is need to do further studies on the fertility of those addicted to its consumption as to confirm the correlation between the results obtained in animal studies and that of human.

**REFERENCES**


Ekanem AP, Obiekezie AI (2000). Antiparasitic effects of leaf extract of *Piper guineense* (Husani) on the juveniles of *Heterobranchus longifilis* (Cuvier and Valencieness). Afr. J. Fisher Aquacult., 8: 74.

Ekanem AP, Wang M, Simon JE, Obiekezie AI, Morah F (2004). In vivo and In vitro acivities of seed extract of *piper guineense* Schum. and Thonn. Against skin and gill Monogenean parasites of Gold fish (*Carassius auratus auratus*),Phytother. Res., 3: 97.

Johnson MH, Everitt BJ (1988). Essential reproduction (3rd Edn). Great Britain: Black Well Scientific Publication. pp.1-34.

Macmillan HF (1984). A handbook for Tropical and Gardening. Macmillan Sientific Publishers, London. p. 326.

Mba MA (1994). Effect of dietary intake of *Piper guineense* on growth and indices of fitness in Rattus rattus,isc.Innoa., 4: 383-388.

Mbongue FGY, Kamtchouing P, Essame OJL, Yewah PM, Dimo T, Lontsi D (2005). Effect of the aqueous extract of dry fruits of *Piper guineense* on the reproductive function of adult male rats, Indian. J. Pharmacol., 7: 30-32.

Ngane AN, Biyiti L, Bouchet PH, Nkegfact A, Zolo PHA (2003). Antifungal activity of Piper guineense of Cameroun. Fitoter., 4(5): 464-468.

Noumi E, Amvam ZPH, Lontsi D (1998). Aphrodisiac plants used in Cameroon. Fitoter. 69: 5-34.

Negbenebor CA, Godiya AA, Igene JO (1999). Evaluation of *Clarias anguillains* treated with spice (*Piper guinnense*) for washed mice and kama book type product, Food Composit. Anal., 2: 12-315.

Sofowora A (1982). Medicinal plant and traditional medicine in Africa. John Wiley and sons, New York. p. 44.

Udoh FV, Theodore YL, Braide VB (1999). Effects of extract of seed and leaf of *Piper guineense* on skeletal muscle activity in rat and frog, Phytother. Res., p.110.