European Journal of Medicinal Plants
5(3): 297-303, 2015, Article no.EJMP.2015.029
ISSN: 2231-0894

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effects of Crude Extract of Dry Fruits of *Piper guineense* on Male Fertility Parameters of Adult Sprague Dawley Rats

A. E. Memudu[1*], I. D. Akinrinade[1], O. M. Ogundele[2] and B. J. Dare[1]

[1]Department of Anatomy, College of Medicine, Bingham University, Karu, Nassarawa State, Nigeria.
[2]Afe Babalola University, Human Biology Department; Ado – Ekiti Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AEM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. She is the major author and contributor as she provided lab space equipments and initiated design. She also drafted the manuscript. All other authors took part in the analyses of the study and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/9466
Editor(s):
(1) Thomas Efferth, Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Germany.
(2) Marcello Iriti, Faculty of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Flaviane Gomes Pereira, Federal University of Rio de Janeiro / Brazil.
(2) Anonymous, Kurukshetra University, India.
(3) Anonymous, University of KwaZulu-Natal, Durban, South Africa.
(4) Anonymous, Bharati Vidyapeeth Deemed University, Mumbai, Maharashtra, India.
(5) Anonymous, Herbal Medicine Research Centre, Institute for Medical Research, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=791&id=13&aid=7244

Original Research Article

Received 11th February 2014
Accepted 13th June 2014
Published 15th December 2014

ABSTRACT

Objective: To study the effect of aqueous extract of dry fruits of *Piper guineense* on male fertility parameters of adult male Sprague Dawley rats.
Materials and Methods: 30 adult male Sprague Dawley rats weighing between 120g -150g were used. They were divided into three (3) groups. The control (group A), Short term treatment Group A (Group B) received 200 mg/kg of extract for 4 weeks (28 days) and long term treatment (Group C) received 200 mg/kg of extracts for 8 weeks (56 days). Extract was given via gastric intubation. The controls as well as treated animals were allowed free access to pelleted feed and water *ad libitum.*

**Corresponding author: Email: jokememudu@gmail.com;*

The animals were sacrificed and blood taken for serum biochemical analysis. Testicular weight was taken and recorded. Caudal epididymis excised for semen analysis.
Results: There was an increase in animal body weight gained throughout the duration of the experiment as compared with the control. There was also a significance increase in testicular weight. Serum testosterone level increased in treated groups.
Semen analysis reveals normal parameters no oligospermic or azoospermic condition was detected. Sperm morphology was normal and no teratozoospermic sperm found.
Conclusion: The aqueous extract of *Piper guineense* at dose of 200mg/kg for 4 weeks and 8 weeks respectively had a positive impact on male fertility parameters. From results obtained, *Piper guineese* enhances testicular hormone secretion. This study revealed no deleterious effects of aqueous extraction of dry fruits of *Piper guineense* on male fertility.

Keywords: Oligospermic; azoospermic; teratozoospermic; testosterone; Piper guineense.

1. INTRODUCTION

In developing countries, traditional medicines are widely used to treat various ailments. Some of these herbal medicines come in form of spicy plants which also have culinary purpose. Varieties of these plants have been used for treating ulcer, diabetes and male fertility problems. *Litsea chinensis* and *Ochis maculate* have been used in averting male fertility problems because of their androgenic and spermatogenic effects or potentials [1]. Dietary supplements and botanical/ herbal medicine has revived growing area of interest in the treatment of diseases; because current / modern medical therapy and facilities are insufficient in remote area and the cost of procuring pharmaceutical drugs has increased [2].

The used of medicinal herbs or spices for treating various diseases dates back to centuries, but their therapeutic components concerning their pharmacokinectic, pharmacodynamics as well as mechanism of action has been tripled over the last decades [3]. For many years, the search for aphrodisiac product has focused on plants. Most fertility disorders are associated with secretion from sex organs and have been can be treated using herbs [3]. Aphrodisiac agents of plant origin have been used in alternative medicine to combat male and female infertility [4]. Some of the factors responsible for this infertility are associated/ traced to hormonal secretion, erectile impotency, disorders of ejaculation, toxic effects of substances (reactive oxygen species, ROS) on the testes and accessory sex organs. Leaves of *Piper guineense* are used for treating respiratory infections as well as female fertility disorders while its fruits / berry are use as aphrodisiac in males. *Piper guineense*, commonly known as black pepper, is the fruit of a tropical herbaceous climber plant belonging to the family Piperaceacea. This herbaceous climber plant, 4m-10m in length, is commonly found in the African Tropical forest zone such as Guinea, Guinea Bissau, Northern and Southern part of Nigeria [4]. In Nigeria, its local names are "*Ata- Iyere*" (in Yoruba), "*Uziza*" (in Igbo) and *Monsoro* (in Hausa). In other West African Countries it is called *Ashanti pepper*, *Guinea Cubeb*, *Benin pepper* or *Bush pepper*. The fruits or berries and the leaves are commonly sold in Nigeria as condiments for food flavoring [5].

Piper guineense is native to the India community where it is use in traditional medicine such as Ayureda, Siddah and Unani medical practices. In West Africa, herbal practitioners make use of it as an aphrodisiac and treatment of respiratory diseases [4].

Its therapeutic components/ chemical constituents are *piperine* (active component), biopiperine, zinc, essential oils such as terpenes, pinene, sabiene, limonene, caryophyllene and linalol; alkaloids such as chaivicine and piperidine. Its active component is *piperine* that accounts for its pungency [6].

Research proved that medicinal properties of *Piper guineese* are due to piperidine and piperine compound. Leaves have been useful in combating female infertility; fruits help to boost male fertility. It has also been reported to act as an antipyretic, analgesic, anti – inflammatory and use in the treatment of liver problems, sunburn, heart diseases, indigestion, constipation, diarrhea etc. [4,7].

Reports about *Piper guineense* therapeutic effects on male reproductive parameters, has been ascertained either by extraction of its active compound, its aqueous extraction or combining it

with other herbs or plants. It was reported that combination of *Piper guineense*, Borax and Embella induced sterility in male rats [8]. A study reported that oral administration of piperine in 1 mg/kg, 10mg/kg and 100mg/kg respectively to adult male Sprague Dawley rats for 30 days resulted in reduction in epididymal weight of rats as dose given increases [9]. A similar study at doses of 5 mg/kg and 10 mg/kg of piperine for 30 days; result obtained revealed a decrease in testicular weight, accessory sex organ, severe damage to seminiferous tubules, Leydigs cells nuclear and desquamation of the spermatocytes [10]. Serum and testicular testosterone level increased after oral administration of the aqueous extracts of black pepper at doses of 122.5 mg/kg and 245 mg/kg for 8 and 55 days respectively [11].

Other reports have been made on the effects of ground fruits of black pepper (*Piper guineense*) on the kidney and liver. Mixing of 3 g of ground black pepper with pellet feed of adult rabbits for 21 days show few distinct round basophilic bodies in the renal interstitium of renal cortex. Presence of mast cells show an inflammatory reaction that indicates the black pepper can trigger renal fibrosis [12].

In view of this divergent studied of the effects of *Piper guineense* as an aphrodisiac agent that affects male fertility potential at different doses, this research was carried out to investigate the effect of a dose of 200 mg/kg; in which animals were subjected to its acute and chronic treatment. At the end of the duration of study, male fertility parameters were studied to understand its aphrodisiac effects and investigate it effect on sperm morphology another determinant of male fertility.

2. MATERIALS AND METHODS

2.1 Plant Material

The dry fruits of black pepper for this experiment was procured from Lawanson market; Surulere, Lagos. They were identified and authenticated by Taxonomists in the Department of Botany, University of Lagos, Nigeria; where voucher specimen of the dry fruits were kept in the herbarium.

2.2 Aqueous Extract of Dry Fruits of Black Pepper Preparation

The dry fruits of black pepper were sun – dried and crushed into fine powdered form using a dry blender. A portion of dried powder been weighed as 10g (which is equivalent to 10,000 mg) was macerated in 50 ml of distilled water. Final extract concentration obtained is 200 mg/ml. The aqueous extract was stored in air – tight containers refrigerated throughout the course of the experimental design. Fresh sample of the same weight and dilution factor was prepared every week; this method was adopted to maintain pungency of the spices. The aqueous extract was given orally via oral canula. 1 ml of the extract was given daily.

2.3 Experimental Animals

Ethical approval was gotten from the Department of Anatomy, University of Lagos, Nigeria; before commencement of the research. 30 adult male Sprague Dawley rats with average weight of 120 g were used. They were purchased from Covenant Farm (Nig.) Enterprises, Gbolasire Estate; Iwo road Ibadan, Oyo state. The animals were authenticated in the Department of Zoology, University of Lagos, Nigeria. They were housed in well ventilated wire- mesh cages in the Animal room of the Department of Anatomy, College of Medicine, Idi – Araba, Lagos. They were kept at room temperature of 28°C-38°C and exposed to 12:12 hr (natural light – dark) cycle. They were fed with pelleted rats' feed (UAC feeds, Lagos Nigeria.) and given water *ad libitum*; and allowed to acclimatize for three weeks before commencement of experiment. They were randomly weighed and allocated into two (2) groups; Group A serves as the control while Group B serves as the treated group further subdivided into two. The first Experimental Group B was treated for a short term basis; they were given 200mg/kg of the aqueous extracts for 28 days while the second experimental group in Group B were treated on a long term basis for 56 days, and were given 200 mg/kg of the extract as shown in Table 1. The extracts was given via oral cannula.1ml of the extract was given per day for 28 and 56 days respectively between 8 – 9 A.M throughout the period of experiments.

2.4 Blood Collection for Serum Hormonal Analysis

At the end of each experimental phase, the experimental animals were anaesthesized via in intraperitoneal injection of Ketamine was used for animal euthanization. Blood samples were collected via cardiac puncture and kept in dried

Table 1. Experimental protocol

Groups	Treatment	Duration
Group A (Control) N= 10	Given rat feed and water *ad libitum.*	28 days and 56 days respectively
Group B (short term treatment) N= 5	Given one ml of 200 mg/kg aqueous extract of *Piper guineense via* gastric intubation*;* Given rat feed and water *ad libitum.*	28 days
Group C (long term treatment) N = 5	Given one ml of 200 mg/kg aqueous extract of *Piper guineense via* gastric intubation*;* Given rat feed and water *ad libitum.*	56 days

air – tight tubes. The blood samples were spin at 2,500 r.p.m for 10minutes in a desktop centrifuge (Heraeus Megafuge 1.0, Germany) to obtain the serum from the whole blood. The serum was then aliquoted and frozen for serum biochemical analysis. The serum sample was assay for testosterone level using the Enzyme linked immunoassay (ELISA) technique. Test kit used was made by the BIOTEC Lab. Ltd, UK.

2.5 Semen Analysis

The caudal part of the epididymis is excised and transferred into normal saline for about 3 minutes for spermatozoa to swim out after which a drop of saline is transferred to the Neubauer Counting Chamber (haemocytometer) for semen analysis under the light microscope (Olympus, Germany). Semen analysis for each group was done as well as sperm count according to the recommended protocol of the WHO manual/ criteria [13].

- Sperm motility: Sperm motility was access using the WHO classification system. Each sample was assessed twice. For consistency all readings were carried out at 37°C [13] under a light microscope (Olympus, Germany).
- Sperm count: microscopic sperm count done according to the recommended protocol of the WHO manual/ criteria [13] with the aid of Light Microscope (Olympus, Germany).
- Sperm morphology: the morphology of the sperm was also evaluated, using WHO criteria as described in the old manual of 1989. A sample is normal if 30% or more of the observed sperm have a normal morphology. The Tygerberg criteria for sperm morphology assessment are recommended in the most recent WHO manual on semen analysis where sample are taken as normal if 14% of sperm has normal morphology.

2.6 Testicular Weight

The testes were weighed using an analytical weighing balance obtained from the Department of Physiology, College of Medicine of the University of Lagos.

2.7 Statistical Analysis

Data were analyzed using SPSS statistical software and Microsoft Excel. The statistical analysis was carried out by one way analysis of variance (ANOVA). Data were expressed as mean ± standard error of mean (SEM). Test for significance was done using student t- test. Differences between mean was considered statistical significant when p – value is < 0.05.

3. RESULTS

Body weight and testicular weight:

- Group B (short term treatment group): there was a significant increase in body weight gain of animals in control and *Piper guineense* treated groups.
- Group C (Long term treatment group): there was a significant increase in body weight gain of animals in control and *Piper guineense* treated groups. In this category the organ and body weight gain is highly significant as compared with the short term treated group.

4. DISCUSSION

The present study showed a significant increase in body weight of *Piper guineense* treated rats for both the acute and the chronic treatment. The increased in body weight as shown in Table 2a and 2b; is traced to the androgenic property of black pepper, since androgen has anabolic property [4]. An increase in serum testosterone level signifies that it influences the steriodogenic

pathway (involved in the production of testosterone using cholesterol as a starter), similar effect was seen in a study using *Hibiscus macranthus* and *Basella alba* [12]. In this study, long – term administration as displayed in Table 3 significantly increased the serum testosterone level more, this show that it stimulates testosterone biosynthesis and there is a likelihood of a significant increase in serum and testicular cholesterol level since it is a starter for testosterone synthesis. This reports correlated with previous findings that demonstrate herbal plants and *Piper guineense* causing an increased in serum cholesterol level a biochemical compound needed for testosterone biosynthesis, thereby increasing serum testosterone level [4,11].

Testicular weights of the treated groups increase significantly when compared with the control at P< 0.05 as shown in Table 3. This reports caused an increased body weight gain which contradicts a study that black pepper causes a reduction in weight gain by inhibiting lipogenesis [14]. Serum testosterone level increased significantly as compared with the control as displayed in Table 4. The increase in serum testosterone level of rat feed 200 mg/kg *Piper guineense* is traced to zinc one of its chemical component [6]. Zinc supplementation results in increased serum testosterone levels; because zinc supplements has being therapeutic again androgenic deficiency [15,16]. Zinc is a co-essential factor for the endogenous production of testosterone since it is capable of inhibiting the conversion of testosterone to dihydrotesterone (by inhibiting the 5- alpha reductase enzyme); this explains its influence in steriodeogenic pathway that mediates testosterone production. In addition results from this study support findings that leave and fruits of black pepper are capable of increasing testosterone levels by stimulating steriodogenesis [11].

Table 2. Mean changes in body and testicular weight of adult male Sprague Dawley rats

Table 2a. Mean changes in body and testicular weight of Sprague Dawley rats after short – term treatment with crude extracts of *Piper guineense* for 28days (Group B)

Group n=5	Initial weight(g)	Final weight(g)	Weight difference(g)	% increase	Testicular weight (g)
Control	123.55±1.30	162.26±2.05	38.90	31.49	0.90±0.10
Black pepper (200mg/kg)	123.05±3.04	182.00*±1.86	58.95	47.91	0.96*±0.00

Data expressed as Mean ±SEM. Statistical significant value (*) when P< 0.05.Mean values were compared with the control. Comparing experimental groups A vs B

Table 2b. Changes in body and testicular weight after long – term treatment with crude extracts of *Piper guineense* for 56 days (Group C)

Group n=5	Initial weight(g)	Final weight(g)	Weight difference(g)	% increase	Testicular weight (g)
Control	122.95±6.25	204.00±4.30	81.05	65.92	0.90±0.10
Black pepper (200mg/kg)	122.45±2.91	203.20±2.97	80.75	65.95	0.98±0.00

Data expressed as Mean ±SEM. Statistical significant value (*) when P< 0.05.Mean values were compared with the control. Comparing experimental groups A vs C

Table 3. Mean serum testosterone level (ng/ml) in Sprague Dawley rats of study after short term and long treatment with crude extracts of *Piper guineense* for 28 and 56 days respectively

Group n=5	Treatment (days)	Serum testosterone level(ng/ml)	Treatment (days)	Serum testosterone level(ng/ml)
Control	28	1.49±0.01	56	1.56±0.02
Black pepper (200mg/kg)	28	1.91*±0.01	56	2.07*±0.02

Data expressed as Mean ±SEM. Statistical significant value (*) when P< 0.05.Mean values were compared with the controls.

Table 4. Mean sperm count and sperm motility (semen analysis) in Sprague Dawley rats of study after short term and long treatment with crude extracts of *Piper guineense* for 28 and 56 days respectively

Group n=5	Duration (days)	Sperm counts (millions/ml)	Sperm motility (%)	Duration (days)	Sperm counts (millions/ml)	Sperm motility (%)
Control	28 (Group A)	192.00±5.01	59.00±2.89	56 (Group A)	223.00±0.01	70.01±2.67
Black pepper (200mg/kg)	28 (Group B)	206.00*±1.22	67.06*±3.94	56 (Group C)	235.00*±0.02	76.00*±1.58

Data expressed as Mean ±SEM. Statistical significant value (*) when P< 0.05.Mean values were compared with the control.

Semen analysis showed no evidence of azoospermia (absence of sperm), oligospermia (low sperm count), asthenozoospermia (poor sperm motility) and teratozoospermia (abnormal/ morphological defected sperms). Sperm counts in the long term *Piper guineense* treated rats increased above the control; sperm motility values in both control and treated groups. This finding also support reports made on *Piper guineense's* ability to increase sperm count and motility attributed to its antioxidants and aphrodisiac properties [16,17].

Hence, this study showed that 200mg/kg aqueous extract of *Piper guineense* aid male reproductive functions. It effects on sperm motility, sperm functions, testicular steroidogenesis and testicular weight could be attributed to its androgenic and aphrodisiac properties.

5. CONCLUSION

This scientific evidences show that *Piper guineense* recommended as a remedy for male fertility disorders associated with hormone secretion. Since, its likely mechanism of action can be trace to its phyto-chemical compounds and their influence on steriodiogenic pathway.

CONSENT

No applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

FURTHER STUDIES

Further studies will be carried out on testicular tissue to investigate effect on spermatogenic cell lineage. Addition biochemical assay will be carried out on its influence on glutathione, Glucose -6- phosphate activity at this dose. Dose variation study will be done to ascertain its beneficial and harmful doses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ageel MA, Islam MW, Ginawi, OT, Al-Yahya O. Evaluation of the Aphrodisaic activity of *Litsea* (lauraceace) and *Orchis maculate* (Orchidaceae) extracts in rats. Phytother Res. 1994;8:103-105.
2. Echard BW, Talpur NA, Bagchi D, Preuss HG. Heptaoprotective ability of a novel botanical formulation onmild liver injury in rats produced by acetomenophen and/or alconol ingestion. Res. Commum. Mol. Pathol. Pharma. 2001;110:73-85.
3. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Van rompay M, Kesseles RC. Trends in alternative medicine use in the United States: Result follow- up national survey. JAMA 1998;280:1569-1575.
4. Noumi E, Amvam ZPH, Lontsi D. Aphrodisiac plants used in Cameroon. Fitoterapia. 1998;LXIX:125-134.
5. Mwinnka NM, Ibeh GO, Ekeke GI. Piper Guineese. Journal of Applied Science and

Environment Management. 2005;9(1):150-155.
6. McGee H. On food and cooking. 2004;427-429.
7. Lee EB, Shin KH, Woo WS. Pharmacological study of piperine. Arch. Pharm. Res. 1984;7:127-132.
8. Munshi SR, Rao SS. Antifertility of an indigenous plant preparation on reproducetion. Indian J. Med. Res. 1972;60:1220-1226.
9. D´Cruz SC, Mathur PP. Effects of piperine on the epididymis of adult male albino rats. Asia Journal of Andrology. 2005;7(4):363-368.
10. Malini TM, Arunakaran J, Arudhasmm A, Govindarajulu P. Effects of piperine on the lipid composition of pyruvate – malate cycle in testis of rats in vivo. Bio Chem. Mol Intel. 1999;147(3):537-545.
11. Mbongue FGH, Kamtchoung P, Lessane OJ, Yewah PM, Dimo T, Lontsi D. Effects of the aqueous extract of dry fruits of *Piper guineense* on the reproductive function of adult male rats. Indian J. Pharmacology. 2005;37(1):30-32.
12. Moundipa FP, Kamtchouing P, Koueta N, Tantchou J, Foyang, NPR, Mbiapo, FT. Effects of aqueous extracts of *Hibiscus macranthus* and *Basella alba* in mature rats testis function. J. Ethnopharmacol. 1999;65:133-139.
13. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. Cambridge: Cambridge University Press; 1999.
14. Park, UH, Jeng HS, Jo EY, Park T, Yoon SK, Kim EJ, Jeong JC, Um SJ. Piperine a component of black pepper inhibits adipogenesis by antagonizing PPARY activity in 3T3 – LI cells. J. Agric. Food. Chem. 2012;60(15):3853-3860
15. Parasad AS, Abbasi AA, Rabbani P, Dumouchelle E. Effect of zinc supplement on serum testosterone level in adult male sickle cell anemia subjects. Am. J. Hemato. 1981;10(2):119-127.
16. Paila KJ, Singh R, Singh S, Singh, K, Singh, MF, Singh V. Aphrodisiac agents from medicinal plants. A Review. J. Chem. Pharm. Res. 2011;3:911-912.
17. Shafiei NL, Gacini AA, Choobinch S. Effect of zinc and selenium supplementation on serum testosterone and plasma lactate in cyclist after exhaustive exercise. Biol. Trace. Elem. Res. 2001;144(1-3):454-462.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=791&id=13&aid=7244