Impact of oral administration of ethanol leaf extract of *Piliostigma thonningii* on fertility hormones of male Wistar rats

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ABSTRACT

Introduction

The quest for the safe herbal remedy for the management of erectile dysfunction and their relationship with male sexual hormones prompted this research.

Aim of the study:

It is aimed at determining the effect of orally administered ethanol leaf extract of *P. thonningii* on some reproductive hormones of male Wistar rats.

Materials & Methods:

Ethanol extract of the leaf were first screened for the presence of bioactive phyto-constituent using standard methods. Twenty (20)Wistar rats were randomly assigned on the basis of average body weight into four (4) groups of five rats each following acclimatization to laboratory and handling conditions. Animals in group A (control) were administered placebo (1ml) of olive oil. Group B, C and D were administered graded doses of 50, 100 and 200mg/kg body weight of the extract respectively. Extract administration lasted for twenty one (21) days. Water and standard feeds were allowed *ad libitum*. At the end of dose administration, animals were sacrificed and blood obtained by cardiac puncture for reproductive hormonal profile using ELISA method and testes were removed for histological examination.

Results: The bioactive phyto-constituents identified were tannins, saponins, glycosides, flavonoids, alkaloids, carbohydrate, anthraquinones, cardiac glycosides, steroids and triterpene. The extract also produced dosage dependent increase *(P<0.05)* in follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone respectively following the administration of the extract with no evidence of pathological lesion on the testes.

Conclusion: It appears that the alteration in fertility hormones in the serum of treated animals might be due to the presence of bioactive constituents. It is speculated that this extract might improve sexual health, sexual activities and libido.

Keywords: Dysfunction, *Piliostigma thonningii,* Reproductive hormones, Wistar rats

INTRODUCTION

Erectile dysfunction is a worldwide medical and social problem. It affects above 10-15% of married couples globally. Erectile dysfunction itself may not threaten physical health but it can certainly have a seriously impact on the mental and social well-being of infertile couple. In many countries the stigma of infertility often leads to marital disharmony, divorce or ostracism[1]. The dysfunction could also affect all levels of intimacy, like emotional, social, sexual, recreational and intellectual intimacy. Research during the past two decades has an unfolded focus on impotence (erectile failure), premature ejaculation and male infertility. The estimated global prevalence has been on the increase. It is projected that the number of men with this condition will rise to 322 million by the year 2025[2,3] and 35-47% in Nigeria .There are a number of drugs which may act as sex stimulant and enhancing the sexual desire and activity in both men and women. Although, the use of allopathic medicines have shown significant improvement in managing sexual disorders, but at the same time there are large number of side effect which include irregularities of the rhythm of the heart, suicidal tendencies, mental disorders and tremors. The use of synthetic aphrodisiacs result in the dilation of blood vessels in other parts of the body causing headache and fainting. Other side effect include facial flushing, stomach upset, burned vision and sensitivity to light which usually occur at higher doses[4].

In Africa, a wide variety of plants are of great medicinal and nutritional importance. It has been documented that from time immemorial, plants have been used for medicinal purpose[5]. Many plants or plant extracts have been used as fertility agents in folklore and traditional medicines without producing apparent toxic effects[6]. Erectile dysfunction is a major clinical problem affecting people medically and psychologically[7]. The management options available for the

treatment of erectile dysfunction/infertility in males include the use of drugs and a variety of surgical procedures[8]. Thus, having a balanced level of hormone is essential to proper fertility in the reproductive health of both men and women. On this account, many plants derived chemicals that influence endocrine activities in both humans and animals have received a great deal of attention due to their possible benefits as well as less adverse effect .Example of this plant are the *Piliostigma thonningii. Piliostigma thonningii* is a plant with numerous ethno medicinal importancewhich ranges from heamatopoietic, antilipidaemic, hepatoprotective, aphrodisiac, antimalarial, anti-venom [5,9]. Therefore this present research is aim at accessing the effect of ethanol extract of *Piliostigma thonningii* on male reproductive hormones of Wistar rats.

METHODS AND MATERIALS

Plant Material

Fresh leaves of *P.thonningii* were collected from Igoli Road, Cross River University of Technology Okuku, Cross River State, Nigeria. The leaves were taken to Federal College of Forestry (FCOFJ) Jos in Plateau State, Department Herbarium for identification and authentication with the Voucher number #25 has been deposited for future reference at the department's (FCOF J) Herbarium.

Preparation of Plant Material

Fresh leaves of *P. thonningii* were air-dried at room temperature for twenty one (21) days, macerated and pulverized into powdery form using the blender and then sieved.

Aqueous/Ethanol Extraction

Three hundred (300) g of powdered *P. thonningii,* leaves were dissolved into 1200mls of distilled water for 24 hours in a refrigerator. Thereafter, it was filtered with muslin cloth and filtered using Whatman filter No1.The filtrate was evaporated to dryness to obtain the slurry and was used to calculate the percentage yield.

Methods

Serum testosterone was estimated using Rapid lab kit (United Kingdom) for the enzyme linked immunosorbent assay (ELISA) while serum FSH, LH was estimated using Dialab Kit (Austria) for ELISA for quantitative determination of FSH in the serum[10,11].

RESULTS

The ethanol extract of *P.thonningii* shows a significant ($P<0.05$) dosage increase following extract for serum FSH, LH and serum TH, at 50, 100, and 200 mg/kg body weight when compared with the control (Fig 1-3).

The phytochemical screening of the *P. thonningii* (for qualitative and quantitative) indicates the presence of carbohydrates, glycosides, cardiac glycosides, saponins, steroids, triterpene, tannins, flavonoids and anthraquinones (Table 1 and 2).

TABLE 1: Qualitative Phytochemical screening of aqueous and ethanol extract of *P. thonningii*

S/N	Constituents	Test	Aqueous Extract	Ethanol Extract
1	Carbohydrates	Molisch Test	+	+
2	Glycosides	Fehlings Test	+	+
3	Anthraquinones	Bontragers Test	+	+
4	Cardiac glycosides	KelleKillani Test	+	+
5	Saponins	Frothing Test	+	+
6	Steroids	LibermanBuchard	+	+
7	Triterpene	LibermanBuchard	+	+
8	Tannins	Ferric Chloride Test	+	+
9	Flavonoids	Schinoda Test	+	+
10	Alkaloids	Dragendorff and Meyers Test	+	+

KEY

+ - Present

- - Absent

Table 2: Quantitative phytochemical screening of aqueous and ethanol extract of *P. thonningii*

S/N	Sample	Aqueous extract(mg/ 100ml)	Ethanol extract (mg/100ml)
1	Alkaloid	2.1	2.4
2	Flavonoids	2.3	2.2
3	Saponin	3.2	3.4
4	Tannin	1.2	1.5
5	Phenol	1.7	2.4

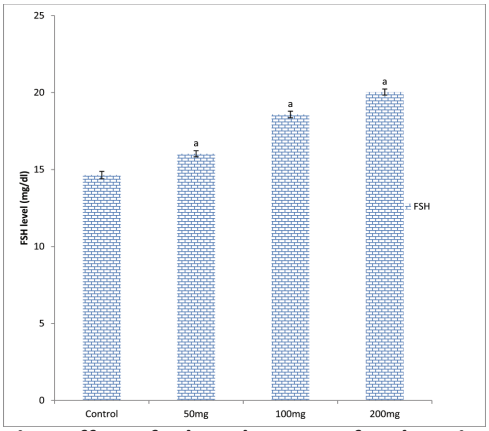


Fig-1.Effect of Ethanol extract of *P. thonningii* on serum FSH of Wistar rats.

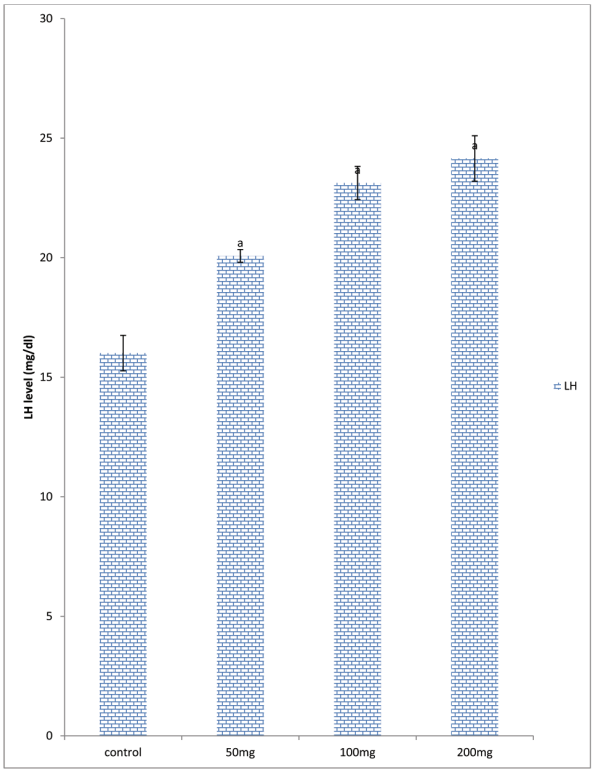


Fig-2. Effect of Ethanol extract of *P.thonningii* on serum LH of Wister rats.

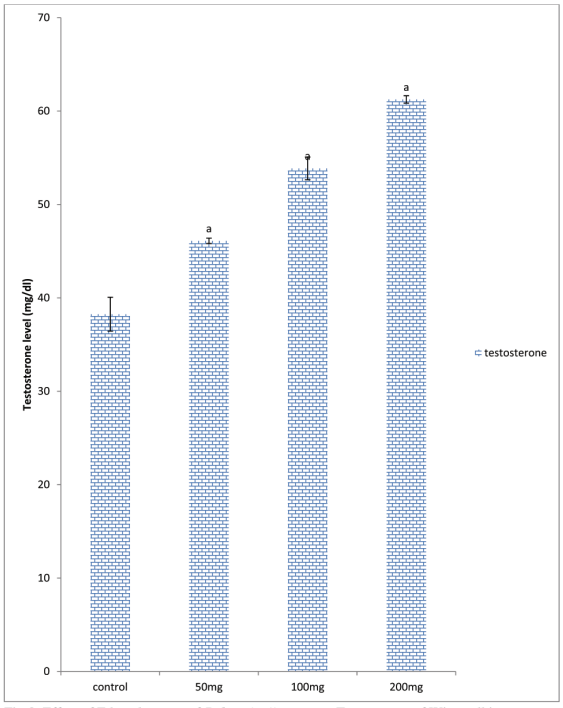
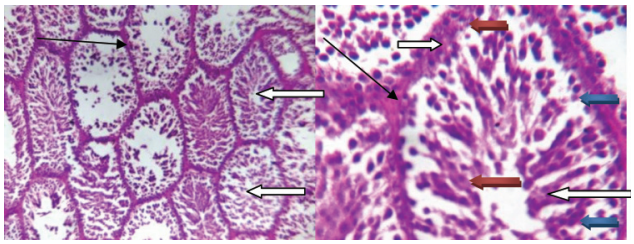


Fig-3. Effect of Ethanol extract of *P.thonningii* on serum Testosterone of Wister albino rats.

Fig-3. Effect of Ethanol extract of *P.thonningii* on serum Testosterone of Wister rats.

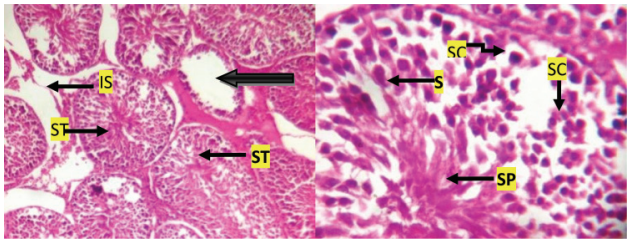
Testis(Control)



X100 x400

Photomicrographs of testicular sections showing normal seminiferous tubules containing normal maturing germinal cells; the spermatogonia cells (blue arrow)and sertoli cells (red arrow) appear normal, the lumen also appear normal (white arrow), the interstitial spaces and interstitial cells appear normal(slender arrow).H&E

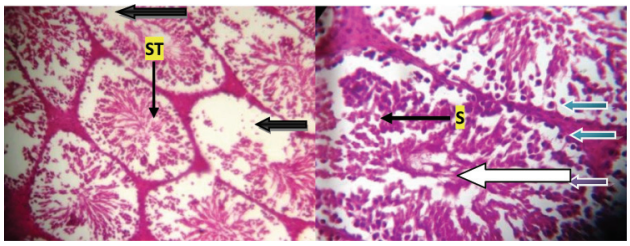
Testis (50mg/kg bwt)



X100 x400

Photomicrographs of testicular sections showing several normal seminiferous tubules **(ST)** containing normal maturing germinal sperm cells **(SP)**; the spermatogonia cells **(S)** and sertoli cells **(SC)** appear normal. However, there are few seminiferous tubules with loss of germ cell layer (black arrow).The interstitial spaces **(IS)** appear normal.H&E

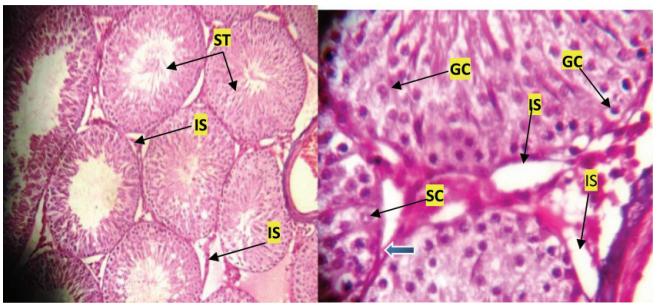
Testis (100mg/kgbwt)



X100 x400

Photomicrographs of sections of a testis showing artefarctual sloughed germ cells, several normal seminiferous tubules (ST) containing normal maturing germinal cells are seen; sertoli cells (S) appear normal. However, there are few seminiferous tubules (ST) with loss of germ cell layer (black arrow). H&E

TESTIS (200mg/kgbwt)



X100 x400

Photomicrographs of sections of the testis showing normal seminiferous tubules (ST) containing normal maturing germinal cells (GC); sertoli cells (SC) appear normal. The interstitial spaces (IS) appear normal.H&E

DISCUSSION

The use of plant extracts as fertility enhancer in animals is now on the increase because of the shifting attention from synthetic drugs to natural plant products [12]. Hormones play a vital role in semen production and men's fertility [13]. Due to chemical agents contained in plant extracts. Phytochemical screening has revealed many bioactive agents of plant extracts that can affect reproduction [14,15]. Alkaloids and flavonoids have been shown to alter plasma concentrations of some fertility hormones [13, 16]. Therefore, the presence of these phytochemicals may account for the alterations in the levels of the circulating hormones observed in this study. Testosterone is a male hormone has significant impact on spermatogenesis [17]. It is secreted by the leydig cells of the testicles, the adrenals and the ovaries, and is the most important androgen secreted into the blood[18, 19, 20].

A low sperm count may indicate a problem with testosterone levels. In this study, the significant increase in serum testosterone could be as a result of some potent phytoandrogen in the ethanol extract of *P. thonningii* that stimulates the synthesis and subsequent release of this hormone in the anterior pituitary glands or by increasing androgen hormones may regulates and/or controls testosterone secretion via feedback effects or affects hypothalamus and controls lutein releasing hormone and partly follicle stimulating hormone via negative feedback mechanism.

More so, Follicle stimulating hormone regulates the growth of seminiferous tubules and maintenance of spermatogenesis in males. It is also critical for sperm production and may support the function of sertoli cells, which in turn enhances sperm cell maturation[20]. Therefore, the significance increase in FSH following the administration of the extract may indicate that the extract stimulates or increases cAMP that might lead to the secretion of ABP (androgen binding protein) which might bind to the sertoli cell to enhance spermatogenesis.

Though, diminished secretion of LH or FSH can result in failure of gonadal function (hypogonadism). This condition is typically manifest in males as failure in production of normal numbers of sperm. In the male, LH acts upon the Leydig cells of the testis and is responsible for the production of testosterone, an androgen that exerts both endocrine activity and intra-testicular activity on spermatogenesis [21,22]. The result shows that the ethanol extract of *P. thonningii* boosted the reproductive hormones levels in the experimental animals in this present study.

CONCLUSION

From all observation shown in the result above, it will be very logical to conclude that the ethanol leaf extract of *P. thonningii* improves sexual health by enhancing spermatogenesis possibly due to the presence of the bioactive constituent or phyto androgens present in the plant extract in a mechanism that is not yet fully unraveled.

REFERENCES

1. Badami S, Desai V B, Suresh B. Drugs play a major role in male fertility. Express Pharma Pulse 2000; 2: 18-25.
2. Ariba A.J,Oladopo O.T,Iyaniwura C.A,Dada O.A. Management of erectile dysfunction:perception and practices of Nigerian primary care clinicians .S.A Fam.Pract.2007; 49(9):16-29.
3. Nooshin Noktedan, Mehrdad Modaresi, Akbar Karimi and Aliasghar Pilevarian. The effects of hydro alcoholic extract of Boswelliacarteri on reproductive hormones in male mice. *Journal of Chemical and Pharmaceutical Research* 2016, 8(2):701-704.
4. Kulkarni S K, Reddy D.S. Pharmacotherapy of Male Erectile Dysfunction with Sildenafil. *Ind.J.Pharmacol* 1998; 30: 367-378.
5. Dasofunjo K;AsukA.A;EzugwuH.C;NwodoO.F.C and Olatunji T.L. The Aphrodisiac Effect of *Piliostigma thonningii leaf* on male wistar albino rats.*Journal of Applied Pharmaceutical Science* 2013,3(10):130-135.
6. Balamurugan K., SakthideviG.and.Mohan V.R .Stimulatory effect of the ethanol extract of *Melastomamalabathricum L. (Melastomataceae)* leaf on the reproductive system of male albino rats. *Journal of Applied Pharmaceutical Science* 2013; 3 (02): 160-165.
7. Akomolafe, S.F., Ganiyu, O.A., Akindahunsi, A.J and Akinyemi, O.A. Inhibitory Effect of Aqueous Extract of Moringaoleifera and Newbuoldialaevis Leaves on Ferrous Sulphate and Sodium Nitroprusside Induced Oxidative Stress in Rat's Testes in Vitro. *Open Journal of Medicinal Chemistry* 2012; 2: 119-128.

8. Egba, Simeon I; Sunday, Godwin I. and Anaduaka, EmekaG.The effect of oral administration of aqueous extract of Newbouldialaevis leaves on fertility hormones of male albino rats .*IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)* 2014; 9(3): 61-64.

9. Dasofunjo K, Ipav SS and Ezugwu C.H.Antimicrobial and Bioactive Potential of *Piliostigma thonningii* leaf. Journal of Pure and Applied Sciences 2014; 5 (1):135-141 .

10. Harborne, J.B.Phytochemical methods, a guide to modern techniques of plant analysis. 4th ed1984, Pp.1 – 5

11. Trease GE, Evans WC. A textbook of Pharmacognosy, 13th Edition, BailliereTindall Ltd. London, 1989,pp. 19-21.

12. Dada, A.A. and Ajilore, V.O.. Use of ethanol extracts of Garcinia kola as fertility enhancer in female catfish Clariasgariepinusbroodstock. *Int. J. Fish. andAquacul.,* 2009;1 (1): 005-010.

13. Browning, R., Schrick, F.N., Thompson, F.W. and Wakefield, T. Reproductive hormonal responses to ergotamine and ergonovine in cows during the luteal phase of the estrous cycle. *Journal of Animal Sciences* 1998: 76: 1448-1454.

14. Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. Phytochemical constituents of some Nigeria medicinal plants. *Afr J Biotechnol* 2005; 4: 685-688.

15. Yakubu, M.T., Akanji, M.A. and Oladiji, A.T. Aphrodisiac potentials of the aqueous extract of Fadogiaagrestis (Schweinf.ExHiern) stem in male albino rats. *Asian J Androl* 2005; 7: 399-404.

16. Bianco, F., Basini, G. and Grasselli, F. The plant alkaloid Sanguinarine affects swine granulose cell activity. Reproductive Toxicology 2006; 21: 335-340.

17. Bratoeff E, Cabeza M, Ramirez E, Heuze Y, Flores E, : Recent advances in the chemistry and pharmacological activity of new steroidal antiandrogens and 5 alpha-reductase inhibitors. *Current Medicinal Chemistry* 2005; 12 (8): 927–43.

18. Amanatkar H.R, Chibnall J.T, Seo B.W,Manepalli J.N, Grossberg G.T. Impact of exogenous testosterone on mood: a systematic review and meta-analysis of randomized placebo-controlled trials. *Annals of Clinical Psychiatry* 2014; 26 (1): 19–32.

19. MansourehEmadi,MehrdadModaresi, Akbar Karimi and AliasgharPilevaria De .The effects of frankincense's hydro alcoholic extract on pituitary-gonadal axis in female mice *Journal of Chemical and Pharmaceutical Research* 2016;8(2):697-700.

20. Harding S.M, Velotta J.P . Comparing the relative amount of testosterone required to restore sexual arousal, motivation, and performance in male rats. *Hormones and* Behavior 2011; 59 (5): 666–73.

21. Colao, A., Vitale, G., Di Sarno, A., Spiezia, S., Guerra, E., Ciccarelli, A. and Lombardi, G. Prolactin and prostate hypertrophy: a pilot observational, prospective, case-control study in men with prolactinoma. *Journal of Clinical Endocrinology and Metabolism* 2004; 89: 2770–2775.

22. Nguyen T.V, McCracken J.T, Albaugh M.D, Botteron K.N, Hudziak J.J, Ducharme S. A testosterone-related structural brain phenotype predicts aggressive behavior from childhood to adulthood. *Psycho neuroendocrinology* 2016;63: 109–18.

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