ORIGINAL ARTICLE

Effect of Guava Leaf Extract on Reproductive Hormone Profile in male Wistar rats

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Abstract

Background: Guava plant is a common tropical plant with a long history of traditional usage for a variety of health care needs, including reproductive/fertility need. It is particularly believed to improve erection, treat impotency and sexual dysfunctions in males. This study aims to examine the effect of aqueous guava leaf extract on male reproductive hormone profile in Wistar rats.

Methodology: Fifteen male Wistar rats were randomized into 3 experimental groups of 5 rats each and were administered water, 250mg/kg body weight and 500mg/kg body weight of oral guava leaf extract respectively for 21 days. The rats were weighed before and at the end of treatments. Blood samples were obtained for ELISA based hormonal analysis.

Results: The aqueous leave extract of Psidium guajava significantly ((p<0.05) increased serum FSH concentrations (122.50±10.40μ/ml and 135.50±5.44μ/ml) at dose dependent manner relative to the control value of 107.50±6.45μ/ml. Similarly, serum testosterone concentration showed significant increases at 2.93±0.07ng/ml and 4.71±0.27ng/ml in response to treatment doses of 250mg/kg and 500mg/kg body weights respectively to the control value 0.96±0.02 ng/ml.

Conclusion: The administration of varied doses of the guava leaf extracts caused a graded increase in the studied parameters of FSH, LH and testosterone (especially for testosterone and FSH), implying that Guava leaf extract has a stimulatory effect on the male reproductive hormones and potentially boosting male fertility.

Key words: Psidium guajava, Gonadotropins, spermatogenesis, male fertility

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Introduction

The incidence of infertility in a population has important demographic and health implications. The prevalence of infertility varies widely, being less in developed countries and more in developing countries where limited resources for investigation and treatment are available.[1] Infertility is an important medical and social problem in the world as 15% of couples are infertile. Forty percent of infertility is attributable to male factor alone, while the remaining 60% is shared between factors attributed to female only, both couples and unknown causes.[2] The reproductive system is regulated by hormones in both male and female sexes. Little wonder that hormonal disturbances account for infertility in approximately 15% of married couples, with male hormonal disturbances accounting for 50% of this group of couple.[3] Measurement of serum sex hormones profile is therefore very useful in assessing the reproductive integrity in males and females, both in animals as well as humans. The suppression of all or any of the reproductive hormones may be a pointer to the existence of deranged reproductive functions.

In the male, the principal function of the testes (spermatogenesis) is regulated by male reproductive hormones. The pituitary gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)) are produced by the gonadotrophs in the anterior pituitary gland while the androgen (testosterone) is produced by the Leydig cells (LCs) and locally sequestered by the Sertoli cells (Scs) all in the testis. Hormonal control of spermatogenesis varies among species characterized by different cycle lengths.[4] In humans, initiation of spermatogenesis occurs at puberty due to the activities of hypothalamic gonadotropin releasing hormone (GnRH), pituitary gonadotropins (Gns), testicular androgen (testosterone), SCs and LCs. Studies from rodent models suggest that both FSH and LH (gonadotropins) support the process of spermatogenesis by suppressing the proapoptotic signals and thus promoting spermatogenic cell survival; the SCs express receptors for FSH and mediate parts of spermatogenesis through production of estradiol, inhibin and anitimullerian factor, as well as providing nourishment and general support to the maturing sperm cells.[5] LCs, on the other hand, expresses functional LH receptorsand are responsible for the steroidogenic synthesis of testicular testosterone.[2,6]

Psidium guajava, guava, is a member of the Myrtaceae family, is a common tropical plant with a long history of traditional usage. It is used not only as food but also as folk medicine, and various parts of this plant have a number of medicinal properties.[7] It has about 133 genera and more than 3,800 species. *P. guajava* is a large tropical evergreen shrub or small shade tree that grows

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up to10 - 15 m in height. It is native to and widely distributed in Mexico and Central America. However, the plant is cultivated today from the west coast of Africa to the Pacific region, including India and China, with varieties originally introduced over the past 300years from the United States. The guava berry is an important tropical fruit that is mostly consumed fresh.

Guava (*Psidium guajava*) has been extensively studied in terms of the pharmacological activity of its major components. Phytochemical analyses revealed that more than 20compounds can be isolated from guava leaf products, including alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, lectins, Phenols, saponins, tannins, triterpenes, and about 80mg of Vitamin C (Ascorbic Acid) per 100g of guava.[8] The essential compounds contain alpha pinene, caryophyllene, cineol, D-limonene, eugenol, and myrcene. The major constituents of the volatile acids include (E)-cinnamic acid and (Z)-3-hexenoic acid. Carbohydrate esters have also been isolated from the fruit, however, the main active constituent been quercetin.[9] The spasmolytic and anti-diarrhoea effects are reported to be associated with its quercetin-derived flavonoid glycosides, which support use of this ancient leaf remedy in treating gastrointestinal disorders.[10] In guava leave essential oil, compounds present include α-pinene, Limonene, β-Pinene, Isopropyl alcohol, Menthol, Terpenylacetate, Caryophyllene, Longicyclene, β-Bisabolene as well as Oleanolic acid.[11] Quantitatively, the leaves have high content of limonene (about 42.1%) and caryophyllene (about 21.3%).[12]

Guava leaf extract usage as an anti infective agent[13,14] is largely due to the concentrations of condensed tannins (catechin)[15] present in it. Furthermore, its activity against bacterial and fungal pathogens was traced to betulinic acid and lupeol.[16] Reported haematological activities of guava leave extract include anticancer effect,[17]anti anaemic effect[10] and anti inflammatoryeffect[15] in animal studies.

The leaf decoction has long been consumed as remedy for throat and chest ailments/pulmonary diseases, anti-inflammatory and haemostatic agent. Similarly, It has been used as an emmenagogue and treatment of leucorrhea.[10] In folk medicine, especially in some traditional African settings, decoctions from guava leaves are used as traditional medicine against diabetes, malaria, in addition to its usage as tonic to treat digestive conditions and gastrointestinal disorders. Crushed leaves are applied on wounds, ulcers and rheumatic spots. The leaves are also chewed or gargled to relieve oral ulcer, toothache and inflammed gums.[8]

Guava plant is one of the major herbs that over 80% of the populations in some Asian and African countries depend on for a variety of health care needs, including reproductive/fertility need,[3] as guava leaf extracts are particularly believed to improve erection, treat impotency and sexual dysfunctions in males.[10] Toxicity studies of guava (*Psidium guajava*) in mice and other animal models as well as controlled human studies have shown that guava fruit, leaf and root are safe and without side effects,[18] hence its widespread use in many African countries and in Nigeria particularly.

This study, therefore, was designed to investigate if guava leaf extract has any effect or not on male reproductive hormones of the pituitary or testicular origin selectively or combined.

Materials and Methods

Guava leaves were obtained from a botanical garden in Makurdi, Benue state. They were authenticated by a taxonomist and a voucher specimen deposited with a number at the Botany Department, Benue State University, Makurdi.

The guava leaves were sorted to eliminate any dead matter and other unwanted particles. The leaves were air-dried for 2 weeks and then grounded into fine powder using an electric dry mill. 200g of the grounded powder was soaked in 1.01 of distilled water for 48hours at room temperature. The mixture was filtered into 500ml conical flask with Whatman filter paper (No.1). The filtrate was subjected to water extraction technique at 60^0c to obtain the guava leaf extract paste. A stock concentration of 100mg/ml was made, from which the appropriate doses of 250mg/ml and 500mg/ml were derived.

A total of fifteen (15) male Wistar albino rats, weighing between 150-300g, procured from the disease-free stock of the animal house of College of Health Sciences, Benue State University, Makurdi, were acclimatized for one week before the commencement of treatment. They were exposed to free water ad libitum, growers chore, and maintained at average room temperature and relative humidity of 29^0C and 38% respectively. The animals were handled in accordance with the standard proctocol.[19] The 15 rats were then randomly assigned to 3 experimental groups 1, 2 and 3 (group n = 5). Group 1 served as Control while groups 2 and 3 were administered guava leaf extract treatments based on the following proctocols:

Group 1: Received 1mL of distilled water only
Group 2: Received 250mg/kg bw of *P.guajava extract*
Group 3: Received 500mg/kg bw of *P.guajava extract*

All treatments were administered orally daily for a duration of 21 days.

At the end of the treatment period, the rats were fasted overnight and weighed on the morning of specimen extraction. Using cardiac puncture, 5mL of blood was obtained from each animal under chloroform

anesthesia into a into plain (non EDTA) bottles while ensuring an aseptic environment. The specimen was allowed to stand and coagulate for 30 minutes before centrifuging at 800g for 5minutes. The supernatant was collected into a clean plain bottle and used for hormonal analysis.

Hormonal assay for testosterone, FSH, LH were performed using the Finecare(R) rapid test kits for each hormone based on the immunoflorescence assay technology and using the ELISA micro wells and micro plate assay principle respectively. The assay was read using Statfax-2100 micro plate reader.

Data obtained were entered into Microsoft office excel spreadsheet version 2019, expressed as Mean ± SEM, and analyzed using SPSS Version 25.0 (2019). One-way analysis of variance (ANOVA) was employed to test the existence of any significant differences between and within group means. Student T-test was used to determine levels of significant variation in means at probability level of 0.05. Where significant differences exist among means, Turkey Post Hoc test was thereafter applied to the significant mean differences to define specific group with mean differences.

Results

After administering guava leaf extract to the 15 Wistar rats, the body weight were measured and sex hormones (FSH, LH and testosterone) were assayed and presented in tabular form.

Table1: Effects of Treatment on mean body weights (g) of male Wistar rats

	Weeks			
Groups	1	2	3	Grand Mean
1	160.40±6.79	132.60±34.21	140.40±36.31	144.47±25.77
2	288.40±25.81	266.00±19.93	184.80±47.11	246.40±30.98
3	250.00±21.49	222.40±6.67	168.40±42.41	213.60±23.52

NS=No Significant difference at (p>0.05)

From the Table 1, it was observed that there were progressive decreases in the body weight from the beginning to the end of treatment in each group. However, these body weight changes were not statistically significant, indicating that guava leaf extract has no significant effect on total body weight.

Effect of guava leaf extract on sex hormones of male rats showed significant difference ($p<0.05$) in the mean of selected hormones in the various groups.

In table 2 Figure 1, it was observed that serum FSH increased significantly ($p<0.05$) with guava leaf extract treatment at doses of 250mg/kg and 500mg/kg bwat concentrations of 122.50±10.40µ/ml and 135.50 ±5.44µ/ml respectively when compared to that of Control group concentration of 107.50 ±6.45µ/ ml. This observed effect is dose dependent. This indicates that guava leaf extract increases serum FSH concentration.

Table 2: Effect of guava leaf extract on testosterone concentration in male Wistar rats

Treatment	Mean FSH(µ/ml)	Mean LH(µ/ml)	Mean Testosterone (ng/ml)
Control	107.50±6.45[a]	3.23±0.04	0.96±0.02[a]
250mg/kg	122.50±10.40[b]	0.98±0.01	2.93±0.07[b]
500mg/kg	135.50±5.44[c]	0.99±0.01	4.71±0.27[c]

Means tagged with different alphabet under the same column are significantly different ($p<0.05$)

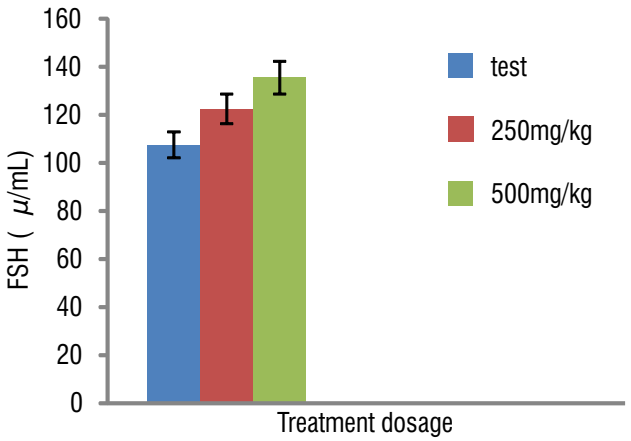


Figure 1: Effect of guava leaf extract on FSH of male rats

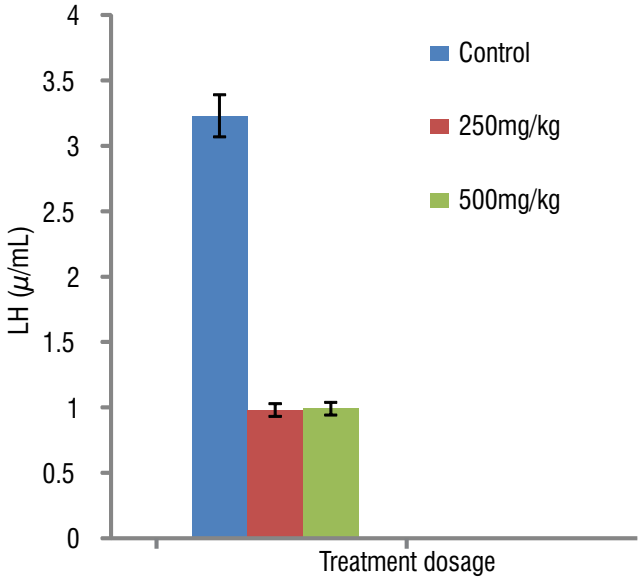


Figure 2: Effect of guava leaf extract on LH of male rats

In table 2 Figure 2, guava leaf extract treatments induced a significant reduction ($p<0.05$) in serum LH

concentration in the treatment groups compared to the control value. However, there was no significant difference in mean serum LH concentration with the different treatment doses. This indicates that the treatment potentially suppresses the secretion of LH in serum and by implication, reduces leutinization in the LH dependent tissues.

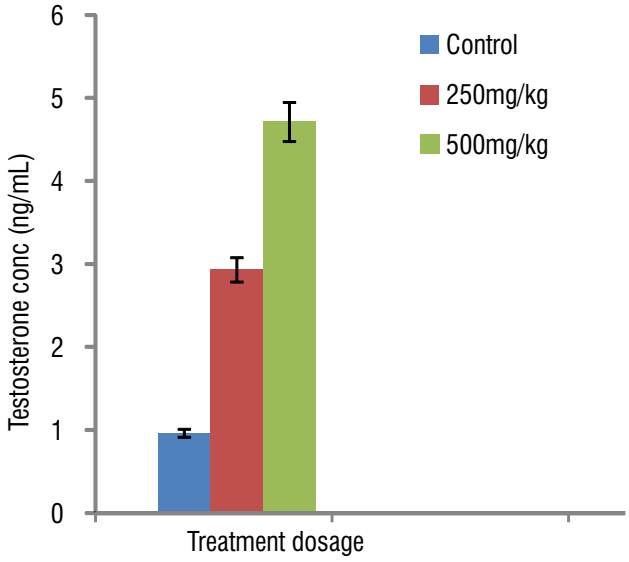


Figure 3: Effect of guava leaf extract on Testosterone of male rats

In table 2 Figure 3, it was observed that serum testosterone concentration showed significant increase ($p<0.05$) at both 250mg/kg (2.93 ± 0.07 ng/ml) and 500mg/kg (4.71 ±0.27 ng/ml) when compared to the control value (0.96 ± 0.02 ng/ml). Highest effect was observed at 500mg/kg compared to 250mg/kg. This indicates that guava leaf extract increases serum testosterone concentration on dose dependent basis.

Discussion

In this study, it was observed that there was no significant change in the body weight of the rats. This finding differs from findings of similar studies performed on male Wistar rats that utilized guava leaves essential oil,[20] ethanolicextracts of Xylopia aethiopica fruit[21] as well as essential oil of Syzygium aromaticum[22] where the authors observed significant testicular, epididymal, and seminal vesicular weight gains among the study animals with increasing doses of treatments apparently due to the anabolic action of androgens, especially testosterone since these studies observed a stimulatory effect of these guava leaf effects on testosterone secretion. It is thought that the increase in weight of these structures eventually added to the overall body weight of the animals. The relative insignificant body weight changes observed in this study in the male Wistar in response to the treatment relative to the Control maybe due to the nature of guava leave extract used as the essential oil appears to be a better weight gain influencer than the water extract used.[23]

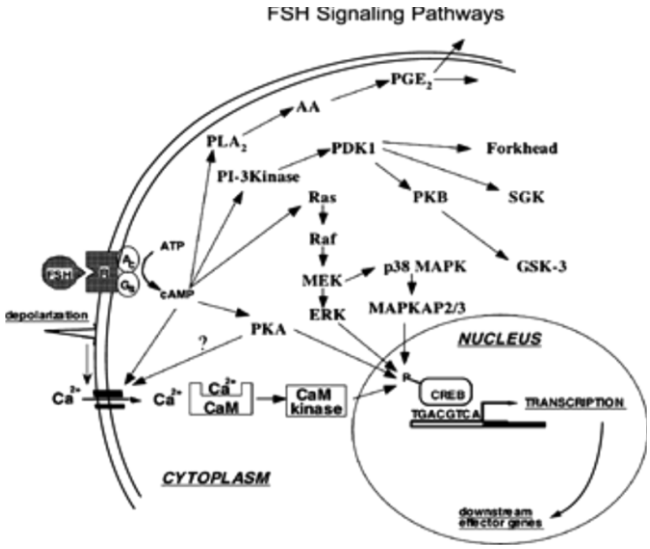
This study equally showed a dose dependent increases in serum FSH, LH and testosterone concentrations in response to treatment with guava leaf extract. Similar findings have been documented where guava leaf extract was noted to upregulate the serum Gn concentration.[24] The aqueous extract of guava leaf has been shown to have a higher potency in enhancing the release of LH, FSH and testosterone into the blood stream; a function attributed to its effect on the accessory sex organs and positive influence on the gonadotropin releasing hormones (GnRH) in stimulating the anterior pituitary to produce the gonadotropin hormones (FSH and LH/ICSH). FSH and LH stimulate the Leydig cells in the testes to synthesize testosterone. These chain of hormonal activities positively impacts on spermatogenesis in the testes. These actions of *P.guajava* may be attributed to stimulatory effect of some of its phytochemical agents on the reproductive function in male rat models. Guava leave is known to be reach in electrolytes, ascorbic acid, carbohydrate, phenols and supfates[25] among 50 other compounds,[26] among which caryophyllene was found to be predominantly present. Caryophyllene possesses antioxidant, anticancer, anti-inflammatory, and antimicrobial properties; accounting for why guava leave extract exerts positive influence on reproductive hormonal functions. However, and unlike the findings of this study, a 2010 study reported that guava leaf extract induced increase testosterone concentration, but caused no change in the level of LH and FSH in rats.[10]

The physiology spermatogenesis show the number of Sertoli and spermatogenic cells up to pathytene spermatocytes increased in parallel with the circulating blood levels of FSH up to days 33 or 35 in the mice. In animal model treated with a GnRH antagonist to suppress pituitary gonadotropin production, the administration of FSH alone was sufficient to maintain spermatogenesis, at least in part.[27] However, the absolute necessity of FSH in spermatogenesis is in doubt. The authors observed in their study carried out on normal men, that normal levels of FSH are not required for the maintenance of qualitatively normal spermatogenesis, but for the maintenance of quantitatively normal spermatogenesis.[28]

An equally significant activity of FSH is the prevention of apoptosis of type A spermatogonia, thereby increasing the production of spermatozoa. Studies from rodent models suggest that both LH and FSH support the process of spermatogenesis by suppressing the proapoptotic signals and therefore promoting spermatogenic cell survival.[29]Further insight to the activities of FSH suggest that FSH may play a role

in stimulating mitotic and meiotic DNA synthesis in type B spermatogonia and preleptotene spermatocytes as well as preventing apoptosis of pachytene spermatocytes and round spermatids.[30] In the rat, the role of FSH is more difficult to define, but this hormone may modulate the number of germ cells proceeding successfully through the mitotic and meiotic phases of spermatogenesis. In a study in which the FSH p-subunit gene was knocked out in mice, rendering them FSH deficient, spermatogenesis proceeded to completion, albeit in the presence of smaller testes, with the mice noted to be fertile,[27] further lending support to the fact that FSH is not an absolute necessity for qualitative spermatogenesis.

The actions of FSH are mediated via binding to the FSH receptor (FSHR), a G-coupled transmembrane receptor on the surface of Sertoli cells that is capable of activating numerous signalling pathways. Five (5) Classical Signalling Pathways of cAMP-PKA, MAP kinase, Calcium (Ca^{2+}), Phosphatidylinositol 3-kinase (PI3-K) and Phospholipase A_2 (PLA_2) pathways have been identified (outlined in the schema below) induced by binding of FSH to Sertoli cells.[31]



Signaling pathways activated by FSH
(Adapted from Reproduction journal, July 1, 2005 vol. 130no. 1 15-28).

Initially FSH binding to the FSH receptor causes receptor coupled G proteins to activate adenylate cyclase (AC) and increase intracellular cAMP levels. Multiple factors can be activated by cAMP in Sertoli cells including PKA that can phosphorylate a number of proteins in the cell and also regulate the expression and activity of numerous transcription factors including CREB. FSH also causes Ca^{2+} influx into Sertoli cells that is mediated by cAMP and perhaps PKA modification of surface Ca^{2+} channels. Depolarization of the cell is also involved in Ca^{2+} influx. Elevated Ca^{2+} levels can activate calmodulin and CaM kinases that have multiple potential downstream effects including the phosphorylation of CREB. During puberty, FSH activates the MAP kinase cascade and ERK kinase in Sertoli cells most likely via cAMP interactions with guanine nucleotide exchange factors (GEFs) and activation of Ras-like G proteins. ERK is capable of activating transcription factors including SRF, c-jun and CREB. In granulosa cells, FSH also activates the p38 MAP kinase. FSH and cAMP also likely act through GEFs to activate PI3-K and then phosphoinositol dependant protein kinase (PDK1) and PKB in Sertoli cells. Studies of granulosa cells identified Forkhead transcription factor (Forkhead), SGK (glucocorticoid-induced kinase) and GSK-3 (glycogen synthase kinase-3) as additional downstream targets of the PI3-K pathway. FSH also mediates the induction of PLA2 and the subsequent release of arachadonic acid.

Even though the study was well designed to satisfy the overall aim, it was limited in scope owing to brevity of time.

The outcome of this study therefore, has shown that exposure to oral aqueous guava leaf extract caused a dose dependent increases in the gonadotropins (especially FSH) as well as testosterone, with insignificant effects on body weight. This implies that guava leaf extract potentially enhance spermatogenesis, thereby improving male fertility in the Wistar rats. However, it is recommended that this study be replicated both on rats and on higher animals in order to isolate the exact phytochemical agent in guava leaf extract responsible for this reproductive effects and to determine the suitability of the extract as remedy for hypofertility in the males.

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Conflict of Interest: None declared

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