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Comparative Effect of Aqueous Extract of *P. Guajava* Leaves and Ascorbic Acid on Serum Sex Hormones Levels in Male and Female Rats

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Abstract: *Psidium guajava* has a rich ethnomedicinal history, particularly in the treatment of various gastrointestinal disorders. In this study, 200mg/kg body weight of aqueous extract of *P. guajava* leaves(AEPGL) and ascorbic acid(vitC) were administered orally to male and female rats daily, for 30 days, to assess their respective effect on the male and female reproductive functions, using serum levels of follicle stimulating hormone(FSH), luteinizing hormone(LH), estradiol, progesterone and testosterone. The results of this study showed that treatment of rats with AEPGL and vitC, respectively, had insignificant effect ($P \geq 0.05$) on the serum levels of FSH and LH in male and female rats, and a significant increase ($P \leq 0.05$) in the serum estradiol and progesterone in females, and testosterone in males. AEPGL increased serum estradiol and progesterone levels by 17.6 and 34.6 percents, respectively, in females, and testosterone by 38.6 percent in males, while vitC increased serum estradiol and progesterone levels by 15.2 and 30.0 percents, respectively, in females, and testosterone by 28.0 percent in males rats. From these results, it was observed that the effect of AEPGL on the levels of these hormones was higher than that of vitC in male and female rats, and that the percentage increase in male sex hormones was significantly higher than the percentage increase in female sex hormones. These observations indicated that AEPGL possess a higher potency in stimulating the secretion of sex hormones than vitC, and that the effect is more significant in male than female rats; hence, AEPGL may be recommended for males with various reproductive dysfunctions.

Key words: *P. guajava*, ascorbic acid, sex hormones.

INTRODUCTION

Psidium guajava, guava, is a member of the Myrtaceae family, with about 133 genera and more than 3,800 species. *P. guajava* is a large tropical evergreen shrub or small shade tree that grows up to 10 - 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 300 years from the United States. Generally, guava plant has spread widely throughout the tropics because it thrives in a variety of soils, propagates easily, and bears fruit relatively quickly. The tree is easily identified by its distinctive thin, smooth, copper-colored bark that flakes off, showing a greenish layer beneath. The guava berry is an important tropical fruit that is mostly consumed fresh. The fruit contains several small seeds and consists of a fleshy pericarp and seed cavity with pulp [1-3]. The guava fruits are either eaten fresh, or made into drinks, ice cream, and preserves. Guava fruit is still enjoyed as a sweet treat by indigenous peoples throughout the rainforest, and the leaves and bark of the guava tree have a long history of medicinal uses that are still employed today.

Photochemical analyses revealed that more than 20 compounds can be isolated from guava leaf products, including alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, lectins, phenols, saponins, tannins, triterpenes, and about 80 mg of vitamin C (ascorbic acid) per 100 g of guava[4-10]. The essential oil contains 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[5,8]. However, the main active constituent in the plant is reported to be quercetin. The spasmolytic and antidiarrheal effects are reported to be associated with its quercetin-derived flavonoid glycosides, which support use of this ancient leaf remedy in treating gastrointestinal disorders[3].

Scientific investigations on the medicinal properties of guava leaf products date back to the 1940s. Some

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investigations examined antiamebic, antibiotic, antidiarrheic, antihyperglycemic, antimutagenic, antispasmodic, and sedative effects, as well as anticough and narcotic-like activities of the plant. However, most scientific evidence relates to the clinical efficacy of guava in treating gastrointestinal disorders[3,5]. The young leaves of the plant have been used as a tonic to treat digestive conditions such as cholera and diarrhea in Brazil and Mexico. Current Mexican medicinal data document the treatment of acute diarrhea, flatulence, and gastric pain by using a guava leaf water decoction for oral administration 3 times daily. A decoction of young leaves and shoots is prescribed as a febrifuge and a spasmolytic. In Bolivia and Egypt, guava leaves are used to treat cough and pulmonary diseases. Young guava leaves are used as an anti-inflammatory and hemostatic agent, as well as an agent for the treatment of cough in Bolivia, Egypt, India, and China[2,4,5,11,12]. Also, aqueous extracts from *P. guajava* is reported to have antioxidant or radical-scavenging activity. Most of the activity is associated with the polyphenols; however, the guava extracts also contain antioxidants such as ascorbic acid and carotenoids[2,13,14].

Several plant extracts are known to possess stimulatory activity on the reproductive functions. For instance, Icariin ($C_{33}H_{40}O_{15}$, molecular weight: 676.67), a flavonoid isolated from the plant *Herba epimedii*[15,16], has been used as a tonic drug in traditional Chinese medicine (TCM) for centuries to improve erectile function[17,18]. Also, it increases the weights of the pituitary gland, testis and epididymis and the testosterone concentration in immature male rats, and has testosterone mimetic properties[19-21]. In folk medicine, especially in some traditional African localities, decoctions from *P. guajava* leaves are commonly employed to treat a wide range of reproductive disorders. In the practice of local herbal medicine, *P. guajava* leaf extracts are particularly believed to improve erection and impotency in males. However, there is paucity of documented evidence to support this practice.

Sex hormones are known to regulate the reproductive functions and characteristics in both male and female organisms[22,23]. Measurement of serum sex hormones profile is therefore very useful in assessing the reproductive integrity in both animals and humans. According to Jenner *et al*.[24], serum estradiol measurement is a valuable index in evaluating a variety of menstrual dysfunctions in females. This indicates that suppression of reproductive functions may evidenced in reduced serum sex hormone profile and vice versa. This study assessed the effect of *P. guajava* leaf extracts and vitamin C on serum sex hormonal profile in male and female rats, with the aim of investigating the vitamin C- property, and the validity or otherwise of the use of the leaf extract in reproductive disorders in folk medicine.

MATERIALS AND METHODS

Identification and Preparation of Plant Materials: Fresh leaves of *P. guajava* were collected in May 2009 from local garden at the University of Calabar, Calabar, Nigeria. The sample of the plant specimen was identified and authenticated by a Botanist from the botanical garden, and the Voucher specimen was deposited in the herbarium of the same University.The leaves were sorted to eliminate any dead matter and other unwanted particles. The leaves were air-dried for 2 weeks and then ground into fine powder using an electric dry mill (Moulinex). 200g of the ground powder was soaked in 1.0l of distilled water for 48 hours at room temperature. The mixture was filtered into 500ml conical flask with Watman filter paper (No.1). The filtrate was dried at a temperature of 30ºC for 10 hours to produce a gel-like extract, which weighed 20.5g. Appropriate concentration of the extract was then subsequently made by dilution with distilled water into 200/mg/kg body weight and administered to the animals.

Handling and Treatment of Animals: A total of 36 adult albino rats (18 males and 18 females) weighing between 150-300g obtained from the disease free stock of the animal house, Biochemistry Department, College of Medical Sciences University of Calabar, Calabar Nigeria, were used for the study. The rats were divided according to sex into six groups with six rats each, as follows:

Group I. Mc (Male control group receiving distilled water as placebo),
Group I. Fc (Female control group receiving distilled water as placebo),
Group II. MvitC (Male test group receiving vitamin C), Group II.FvitC (Female test group receiving vitamin C),
Group III. MPg (Male test group receiving aqueous extract of *P. guajava* leaves),
Group III. FPg (Female test group receiving aqueous extract of *P. guajava* leaves).

The rats were acclimatized in the experimental animal house for one week before the commencement of the experiment. The animals, housed in stainless steel cages under standard conditions (ambient temperature, 28.0±2.0º C and humidity,46%, with a 12 hr light/dark cycle), were fed with the normal rat pellets. All the rats in both test and control groups

were allowed free access to food and water *ad libitum*, throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feed from cages daily.

The animals in test groups II.MvitC, II.FvitC and III.MPg, III.FPg received 200/mg/kg body weight oral daily doses of vitamin C and aqueous extract of *P. guajava* leaves, respectively, using orogastric tubes and syringes. This lasted for a period of 30 days and the experiments were conducted between the hours of 09.00 am and 10.00am daily. Rats in the control groups I.Mc, I.Fc were administered, by oral gavage, with 5ml of distilled water (placebo).

Synthetic vitamin C was obtained from the Sigma Chemicals, Poole England and used for the study. A stock solution of vitamin C was prepared by dissolving 20g of vitamin C powder in 500ml of distilled water out of which a dose of 200mg/kg body weight was administered to animals in 5ml of vehicle daily for 30 days. All the animal experiments were carried out in accordance with the guidelines of the Institution's Animal Ethical Committee.

Collection and Analysis of Blood: All the animals were anaesthetized with chloroform vapour, twenty-four hours after last day of extract and vitamin C administration, and dissected for blood collection. Blood samples were collected by cardiac puncture into a set of plain sample bottles, and allowed to clot. The clotted blood samples were spun in a bench top centrifuge (MSE, England) to obtain sera. The serum samples were separated into another set of plain sample tubes. The separated serum samples were stored in the refrigerator until required for the hormonal assay. All assays were done within 24 hours of the sample collection. The serum samples were assayed for FSH, LH, estradiol, progesterone and testosterone using enzymes immunoassay methods. The respective immunoassay reagent kits were obtained from Diagnostic Automation Inc., 23961 Craftman Road, Suite E/E, Calabasas, CA 91302. Microplate reader (Dialab Instruments Ltd.) was used in taking the absorbance. Calculations of the concentrations of hormones were made according to the method given in the kits handbook.

Statistical Analyses: The results obtained from this study were analyzed by one-way analysis of variance (ANOVA), followed by Student's t-test to evaluate the significance of the difference between the mean value of the measured parameters in the respective test and control groups. A significant change was considered acceptable at $P < 0.05$.

RESULTS AND DISCUSSION

Results: The results obtained from this present study are summarized in Table 1. From these results, it was discovered that treatment of rats model with aqueous extract of *P. guajava* leaves and vitamin C (chemical test agents), respectively, caused no significant effect ($P \geq 0.05$) on the levels of serum FSH and LH in both male and female rats (Table 1). However, it was also observed that treatment of the animal model with the respective chemical test agents caused a significant increase ($P \leq 0.05$) in the levels of serum estradiol and progesterone, in females, and testosterone in male, when compared respectively with the serum FSH and LH levels obtained for the respective control groups (Table 1).

In this study, it was interesting to noticed that while treatment of rats with the aqueous extract of *P. guajava* leaves increased serum estradiol and progesterone levels by 17.6 and 34.6 percents, respectively, in females, and testosterone by 38.6 percent in males; treatment of the animals with vitamin C increased serum estradiol and progesterone levels only by 15.2 and 30.0 percents, respectively, in females, and testosterone by 28.0 percent in males.

From these observations, it was noted that the effect of the aqueous extract of *P. guajava* leaves on the levels of these sex hormones released into circulation was higher than the effect of vitamin C in both male and female rats model. Moreover, the percentage increase in male serum sex hormones was observed to be significantly higher than the percentage increase in female serum sex hormones following treatment of the animal model with aqueous extract of *P. guajava* leaves and vitamin C. These observations indicated that the aqueous extract of *P. guajava* leaves possess a higher potency in stimulating the secretion of sex hormones than vitamin C, and that the effect seems to be more significant in male than female rats model.

Discussion: Endocrine changes and decline in endocrine function involve tissue responsiveness, reduced secretory output from peripheral glands and alterations in the central mechanism controlling the temporal organization of hormonal release[25]. Hypogonadism is a clinical condition in which low level of serum sex hormones, including testosterone in males, as well as estradiol and progesterone in females, is found in association with specific signs and symptoms. These signs and symptoms may include diminished libido and sense of vitality, erectile dysfunction, dysmenorrhoea, reduced muscle mass and bone density, depression and anemia. By restoring these serum sex hormones level to the normal range using such agents as hormone supplement therapy, many of

Table 1: Serum sex hormones levels of male and female rats treated with aqueous extract of *P. guajava* leaves and vitamin C

Group	FSH(mIU/ml)	LH(mIU/ml)	Progesterone(ng/ml)	Estradiol(ng/ml)	Testosterone(ng/ml)
I.Mc	2.4±0.6	3.8±1.1	-	-	13.2±2.3
II.MvitC	3.0±1.0	4.0±2.3	-	-	16.9±2.4*
III.MPg	2.8±1.3	4.2±2.1	-	-	18.3±3.1*
I.Fc	2.6±0.2	4.3±0.2	28.0±1.6	68.3±2.7	-
II.FvitC	2.9±0.1	4.4±0.2	36.4±1.4*	78.7±1.4*	-
III.FPg	2.6±0.4	4.5±1.1	37.7±1.7*	80.3±2.6*	-

Values are presented as mean ± SEM, n = 6, *P < 0.05 compared with the control. I.Mc = Male control group receiving distilled water as placebo, I.Fc = Female control group receiving distilled water as placebo, II.MvitC = Male test group receiving vitamin C, II.FvitC = Female test group receiving vitamin C, III.MPg = Male test group receiving aqueous extract of *P. guajava* leaves, III.FPg = Female test group receiving aqueous extract of *P. guajava* leaves.

these symptoms can be relieved[26,27]. Reports indicate that testosterone supplement therapy and Icariin, from the plant *Herba epimedii,* might produce a wide range of benefits for men with hypogonadism that include improvement in libido, bone density, muscle mass, body composition, mood, erythropoiesis and cognition[17-21,28,29].

Sex hormones, particularly estradiol and progesterone in females, and testosterone in males, are produced primarily in the gonads under the influence of FSH and LH. The increases in the concentrations of sex hormones are known to exert positive feedback influence at the level of the pituitary gland, where they regulate the secretion of gonadotropins[30,31]. In non-pregnant females with a normal menstrual cycle, the progesterone level remains relatively constant throughout the follicular phase of the menstrual cycle and the increases rapidly following the ovulation, while the estradiol secretion follows a cyclic biphasic pattern, with the highest concentration found immediately prior to ovulation[32-34].

In this study, we found that treatment of male and female rats with *P. guajava* leaf extract and vitamin C increased the circulating level of testosterone in males, as well as estradiol and progesterone in females. However, in the present study, there was no significant difference in serum levels of LH and FSH between the rats in control group and those in the groups treated with *P. guajava* leaf extract and vitamin C, respectively. From the results of this study, it seems that *P. guajava* leaf extract and vitamin C have a positive effect mainly on the gonads, stimulating the secretion of these gonadal hormones into circulation in male and female rats. On the whole, this gonadal hormones secretion stimulating effect was observed to be higher in rats treated with *P. guajava* leaf extract than those treated with vitamin C. Moreover, this effect was observed to be remarkably greater in male than the female rats for both *P. guajava* leaf extract and vitamin C treated groups. The observation made from the results of this study supported the report that *P. guajava* leaf extracts contain vitamin C, in addition to other chemical agents[4-10].

The results of this present study revealed that some of the chemical agent(s) which are contained in *P. guajava* leaf extracts possess stimulatory activity on the reproductive functions in rats model, mainly the males. This assertion hypothesized and documented, in support of the practice of African folk medicine, that *P. guajava* leaf extracts may be strongly recommended to males, and mildly to females with various degrees of reproductive dysfunctions. However, the specific chemical agent(s) responsible for the enhanced effect of *P. guajava* leaf extract over vitamin C and the mechanism(s) of gonadal hormones stimulating effect are subjects for further investigation.

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