

## ENDOCRINE AND ORGANOMETRIC EVALUATION OF *PSIDIUM GUAJAVA* (GUAVA) LEAF EXTRACT IN ALBINO RATS' MODEL WEIGHT LOSS

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### ABSTRACT

Folkloric assertions stipulate that *Psidium guajava* (Guava) leaf extract is effective in weight loss. This research was set up to evaluate the efficacy of *Psidium guajava* leaf (PGL) extract in weight loss as well as investigate its effect in reproductive hormone profile and organ metrics of albino rats. Methods: Sixty albino rats weighing between 100-180 grams were used for the experiment. Twenty eight rats were used for toxicity testing, while the remaining 32 rats were divided into male and female groups of 16 rats each. The 16 males were divided into 4 groups as M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> while the 16 females were also divided into F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> and F<sub>4</sub>. The control (M<sub>1</sub> & F<sub>1</sub>) were administered 2 ml/kg distilled water as control, while the remaining 6 groups were treated with graded doses (200mg/kg, 400mg/kg and 800mg/kg) body weight of PGL extract orally for 30 days. At day 31, Blood was collected through the retro-bulba plexus into plain sample bottles for hormonal assay (Testosterone, Oestrogen & Progesterone). Result: Treatment with PGL extract significantly ( $P \leq 0.05$ ) increased sera testosterone in all the treatment groups while, Sera oestrogen were significantly reduced. Furthermore, Weight loss (least weight gain) was observed in 800 mg/kg PGL extract treatment in males but no weight loss in females. Conclusion: *Psidium guajava* leaf extract can be used by males to improve testosterone secretion and in weight loss but not in females since PGL extract treatment not only reduced sera oestrogen but also did not lead to significant weight loss.

**Keywords:** Guava leaf, Oestrogen, Testosterone, Weight loss

### INTRODUCTION

*Psidium guajava* (Guava) belongs to the order; *Myrtales*, family; *Myrtaceae*, genus; *Psidium* and species *Psidium guajava* (Hussain *et al.*, 2021). Guava is a berry and has high quantities of macro and micro nutrients (Sameena-Begum *et al.*, 2019).

*Psidium guajava* is an important fruit in tropical areas like India, Indonesia, Pakistan, Bangladesh, and South America due to plethora of phytochemicals found in the leaves as well as the fruit which has among other things antioxidant properties (Joseph & Priya, 2011).

Guava is a fast growing evergreen shrub and grows to a height of 3-10 m. It has a shallow root system. The branches are low and drooping. Guava suckers from the roots. The trunk is about 20 cm in diameter. The trunk is covered with smooth green to red brown bark that peels off in thin flakes.

The leaves grow in pairs and are opposite each other. The leaf blade is 5-15 cm long and 3-7 cm broad. It is elliptic to oblong in shape. The flowers are solitary or in clusters and are normally white in colour (Asim *et al.*, 2022).

According to Sharma *et al.* (2023), guava leaf has anti-lipidemic activity. Cholesterol, however is lipophilic and is essential for life. Cholesterol functions as precursor for the synthesis of steroid hormones of which reproductive hormones (Testosterone, Oestrogen, and Progesterone) are key (Huff *et al.*, 2023).

Reproductive hormones are essential for the posterity of animal species. Testosterone is mostly expressed by males while oestrogen and progesterone are mostly expressed by females (Smith & Walker, 2015; Rosner *et al.*, 2024). Heightened testosterone secretion is synonymous with increased spermatogenesis (Nasser & Leslie, 2023) whereas

increased secretion of oestrogen and progesterone is indicative of ovulation and maintenance of pregnancy (Vigil *et al.*, 2017). Many plants and plant products used in ethno-medicine has proved to have adverse effects on some organs or reproductive hormone secretions. Some plant products consumed often lead to reproductive hormonal stasis, even miscarriages or stunted growth of the foetus (Nwankudu & Ifenkwe, 2023).

It has become necessary to assess the physiological safety of plant or plant products used for treatment in animals in order to advise the public about the advantages or potential hazard the use of each plant of interest poses.

This research was set up to ascertain the effect of *Psidium guajava* (Guava) leaf extract on reproductive hormones. This is because anything consumed to watch weight often targets cholesterol precursor for reproductive hormones' secretions. Cholesterol is essential for posterity. However, excess of cholesterol may lead to cardiovascular issues (Atherosclerosis) (Roth *et al.*, 2020).

## MATERIALS AND METHODS

### PLANT COLLECTION AND EXTRACTION

Fresh guava leaves were harvested early in the morning in Michael Okpara University of Agriculture Umudike, Abia State, Nigeria on the 16<sup>th</sup> of October, 2021. They were taken to the Department of Forestry for identification and confirmation as guava leaves in the same institution. The herbarium number in the Department of Veterinary Physiology and Pharmacology is MOUAU/VPP/15/011. The leaves were washed with clean running water and dried on laboratory bench for 5 days (Figures I&II).



**Figure I: Photomicrograph of Fresh Guava leaves**



**Figure II: Photomicrograph of dried guava leaves**

The leaves were then pulverised. A total of 220g of the pulverized leaf was subjected to extraction using 98% methanol with intermittent shaking for 48hours. The filtrate was dried using hot air oven at 40°C. The percentage yield was calculated and the resulting extract was stored at -4° C in a freezer until needed for experimentation.

### EXPERIMENTAL ANIMALS

Sixty albino rats weighing between 100-180 grams, were obtained from the laboratory animal unit of Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. The animals were kept in aluminium cages at room temperature. They were fed with commercial poultry feed and clean water *ad libitum*.

### ACUTE TOXICITY TEST OF PSIDIUM GUAJAVA (GUAVA) LEAF EXTRACT

Acute toxicity test was conducted using Lorke's method, 1983. Twenty-eight Albino rats of both sexes were grouped into seven (1-7) of four rats per group.

Group 1; (control) was given 2ml/kg of distilled water while Groups 2, 3 and 4 were given 10 mg/kg, 100 mg/kg, 1000 mg/kg of guava leaf extract (GLE) respectively.

When no toxicity was observed, 1600mg/kg, 2900mg/kg and 5000mg/kg of GLE was given to groups 5, 6 and 7 respectively through the oral route using gastric gavage.

The animals were observed for 30minutes, 1hour, 3hours, 12hours, 24hours, 48hours, 72hours for signs of acute toxicity and probably death. The animals were allowed extra 7days to observe signs of delayed toxicity.

The LD<sub>50</sub> was calculated using:

$$LD_{50} = \sqrt{\text{(least dose with mortality)} \times \text{Highest dose without mortality}}$$

### IN VIVO EXPERIMENTS WITH GUAVA LEAF EXTRACT

A total of 32 albino rats separated into 16 males and 16 females were used. The 16 males were divided into 4 groups as M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub> and M<sub>4</sub> while the 16 females were also divided into F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> and F<sub>4</sub>. M<sub>1</sub> and F<sub>1</sub> were control.

Treatment was done through the oral route. Control group in each gender was treated with 2ml/kg body weight of distilled water while the remaining 3 groups of each gender were treated with graded doses (200mg/kg, 400mg/kg and 800mg/kg) body weight of guava leaf extract (GLE). Treatment lasted for 30 days. At day 31, blood was collected through the retro-bulba plexus into plain sample bottles for hormonal assay (Testosterone, Oestrogen & Progesterone) using Elisa kits for the individual hormones.

Thereafter, a graph for known quantities of these hormones was used to extrapolate the levels of the individual hormones found in each rat.

### RESEARCH ETHICS

This research received MOUAU ethical clearance: MOUAU/CVM/REC/202407.

Moreover, We ARRIVE guidelines for in vivo experimental animal research and reporting which stresses that the methods used in research and reporting should be easy to replicate (PLOS BIO. B (6), E1000412 2010) was adhered to.

### STATISTICAL ANALYSIS

The data collected were analyzed using Statistical Package for Social Sciences (SPSS), version 20. Analysis of variance was done and values at ninety-five percent confidence interval ( $p < 0.05$ ) were considered significant.

Variant means were separated using Turkey HSD.

## RESULTS

### RESULT OF ACUTE TOXICITY TESTING IN *PSIDIUM GUAJAVA* LEAF (PGL) EXTRACT TREATED RATS

The result observed in highest dose (5000 mg/kg) of guava leaf extract administered to albino rats showed toxicity and death post administration.

The higher dose of 2900 was also toxic to the animals. Sign of toxicity observed were: Gasping for air, lethargy and death.

The lethal dose observed was: 4161.28 mg/kg (Table I).

**TABLE I: RESULT OF ACUTE TOXICITY TEST IN *PSIDIUM GUAJAVA* (GUAVA) LEAF EXTRACT TREATED RATS**

Groups	Dose mg/kg	Number of deaths	Percentage mortality
1	2ml/kg	0/4	0.00
2	10	0/4	0.00
3	100	0/4	0.00
4	1000	0/4	0.00
5	1600	0/4	0.00
6	2900	0/4	0.00
7	5000	4/4	100

Table I shows guava leaf extract is toxic at very high dose using Lorke's method.

### SOME HORMONAL PROFILE OF MALE AND FEMALE ALBINO RATS TREATED WITH *PSIDIUM GUAJAVA* LEAF EXTRACT

From the research, testosterone profile of male albino rats treated with PGL extract most significantly ( $P \leq 0.05$ ) increased in higher dose of (400 mg/kg) body weight, but began to decline at 800 mg/kg body weight PGL extract treatment. Contrarily, hormonal profile of female albino rats treated with PGL extract showed that estrogenic profile most significantly ( $P \leq 0.05$ ) reduced in 200 mg/kg PGL extract treatment when compared to control but in higher doses, the reduction though significant were minimal. Furthermore, progesterone profile show fluctuations of down, up and normal down.

Sera progesterone was significantly reduced in 200 mg/kg PGL extract treatment but significantly increased in 400 mg/kg PGL extract treatment when compared to control (Table II).

Table II shows that *Psidium guajava* leaf extract treatment enhanced testosterone secretion in male which may lead to enhanced spermatogenesis and fertility. Moreover, the table shows that treatment with PGL extract led to depletion in oestrogen secretion in all the treatment groups when compared to control. Depletion in oestrogen secretion may lead to infertility and in older females, may lead to osteoporosis. However, the mean difference between PGL extract treatment and control was not so elaborate and may not really affect fertility.

### WEIGHT LOSS IN *PSIDIUM GUAJAVA* (GUAVA) LEAF EXTRACT TREATMENT

The mean weights at commencement of research for females treated with distilled water as control and guava leaf extract treatment groups (200mg/kg, 400 mg/kg and 800 mg/kg) include:

**TABLE II: SOME HORMONAL PROFILE OF MALE AND FEMALE RATS TREATED WITH *PSIDIUM GUAJAVA* LEAF (PGL) EXTRACT**

Hormone	Gender	Treatments			
		2ml/kg DW (Control)	200mg/kg PG	400mg/kg PG	800mg/kg PG
Testosterone (ng/ml)	Male	2.50±0.65	6.50±0.65c	10.50±0.65a	8.50±1.19b
Oestrogen (pg/ml)	Female	87.35±0.42	79.88±0.75a	85.50±1.37b	83.25±0.32b
Progesterone (nmol/ml)	Female	9.4±0.18	4.85±0.14a	11.28±0.19b	8.93±0.09

The superscripts show significant ( $P \leq 0.05$ ) difference in a row

114.50, 111.00, 112.50 and 113.50 while the males in the same order include: 137.25, 136.75, 134.25 and 134.75. The weight loss/gained is in Table III. However, weight loss is synonymous with least weight gained during the period of treatment. For a growing animal, less weight gain when compared to control is equal to weight loss. From the research, in the male, 800 mg/kg body weight treatment of *Psidium guajava* leaf (PGL) extract led to significant ( $P \leq 0.05$ ) weight loss when compared to control. In contrast, there were no significant weight loss in PGL extract treated females in all the groups when compared to control (Table III).

**TABLE III: WEIGHT LOSS IN *PSIDIUM GUAJAVA* LEAF EXTRACT TREATED ALBINO RATS**

Treatments	Weight gain/loss (grams)	
	Male	Female
2ml/kgDW (Control)	22.83±6.56	17.58±2.42
200mg/kg PG	17.84±7.07	13.42±4.62
400mg/kg PG	19.83±4.83	13.83±3.97
800mg/kg PG	08.92±5.44*	13.17±1.55

Superscript indicates significant difference ( $P \leq 0.05$ ) in a column.

Table III shows that the males least weight gain (weight loss) was at 800 mg/kg *Psidium guajava* leaf extract treatment which was significantly different from distilled water treatment which served as control. In the females, there were no significant different between PGL extract treated groups and control which shows that PGL extract treatment does not lead to weight loss in females.

### ORGAN METRICS IN FEMALES AND MALES TREATED WITH *PSIDIUM GUAJAVA* LEAF (PGL) EXTRACT

From the research, these organ (kidney, liver, heart, testis and spleen) weights relative to body mass in males were not significantly ( $P \leq 0.05$ ) different from control.

Moreover, in females, (Kidney, Heart, Liver, Spleen, Ovary and Uterus) mass in PGL extract graded dose treatments, were not significantly different from distilled water treatment which served as control (Tables IVa & IVb)

Table IVa shows that there is no significant ( $P \leq 0.05$ ) difference in organ metrics of female albino rats treated with graded doses of PGL extract and control. This shows that *Psidium guajava* (Guava) leaf extract is organ safe.

Table IVb shows that there is no significant ( $P \leq 0.05$ ) difference in organ metrics of male albino rats treated with graded doses of PG extract and control. This shows that *Psidium guajava* (Guava) leaf extract is male organ safe.

### DISCUSSION

*Psidium guajava* (Guava) leaf extract does not lead to weight loss in females treated for 30 days. However, the use of *Psidium guajava* leaf (PGL) extract treatment for weight loss in males should be at a dose of 800 mg/kg.

Moreover, *Psidium guajava* leaf (PGL) extract treatment in male rats caused significant increase in testosterone secretion in dose dependent manner but began to decline at highest dose (800 mg/kg) body weight for the treatment.

Therefore, to enhance fertility in male, PGL extract should be used at a dose of 400 mg/kg which was the optimum according to the research. Testosterone is a male hormone that primes spermatogenesis and maintains male characteristics (Walker, 2011).

**TABLE IVB: ORGAN METRICS IN MALE RATS TREATED WITH *PSIDIUM GUAJAVUM* (PG) LEAF EXTRACT**

Treatment	Organ			Metrics	
	Liver	Kidney	Spleen	Heart	Testis
2ml/kg DW	8.16±1.08	1.36±0.12	1.15±0.32	1.09±0.14	2.73±0.05
200 mg/kg PG	7.06±0.45	1.27±0.05	0.89±0.13	0.88±0.05	2.49±0.06
400 mg/kg PG	7.53±0.49	1.57±0.34	0.98±0.14	0.83±0.05	2.56±0.18
800 mg/kg PG	6.34±0.29	1.26±0.10	1.01±0.97	0.89±0.03	2.47±0.08

Superscript show significant ( $P \leq 0.05$ ) difference in column

**TABLE IVA.: ORGAN METRICS IN FEMALE RATS TREATED WITH *PSIDIUM GUAJAVUM* (PG) LEAF EXTRACT**

Treatment	Organ			Metrics		
	Liver	Kidney	Spleen	Heart	Ovary	Uterus
2ml/kgDW (Control)	6.03±1.34	1.08±0.08	0.91±0.14	0.59±0.06	0.17±0.03	0.79±0.22
200 mg/kg PG	5.04±0.86	1.09±0.12	0.81±0.19	0.55±0.02	0.20±0.10	0.52±0.33
400 mg/kg PG	5.52±0.50	1.04±0.12	0.83±0.17	0.66±0.13	0.14±0.02	0.62±0.28
800 mg/kg PG	5.41±0.94	1.07±0.05	0.73±0.11	0.63±0.07	0.21±0.12	0.39±0.10

Superscript show significant ( $P \leq 0.05$ ) difference in column

In contrast, PGL extract treatment in female albino rats caused a significant decrease in sera oestrogen and progesterone at a dose of 200 mg/kg body weight when compared to control. But, at 400 mg/kg body weight, there were significant increase in sera oestrogen and progesterone which began to decline at 800 mg/kg body weight treatment. Oestrogen and progesterone are hormones for female reproductive capacity (Rosner *et al.*, 2024). Fluctuation in these hormones which are not cyclic induced could lead to abnormal fertility. Therefore, from the research, sub-normal dose and prolonged (sub-acute) use of PGL extract treatment in females should be discouraged.

Organ metrics: The research showed that PGL extract does not significantly reduce the size of the heart, liver, kidney, spleen and testis of male rats. Furthermore, *Psidium guajava* leaf extract treatment does not significantly reduce the size of kidney, liver, heart, spleen, ovary and uteri of female albino rats treated with the extract.

Therefore, PGL extract is safe for ethnomedical pharmacotherapy, since it is not only organ safe, but also reproductive hormone enhancer in male.

## CONCLUSION

*Psidium guajava* (Guava) leaf extract is sex variant in weight loss and reproductive hormone profile. It is user friendly in males as opposed to females. However, organ metrics of *Psidium guajava* leaf extract is non sex distinctive. It is

organ safe. Therefore, PGL extract can be used in treatments in male and female for any other course, but sub-acute therapy is discouraged in females.

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