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## Original Article

# Biochemical parameters and reproductive traits in female rabbits (*oryctolagus cuniculus*) exposed to *psidium guajava* leaf aqueous extract

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**ABSTRACT**

**Background:** The potential impact of aqueous extracts from *Psidium guajava* leaves on the reproductive system of female rabbits was evaluated.

**Methods:** Twenty-eight rabbits, aged five to six months were utilized. Rabbits were divided into four groups and were randomly assigned to receive one of the following oral doses of the guava leaf extracts: 0 (control group), 10, 20, or 30 mg/kg of body weight. After a treatment period of 30 days, blood was collected via jugular venipunture and the serum was extracted for the assessment of serum biochemical traits levels. The females were bred and monitored throughout their pregnancy to ascertain reproductive outcomes.

**Results:** The results indicated that the guava leaf extract significantly increased the body weight of the rabbits during both pre- and post-pregnancy compared to the control group ($p < 0.05$). The litter size at three weeks post-birth, prolificity rate, FSH, LH, and protein levels were notably higher ($p < 0.05$) at a dose of 20 mg/kg of body weight. The viability rate three weeks post-birth increased with escalating extract doses, and the highest values were observed at doses of 20 and 30 mg/kg of body weight ($p < 0.05$).

**Conclusions:** This study demonstrated that, the aqueous extract of guava leaves appears to stimulate the production of FSH, LH and enhance body weight, prolificity, and pregnancy outcomes in mammals. As such, it is suggested that a dose of 20 mg/kg body weight could be beneficial in improving the reproductive performance of female.

**Keywords:** aqueous extract, body weight, *psidium guajava* leaf, rabbits, reproductive parameters

## INTRODUCTION

Plants have served as critical resources for medicinal compounds throughout human history. Even in today's modern era, plants continue to play a pivotal role in healthcare due to their wide range of therapeutic properties. Increasingly sophisticated scientific research, coupled with heightened public awareness, has underscored the diverse beneficial effects of plants. These effects extend not just to treating a variety of ailments, but also to augmenting culinary experiences and improving reproductive health in humans and animals alike (Ibe and Nwufo, 2005). A case in point is *Psidium guajava*, more commonly known as guava.

The guava tree, native to South America and a member of the Myrtaceae family, is well-suited to tropical climates. Its various parts, including roots, fruits, and leaves, are utilized in traditional medicine for treating a multitude of diseases. These range from common gastrointestinal and respiratory disorders to more severe conditions like diabetes and cancer (Hernández et al., 2004; Mathlouthi et al., 2009). Moreover, extensive research has demonstrated that extracts from guava leaves and essential oils display a plethora of advantageous properties. These include anti-inflammatory, antibiotic, analgesic, hepatoprotective, antiinfectious, antifungal, antiparasitic, and antioxidant effects (Konstantopoulou et al., 1992; Recoquillay, 2009). Given these wide-ranging benefits, it's clear to see the potential value of such extracts in animal husbandry, where they could foster growth and optimize reproductive efficacy.

Several researchers, such as Adebayo et al. (2005), Kullu et al. (2013), and Ferdinand et al. (2014), have highlighted the positive impact of aqueous and methanol extracts of *Psidium guajava*, along with its essential oil, on male reproductive health in rats. Specifically, these studies have found improvements in the weight of testes and epididymis, motility, concentration epididymal sperm, and white blood cell count. Further research has corroborated these findings, with studies showing an increase in levels of reproductive hormones (testosterone, LH, FSH), testes and epididymis weight, motility, and concentration epididymal sperm in rats treated with guava leaf extracts (Uboh et al., 2010a; Ekaluo et al., 2013b).

Despite this extensive body of research centered on male reproductive health, a conspicuous knowledge gap exists when it comes to understanding the effects of guava leaf extract on female reproductive performance. Most studies to date have been male-centric, primarily because male reproductive variables are easily quantifiable, such as sperm count and testosterone levels. However, this trend overlooks the fact that female reproductive health is equally vital for effective animal reproduction and production. With numerous factors influencing female fertility - from ovarian function and hormonal balance to gestation and litter size - it is crucial to assess the potential benefits of natural therapies like guava leaf extract in this context.

The lack of comprehensive studies on female animals also represents a missed opportunity in developing sustainable and natural approaches for animal health management. Particularly in the context of antibiotic resistance and the drive towards organic farming, research in this field can potentially open new avenues in improving animal reproduction while minimizing chemical intervention.

Therefore, this study aims to bridge this significant research gap by investigating the effects of aqueous guava leaf extract on multiple reproductive parameters in female rabbits. This research aims to provide comprehensive data that will help to understand and fully exploit the potential benefits of guava leaf extract in enhancing female reproductive performance in animal production.

## MATERIALS AND METHODS

### Plant material and extract preparation

Fresh guava leaves were collected from the campus of Dschang University, situated in the western region of Cameroon with latitude 5°26'38''Nord, Longitude 10°03'11''East and altitude above sea level 1.345 m. The leaves were dried at ambient temperature in a shaded area to maintain their medicinal properties and subsequently crushed into a fine powder using an electric grinder. The aqueous extraction was performed following the procedure described by Yakubu et al. (2005) in the Laboratory of Animal Physiology of the Faculty of Agronomy and Agricultural Sciences.

A quantity of 1.000 g of the powdered leaves was steeped in six liters of cold distilled water for 48 hours at room temperature. The extract was then filtered using Whatman No.1 filter paper. The resulting filtrate was further concentrated on a steam bath at 50℃ until it re-

duced to a residue (powder). Appropriate quantities of the residue were reconstituted in distilled water to produce the required doses of 10, 20, and 30 mg per kg of body weight, respectively, for subsequent experimental use.

## Animal subjects

Twenty-eight female rabbits (Oryctolagus Cuniculus) of the Buscat's White breed were used in the study. These rabbits were aged between 4 and 5 months and had an average weight ranging from 2.30 to 2.40 kg. All rabbits were bred and raised at the Teaching and Research Farm of the University of Dschang to ensure a consistent genetic and environmental background.

## Experimental design

The rabbits were randomly divided into four groups and housed in four identical enclosures to ensure uniform living conditions. Over a period of 60 days, the animals in the first enclosure (control group) were orally administered distilled water, while the animals in the remaining three enclosures received daily doses of 10, 20, and 30 mg/kg body weight of the aqueous extract of guava leaves through oral gavage. Throughout the experimental period, all animals were provided with a standardized diet (Table 1) and water *ad libitum*, using appropriate feeding equipment to ensure their well-being and uniform access to resources.

Table 1. Centesimal composition and chemical characteristics of the ration

| Ingredients | Amount (kg/100 g) |
|---|---|
| Centesimal characteristics | |
| Corn | 20.00 |
| Bran wheat | 39.50 |
| Palm kernel cake | 15.00 |
| Cotten seed meal | 8.00 |
| Soybean meal | 3.00 |
| Fishmeal | 1.00 |
| Bone meal | 1.00 |
| Premix 10% | 2.00 |
| Salt | 2.00 |
| *Pennisetum purpurum* | 0.50 |
| Total | 100 |
| Chemical characteristics | |
| Crude protein (%) | 17.72 |
| Metabolizable energy (kcal/kg) | 2014.5 |
| Crude fiber (%) | 12.11 |
| Calcium (%) | 1.15 |

## Data collection

### 1) Blood sample collection

After 30 days of treatment, blood samples were collected from each rabbit via jugular venipunture while under ether anesthesia. The samples were left undisturbed for 24 hours to allow clotting. Subsequently, the supernatant (serum) was isolated through centrifugation at 2.000 rpm for 15 minutes. These purified serum samples were then stored in 1.5 mL Eppendorf tubes at -20℃ until required for further analysis.

### 2) Protein and hormonal assay

The total protein content of the serum was determined using the Biuret method (Gornall et al., 1949). The hormone levels, including follicle stimulating hormone (FSH), luteinising hormone (LH), and estradiol, were assessed using commercial ELISA kits, following the manufacturer's instructions outlined in the hormone assay commercial kits (ELISA AccuDiag™, Diagnostic Automation Inc.).

### 3) Reproductive traits

Several reproductive indices were calculated as follows:

- Delay of receptivity: This was measured as the time taken by the female rabbit to accept mating with a male rabbit.
- Fertility index: This was calculated as the number of pregnant females divided by the number of females that successfully copulated, multiplied by 100.
- Gestation index: This was defined as the number of females with live offspring divided by the number of pregnant females, multiplied by 100.
- Pregnancy duration: This was noted as the time elapsed between the mating of the female rabbit and the birth of the litter.
- Litter size: This was defined as the number of live offspring present at birth.
- Post-natal viability index: This was calculated as the number of live offspring at birth divided by the total number of offspring born, multiplied by 100.
- Weaning viability index: This was measured as the number of live offspring at day 21 post-birth divided by the number of live offspring at birth, multiplied by 100.

### Statistical analysis

Data were analyzed using one-way ANOVA with a significance level set at $p < 0.05$. If significant differences were identified between means, these were further explored using the Duncan test to separate the means. All statistical analyses were conducted using SPSS 20.0 software.

## RESULTS

### Impact of oral administration of aqueous extract of *Psidium guajava* on body weight

As depicted in Table 2, the oral administration of the aqueous extract of *Psidium guajava* led to a significant ($p < 0.05$) increase in the body weight of the rabbits. This significant increase was notably observed in rabbits that received a dose of 20 mg/kg of body weight, specifically during the mating and birthing periods, in comparison to the other groups.

### Effects of oral administration of aqueous extract of *Psidium guajava* on reproductive indices

Table 3 reveals significant effects of the aqueous extract of *Psidium guajava* on several reproductive indices. Specifically, both the litter size three weeks post-birth and the prolificity rate at birth were significantly higher ($p < 0.05$) in rabbits administered a dose of 20 mg/kg body weight.

Furthermore, the viability rate three weeks post-birth demonstrated an increasing trend with escalating doses of the extract. Notably, the highest ($p < 0.05$) values were recorded in rabbits treated with doses of 20 and 30 mg/kg body weight.

Moreover, the individual weight of young rabbits at three weeks post-birth was significantly higher ($p < 0.05$) when the parent rabbits received doses of 10 and 20 mg/kg body weight. Conversely, the untreated control group (T0) exhibited a significantly greater ($p < 0.05$) delay of receptive.

### Biochemical parameters

The impact of the oral administration of the aqueous extract of *Psidium guajava* on serum reproductive biochemical parameters is summarized in Table 4. The results indicate a significant increase ($p < 0.05$) in serum follicle stimulating hormone (FSH), luteinising hormone (LH), and protein levels at the dosage of 20 mg/kg compared to the control group. However, it is noteworthy that the follicle stimulating hormone (FSH) level exhibited a decreasing trend with increasing doses of the extract.

Table 2. Effect of oral treatment of aqueous extract of *Psidium guajava* on body weight

| Periods | Doses of *Psidium guajava* (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 10 | 20 | 30 |
| Initial body weight (kg) | 2.34 ± 0.55[a] | 2.36 ± 0.42[a] | 2.35 ± 0.34[a] | 2.33 ± 0.53[a] |
| Body weight at mating (kg) | 2.53 ± 0.49[a] | 2.79 ± 0.50[a] | 3.55 ± 0.29[b] | 3.25 ± 0.45[a,b] |
| Body weight at caving (kg) | 2.78 ± 0.47[a] | 2.89 ± 0.54[a] | 3.87 ± 0.52[b] | 3.81 ± 0.37[b] |

([a,b]) on the same line, values affected with the same letter don't different significantly ($p > 0.05$).

Table 3. Effects of oral administration of aqueous extract of *Psidium guajava* on some reproductive traits

| Reproductive parameters | Doses of extrait (mg/kg of body weight) | | | | *p*-value |
|---|---|---|---|---|---|
| | 0 (control) | 10 (n = 7) | 20 (n = 7) | 30 (n = 7) | |
| Limit of receptivity (D) | 1.60 ± 0.36[b] | 1.52 ± 0.26[a,b] | 1.43 ± 0.13[a] | 1.34 ± 0.25[a] | 0.02 |
| Duration of pregnancy (D) | 28.00 ± 1.29[a] | 28.50 ± 1.19[a] | 29.00 ± 1.00[a] | 30.33 ± 0.58[a] | 0.4 |
| Litter size at birth | 6.00 ± 2.16[a] | 6.33 ± 2.52[a] | 6.67 ± 2.14[a] | 6.00 ± 1.91[a] | 0.3 |
| Litter size 3 weeks after birth | 4.25 ± 1.50[a] | 5.00 ± 3.60[a] | 6.67 ± 2.51[b] | 5.67 ± 0.59[a,b] | 0.03 |
| Rate of prolificity (%) | 4.56 ± 1.00[a] | 4.78 ± 2.66[a] | 8.00 ± 4.22[b] | 5.29 ± 1.70[a,b] | 0.02 |
| Individual weight of young rabbit at birth (g) | 51.28 ± 5.05[a] | 57. 40 ± 8.65[a] | 59.50 ± 9.66[a] | 53.01 ± 5.46[a] | 0.3 |
| Individual weight of young rabbit 3 weeks after birth (g) | 168.50 ± 27.10[a] | 205.53 ± 34.80[b] | 218.99 ± 54.61[b] | 176.44 ± 31.16[a,b] | 0.02 |
| Rate of viability at birth (%) | 96.87 ± 7.32[a] | 96.88 ± 9.62[a] | 100.00 ± 0.00[a] | 100.00 ± 0.00[a] | 0.3 |
| Rate of viability 3 weeks after birth (%) | 77.78 ± 14.50[a] | 80.35 ± 18.31[a,b] | 87.77 ± 16.51[b] | 94.44 ± 9.62[b] | 0.02 |

n, number; D, days; %, percentage; g, gram. ([a,b]) on the same line, values affected with the same letter don't different significantly ($p > 0.05$).

Table 4. Effects of aqueous extract of *Psidium guajava* on some reproductive biochemicals parameters in rabbit after 30 days of feeding

| Biochemical parameters | Doses of extrait (mg/kg of body weight) | | | | *p*-value |
|---|---|---|---|---|---|
| | 0 (control) (n = 7) | 10 (n = 7) | 20 (n = 7) | 30 (n = 7) | |
| Protein (mg/mL) | 101.46 ± 15.08[a] | 119.13 ± 11.16[a,b] | 130.45 ± 9.66[b] | 135.42 ± 17.6[b] | 0.02 |
| Estradiol (mIU/mL) | 0.47 ± 0.01[a] | 0.48 ± 0.05[a] | 0.47 ± 0.06[a] | 0.47 ± 0.01[a] | 0.3 |
| FSH (mIU/mL) | 7.25 ± 2.5[a] | 8.75 ± 3.09[a,b] | 13.00 ± 4.24[b] | 12.5 ± 1.91[b] | 0.03 |
| LH (mIU/mL) | 4.56 ± 1.00[a] | 4.78 ± 2.66[a] | 8.00 ± 4.22[b] | 5.29 ± 1.7[a,b] | 0.04 |

n, number; FSH, follicle-stimulating hormone; LH, luteinising hormone. ([a,b]) on the same line, values affected with the same letter don't different significantly ($p > 0.05$) .

Furthermore, as detailed in Table 4, the highest protein levels were observed in rabbits treated with the maximum administered dose (30 mg/kg body weight) of the aqueous extract of *Psidium guajava* leaves when compared to other treated groups.

## DISCUSSION

The current investigation substantiates the premise that aqueous extract of guava leaves engenders a significant increase in body weight, corroborating the results observed by Ugwah et al. (2011) and Adaay and Mosa (2012). The observed weight augmentation could be attributed to the bioavailability of amino acids and androgens in the guava leaf extract. These bioactive compounds have the potential to boost protein synthesis, a process fundamental to cell growth, architecture, and metabolism (Mommsen and Walsh, 1991).

Additionally, the anabolic effects of androgens may precipitate an enhancement of muscle mass through the promotion of protein synthesis (Hazard et al., 2000; Gayrard, 2007). This elucidates a potential mechanism by which guava leaf extract could be harnessed as a natural growth stimulator, offering considerable implications for nutritional strategies in both animal husbandry and human health.

The observed weight loss during the first three weeks of lactation might be attributed to the energy expenditure associated with milk production, necessitating the utilization of bodily reserves (Rivière, 1991). This physiological response underscores the metabolic adaptations that occur in females during lactation, which warrant further investigation to inform nutritionally supportive strategies for lactating animals.

Furthermore, the notable increase in serum FSH and LH levels is indicative of the potential endocrine modulating properties of guava leaf extract. This concurs with the findings of Slaughter et al. (2009) and implies that the extract, through its saponin content, could stimulate hormone synthesis by acting on the hypothalamic-pituitary-ovaries axis (Lieberman, 1996; Couchman and Hammond, 1999). These insights could potentially inform the development of novel, plant-based interventions for fertility and reproductive health disorders.

The augmentation of litter size and prolificacy rate in treated groups could be ascribed to the FSH stimulated growth and development of ovarian follicles (Natumanya et al., 2008). These results contribute to a broader understanding of how plant extracts can influence reproductive processes, offering potential applications for livestock breeding programs and reproductive health research.

Lastly, the guava leaves' antioxidant properties, conferred by their alkaloids, phenolics, flavonoids, and tannins content, could play a crucial role in mitigating the adverse effects of reactive oxygen species on the structure of fetal membranes (Grignard, 2005; Ryu et al., 2012). This area of investigation may prompt further research into natural antioxidants' role in promoting fetal health and ensuring successful pregnancies.

The outcomes of this study provide compelling evidence of the multifaceted benefits of guava leaf extract, contributing to the understanding of its potential application in enhancing animal growth, reproductive performance, and overall health. This novel evidence lays the groundwork for further exploration, with implications that extend from agricultural practices to livestock health management and potentially to human health.

## CONCLUSION

The evidence from this investigation suggests that the guava leaf extract at 20 mg/kg bw notably stimulates

the synthesis of follicle stimulating hormone (FSH) and luteinizing hormone (LH), both key hormones involved in regulating fertility and overall reproductive health. Furthermore, the extract has been observed to enhance the receptivity rate and prolificacy in mammals, fostering successful pregnancies and contributing to enhanced reproductive performance. These outcomes align with existing scientific literature, endorsing the role of phytochemicals as modulators of reproductive health.

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