

ASSESSMENT OF *Phyllanthus amarus* Leaf Extract on Sexual Behaviour and Physiological Responses of rabbit bucks

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ABSTRACT

This study aimed to investigate the impact of Phyllanthus amarus aqueous leaf extract on some physiological parameters of growing rabbits. Thirty-eight rabbits were divided into four groups receiving different doses of the extract for 12 weeks. Physiological parameters such as respiratory rate, rectal temperature, reaction time, and libido were assessed. No significant ($p>0.05$) differences were observed in rectal temperatures and respiratory rates of rabbits exposed to different doses of the extract. Rephrase (Higher dosages were associated with longer reaction times and lower libido scores suggesting a possible undesirable effect on these reproductive aspects. The study revealed distinct responses based on dosage levels, highlighting the potential influence of Phyllanthus amarus extract on various physiological aspects in rabbits. However, further research is needed to understand the mechanisms behind these observed effects.

Keywords: *Phyllanthus amarus*. Extract, Rabbits, Libido, Physiology

INTRODUCTION

Rabbit farming in Nigeria is gaining significant recognition as a promising and sustainable agricultural practice in recent years. This effort holds the potential to address pressing issues of food security, economic diversification, and environmental sustainability among others (Para *et al.*, 2015). Various nutritional strategies have been explored to enhance productivity in rabbits. One of these strategies involves the use of natural supplements, such as plant extracts, essential oils, and ethnobotanical plant parts (Eweka and Adaze, 2011). *Phyllanthus amarus* is a widely distributed perennial herbal shrub (Joseph and Raj, 2011). It is commonly called several names, including gale of the wind, stonebreaker, carry-me-seed, and so on (Sarin, *et al.*, 2014). This plant exhibits a diverse range of beneficial properties, including antiviral, anti-inflammatory, hepatoprotective, and antioxidant activities (Zubair *et al.*, 2017). However, the specific effects of *Phyllanthus amarus* aqueous leaf extract on the physiological response of rabbit bucks remain relatively insufficient, hence this research. This study aims to ascertain its level of safe use in rabbit production.

MATERIALS AND METHODS

Experimental location

This trial was carried out at University of Abuja, Teaching and Research Farm. The site lies between latitude 8°56'29" North, 70°5'31" East, and longitude 7°20' North and 7°51' East North and 0070 51' East, (Balogun, 2001)

Collection and Preparation of *Phyllanthus amarus* Leaf extract

The *Phyllanthus amarus* plant was collected from within University of Abuja premises. The leaves were cleaned, air dried and milled into fine powder which was processed into aqueous solution. The dried material was soaked in distilled water for 48 hours kept at 4°C in a refrigerator. The extract was sieved and the juice was filtered using Whatman grade 1 filter paper and the resultant extract was stored (Eweka and Enogieru, 2011).

Animal care and experimental design

A total of 38 New Zealand rabbits of average weight of 500±0.5 g and within the age of 10 weeks were used for this experiment. The hutchers were cleaned and disinfected before their arrival. The rabbits were divided into 4 treatments: T1 (0 mL extract), T2 (2 mL extract), T3 (4 mL extract), and T4 (6 mL extract) in a completely randomized design. The rabbits were allowed to acclimatize for one week before the commencement of the experiment, and were fed the same diet throughout the experimental period which lasted for 12 weeks.

Rectal Temperature

The rectal temperature was measured using a digital thermometer. The thermometer's bulb was cleaned for hygiene and to prevent cross contamination, it was gently inserted into the rabbit's anus at about 2-3 cm deep, and the resulting temperature was recorded after a beep or reading was provided (Robinson, and Rubenstein, 2010).

Respiratory Rate

The respiratory rate of the rabbits was measured in a relaxed state using a stopwatch. The rise and fall of the nose were observed and counted within a 15-second interval, with the resulting count multiplied by 4 to obtain the number of breaths per minute (Ewuola *et al.*, 2022).

Reaction Time and Libido

The reaction time and libido of the rabbit bucks were assessed and recorded weekly through the introduction of a teaser doe to the bucks and monitoring their sex drive. Reaction time was measured using the time it took the rabbit buck to sniff, groom, and mount the rabbit doe, while libido score was measured as the number of times the buck attempted to mount per minute. Both parameters were measured with the aid of an electronic stopwatch (Verstegen *et al.*, 2002)

RESULTS AND DISCUSSION

The phytochemical compositions of *Phyllanthus amarus* leaf extract are presented in Table 1. The results from this experiment showed the presence of flavonoids, terpenoids, tannins, oxalates, steroids, phytates and phenols which is the most abundant. These phytochemicals possess strong antioxidant activities and exhibit antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral activities (Sharma *et al.*, 2018). Phenols can scavenge free radicals; donate hydrogen atoms, electrons, or other antioxidant properties, contributing to the reduction of oxidative stress (Minatel *et al.*, 2017). These activities put together are evident on the improved overall health observed in the experimental rabbits.

Table 1: Phytochemical composition of *Phyllanthus amarus* leaf extract

Components identified	Quantity Present (%)
Flavonoids	5.77
Terpenoids	3.60
Tannins	3.11
Phenols	7.08
Oxalates	0.12
Steroids	1.14
Phytates	0.87

Table 2 shows the rectal temperature of rabbits exposed to varying doses of *Phyllanthus amarus* aqueous leaf extract, there were no significant ($p > 0.05$) difference observed among the treatments.

Table 2: Rectal Temperature of Rabbit fed *Phyllanthus amarus* aqueous leaf extract

DAYS	T1 (0ml)	T2 (2ml)	T3 (4ml)	T4 (6ml)	SEM
1	39.01	38.87	39.04	39.18	0.12
2	39.38	39.43	39.30	39.42	0.10
3	38.80	39.29	39.14	38.98	0.12
4	38.57	39.51	39.12	38.97	0.13
5	39.00	39.10	39.24	38.50	0.09
6	39.38	39.40	39.40	39.13	0.10
7	38.73	38.67	38.64	39.20	0.08
8	38.53	38.74	38.56	39.20	0.08
9	38.42	38.63	38.14	38.68	0.10
10	39.32	39.10	39.02	39.50	0.12
11	38.95	38.71	38.82	39.37	0.10
12	38.88	39.07	38.94	38.88	0.11
13	38.77	38.70	38.52	38.75	0.08
14	38.73	38.71	38.64	39.20	0.07
15	38.98	39.07	38.96	38.90	0.11

SEM=standard error of means.

Generally, the result shows that the plant extract did not exert any influences on the body temperature of the experimental rabbits when compared with the control groups. Temperature is a crucial vital sign that checks the overall health and well-being of the rabbits, abnormalities in body temperature can be a sign of a health issue. Rabbits are very sensitive animals, change in environment, disease and stress can affect their temperature, so it is important to monitor the temperature for early detection of distress or disease. This highlights a stable thermoregulatory response to the administered doses, implying that the extract has no adverse effect on the rabbits. These findings align with the work of Saleem *et al.*, (2017) that also had similar effect on temperature with of aqueous extract of *Terminalia citrina* fruit in mice.

Table 3 shows the reading of respiratory rate of rabbits exposed to varying doses of *Phyllanthus amarus* aqueous leaf extract. Variations were observed across the treatment groups compared to the control. The respiratory rates fluctuated inconsistently during the experiment. The level of variation was however not significantly ($p>0.05$) influenced. This suggests that the rabbits were not affected by the treatment. Various factors can be responsible for the inconsistency in the respiratory rate as reported here. These may include including age, temperature, health status and the activity level of the rabbit at the time of examination.

Table 3: Respiratory Rates of Rabbit fed *Phyllanthus amarus* aqueous leaf extract

DAYS	T1 (0ml)	T2 (2ml)	T3 (4ml)	T4 (6ml)	SEM
1	72.40	66.00	58.40	71.20	2.59
2	74.40	67.20	70.40	73.60	2.41
3	77.60	73.60	66.40	94.40	4.08
4	72.80	72.00	73.20	70.40	2.53
5	70.60	78.00	79.20	71.20	5.72
6	87.20	90.00	89.60	91.60	4.33
7	90.40	87.60	91.00	92.60	5.37
8	70.00	99.20	99.60	98.80	5.72
9	68.00	80.40	87.20	88.00	6.29
10	81.60	80.40	83.20	80.20	6.11
11	72.40	66.00	58.40	73.60	2.65
12	72.81	72.05	73.11	70.40	2.52
13	71.40	66.00	59.40	61.20	2.57
14	70.20	70.00	79.60	71.60	4.33
15	81.20	80.00	79.60	81.60	4.43

SEM=standard error of means.

Table 4 shows the seven-day reaction time of rabbit bucks fed to varying doses of *Phyllanthus amarus* aqueous leaf extract. In day 1, T4 has a significantly higher RT than T2 and T3 with T1 having the significantly least value. The reproductive developmental traits such as testicular development, mounting tendencies, heightened aggression, and attraction to the opposite sex were all manifested concurrently around 8 weeks into the experiment.

Table 4. Reaction time of Rabbit fed *Phyllanthus amarus* aqueous leaf extract

DAYS	T1	T2	T3	T4	SEM
1	6.80 ^b	9.35 ^a	9.05 ^a	10.01 ^a	1.30
2	6.32 ^c	3.81 ^b	9.60 ^a	9.27 ^a	1.12
3	6.80 ^b	6.24 ^b	9.73 ^a	10.96 ^a	1.41
4	6.55 ^b	8.73 ^b	16.78 ^a	11.47 ^a	2.13
5	7.86 ^c	11.68 ^a	11.73 ^a	11.68 ^a	1.54
6	6.59 ^c	8.80 ^b	14.72 ^a	11.48 ^a	1.05
7	8.22 ^b	7.92 ^b	13.34 ^a	10.29 ^a	1.64

SEM=standard error of means.

Table 5 presents a 7-day reading on the impact of varying concentrations of *Phyllanthus amarus* aqueous leaf extract on rabbit libido. Across treatments (T1, T2, T3, T4), involving different extract levels in the diet, libido values exhibit fluctuations with no clear linear trend. The control group (T1) shows relatively stable libido values, while T2, T3, and T4, corresponding to increasing extract concentrations, display variations. Day-to-day, libido values fluctuate within each treatment group. Notably, higher concentrations (T3 and T4) suggest potential divergent libido responses compared to lower concentrations (T2) or the control (T1), and overall, lower concentrations of *Phyllanthus amarus* aqueous leaf extract are associated with higher libido values compared to

higher concentrations. This observation implies a potential concentration-dependent effect on rabbit libido, where a lower supplement concentration may be more beneficial to a heightened libido response.

Table 5: Libido of Rabbit fed *Phyllanthus amarus* aqueous leaf extract

DAYS	T1	T2	T3	T4	SEM
1	6.50	5.33	5.67	5.5	3.19
2	4.00	3.00	1.00	1.00	2.14
3	2.00	1.33	1.33	1.50	1.78
4	8.00	5.00	8.33	15.50	1.80
5	7.00	6.67	6.33	5.50	1.94
6	5.00	4.33	1.33	3.50	1.14
7	8.00	5.67	2.00	3.00	1.67

SEM=standard error of means.

Phytochemical composition of *Phyllanthus amarus* shows that phenols had the highest concentration (7.08%), along with flavonoids with (5.77%). These phytochemicals are known for their pharmacological activities such as oxidative stress reduction, antioxidant, anti-inflammatory, and antimicrobial activities (Danladi *et al.*, 2018). In a study conducted by Alagbe *et al.* (2019), terpenoids demonstrate Tannins, identified as naturally occurring complex compounds, exhibit nitrogen-free polyphenols with diverse pharmaceutical effects such as antimicrobial, cytotoxic, antioxidant, and hepato-protective properties, as documented by Caprarulo *et al.* (2021). Flavonoids possess a number of medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties. They also have neuroprotective and cardio-protective effects (Bondonno *et al.*, 2019). Phenols are another class of compounds with antioxidant properties. In *phyllanthus amarus* leaf extract the concentration of phenols at 7.08% suggests a substantial presence of these bioactive compounds. Phenols are known for their ability to neutralize free radicals, contributing to the plant's defense mechanisms against oxidative stress. It is safe to agree that the higher concentration in phenols values among other constituents constitutes the amount of antioxidant, hepato-protective, immuno-stimulatory properties present in the extract.

Understanding the physiological and behavioral responses of animals to various stimuli is crucial for gaining insights into their overall well-being and performance. Focusing on animal behavior research, reaction time and libido serve as important indicators, providing valuable information about an animal's responsiveness and reproductive health. According to the results obtained from this research, lower concentrations are associated with shorter reaction times and higher libido in rabbits. This indicates a potential dose-dependent influence, implying that lower supplementation levels may positively affect both reaction time and libido, while higher supplementation may have adverse effects. This finding is similar with Iwuji *et al.*, 2020, where higher inclusion levels of *Dialium guineense* leaf meal had an adverse effect on reaction time and libido of rabbit bucks. Since libido scores were lower in treatments where higher dosages of *phyllanthus amarus* aqueous leaf extract were administered, this could imply a reduced testosterone production.

CONCLUSION

It can be concluded from this research that *phyllanthus amarus* aqueous leaf extract had no negative effects on the body temperature and respiratory rates of rabbits. However, it effects on libido and the reaction time of buck are highly dose dependence as lower dosages of the extract demonstrated more favorable effects compared to higher dosages. Further studies are recommended to ascertain the dosage appropriate for reproductive responses of rabbit, particularly in bucks for optimal performance.

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