EFFECT OF *Phyllanthus amarus* AQUEOUS LEAVES EXTRACT ON GUT HISTOMOPHORMETRY OF GROWING RABBIT

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ABSTRACT
The study was carried out to determine the effects of Phyllanthus amarus leaves extract on gut histology of rabbits. Thirty-two weaner rabbits of about 7-8 weeks were randomly divided into four treatments groups, each with eight rabbits in a completely randomized design (CRD). Rabbits in treatment T1 were given only water as placebo, T2, T3 and T4 were given oral administration of 2ml, 4ml and 6ml of Phyllanthus amarus aqueous leaf extract respectively and rabbits in all the treatment were offered the same basal diets which was formulated to meet their nutritional requirements. Phyllanthus amarus leaf powder were taken to the laboratory for phytochemical screening. After 8 weeks feeding trial, the rabbits were slaughtered and dissected, samples from the small intestine were taken for histological evaluations. The phytochemical analysis revealed the presence of tannins (2.05%), flavonoids (5.77%), terpenoids (3.60%), phytates (0.87%), phenols (7.08%), steroids (1.14%) and oxalates (0.12%) respectively. There was a significant higher value ($p<0.05$) in the duodenum villus height of rabbit in treatment 3 (4ml) and width in the control group. The crypt depth was highest ($p<0.05$) in the T4 while lowest was recorded in T3. The villus height and width of the ileum were significantly higher ($p<0.05$) in T2 while the crypt depth was higher in T4 and lowest in T1. In the jejunum, the best results were recorded in T2 with higher values ($p<0.05$) for villus height and width, while T1 and T3 had lowest and higher crypt depth respectively. Histological study also revealed different forms of cellular growth and development of villi epithelium, crypt depth development in the treated groups. The results showed a dose dependence benefit of the plant. This study concluded that excessive administration of aqueous extract of Phyllanthus amarus has potential adverse effect on the microanatomy of the intestinal integrity of rabbits.

Keywords: *Phyllanthus amarus, gut histomorphometrics, rabbits, aqueous extract*

INTRODUCTION
Medicinal plants have long been utilized in the management of various diseases due to the presence of biologically active compounds that can be used directly for therapeutic purposes or as precursors for drug synthesis (Sofowora, 2008; Olafadehan *et al*., 2020). The pharmacological effects of these plants are mainly attributed to phytochemicals, which occur as primary and secondary metabolites. While primary metabolites include proteins and carbohydrates, secondary metabolites such as alkaloids, flavonoids, saponins, phenolics, terpenoids, and tannins are largely responsible for biological activities (Musa *et al*., 2020). These phytochemicals have been reported to exhibit antimicrobial, antioxidant, immunomodulatory, hepatoprotective, and

chemopreventive properties (Aliyu and Adeyina, 2022, Alagbe, 2021).

The World Health Organization (WHO, 1991) reported that over 25,000 plant species are traditionally used worldwide for the treatment of ailments such as malaria, gastrointestinal disorders, anaemia, wounds, and respiratory diseases (Shittu and Alagbe, 2020), the activities of which are mostly linked with presence of these secondary metabolites. The phytochemical composition of various medicinal plants varies depending on factors such as plant age, extraction method, geographical location, harvesting season, storage conditions, and plant part used (Alagbe, 2021). Many medicinal plants are considered relatively inexpensive, effective, and safe, making them attractive for treatment of diseases in man and alternative feed ingredients as well as feed additives in animal production and health management (Wong *et al*., 2008).

One of such medicinal plants is *Phyllanthus amarus*, a member of the Euphorbiaceae family, which is widely distributed across tropical and subtropical regions of the world, including Nigeria, India, Brazil, Ghana, and Kenya (Jantan *et al*., 2019). The plant typically grows as a small, erect, and branched perennial herb, often found in farmlands, roadsides, and forest margins, where it thrives under warm climatic conditions. *Phyllanthus amarus* has gained considerable attention in ethnomedicine due to its extensive use in folkloric medicine, where various parts have been used either as decoctions, infusions (Aliyu *et al*., 2024) or powders.

Traditionally, *Phyllanthus amarus* is used in the management of a wide range of ailments, including liver disorders such as jaundice and hepatitis, diabetes mellitus, urinary tract infections, gastrointestinal disturbances, and certain viral infections (Okafor *et al*., 2008). These uses has also been evaluated in scientific studies and have demonstrated that the plant exhibits significant hepatoprotective and immunostimulatory activities, which may be attributed to its rich phytochemical composition, including lignans, flavonoids, alkaloids, tannins, and phenolic compounds (Negi *et al*., 2008). These bioactive constituents are believed to confer antioxidant, anti-inflammatory and antimicrobial properties, thereby supporting its traditional applications and highlighting its potential as a valuable resource in both human and livestock. Despite these various reported medicinal benefits of *Phyllanthus amarus*, information on the effect of aqueous leaf extract on gut histology of rabbits is limited. Therefore, this study was conducted to evaluate the effects of aqueous leaf extract of *Phyllanthus amarus* on the gut histology of rabbits as its crucial to the growth performance and overall health status of rabbit.

Materials and methods

Experiment Site

This experiment was carried out at the Faculty of Agriculture, Teaching and Research Farm, University of Abuja, along Airport Road, Gwagwalada, Abuja, Nigeria. It is along Latitudes 8^0 57^1 and 8^0 551N and Longitudes 7^0 051 and 7^0 061E. (NASRDA, 2023).

Collection and Processing of the *Phyllanthus amarus* leaf

The leaf of *Phyllanthus amarus* plant was collected within the University of Abuja premises. Taxonomic authentication was conducted in the Department of Crop Science, University of Abuja. The fresh leaves were cleaned, rinsed, and air-dried until completely dried (constant weight was obtained). The dried leaf was grinded into powder using a blender, and the powder was stored in an air-tight container until needed for further analysis.

Experimental animals and managements and design

A total of 32 weaner rabbits (8 weeks old) were

used for this experiment. They were randomly divided into four treatment groups, each consisting of eight (8) rabbits in a completely randomized design. The rabbits were housed individually in a wired cage where water and feed were supplied *ad libitum*. All routine management practices were strictly maintained throughout the experimental period. The four treatment groups were administered varied level of *Phyllanthus amarus* aqueous leaf extract: T1 (0ml of PALE), T2 (2ml of PALE), T3 (4ml of PALE), T4 (6ml of PALE). The control (T1) group were given 2 ml of water as placebo. All the group were fed the same basal diet formulated to meet their nutritional requirements as presented in table 1.

Table 1. Composition of the Experimental Diet

Ingredients	Quantity%
Maize	25.00
Soyabean	20.00
Wheat offal	15.00
Wheat offal	7.00
Rice husk	29.00
Fish meal	1.00
Salt	0.50
Dicalcium phosphate	2.00
Lysine	0.05
Premix	0.45
Total	100.00

Gut Histomorphometry and Organ Assessment

Four rabbits per treatment were selected for organ and gut histomorphometry evaluation. At the end of the 8-week feeding trial, rabbits were fasted for 12 h, stunned and slaughtered. Slaughtering, evisceration, and organ collection were carried out in accordance with the World Rabbit Science Association guidelines as reported by Blasco and Ouhayoun (1996). The gastrointestinal tract was removed and samples of duodenum, jejunum and ileum were collected and fixed in 10% buffered formalin for 24 h prior to histological evaluation (Bancroft and Stevens 2008).

Tissues were dehydrated in graded alcohol series, cleared in xylene, infiltrated and embedded in paraffin wax, thereafter sectioned at 5 µm thickness using a rotary microtome (Leica RT 25). Sections were mounted on slides and stained with hematoxylin and eosin (H and E).

Photomicrographs were captured using a digital microscope camera, and histomorphometric measurements were performed using Digimizer image analysis software (version 4.5). Villus

height was measured from the villus tip to the villus–crypt junction, while crypt depth was defined as the depth of the invagination between adjacent villi. Prepared slides were examined under a binocular light microscope for histological evaluation.

Statistical analysis

All data were subjected to analysis of variance using the general linear models (GLM) procedure of SAS (2004). The significant differences between treatment means were separated using Duncan's multiple range test of the same software (Duncan, 1955).

RESULTS

The phytochemical composition of *Phyllanthus amarus* leaves is presented in Table 2 Phytochemical analysis revealed the presence of tannins (20.5 mg/g), flavonoids (57.7 mg/g), terpenoids (36.0 mg/g), phytates (8.7 mg/g), phenols (70.8 mg/g), steroids (11.4 mg/g) and oxalates (1.2 mg/g).

Table 2. Phytochemical composition of *Phyllanthus amarus* leaves

Parameters	Concentration (mg/g)
Tannins	20.5
Flavonoids	57.7
Terpenoids	36.0
Phytates	8.7
Phenols	70.8
Steroids	11.4
Oxalates	1.2

The duodenum villi morphometric parameters of weaner rabbits supplemented with *Phyllanthus amarus* aqueous leaf extract are shown in table 3. Duodenum villi area (DVA) was significantly higher ($p<0.05$) in T2 and T3 groups. The lowest value was obtained in T4. Duodenum villi height (DVH) followed a similar pattern, with T3 having the highest value ($p<0.05$) though comparable with T1, both significantly higher than T4. The duodenum villi width (DVW) was significantly larger ($p<0.05$) in T1 and similar to T3, however T2 and T3 are also comparable, while T4 had the smallest villi width. Duodenum crypt depth (DCD) was significantly higher ($p<0.05$) in T4, T1 and T2 had similar results while T3 recorded the lowest value.

Table 3. Duodenum villi morphometry of weaner rabbit supplemented with aqueous leaf extract of *Phyllanthus amarus*

Parameter	T1	T2	T3	T4
DVA	23412.92±9493.21^{b}	24329.62±2993.50^{ab}	26679.60±9450.21^{a}	18216.88±2568.81^{c}
DVH	518.94±40.49^{ab}	498.51±23.55^{b}	552.31±44.14^{a}	162.43±25.90^{c}
DVW	131.66±22.26 ^{a}	99.28±3.50 ^{b}	119.02±53.21 ^{ab}	83.88±10.50^{bc}
DCD	251.53±31.81^{b}	250.27±5.92^{b}	236.15±31.12^{c}	260.21±23.24^{a}

a, b, c – means with different superscript are significant along the column, DVA = duodenum villi area; DVH = duodenum villi height; DVW = duodenum villi width; DCD = duodenum crypt depth

The Jejunum villi morphometry of rabbit treated with aqueous leaf extract of *Phyllanthus amarus* is presented in table 4. The jejunum villi area of rabbits on T1, T2 and T3 were similar ($p<0.05$) while T4 had the lowest value. The villi height was significantly ($p<0.05$) higher in T2 while T4 had the lowest value. The jejunum villi width was significantly ($p<0.05$) higher in T1 and T2 with T4 having the least value. There was no significant difference ($p>0.05$) in jejunum crypt depth showing that the rabbit was not influence by the experimental treatments.

Table 4. Jejunum villi morphometry of weaner rabbit supplemented with aqueous leaves extract of *Phyllanthus amarus*

Parameters	T1	T2	T3	T4
JVA	19384.49±4872.52^{b}	23692.73±4610.63^{a}	19857.48±3129.41^{b}	14597.87±254.44^{c}
JVH	459.84±26.15^{b}	499.73±67.41^{a}	457.40±36.51^{b}	383.33±16.73^{c}
JVW	101.98±32.08 ^{a}	99.51±19.13 ^{a}	95.01±7.36 ^{b}	83.33±16.73^{c}
JCD	131.14±33.96 ^{a}	132.58±27.99 ^{a}	103.25±22.78 ^{b}	133.37±20.13 ^{a}

a, b, c – means with different superscript are significant along the column, JVA = jejunum villi area; JVH = jejunum villi height; JVW = jejunum villi width; JCD = jejunum crypt depth

Table 5 shows the ileum villi morphometry of weaner rabbit supplemented with aqueous leaf extract of *Phyllanthus amarus*. The ileum villi area, ileum villi width and ileum crypt depth show a similar pattern as they are significantly ($p<0.05$) higher in treatment 2 compared to other treatments. However, the ileum villi height was significantly ($p<0.05$) higher in T4 and T2 compared with other treatments. The crypt depth was higher in T2 while lowest value was recorded in the control group.

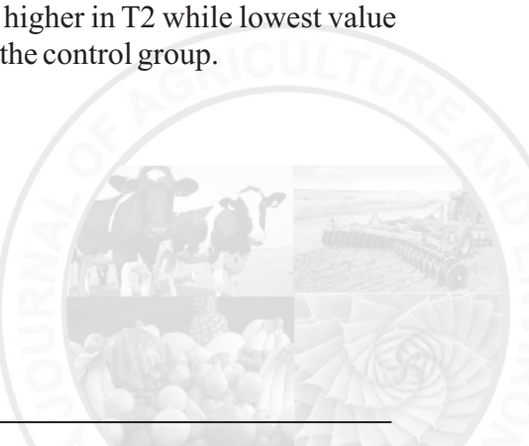

Table: 5 Ileum villi morphometry of weaner rabbit supplemented with aqueous leaf extract of *Phyllanthus amarus*

Parameters	T1	T2	T3	T5
IVA	11756.23±2608.2^{c}	23464.58±24.74^{a}	20599.93±5484.06^{b}	10599.53±5403.45^{c}
IVH	349.81±46.40 ^{b}	387.56±83.35 ^{a}	340.84±54.64 ^{b}	334.64±46.57 ^{c}
IVW	87.75±10.53^{b}	105.82±8.47^{a}	95.27±21.45^{ab}	62.47±29.53^{c}
ICD	98.03±35.23^{c}	113.00±13.47^{bc}	131.03±22.68^{a}	116.87±2953^{b}

a, b, c – means with different superscript are significant along the column, IVA = ileum villi area; IVP = ileum villi parameter; IVH = ileum villi height; IVW = ileum villi width; ICD = ileum crypt depth

DISCUSSION

Phyllanthus amarus leaf revealed richness in phytocompounds such as alkaloids, tannins, flavonoids, terpenoids, phytates, phenols, steroids, and oxalates (table 2). These phytochemicals are important and plays a vital role in animal health. They possess strong antioxidant, anti-inflammatory, immune modulatory and anti-microbial properties among others, making them capable of scavenging free radicals, thus strengthening the immune system and preventing diseases (Shittu *et al*., 2021). Wide range of plant materials contains phytochemicals in varied concentration (Aliyu and Adeyina, 2022, Alagbe *et al*. 2025) and thus have different functionalities (Adewale *et al*., 2021). In this study, phenolics and flavonoids were identified as the predominant phytochemical in *Phyllanthus amarus* leaf. These compounds have been widely reported to enhance intestinal morphology, particularly villus height and width (Awad *et al*., 2011), which by implication could lead to improved absorptive capacity in growing animals. This is achieved with rapid epithelial cell proliferation, leading to an expanded absorptive surface area, improved nutrient utilization, and consequently better growth. The use of *Phyllanthus amarus* aqueous leaf extract in this study proved that it effect is dose dependent. According to the results obtained, rabbits on treatment 3 (4 mL PALE) presented the best performance as seen in the duodenum villi height, width and the crypt dept. Jejunum, the site of absorption of nutrient was best in the group treated with 4 mL PALE as they presented higher values for villi height and width even though the crypt dept were similar to other treatments. The ileum also presented similar results as the jejunum. These results may be attributed to the various functionalities of phytocompounds present in the plant. Their therapeutic properties are capable of performing multiple biological functions such as antibacterial, antiviral, anti-inflammatory, anti-helminthic, antidiuretic, antioxidants, antifungal, antispasmodic, immune-modulatory, neuro-protective, anti-inflammatory and neuroprotective functions (Adewale *et al*., 2021; Singh *et al*., 2021). Phytochemicals have also been reported to be safe and effective without causing any negative effective on animals once administered in right doses and concentrations (Oluwafemi *et al*., 2021).

The results support previous work of Pelicano *et al.,* (2003) and Loddi *et al.,* (2004) who reported that *Phyllanthus amarus* play regenerative and absorptive role of intestine by increasing surface areas of gut cells. Other similar finding in birds by Laudadio *et al.,* (2012) suggested that a level of 20.5% concentration in the diet is optimal for maximizing growth performance and absorptive capacity in broiler chickens.

Similarly, Pelicano *et al.,* (2007) reported that the use of growth promoters increased intestinal villi height when *Bacillus subtilis* was associated to prebiotics; suggesting that feeding and introduction of feed additives in animal diets can influence intestinal mucosa development hence improve growth performance.

CONCLUSION AND RECOMMNDATION

The application of *Phyllanthus amarus* aqueous leaf extract in growing rabbit revealed that it can positively influence the small intestine mucosa lining. However, the application requires caution since the performance observed was dose dependent as also observed by Okanlawon *et al*., (2020). Overall, the rabbits treated with 4ml *Phyllanthus amarus* aqueous leaf extract had the best intestinal villi development and moderate crypt depth.

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