Research Square

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Impact of Phyllanthus amarus on reproductive functions in hemorrhoid-induced male Wistar rats

Oluwakemi Oyelowo

ooyelowo@unilag.edu.ng

University of Lagos

Ruth Ojo

University of Lagos

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Abstract

Background Hemorrhoids represent a widespread anorectal disorder with potential systemic implications that extend beyond localized symptoms. This study investigated the therapeutic efficacy of *Phyllanthus amarus* leaf extract on reproductive parameters in male Wistar rats with jatropha oil-induced hemorrhoids. Twenty-five male Wistar rats (90-160g) were randomly allocated into five groups: Control, Hemorrhoid-induced (H), Hemorrhoid-induced + Pilex 200mg/kg (H+P), Hemorrhoid + Extract 200mg/kg (H+E200mg), and Hemorrhoid + Extract 400mg/kg (H+E400mg). Following hemorrhoid induction, treatments were administered orally for ten days. Reproductive parameters, including sperm count, motility, morphology, serum testosterone levels, oxidative stress markers, and TNF-α, were evaluated.

Results The results revealed a significant increase in the testosterone level in the H+E200mg when compared to the control, H, H+P groups. Hemorrhoidal inflammation significantly compromised sperm motility, count, and morphological integrity, while treatment with *Phyllanthus amarus* extract demonstrated dose-dependent ameliorative effects, with the 400mg/kg dose showing superior restoration of sperm variables and in the CAT and GSH antioxidant defense mechanisms compared to the control, H, H+P, and H+E200mg groups. Inflammatory cytokine (TNF-α) levels and macroscopic hemorrhoidal scores improved substantially following the administration of the extract.

Conclusion According to the findings of this study, Phyllanthus amarus exhibits significant protective potential against hemorrhoid-induced reproductive dysfunction through its antioxidant, anti-inflammatory, and tissue-protective properties, suggesting therapeutic promise for managing reproductive complications associated with chronic anorectal inflammatory conditions.

Background

Hemorrhoids are a common anorectal disorder resulting from dilated vascular structures within the anal canal and are a significant global health issue that increases in incidence with age (Kibret et al., 2021). They are often referred to as piles in many African contexts (Azeez & Isiugo-Abanihe, 2017). Hemorrhoids are thought to be caused by increased abdominopelvic pressure that is uniformly transmitted to the rectal veins, most commonly due to straining with bowel movements from chronic constipation and sitting for long periods of time. Symptoms can present as painless bleeding and itching to painful swelling and discomfort (Azeez & Isiugo-Abanihe, 2017). The clinical symptoms include rectal bleeding, anal itching, mucous discharge, and a feeling of incomplete evacuation (Patcharatrakul & Rao, 2018). Serious complications include thrombosed external hemorrhoids that lead to acute pain and swelling, and chronic complications present with skin irritation, infection, and anemia due to chronic bleeding (Patcharatrakul & Rao, 2018). Each impact of hemorrhoids leads to a decreased quality of life, and for many cultures, the problem is exacerbated by stigma that prevents open discussion and treatment (Azeez & Isiugo-Abanihe, 2017).

Dietary patterns are key contributors to the treatment of hemorrhoids. Historically, traditional African diets typically contained high amounts of fiber due to unrefined carbohydrates, fruits, and vegetables, all

of which provided natural protection against constipation and excessive hemorrhoidal bulging (Azeez & Isiugo-Abanihe, 2017). In recent years, because of urbanization, Westernized diets made of processed foods, sugars and low fiber diets have prevailed (Labidi et al., 2019). Low fiber diets make stools firmer and require straining, which increases anal venous pressure, further worsening hemorrhoidal symptoms (Labidi et al., 2019).

Epidemiological information shows that hemorrhoids affect both sexes, but rates of prevalence and presentation differ. Some studies show a higher incidence in men (Kibret et al., 2021), whereas other studies report a marginally higher prevalence in women, often in relation to pregnancy (D'Alfonso et al., 2024). In many parts of Africa, limited access to healthcare and the cost of formal treatment are factors in seeking traditional treatment (Kacholi et al., 2022). Besides, plants and their derivatives are an ancient foundation to medicinal practices and remain an important source of therapeutic compounds today (Ahmad et al., 2025; Ekpo et al., 2019).

Phyllanthus amarus is an annual herbaceous plant, that belongs to the Euphorbiaceae family, typically growing to a height of between 10 and 50 cm. *Phyllanthus amarus*, a tropical plant, bears small leaves, green capsules on the stems, and minuscule fruits that dehisce when dried (Ekpo et al., 2019), is also commonly referred to as "stonebreaker". In Yoruba (Southwest Nigeria), it is referred to as “Iyin-olobe”; in Ibibio it is called “Oyomo ke iso Amankedem”, while in Asaba it is called, “Buchi oro” (Bose et al., 2022; Ekpo et al., 2019). It has become widely accepted as a traditional medicine for the treatment of digestive disturbances, infections, and inflammations across Africa and Asia (Bose et al., 2022). Most of its pharmacological properties, including hepatoprotective, antioxidant and antimicrobial activity properties, are credited to its bioactive compounds, which include alkaloids, flavonoids and triterpenes, which provide analgesics and help in repairing injured tissue (Nisar et al., 2018).

This research aims to assess the effects of *Phyllanthus amarus* on reproductive variables in male Wistar rats with jatropha oil-induced hemorrhoids. With evidence documenting that some herbal medications may impact male reproductive function and reduce fertility or improve it, this study will seek to establish the capacity of *Phyllanthus amarus* to either protect or cause damage to male reproductive functions.

Materials and methods

Collection and identification of plants

Leaves of *Phyllanthus amarus* were collected from a family farm in Ilorin, Kwara state, North-Central Nigeria in the month of October 2024. The plant was identified and authenticated by Mr Nodza George of the Department of Botany, University of Lagos. The plant was deposited at the Botany Department in the University of Lagos with an identification number of LUH: 100597.

Extraction of plant materials

The leaves were cleaned and air-dried for two weeks and then pulverized into coarse powder using an electric grinder and sieved to control the particle size. 2gm of powdered leaves were added in 20 mL of distilled water in a clean beaker and boiled for 10 minutes, (since it is taken as a tea in folkloric use). The solution was allowed to cool and filtered through a Whatman No. 1 filter paper. The filtrate was stored in an amber bottle and kept in a refrigerator for use.

Experimental Animals

Twenty-five male Wistar rats weighing 110-160g were used in this study. The animals were obtained from the Animal House of the College of Medicine, University of Lagos. The animals were housed in plastic cages with paper bedding, kept at a room temperature of 29 ± 2°C, under a 12-hour light/dark cycle. The animals were fed standard rat chow and provided with clean water *ad libitum* throughout the adoption period of acclimatization. The experiment lasted for 10 days.

Animal Ethics Declaration

Institutional and national guidelines for the care and use of animals were followed. The study received approval from the Health Research Ethics Committee of the College of Medicine, University of Lagos with approval number (CMUL HREC number: CMUL/ACUREC/03/25/1868). Our manuscript adheres to ARRIVE guidelines.

Animal Grouping

Rats were randomly divided into five groups of five animals each:

- **Control**: Standard feed and water
- **H**: Hemorrhoid-induced with jatropha oil; standard feed and water
- **H + P**: Hemorrhoid-induced with jatropha oil and treated with 200mg/kg Pilex tablet
- **H+E200mg**: Hemorrhoid-induced with jatropha oil and treated with 200mg/kg *Phyllanthus amarus* extract
- **H+E400mg**: Hemorrhoid-induced with jatropha oil and treated with 400mg/kg *Phyllanthus amarus* extract

Hemorrhoid Induction

Hemorrhoids were induced in all animals except the control animals by inserting cotton swabs into the anus (recto-anal portion, 20 mm from the anal opening). Sterile cotton swabs (4 mm in diameter) were soaked in jatropha oil (100 µL) once a day for five consecutive days, while restraining the animal for 10 seconds. On day 5 of induction, Evans Blue (EB) dye (30 mg/kg) was administered by injecting into the animals' tail veins, 30 minutes before application of the jatropha oil preparation to quantify plasma

exudation (Takvou et al., 2024). Following the induction, the rats were observed for signs of pile, such as swelling near the base of the tail, sitting hunched, and their treatment with the respective plant extracts or standard drug commenced after the fifth day. From the sixth day, the animals received 10 mL/kg of distilled water daily for 10 days (normal and negative control); 200mg/kg of Pilex tablet (positive control), and the *Phyllanthus amarus* extract at doses of 200 and 400 mg/kg (for the 2 test groups). Body weight was assessed daily in all animals. On the eleventh day, all animals were sacrificed by cervical dislocation.

Body Weight and Food Intake Monitoring

Animal weights were recorded daily for 10 days using an electronic weighing scale. Food intake was monitored daily and measured.

Collection of blood samples and organ collection

At study termination, animals were humanely sacrificed following overnight fasting. Blood samples were collected via ocular puncture into plain sample bottles and centrifuged at 3000 rpm for 10 minutes. Serum was extracted using a micropipette and stored at 2–8°C until analysis. The testes, epididymis, anorectal tissue, seminal vesicle, prostate, liver, and kidneys were carefully excised using sterile instruments, rinsed in cold normal saline, and weighed using a digital analytical balance (accuracy: 0.001g).

Anorectal Coefficient (ARC) Measurement

After euthanasia, the rats were dissected, and tissue samples from the anorectal region were cleaned, trimmed of excess fat, and each measuring 20 mm in length, were collected and weighed. The ARC was then calculated using the following formula:

$$ARC = \frac{\text{Weight of anorectal tissue(mg)}}{\text{Body weight of Rat(g)}}$$

Afterwards, the anorectal tissue samples were homogenized in phosphate-buffered saline and kept in the freezer for further biochemical analysis.

Assessment of Macroscopic Severity Score

The anorectal tissue samples were fixed on ordinary paper, and the macroscopic severity of these tissue samples was assessed and recorded as previously described (Ai et al., 2025). Briefly, the assessment

criteria included the degree of anal swelling, the extent of rectal mucosal damage, color intensity, and tissue elasticity. The scoring system ranges from 0 to 3, detailed as follows:

normal (0), tissue appears normal in color with no abnormal vascular dilation or surface damage; mild symptoms (1), tissue exhibits slight inflammation or swelling with a reddish discoloration; moderate swelling (2), redness, or vascular congestion;

severe symptoms (3), tissue shows significant inflammation, severe hemorrhoidal swelling with prolapse or visible nodules swelling, or even bleeding.

The observations and scoring were conducted by two experienced independent reviewers who were fully blinded to the treatment groups.

Serum Lactate Dehydrogenase (LDH) and Tumor Necrosis Factor-alpha (TNF-α)

Serum lactate dehydrogenase (LDH) was measured by standardized enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Antrim, UK), while the level of

TNF-α was measured using Rat TNF-α ELISA kit based on the sandwich-ELISA principle, with optical density measured at 450nm.

Lipid peroxidation and antioxidant analysis

Anorectal homogenate and serum lipid peroxidation were determined by measuring Thiobarbituric Acid Reactive Substances (TBARS/MDA) produced during lipid peroxidation according to the method of Uchiyama and Mihara, (1978). Serum catalase activity was assessed according to the method of Aebi (1984). Assessment of serum superoxide dismutase (SOD) activity was determined according to the method of Sun and Zigman (1978). The serum reduced glutathione (GSH) level was measured by a spectrophotometric assay kit (Oxford Biomedical Research, USA).

Sperm function analysis

The caudal epipidymal tissue was removed and placed in a Petri dish containing 1 mL normal saline (0.9% NaCl) solution. An incision of about 1 mm was made in the caudal epididymis so as to liberate its spermatozoa into the saline solution. Progressive sperm motility, sperm count and sperm viability were then examined under the microscope using ×40 objective as previously described by Adekunbi et al., (2016). Epididymal sperm motility was determined by calculating motile spermatozoa per unit area and was expressed as percent motility. Epididymal sperm counts were evaluated using the improved Neubauer hemocytometer and were expressed as million/ml of suspension. The sperm viability was also determined using eosin/nigrosin stain. The motile (live) sperm cells were unstained while the non-motile (dead) sperms absorbed the stain. The stained and unstained sperm cells were counted and an average value for each was recorded from which percentage viability was calculated. Sperm morphology was evaluated by staining the sperm smears on microscope slides with two drops of Walls and Ewa stain

after they were air-dried. The slides were examined under the microscope under oil immersion with ×100 objectives. The abnormal sperms were characterized based on the presence of double tails, detached heads, detached tails, mid piece bending and irregular heads. The relative proportions of normal and abnormal sperm were expressed in terms of percentages.

Testosterone analysis

Testosterone was assessed by enzyme-linked immunosorbent assay (ELISA) using ELISA assay kits (Acc-Bind Elisa microwell monoband (USA).

Statistical Analysis

Data are presented as Mean ± SEM. Statistical analysis was performed using GraphPad Prism version 8.0 (San Diego, California, USA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for pairwise comparisons between groups. Statistical significance was set at $p < 0.05$.

Results

Body Weight

On day 1, there was a significant increase in body weight of H, H + P, H + E 200mg and H + E 400mg groups when compared to control group. There was also a significant increase in body weights of H + P, H + E 200mg and H + E 400mg groups when compared to H group. There was a significant increase in the body weight of H + E 400mg group when compared to H + P group. There was also a significant increase in the body weight of H + E 400mg group when compared to H + E 200mg group.

On day 2 there was a significant increase in the body weight of H, H + P, H + E 200mg, and H + E 400mg groups when compared to the control group. There was also a significant increase in body weight of H + P, H + E 200mg, and H + E 400mg groups when compared to H group. There was a significant increase in body weight in the H + E 400mg groups compared to the H + P group.

There was a significant increase in the body weight of H + E 400mg group when compared to H + E 200mg group.

On day 3, there was a significant increase in body weight of H, H + P, H + E 200mg and H + E 400mg groups when compared to control group. There was also a significant increase in body weight of H + P, H + E 200mg and H + E 400mg groups when compared to H group. There was a significant increase in body weight of H + E 400mg group when compared to H + P group. There was a significant increase in body weight of H + E 400mg group when compared to H + E 200mg group.

On day 4, there was a significant increase in the body weight of the H, H + P, H + E 200mg and H + E 400mg groups when compared to control group. There was a significant increase in body weight of the H + P, H + E 200mg and H + E 400mg groups when compared to H group. There was also a significant increase in body weight of the H + E 400mg group when compared to H + P.

A significant increase in body weight was also observed in the H + E 400mg group when compared to H + E 200mg group.

On day 5 there was a significant increase in the body weight of H, H + P, H + E 200mg and H + E 400mg groups when compared to control group. There was also a significant increase in the body weight of H + P, H + E 200mg and H + E 400mg groups when compared to H group. There was a significant increase in the body weight of H + E 400mg group when compared to H + P group. There was a significant increase in body weight of H + E 400mg group when compared to H + E 200mg group.

On day 6, there was a significant increase in body weight of H, H + P, H + E 200mg and H + E 400mg groups when compared to the control group. There was a significant increase in body weight of H + P, H + E 200mg and H + E 400mg groups when compared to the H group. There was also a significant increase in the body weight of H + E 400mg when compared to H + P, H + E and H + E 200mg groups respectively.

On day 7, there was a significant increase in body weight of the H, H + P, H + E 200mg and H + E 400mg groups when compared to control group. There was a significant increase in body weight of H + P, H + E 200mg and H + E 400mg when compared to H. There was also a significant increase in body weight of H + E 400mg group when compared to H + P and H + E 200mg groups

On day 8, there was a significant increase in the body weight of the H, H + P, H + E 200mg and H + E 400mg groups when compared to control group. There was a significant increase in body weight of H + P, H + E 200mg and H + E 400mg groups when compared to H.

There was also a significant increase in body weight of H + E 200mg and H + E 400mg groups when compared to the H + P group.

On day 9, there was a significant increase in body weight of H, H + P, H + E 200mg and H + E 400mg groups when compared to control group. There was a significant increase in body weight of H + P, H + E 200mg and H + E 400mg groups when compared to the H group. There was a significant increase in body weight of H + E 200mg and H + E 400mg groups when compared to the H + P group. There was also a significant increase in the body weight of H + E 400mg group when compared to H + E 200mg group.

On day 10, there was a significant increase in body weight of the H, H + P, H + E 200mg and H + E 400mg groups when compared to control group. There was also a significant increase in the body weight of the H + P, H + E 200mg and H + E 400mg groups when compared to H group. There was a significant increase in body weight of H + E 200mg and H + E 400mg groups when compared to H + P group.

Food Intake Patterns

On day 1, there was a significant increase in the food intake of H, H + P, H + E 200mg and H + E 400mg groups when compared to the control group. There was also a significant increase in food intake of H + P, H + E 200mg and H + E 400mg groups when compared to the H group. There was a significant

decrease in food intake of the H + E 200mg and H + E 400mg groups when compared to H + P group. There was also a significant decrease in food intake of the H + E 400mg group when compared to the H + E 200mg group.

On day 2, there was also a significant increase in food intake of H, H + P, and H + E 200mg groups when compared to the control group, like on day 1. There was a significant decrease in food intake of H + E 400mg group when compared to the control group. There was a significant increase in food intake of H + P and H + E 200mg groups when compared to the H group. There was a significant decrease in food intake of the H + E 400mg group when compared to the H group. There was a significant increase in food intake of the H + E 200mg group when compared to the H + P group. There was a significant decrease in food intake H + E 400mg group when compared to H + P group.

There was a significant decrease in food intake H + E 400mg group when compared to H + E 200mg.

On day 3, there was a significant increase ($P < 0.05$) in food intake of H, H + P, H + E 200mg, and H + E 400mg groups when compared to the control group. There was also a significant increase in food intake of the H + P, H + E 200mg, and H + E 400mg groups when compared to H group. There was a significant decrease in the food intake of H + E 200mg and H + E 400mg groups when compared to the H + P group. There was also a significant decrease in food intake of H + E 400mg group when compared to H + E 200mg group.

On day 4, there was a significant increase in food intake of H, H + P, H + E 200mg and H + E 400mg groups when compared to the control group. There was, however, a significant decrease in food intake of H + P, H + E 200mg, H + E 400mg groups when compared to the H group.

There was a significant decrease in the food intake of the H + E 200mg group when compared to the H + P group. There was a significant increase ($P < 0.05$) in food intake of the H + E 400mg group when compared to the H + P and H + E 200mg groups, respectively.

On day 5, there was a significant increase in the food intake of H, H + P, H + E 200mg, and H + E 400mg groups when compared to the control group. There was also a significant increase in food intake of the H + P, H + E 200mg, and H + E 400mg groups when compared to the H group. There was a significant increase in food intake of the H + E 200mg group when compared to the H + P group. There was a significant decrease ($P < 0.05$) in food intake of the H + E 400mg group when compared to the H + P and H + E 200mg groups, respectively.

On day 6, there was a significant decrease in food intake of the H, H + P and H + E 200mg groups when compared to the control group. There was a significant increase in food intake of the H + E 400mg group compared to the control group. There was a significant decrease ($P < 0.05$) in the food intake of the H + P and H + E 200mg groups when compared to the H group. There was a significant increase ($P < 0.05$) in food intake of the H + E 400mg group when compared to the H, H + P, and H + E 200mg groups,

respectively. There was a significant decrease ($P < 0.05$) in food intake of the H + E 200mg group when compared to the H + P group.

There was a significant decrease, on day 7, ($P < 0.05$) in the food intake of the H + P group when compared to the control group. There was a significant increase in the food intake of the H + E 200mg and H + E 400mg groups when compared to the control group. There was a significant decrease in food intake of the H + P group when compared to the H group. There was a significant increase in the food intake of the H + E 200mg and H + E 400mg groups when compared to the H group. There was a significant increase in the food intake of the H + E 200mg and H + E 400mg groups when compared to the H + P group. There was a significant decrease in the food intake of the H + E 400mg group when compared to the H + E 200mg group.

On day 8, there was a significant increase in food intake of H, H + P, H + E 200mg, and H + E 400mg groups when compared to the control group. There was a significant decrease in food intake of the H + P group when compared to the H group. There was a significant increase in food intake of H + E 200mg and H + E 400mg groups when compared to the H group. There was a significant increase in food intake of the H + E 200mg and H + E 400mg groups when compared to the H + P group. There was a significant decrease in food intake of H + E 400mg group when compared to H + E 200mg group.

On day 9, there was a significant increase in the food intake of H, H + P, H + E 200mg, and H + E 400mg groups when compared to the control group. There was a significant decrease in food intake of H + P and H + E 400mg groups when compared to the H group. There was a significant increase in food intake of the H + E 200mg group when compared to the H group. There was a significant increase in the food intake of H + E 200mg group when compared to H + P group. There was a significant decrease in the food intake of H + E 400mg group when compared to H + P and H + E groups, respectively.

On day 10, there was a significant increase ($P < 0.05$) in food intake of H, H + P, H + E 200mg and H + E 400mg groups when compared to the control group. There was a significant decrease in the food intake of the H + P group when compared to the H group. There was a significant increase in the food intake of the H + E 200mg and H + E 400mg groups when compared to the H group.

There was a significant increase in the food intake of H + E 200mg and H + E 400mg groups when compared to the H + P group. There was also a significant increase ($P < 0.05$) in food intake of the H + E 400mg group when compared to the H + E 200mg group.

Organ Weights

Testis: Significant increases occurred in all hemorrhoid-induced groups versus controls ($p < 0.05$). The treatment groups (H + P, H+E200mg, H+E400mg) showed further elevation compared to hemorrhoid-only animals.

Epididymis: The H + P and H+E400mg groups demonstrated significant increases compared to the control and hemorrhoid-only (H) groups ($p < 0.05$). The epididymal weight in the H + E 200mg was

significantly reduced compared to the H + P group. The organ weight of the H + E 400mg group was significantly increased when compared to the H + E 200mg group.

Seminal Vesicle and Prostate: All treatment groups showed significant increases versus the control and H groups.

Anorectal Tissue: Significant weight increases were observed across groups, with H+E400mg showing the most pronounced elevation ($p < 0.05$) when compared to other groups. A decrease in the tissue weight was observed in the H+200mg group, while an increase was observed in the H+E400mg group when compared to the H + P group. An increase was also observed in the H+E400mg group when compared to the H + E 200mg group.

Liver: There was a significant increase in liver weights in all groups when compared to the control group as well as a significant increase in the weights of the H + P, H+E400mg groups when compared to the H group.

Kidney: There was a significant increase in the kidney weights in all groups when compared to the control group as well as a significant increase in the weights of the H + P, H+E200mg, and H+E400mg groups when compared to the H group.

Table 1
Effect of *Phyllanthus amarus* leaves extract on organ weight in hemorrhoid- induced male Wistar rats

Organ/Group	Control	H	H + P	H+E200mg	H+E400mg
Testis	1.45 ± 0.03	1.64 ± 0.02*	2.10 ± 0.04*#	2.15 ± 0.03*#	2.15 ± 0.03*#
Epididymis	0.10 ± 0.003	0.10 ± 0.003	0.16 ± 0.006*#	0.10 ± 0.003α	0.16 ± 0.006*#β
Anorectal	0.10 ± 0.003	0.12 ± 0.004*	0.14 ± 0.003*#	0.12 ± 0.004*α	0.20 ± 0.003*αβ
Seminar vesicle	0.10 ± 0.004	0.16 ± 0.0003*	0.48 ± 0.01*#	0.36 ± 0.02*#α	0.36 ± 0.02*#α
Prostate	0.10 ± 0.004	0.10 ± 0.004	0.14 ± 0.001*#	0.12 ± 0.001*#α	0.12 ± 0.0006*#α
Liver	2.80 ± 0.02	3.84 ± 0.02*	4.06 ± 0.02*#	3.76 ± 0.02*α	4.12 ± 0.01*#β
Kidney	0.64 ± 0.01	0.80 ± 0.003*	0.88 ± 0.01*#	0.86 ± 0.006*#	0.94 ± 0.0006*#αβ

Values are expressed as mean ± SEM of 5 rats per group, * = $p < 0.05$ when compared to control group, # = $p < 0.05$ when compared to H, α = $p < 0.05$ when compared to H + P, β- = $p < 0.05$ when compared to H + E 200mg. Hemorrhoid (H), Hemorrhoid + Pilex (H + P), and Hemorrhoid + *Phyllanthus amarus* extract (H+E200 and H+E400)

Sperm variables

Effect of Phyllanthus amarus leaves extract on sperm motility in male Wistar rats

There was a significant increase in the sperm motility of the H + P, H + E 200mg and H + E 400mg groups when compared to the control group. There was a significant increase in the sperm motility of the H + P and H + E 400mg groups when compared to H group. There was a significant decrease in the sperm motility of the H + E 200mg group when compared to H + P group. There was a significant increase in H + E 400mg group when compared to H + E 200mg group.

Effect of Phyllanthus amarus leaves extract on sperm count in male Wistar rats

There was a significant increase in the sperm count in all groups when compared to the control group. There was a significant increase in the sperm count in H + P and H + E 400mg groups when compared to H group. There was a significant decrease in the H + E 200mg group and a significant increase in H + E 400mg group when compared to H + P group respectively. There was a significant increase in H + E 400mg group when compared to H + E 200mg group.

Effect of Phyllanthus amarus leaves extract on normal sperm morphology in male Wistar rats

There was a significant increase in the normal sperm morphology of the H and H + E 400mg groups when compared to the control group. There was increase in normal sperm morphology of the H + P, H + E 200mg and H + E 400mg groups when compared to H group. There was a significant increase in normal sperm morphology of the H + E 400 mg group when compared to H + P group. There was a significant increase in normal sperm morphology of the H + E 400mg group when compared to H + E 200mg group.

Effect of Phyllanthus amarus leaves extract on abnormal sperm morhology in male Wistar rats

There was a significant increase in abnormal sperm morphology of the H, H + P and H + E 200mg groups when compared to the control group. There was a significant decrease in abnormal sperm morphology of H + E 400mg group when compared to the control group. There was a significant increase in abnormal sperm morphology of H + E 200mg group when compared to H group. There was a significant decrease in H + E 400mg group when compared to H, H + P and H + E 200mg groups respectively.

Oxidative Stress Markers

There was a significant increase in the SOD level of all groups when compared to the control group. There was a significant decrease in the SOD level of H + P and H + E 400mg groups when compared to H group. There was a significant increase in the SOD level of H + E 200mg group when compared to the H group. There was a significant increase in SOD level of the H + E 200mg and H + E 400mg groups when compared to the H + P group. There was a significant decrease in the SOD level of H + E 400mg group when compared to the H + E 200mg group.

There was a significant decrease in the CAT level of H and H + E 200mg groups when compared to control group. There was however a significant increase in the CAT level of H + E 400mg group when compared to control group. There was a significant increase in the CAT level of H + P and H + E 400mg groups when compared to H group. There was a significant decrease in the CAT level of the H + E 200mg group when compared to H + P group. There was a significant increase in CAT level of H + E 400mg group when compared to the H + E 200mg group. There was a significant increase in the MDA level of H and H + P groups when compared to the control group. There was a significant decrease in the MDA level the H+400mg group when compared to control group. There was a significant decrease in the MDA level H + E 200mg and H + E 400mg groups when compared to the H group. There was a significant decrease in the MDA level of the H + E 200mg and H + E 400mg groups when compared to the H + P group. There was also a significant decrease in the MDA level of the H + E 400mg group when compared to the H + E 200mg group.

There was a significant increase in the GSH level of the H + P, H + E 200mg and H + E 400mg groups when compared to control group. There was a significant increase in the GSH level of the H + E 200mg and H + E 400mg groups when compared to the H group. There was a significant increase in the GSH level of the H + E 200mg group when compared to the H + P group. There was a significant increase in the GSH level of the H + E 400mg group when compared to the H + E 200mg group.

Testosterone and Lactate Dehydrogenase (LDH) Levels

There was a significant increase in the testosterone level of the H and H + E 200mg groups when compared to the control group. There was a significant decrease in the testosterone level of the H + P and H + E 400mg groups when compared to the control group. There was a significant decrease in the testosterone level of the H + P and H + E 400mg groups when compared to H group.

There was a significant increase in the testosterone level of the H + E 200mg when compared to the H and H + P groups respectively. There was a significant decrease in the testosterone level of the H + E 400mg group when compared to the H + E 200mg group.

There was a significant decrease in the LDH level of all groups when compared to the control group. There was a significant decrease in the LDH level of H + P and H + E 400mg groups when compared to H group. There was a significant increase in the LDH level of H + E 200mg and H + E 400mg groups when compared to H + P group. There was a significant decrease in LDH level of H + E 400mg group when compared to H + E 200mg group.

Tumor Necrosis Factor-alpha (TNF-α)

There was a significant decrease in the Tumor Necrosis Factor alpha (TNF-α) level of H, H + E 200mg and H + E 400mg groups when compared to control group. There was a significant increase in TNF-α level of H + P group when compared to control group. There was a significant increase in TNF-α level of H + P group when compared to H group. There was a significant decrease in TNF-α level of H + E 200mg and H + E 400mg groups when compared to H group. There was a significant decrease in the TNF-α level of the H + E 200mg and H + E 400mg groups when compared to H + P group. There was a significant decrease in the TNF-α level of the H + E 400mg group when compared to H + E 200mg group.

Anorectal Tissue Assessment

Anorectal MDA

There was a significant increase in the MDA level of all groups when compared to control group.

There was a significant decrease in the MDA level of H + P and H + E 400mg groups when compared to H group. There was a significant increase in the MDA level of the H + E 200mg group when compared to H group. There was a significant increase in MDA level of H + E 200mg group when compared to H + P group. There was a significant decrease in MDA level of H + E 400mg group when compared to H + E 200mg group.

There was a significant decrease in the anorectal/body weight index of H, H + P and H + E 200mg groups when compared to the control group. There was a significant decrease in anorectal/body weight level of H + E 200mg group when compared to H group. There was a significant increase in anorectal/body weight index of H + E 400mg group when compared to H group. There was a significant decrease in

anorectal/body weight index of the H + E 200mg group when compared to H + P group. There was a significant increase in the anorectal/body weight index of H + E 400mg group when compared to H + P group. There was a significant increase in the anorectal/body weight index of H + E 400mg group when compared to H + E 200mg group.

Anorectal/Body Weight Ratio:

Macroscopic Scoring

There was a significant increase in the macroscopic scoring of all groups when compared to control group. There was a significant increase in macroscopic scoring of H + P group when compared to H group. There was a significant decrease in the macroscopic assessment level of H + E 200mg and H + E 400mg groups when compared to H group. There was a significant decrease ($P < 0.05$) in macroscopic assessment level of H + E 200mg and H + E 400mg groups when compared to H + P group. There was also a significant decrease in the macroscopic assessment level of the H + E 400mg group when compared to the H + E 200mg group.

Discussion

Hemorrhoid-induced animals showed a consistent increase in body weight throughout the experiment duration, which represents the systemic inflammatory response and metabolic strain associated with chronic anorectal inflammation. The pattern of weight gain corresponds to chronic inflammatory conditions that alter energy metabolism and lead to fluid retention due to inflammatory mediators (Wang & Ye, 2015). *Phyllanthus amarus* treatment, particularly at 400mg/kg, demonstrated superior weight normalization compared to standard hemorrhoid treatment, suggesting its capacity to modulate hemorrhoid-induced metabolic dysfunction.

The feeding patterns indicated complex interactions involving hemorrhoidal stress and metabolic demands, as well as treatment implications. The variable consumption patterns observed across treatment days indicate that hemorrhoidal inflammation disrupts normal feeding behaviors through pain, discomfort, and systemic inflammatory responses. *Phyllanthus amarus* extract showed dose-dependent normalization of these feeding disruptions, with treated groups generally demonstrating improved appetite regulation, consistent with previous research of appetite improvements following plant extract administration (Aliyu et al., 2021). Food intake varied considerably across treatment days. These fluctuations suggest complex interactions between hemorrhoidal stress, metabolic demands, and treatment effects on appetite regulation.

Hemorrhoidal inflammation significantly impacted reproductive organ weights, demonstrating the systemic nature of anorectal conditions on male reproductive health. The increases in testicular and epididymal weights likely occurred due to inflammation-mediated swelling of the tissue, and possibly compensatory hypertrophy due to a stressor to reproductive health. Research has established connections between hemorrhoids and erectile dysfunction (Abdelaziz et al., 2019), supporting the

concept that anorectal inflammation affects circulation and function in reproductive organs through shared vascular pathways. The elevated seminal vesicle and prostate weights further confirm the systemic reproductive impact of anorectal inflammation. Males presenting with hemorrhoids exhibit a predisposition for erectile dysfunction (Al-Kubati & Radman, 2024), illustrating the interconnectedness of serial reproductive systems and anorectal health. The *Phyllanthus amarus* extract exhibited normalizing effects on the weight alterations in these organs, possibly due to its demonstrable anti-inflammatory mechanisms that limit tissue swelling and inflammatory responses (Harikrishnan et al., 2018).

Hemorrhoidal inflammation critically compromised sperm motility, establishing a direct relationship between anorectal health and reproductive function. Chronic hemorrhoidal conditions impair sperm motility through systemic inflammatory mediator effects on spermatogenesis and sperm maturation processes (Potiris et al., 2025). Pro-inflammatory cytokines such as IFN-γ, IL-17A, and IL-1β significantly reduce sperm motility through increased oxidative stress and apoptosis (Paira et al., 2022). The hemorrhoid-induced reduction in sperm motility was effectively ameliorated by *Phyllanthus amarus* extract in a dose-dependent manner, with the 400mg/kg dose providing superior improvement. This restoration demonstrates the plant's capacity to counteract inflammation-mediated damage to sperm function, consistent with previous findings of enhanced sperm motility following plant extract treatment (Azubuike et al., 2018). Sperm count alterations reflected disrupted spermatogenesis through inflammation-mediated damage. Cytotoxic substances and lytic enzymes released by leukocytes intensify inflammation, oxidative stress, and tissue fibrosis, impairing testicular function (Agarwal et al., 2018). Inflammation disrupts spermatogenesis and steroidogenesis through cytokine- and reactive oxygen species-mediated damage to testicular cells (Fomichova et al., 2025). *Phyllanthus amarus* extract effectively modulated these hemorrhoid-induced changes, with the 400mg/kg dose showing superior protective effects. Sperm morphological integrity was significantly compromised by hemorrhoidal inflammation, demonstrating a direct impact on developing sperm cells during spermatogenesis. Oxidative stress impairs sperm function through lipid peroxidation, DNA fragmentation, and protein damage (Wang et al., 2025). The chronic inflammatory state increases abnormal sperm morphology through inflammatory mediators and oxidative stress that damage developing sperm cells (Azenabor et al., 2015). Oxidative damage reduces membrane fluidity, impacting motility and fertilizing capacity (Sengupta et al., 2024). *Phyllanthus amarus* extract provided dose-dependent protection against hemorrhoid-induced morphological damage, with the 400mg/kg dose offering superior protection through its antioxidant properties that counteract inflammation-induced.

Hemorrhoidal inflammation triggered substantial oxidative stress, as evidenced by elevated MDA levels, demonstrating that chronic anorectal inflammation generates systemic oxidative damage. Male reproductive function is highly susceptible to oxidative stress arising from imbalances between reactive oxygen species production and antioxidant defense mechanisms (Walke et al., 2023). This lipid peroxidation represents a key mechanism through which hemorrhoidal inflammation damages reproductive tissues and function. *Phyllanthus amarus* extract demonstrated superior antioxidant properties, with dose-dependent lipid peroxidation inhibition. The 400mg/kg dose showed excellent protection, significantly reducing MDA levels below control values. These findings align with previous

demonstrations that *Phyllanthus amarus* extract significantly reduces MDA levels with dose-dependent effects (Saliu et al., 2022). Hemorrhoidal inflammation disrupted antioxidant enzyme systems, indicated by changes in SOD, CAT and GSH levels, suggesting that hemorrhoidal conditions overwhelm normal antioxidant defense mechanisms. Therefore, inflammation produces reactive oxygen species that exert toxic effects on spermatozoa and decrease sperm motility and concentration (Nguyen et al., 2023; Pavuluri et al., 2024). The complex patterns in antioxidant enzyme responses reflect the severity of oxidative stress induced by chronic anorectal inflammation. *Phyllanthus amarus* extract demonstrated the capacity to restore antioxidant enzyme balance with improvements observed in a dose-dependent manner. The extract's influence on glutathione levels plus modulation of catalase and SOD activity provides a net protection against oxidative damage from hemorrhoid development, encompassing the previously published data that presents increased antioxidant enzyme activities with dose-dependent doses of *Phyllanthus amarus* (Ezedom et al., 2018; Iteire et al., 2023).

Hemorrhoidal inflammation significantly disrupted testosterone homeostasis through complex interactions with the hypothalamic-pituitary-gonadal axis. This hormonal disruption reflects intricate interplay between chronic inflammation and hormonal feedback mechanisms, where inflammatory mediators alter normal testosterone production and regulation. Pro-inflammatory cytokines like TNF-α and IL-6 interfere with spermatogenesis, promote oxidative damage, and reduce testosterone production (Hasan et al., 2022; Saad, 2015). The paradoxical reduction in testosterone observed with higher *Phyllanthus amarus* doses and Pilex treatment suggests complex dose-dependent effects on hormonal regulation. The lower dose of 200mg/kg maintained elevated testosterone levels while a higher dose of 400mg/kg significantly reduced testosterone below control levels. This pattern indicates that optimal dosing is crucial for hormonal restoration in hemorrhoid-compromised systems, with different doses exerting varying impacts on the hypothalamic-pituitary-gonadal axis.

In male Wistar rats with induced hemorrhoids, a significant elevation in serum lactate dehydrogenase (LDH) levels is consistently observed. This rise in LDH serves as a reliable biochemical indicator of tissue injury and inflammation associated with the hemorrhoidal condition. LDH is a cytosolic enzyme normally confined within intact cells; therefore, its increased presence in the circulation reflects cellular membrane disruption, tissue damage, or necrosis. In this experimental model, the elevated serum LDH levels are indicative of damage to the anorectal tissues. The increase in LDH occurs in parallel with other established markers of inflammation and oxidative stress. These include enhanced myeloperoxidase (MPO) activity and lipid peroxidation (LPO) in the affected tissues, as well as elevated concentrations of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α). Collectively, these changes confirm the presence of a robust inflammatory response and ongoing tissue injury. Upon treatment with anti-inflammatory and antioxidant interventions such as *P. amarus* as observed in this study, and standard therapeutic formulations like Pilex, a marked reduction in the previously elevated serum LDH levels is observed. This normalization of LDH underscores the effectiveness of these treatments in mitigating tissue damage and suppressing inflammation in the hemorrhoidal model.

Hemorrhoidal inflammation significantly altered inflammatory cytokine profiles, with complex TNF-α patterns demonstrating that chronic hemorrhoids trigger systemic inflammatory responses affecting reproductive function. The association between hemorrhoids and erectile dysfunction in population-based studies (Keller and Lin, 2012) supports the systemic impact of anorectal inflammation on male reproductive health. *Phyllanthus amarus* extract demonstrated superior anti-inflammatory effects compared to standard treatment. The progressive reduction in TNF-α levels with increasing extract doses suggests superior cytokine modulation capacity. These findings align with previous reports of significant reductions in pro-inflammatory cytokines following *Phyllanthus amarus* extract treatment (Harikrishnan et al., 2018). The paradoxical elevation of TNF-α levels with Pilex treatment may suggest inflammatory activation through various treatment components, which is noticeably in contrast to *Phyllanthus amarus*, which possesses anti-inflammatory effects. This differential activation highlights the significance of treatment selection in treating systemic inflammation from hemorrhoids.

Anorectal tissue responses demonstrated significant local oxidative damage and structural changes from hemorrhoidal inflammation. Elevated anorectal MDA levels indicated severe local tissue oxidative damage contributing to systemic inflammatory effects that compromise reproductive function (Hosen et al., 2015). *Phyllanthus amarus* extract provided dose-dependent local antioxidant protection, with the 400mg/kg dose showing superior protective effects in anorectal tissue. The increased anorectal/body weight ratio in the H+E400 group, despite initial hemorrhoidal damage, suggests enhanced tissue recovery and regeneration. This finding indicates that *Phyllanthus amarus* not only prevents further damage but also actively promotes tissue healing and restoration of normal anorectal structure.

Macroscopic assessment revealed substantial visible tissue damage and inflammation from hemorrhoidal conditions. *Phyllanthus amarus* extract administered at higher doses improved tissue healing and recovery, thus improving tissue appearance to almost normal appearance. This tissue-protective effect of *Phyllanthus amarus* extract reinforces the notion that treating hemorrhoids locally has positive implications that restore normal reproductive function, indicative of the resolution of systemic inflammatory cascades demonstrated in this study.

The current study demonstrates that hemorrhoidal inflammation significantly impairs male reproductive function via a variety of mechanisms that include: oxidative stress, inflammatory mediator effects, and hormonal interference. These results provide new evidence for the systemic nature of anorectal inflammatory disease and indicate the potential for broader impacts of hemorrhoidal inflammation on reproductive health.

Conclusion

Hemorrhoids severely compromise male reproductive function through oxidative stress, inflammatory mediator effects and hormonal disturbances. *Phyllanthus amarus* extract has demonstrated significant potency in ameliorating dysfunctional reproductive mechanisms induced by hemorrhoids through its antioxidant, anti-inflammatory, and tissue-protective qualities. Several variables consistently favored the

400mg/ kg dose relative to standard treatment in sperm and tissue repair parameters as well as markers of oxidative stress. Taken together, findings suggest that *Phyllanthus amarus* has the potential remedy for reproductive dysfunction associated with hemorrhoids. Clinical trials in managing the complex relationship between hemorrhoidal conditions and male reproductive health using *Phyllanthus amarus* should be looked into. Further research is thus necessary to optimize dosing regimens and validate these findings in clinical settings to establish effective protocols for managing hemorrhoid-induced reproductive dysfunction.

Abbreviations

P. amarus	*Phyllanthus amarus*
LDH	Lactate Dehydrogenase
MDA	Malondialdehyde
CAT	Catalase
SOD	Superoxide Dismutase
GSH	Glutathione
TNF-α	Tumor Necrosis Factor-alpha
ARC	Anorectal Coefficient

Declarations

Ethics approval and consent to participate

Institutional and national guidelines for the care and use of animals were followed. The study received approval from the Health Research Ethics Committee of the College of Medicine, University of Lagos, with approval number (CMUL HREC number: CMUL/ACUREC/03/25/1868). Our manuscript adheres to ARRIVE guidelines.

Consent for publication

All authors warrant that this research represents original work that has not been published previously and is not under consideration for publication elsewhere. The authors confirm that they have reviewed the manuscript and given their consent for publication in The Journal of Basic and Applied Zoology.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Figures

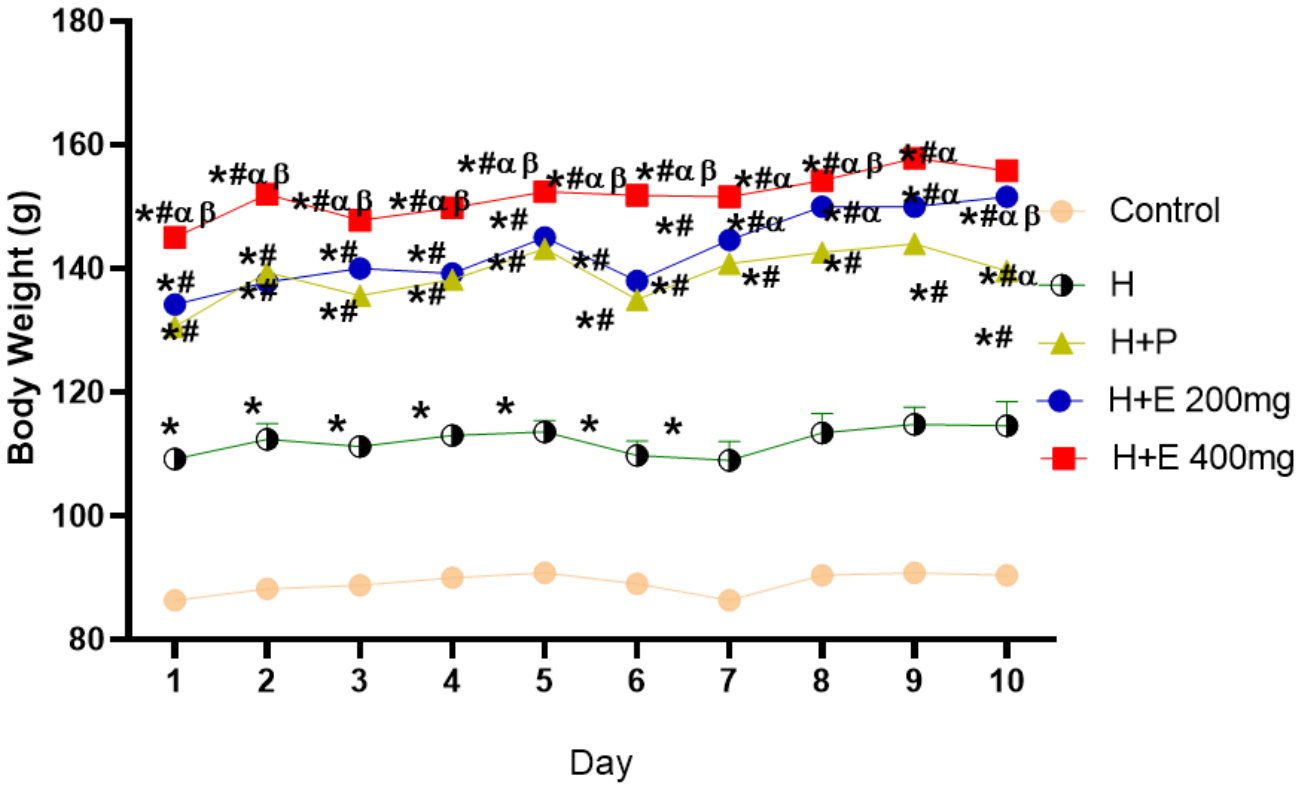


Figure 1

Effect of *Phyllanthus amarus*leaves extract on body weight of hemorrhoid-induced male Wistar rats. Values are expressed as mean ± SEM of 5 rats per group, * = p<0.05 when compared to control group, # = p<0.05 when compared to H, α = p<0.05 when compared to H+P, β- = p<0.05 when compared to H+E

200mg. Hemorrhoid (H), Hemorrhoid + Pilex (H+P), and Hemorrhoid + *Phyllanthus amarus* extract (H+E200 and H+E400)

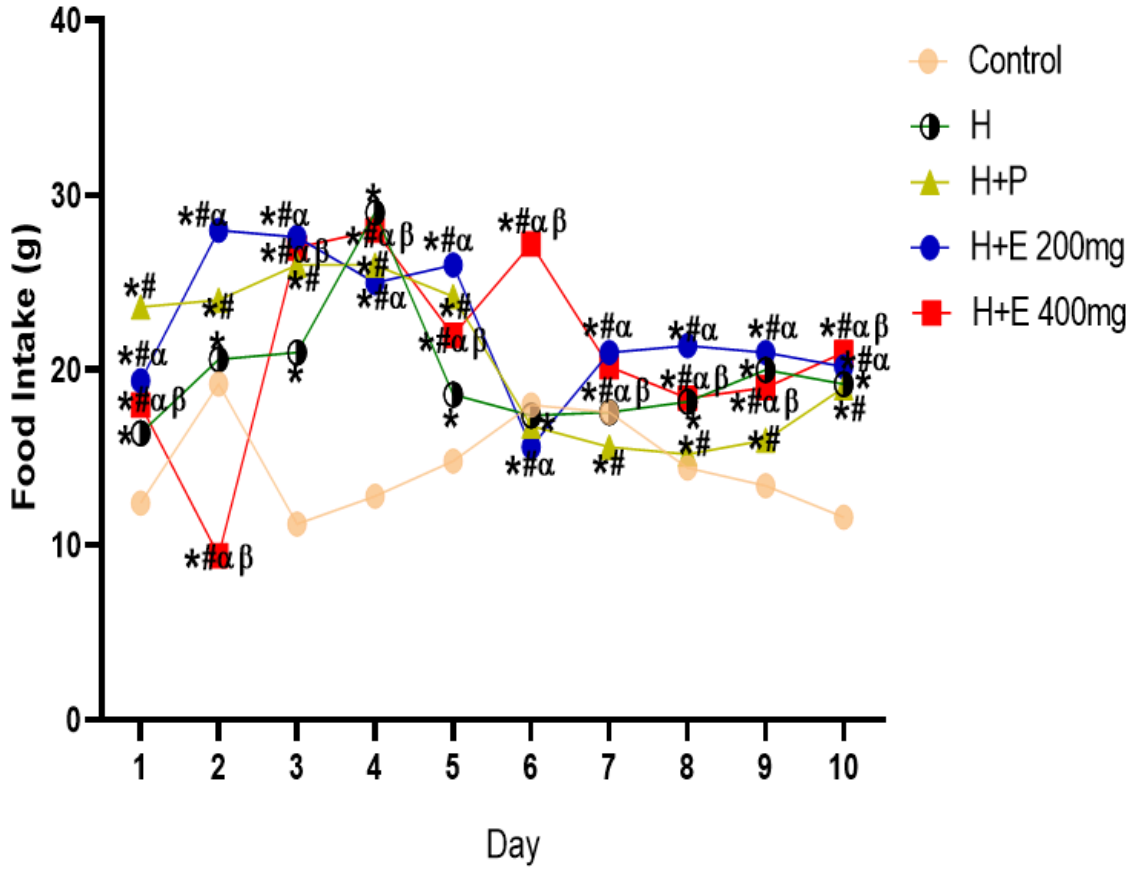


Figure 2

Effect of *Phyllanthus amarus*leaves extract on food intake of hemorrhoid-induced male Wistar rats. Values are expressed as mean ± SEM of 5 rats per group, * = p<0.05 when compared to control group, # = p<0.05 when compared to H, α = p<0.05 when compared to H+P, β- = p<0.05 when compared to H+E 200mg. Hemorrhoid (H), Hemorrhoid + Pilex (H+P), and Hemorrhoid + *Phyllanthus amarus* extract (H+E200 and H+E400)

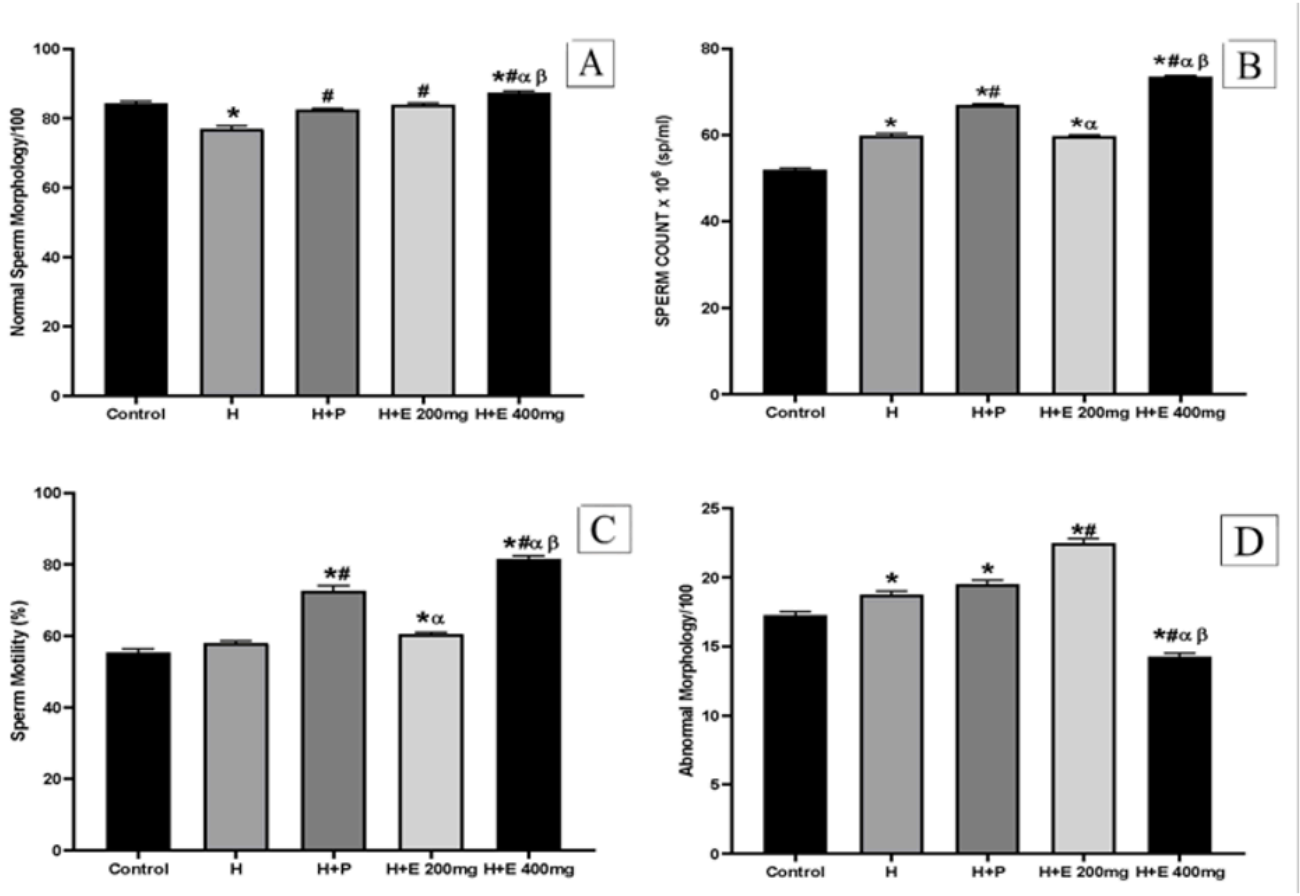


Figure 3

Effect of *Phyllanthus amarus*leaves extract on **A** Normal Sperm Morphology **B** Sperm Count **C** Sperm Motility **D** Abnormal Sperm Morphology of hemorrhoid-induced male Wistar rats. Values are expressed as mean ± SEM of 5 rats per group, * = $p<0.05$ when compared to control group, # = $p<0.05$ when compared to H, α = $p<0.05$ when compared to H+P, β- = $p<0.05$ when compared to H+E 200mg. Hemorrhoid (H), Hemorrhoid + Pilex (H+P), and Hemorrhoid + *Phyllanthus amarus*extract (H+E200 and H+E400)

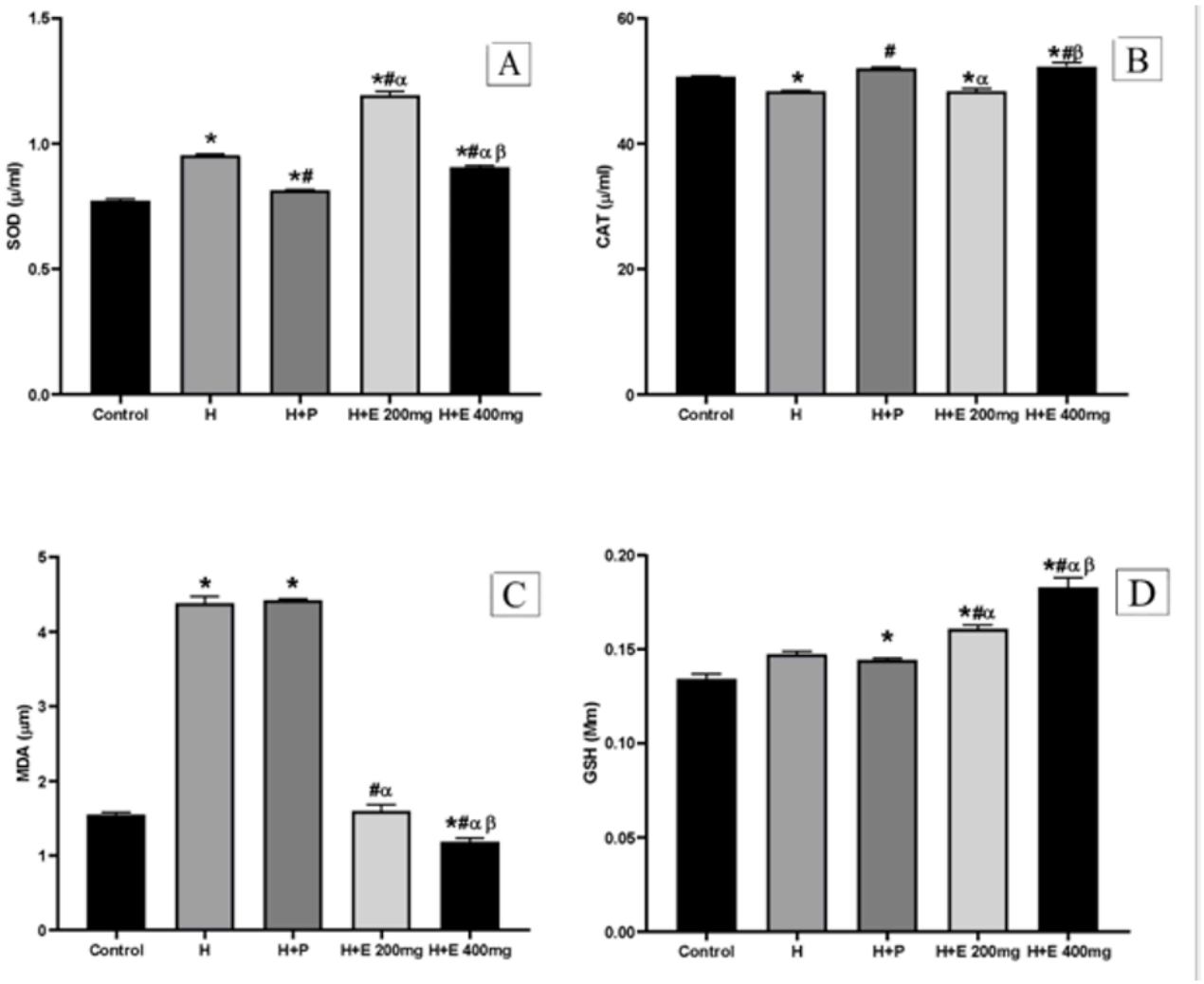


Figure 4

Effect of *Phyllanthus amarus*leaves extract on **A** SOD **B** CAT **C** MDA **D** GSH of hemorrhoid-induced male Wistar rats. Values are expressed as mean ± SEM of 5 rats per group, * = p<0.05 when compared to control group, # = p<0.05 when compared to H, α = p<0.05 when compared to H+P, β- = p<0.05 when compared to H+E 200mg. Hemorrhoid (H), Hemorrhoid + Pilex (H+P), and Hemorrhoid + *Phyllanthus amarus* extract (H+E200 and H+E400)

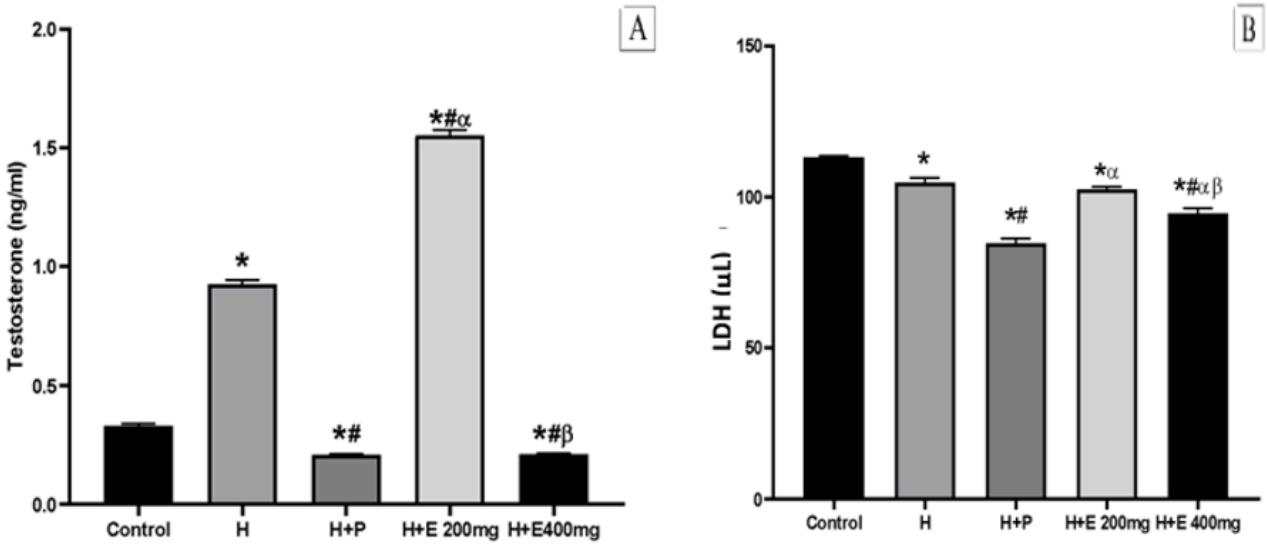


Figure 5

Effect of *Phyllanthus amarus*leaves extract on **A** Testosterone level **B** LDH level of hemorrhoid-induced male Wistar rats. Values are expressed as mean ± SEM of 5 rats per group, * = p<0.05 when compared to control group, # = p<0.05 when compared to H, α = p<0.05 when compared to H+P, β- = p<0.05 when compared to H+E 200mg. Hemorrhoid (H), Hemorrhoid + Pilex (H+P), and Hemorrhoid + *Phyllanthus amarus* extract (H+E200 and H+E400)

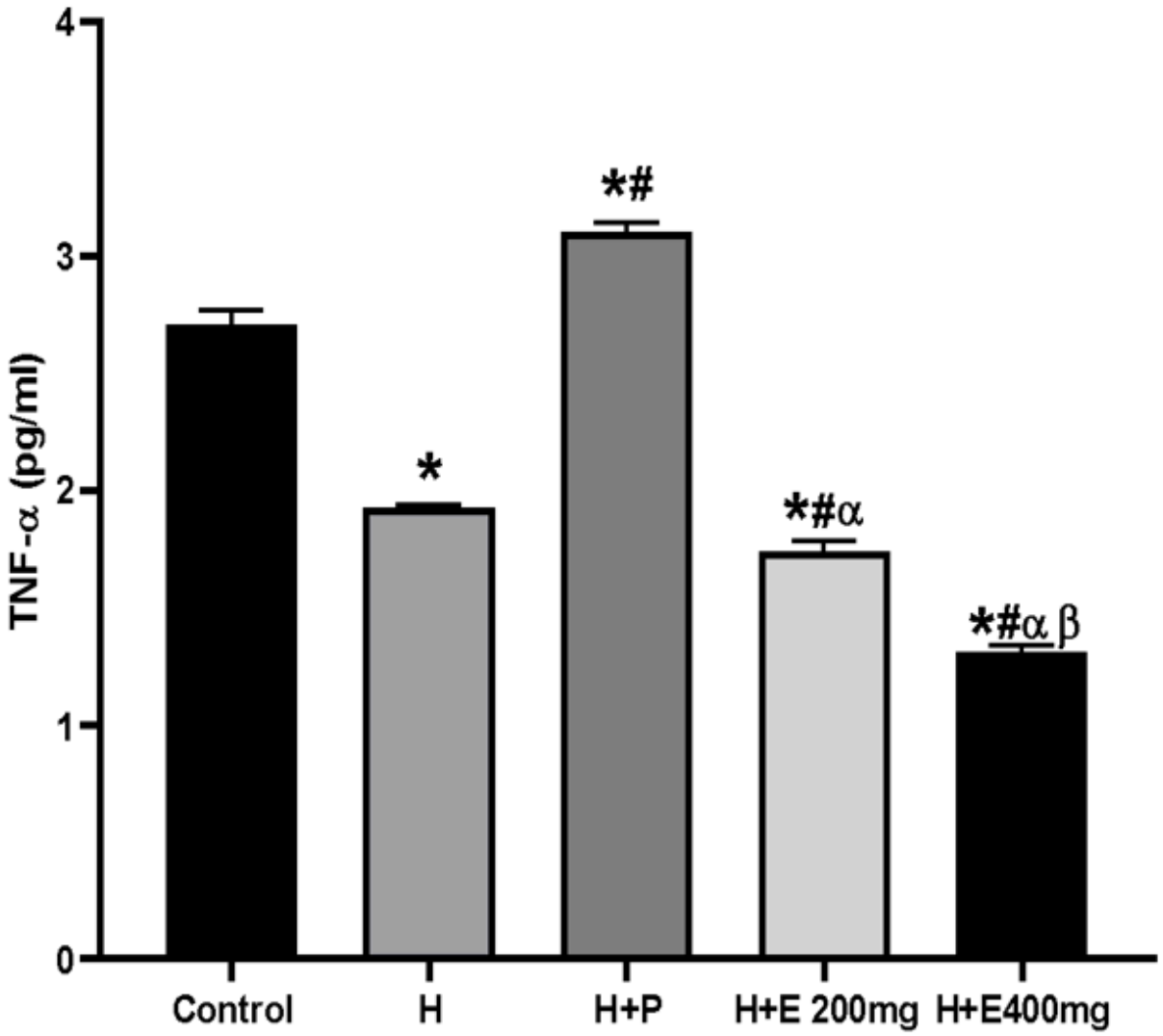


Figure 6

Effect of *Phyllanthus amarus*leaves extract on TNFα level in hemorrhoid-induced male Wistar rats. Values are expressed as mean ± SEM of 5 rats per group, * = $p<0.05$ when compared to control group, # = $p<0.05$ when compared to H, α = $p<0.05$ when compared to H+P, β- = $p<0.05$ when compared to H+E 200mg. Hemorrhoid (H), Hemorrhoid + Pilex (H+P), and Hemorrhoid + *Phyllanthus amarus* extract (H+E200 and H+E400)

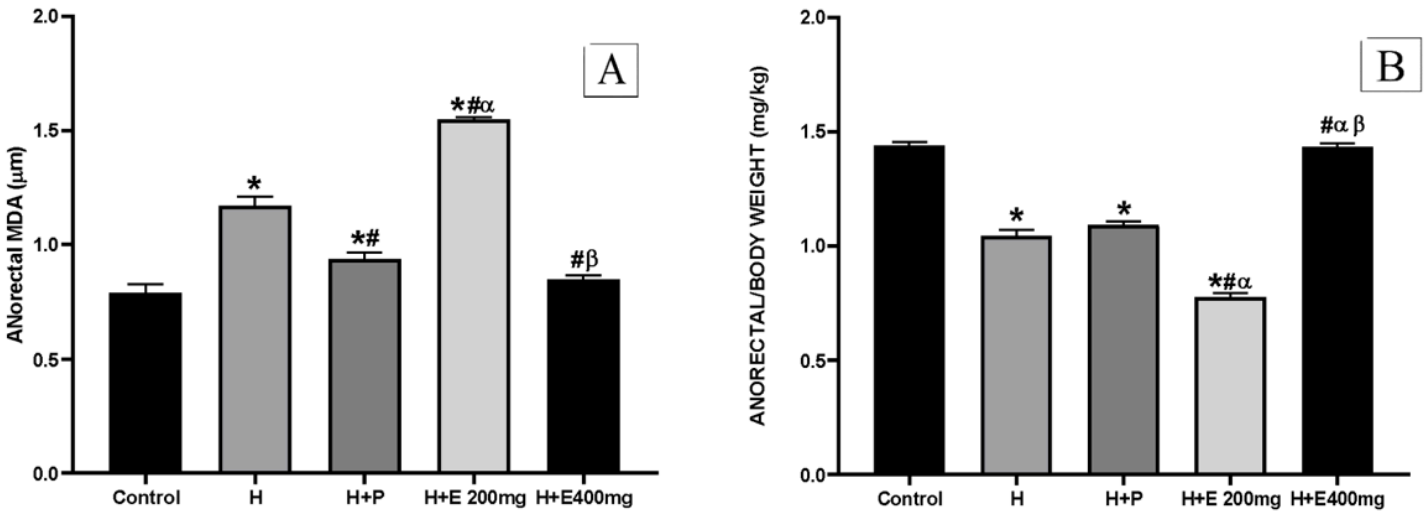


Figure 7

Effect of *Phyllanthus amarus*leaves extract on **A** Anorectal MDA **B** Anoretal/ Body weight index in hemorrhoid-induced male Wistar rats. Values are expressed as mean ± SEM of 5 rats per group, * = $p<0.05$ when compared to control group, # = $p<0.05$ when compared to H, α = $p<0.05$ when compared to H+P, β- = $p<0.05$ when compared to H+E 200mg. Hemorrhoid (H), Hemorrhoid + Pilex (H+P), and Hemorrhoid + *Phyllanthus amarus* extract (H+E200 and H+E400).

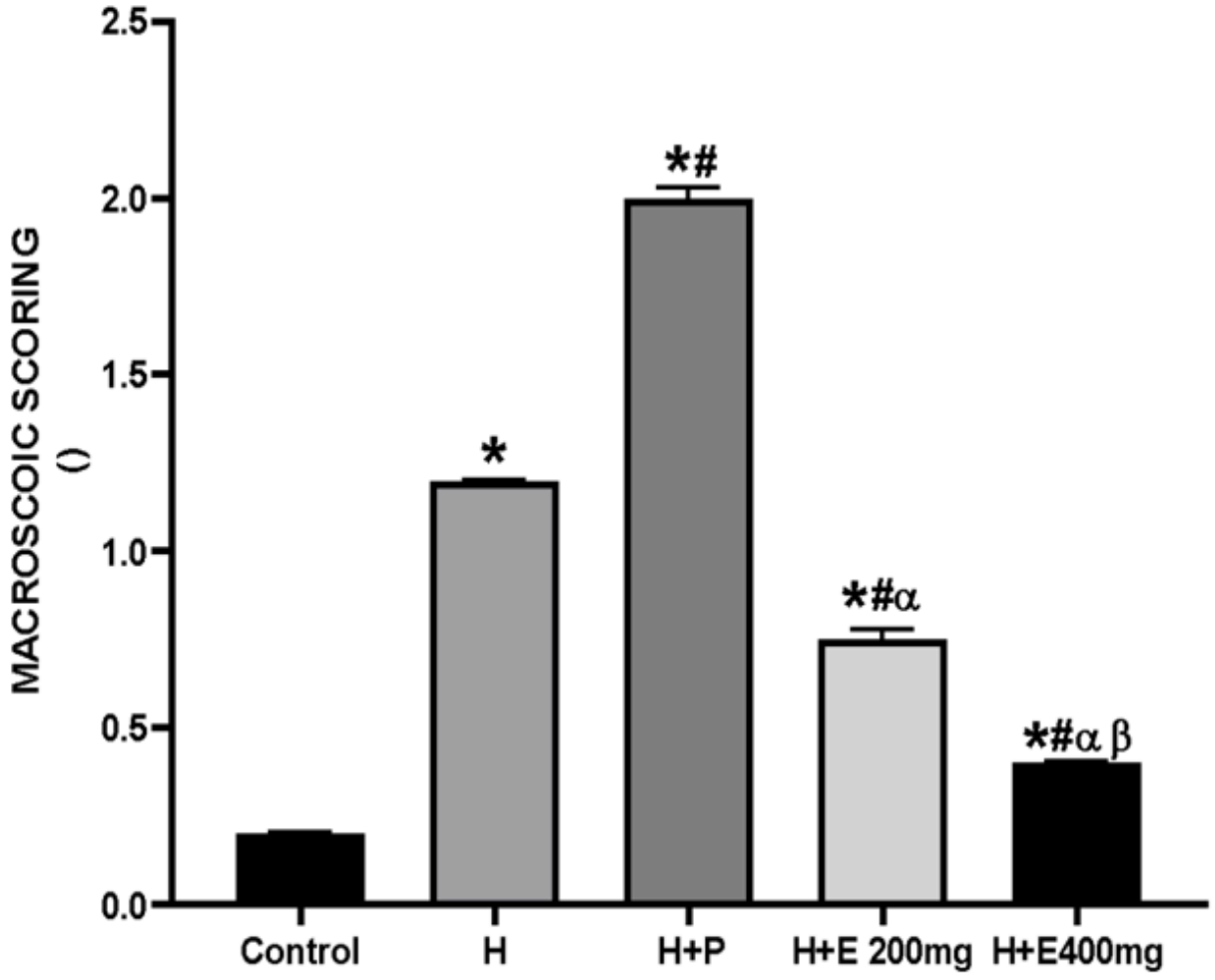


Figure 8

Effect of *Phyllanthus amarus*leaves extract on macroscopic scoring in hemorrhoid-induced male Wistar rats. Values are expressed as mean ± SEM of 5 rats per group, * = p<0.05 when compared to control group, # = p<0.05 when compared to H, α = p<0.05 when compared to H+P, β- = p<0.05 when compared to H+E 200mg. Hemorrhoid (H), Hemorrhoid + Pilex (H+P), and Hemorrhoid + *Phyllanthus amarus*extract (H+E200 and H+E400)