



Characterization and Antiulcerogenic Effect of Different Solvent Extracts of *Verbena hastata* Plant on Indomethacin Induced Ulcer Albino Rats

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DOI: <https://doi.org/10.70382/ajcms.v8i3.005>

Abstract

Peptic ulcers remain a prevalent gastrointestinal disorder with significant morbidity, often exacerbated by non-steroidal anti-inflammatory drugs (NSAIDs) like indomethacin. This study evaluated the antiulcerogenic and antioxidant potential of different solvent fractions of *Verbena hastata* leaf extracts in indomethacin-induced ulcerated Wistar rats. Crude and ethyl acetate extracts were rich in flavonoids (6.59 ± 0.05 and 7.15 ± 0.04 mg/mL) and saponins (4.23 ± 0.06 and 5.28 ± 0.05 mg/mL), with phenols at the lowest levels (0.59 ± 0.15 and 0.79 ± 0.12 mg/mL), respectively. Rats were grouped and treated with 20 mg/kg body weight of n-hexane (NHFVA), ethyl acetate (EAFVA), n-butanol (BFVA), or residual (RFVA) extracts for 7 days post-ulcer induction. Gastric pH in the untreated group was markedly reduced (pH = 3.22), while omeprazole and EAFVA significantly increased pH (6.45 and 6.28, respectively; $p < 0.05$). Mucin content, indicative of mucosal protection, was lowest in the untreated group (0.93 ± 0.03 mg/mL) and significantly restored by EAFVA (2.16 ± 0.02 mg/mL), comparable to omeprazole

(2.29 ± 0.03 mg/mL). EAFVA treatment markedly improved liver function markers with ALP, ACP, ALT, and AST activities reduced to 80.13 ± 2.03 , 77.07 ± 4.61 , 24.56 ± 1.17 , and 17.91 ± 1.08 IU/L respectively, mirroring the omeprazole group. Similarly, serum antioxidant markers (SOD, CAT, GSH, GST) and MDA levels in EAFVA-treated rats showed significant protection against oxidative stress, with stomach MDA levels reduced to 3.11 ± 0.12 μ mol/mg protein versus 6.84 ± 0.25 in the untreated group. Histological assessment confirmed gastric mucosal integrity preservation in EAFVA-treated rats, with minimal edema, necrosis, or inflammatory infiltration. Kidney function indices (serum urea, electrolytes) remained stable across all treatment groups, suggesting low systemic toxicity. In conclusion, *Verbena hastata*, particularly its ethyl acetate extract, demonstrated significant gastroprotective, antioxidant, and organ-preserving properties comparable to standard omeprazole therapy, validating its potential as a phytotherapeutic agent for peptic ulcer management.

Keywords: *Verbena hastata*; Antiulcerogenic; Ulcers; Indomethacin; Antioxidant; Omeprazole; Ethyl acetate

Introduction

Peptic ulcers, one of the most prevalent digestive tract conditions are primarily caused by an imbalance between aggressive and defensive stomach forces (Salunke *et al.*, 2024). It encompasses both gastric and duodenal ulcers and has been a major threat to the world's population over the past two centuries, with high morbidity and substantial mortality (Ofusori *et al.*, 2020; Balaky, 2024). The defensive/protective factors include mucus and bicarbonate barrier, prostaglandins and proper blood flow and the aggressive agents include pepsin and HCl acid of the stomach, esophagus, or duodenum, genetic factors, bile reflux and decreased blood flow (Serafim *et al.*, 2020; Ofusori *et al.*, 2020). Exogenous factors have also been

reported concerning the development of ulceration. These factors include inappropriate eating habits, stress, chemical agents like alcohol, and smoking, prolonged and excessive use of non-steroidal anti-inflammatory drugs (NSAIDs) and infection with *Helicobacter pylori* (*H. pylori*), a bacterium that frequently colonizes the human stomach (Serafim *et al.*, 2020; Awuchi *et al.*, 2023). Prevalence of peptic ulcer is estimated to be 200-250 per 100,000 populations globally, with increased risks of development in developing countries. Peptic ulcers affect both genders equally and can manifest at any stage of life, however, the increased rate of onset development is higher at the age of 10 - 15 years (Gupta, *et al.*, 2021). A

tremendous decrease in morbidity and mortality correlated with peptic ulcer has also been reported. This is possibly due to the emergence of new treatments and better hygiene, which resulted in a reduction of infections caused by *H. pylori* (Balaky, 2024). Although, several conventional treatment approaches are being used to manage ulcer-mediated disorders. However, the side effects associated with these treatment approaches is of concern, with the main adverse effects including gastric disturbance such as nausea, vomiting, constipation, diarrhea, and cardiovascular effects (Gupta, *et al.*, 2021). The use of herbal products (Phytomedicine), that have been widely employed right from ancient times for the treatment and management of various disorders, have been reported to pose little or no toxicity and are highly considered to be safer compared to the conventional treatment such as the use of synthetic drugs (Gupta, *et al.*, 2021).

Phytomedicine is a promising approach to the treatment and management of several diseases. The significance of natural products in modern medicine is well recognized and they continue to be of interest as sources of novel lead compounds (Balaky, 2024). Medicinal plants contain very active ingredients used for treating various diseases. Traditional medical practitioners have provided insight into alternative treatments for ulcers as many plant extracts have been beneficial in their management (Ofusori *et al.*, 2020). Several plant-derived chemical constituents have been reported to possess antiulcer properties in various preclinical studies. These constituents are a class of secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, glycosides, carotenoids, and saponins holding the secret to medicinal properties of several plants (Gupta, *et al.*, 2021; Yokosuka *et al.*, 2021).

Verbena hastata is a flowering and drought resistant plant which belongs to Verbenaceae family. It is called American vervain, blue vervain or swamp verbena (Akuodor *et al.*, 2010). This plant has been employed traditionally for medicinal purposes such as a diuretic, an astringent for wound healing, an expectorant, acne, treatment of headaches antirheumatic due to its rich biochemical constituents (Yokosuka *et al.*, 2021). There is a growing need to develop alternative approaches towards the management and prevention of peptic ulcer. The use of verbena can also be effective in the management of ulcer and the adverse side effects associated with various available conventional drugs used to reduce ulcer (Gupta, *et al.*, 2021). Hence, this study aims to examine the anti-ulcer activity of different solvent extracts of the *Verbena hastata* plant for the treatment of ulcers induced in albino rats.

Materials and methods

Materials

The materials used in this study were *Verbena hastata* plant, Adult Wistar male rats, indomycin (ulcer inducer), and Omeprazole as the reference drug for the treatment of induced ulcers. All other chemicals and reagents were of analytical grades.

Methods

Plant Material Collection and Authentication and preparation

Fresh leaves of *V. hastata* leaves known as “ewe ogangan” were obtained in the Agor market in Ilorin Kwara state. The plant leaves were identified and authenticated by a botanist (Mr. Bolu) at the plant biology herbarium center of the University of Ilorin, Kwara state and the identification number is UILH/001/2020/1396.

Preparation of Plant Extracts

The leaves were washed thoroughly and air dried at room temperature for two weeks. The dried leaves samples were then blended into powder using a blender. Maceration and Soxhlet extraction methods were used in the preparation of the extract. Briefly, 250 grams of sample powder was soaked in 2 liters of hydro-ethanol (50: 50 %) and allowed to stand for 48 hours at room temperature, after which it was filtered with a chess cloth. The resulting solution was further extracted successively with different solvents (n-hexane, ethyl acetate, and n-butanol) using a Soxhlet extractor and the resulting residual was stored for further uses. The solvent extracts were concentrated to dryness under reduced pressure in a vacuum at 40 °C and preserved at 5° C in an air-tight bottle until further use. The final yield was 0.55 g, 5.01 g, 12.30 g and 25.6 g for n-hexane, n-butanol, ethyl acetate and residual respectively.

Phytochemical Analysis of the Crude and Ethyl Acetate Extracts of *Verbena hastata* Extracts

The phytochemical compounds (alkaloid, flavonoids, tannin, phenols and saponin) of the crude and ethyl acetate extracts of *V. hastata* leaves were quantitatively determined according to the following standard procedures: The method of Harborne (1978) was used to determine the total alkaloid contents of the extracts. The total flavonoid content in the sample was estimated by the method of Chang (2002). The Folin-Ciocalteu method of Slinkard and Singleton (1977) was used to estimate the total phenolic content of the extracts with some modifications. The method of Peri and Pompei (1971) was used to determine the total tannins content. Total saponins content of the extract was determined by the method described by Makkar *et al.* (2007).

Experimental Animals

Thirty-five adult male Wistar rats (weighing 120-160 g) were used for the studies. The rats were maintained in the Animal Facility Centre, Tanke, Ilorin, Kwara state. They were allowed free access to water and feeds throughout the experimental periods. They were acclimatized to room temperature and moisture, under a naturally illuminated environment for 7 days on a 12:12h dark/light cycle. They were fed with top formulated feeds and had access to water *ad libitum*. The study protocol was carried out as per the rules of the National Institute of Health's guide for the care and use of laboratory animals (NIH, 2011).

Experimental Design

Animal Grouping and Extract Administration

The rats were randomly selected into seven groups (I - VII) consisting of five rats each. Indomethacin was then used to induce ulcers in the Rats. After confirmation of ulceration, the rats were treated through oral administration for seven days. Group I control (negative control) was given distilled water (20 mg/kg). Group II untreated (Ulcer control) was given distilled water (20 mg/kg). Group III reference (Standard) was given 20 mg/kg body weight of Omeprazole every day. Group IV, V, VI, and VII were given 20mg/kg body weight of n- hexane, ethyl acetate, n- butanol, and residual extracts of *verbena hastata* leaves respectively.

Sacrifice of Experimental Animals

After seven days of treatment with the extracts, the weight of each rat was determined and subsequently anesthetized with diethyl ether in a glass jar. The blood, Organs, and tissues were harvested for further analysis.

Determination of the Effects of *Verbena hastata* Extracts on Biochemical Parameters **Effects of *Verbena hastata* Extracts on total Protein and Carbohydrates concentration**

The total protein concentration in the serum of the animals was assayed, using Biuret reagent as described by Gornall *et al.* (1949). The total carbohydrate content was determined according to the method of Colwel *et al.* (1976). The mucoadhesive activity was then expressed as the TC: PR ratio (Colwel *et al.*, 1976).

Effects of *Verbena hastata* Extracts on Gastric pH of Indomethacin-Induced Ulcer Rats.

Gastric juice pH was determined using a pH meter and a standard cylinder as described by Kurasawa *et al.* (2005). The stomachs of the sacrificed rats were open along the greater curvature. Gastric contents from each rat were then drained into a centrifuge tube containing 5 mL distilled water and centrifuged at 1 000 ×g for 10 min. The pH examination was done to know the acidity of the stomach's content by collecting the supernatant from mucosa weight in a falcon tube. Supernatant in the same group was put in the same falcon tube because of limited volume. The acidity of the collected supernatant was determined using a pH meter (Kurasawa *et al.*, 2005).

Determination of the Effects of *Verbena hastata* Extracts on Antioxidant Activities of Indomethacin-Induced Ulcer Rats.

The method described by Misra and Fridovich (1972) was used to assay for the activity of superoxide dismutase. Catalase activity was assayed as described by Beers and Sizer

(1952). The level of reduced glutathione (GSH) in the liver homogenate was determined using the procedure described by Ellman (1959). Glutathione transferase (GST) activity in the liver was determined using the method of Habig *et al.* (1974). The concentration of malondialdehyde (MDA) was quantified according to the method of Yoshioka *et al.* (1979).

Determination of the Effects of *Verbena hastata* Extracts on Liver Function Indices of Indomethacin-Induced Ulcer Rats

The procedure described by Doumas *et al.* (1971) was employed for the determination of serum albumin of the rat. The alkaline phosphatase activity was assayed using a method described by Roy (1970). Serum acid phosphatase was assayed using the procedure of Gutman and Gutman (1940). The method described by Reitman and Frankel (1957) was used to assay for the activity of alanine aminotransferase. The method described by Reitman and Frankel (1957) was used in the assay of the activity of aspartate aminotransferase.

Determination of the Effects of *Verbena hastata* Extracts on Kidney Function Indices of Indomethacin-Induced Ulcer Rats

The serum urea was determined using the method described by Veniamin and Vakirtzi (1970). Further, the level of serum potassium and sodium ions was measured using flame photometry (Cheesbrough, 1998), while serum bicarbonate and chloride ions and phosphate were determined using the titration/volumetric method (Chapman, 1961).

Histopathological examination of the stomach

Stomachs were preserved in 10% formalin solution for histopathological examination (x 100). The central part of damaged or ulcerated tissue (if present) was cut off along the long diameter. If the stomach was protected from the damage, then the section was taken from the basal part, thickness of about 5 µm was cut and stained with haemotoxylin and eosin. These were examined under the microscope for histopathological changes such as congestion, hemorrhage, necrosis, inflammation, infiltration, erosion, and ulcers.

Statistical Analysis

Data were expressed as mean $\pm$ standard deviation. Comparative analysis was performed between the various groups using analysis of variance (ANOVA) and Duncan multiple range test for the post hoc. Differences were considered at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Contents of *Verbena hastata* extracts

The results of the phytochemical contents (alkaloids, total flavonoids, tannins, total phenols, and total saponins) of the crude extracts and ethyl acetate extract of the *verbena*

hastata leaves are shown in Table 1. The table revealed that the extracts contained alkaloids, total flavonoids, tannins, total phenols, and total saponins. There is no significant difference ($p < 0.05$) between the quantity of alkaloids, total flavonoids, tannins, total phenols, and total saponins of both crude and ethyl acetate extract. The results revealed highest quantity of total flavonoid content (6.59 ± 0.05 and 7.15 ± 0.04 mg/mL) followed by Saponins (4.23 ± 0.06 and 5.28 ± 0.05 mg/mL) and lowest total phenols (0.59 ± 0.15 and 0.79 ± 0.12 mg/mL) in both crude and ethyl acetate extract respectively. These compounds are remarkable for their numerous health benefits such as antioxidant (Irondi *et al.*, 2022) and anti-ulcer activity (Gupta, *et al.*, 2021).

Effects of *Verbena hastata* Extracts on the Biochemical Parameters of Indomethacin-Induced Ulcer Rats

Effects of *Verbena hastata* Extracts on total Protein and Carbohydrates concentration

Figure 1 and 2 revealed the results of the effects of *verbena hastata* extracts on total protein and carbohydrates concentration of the experimental rats. The concentration of protein and carbohydrate are significant lower ($p < 0.05$) in the negative control group when compared with the positive control. The effects of n-hexane (NHFVA), n-butanol (BFVA) and residual (RFVA) extracts of *Verbena hastata* on the protein concentration of indomethacin-induced ulcer rats are significantly comparable ($p > 0.05$). There is no significant different ($p < 0.05$) in the protein and carbohydrate concentration of positive control, omeprazole treated and ethyl acetate (EAFVA) extracts treated groups. Further, BFVA and RFVA have similar ($p < 0.05$) effect on the carbohydrate concentration of indomethacin-induced ulcer rats. Total protein and carbohydrate are indicators of overall protein and carbohydrate status and liver function (Johnson *et al.*, 2013; Nurudeen *et al.*, 2023). The absence of substantial changes in total protein or carbohydrate concentration in the different groups tested in this study may suggests that there may be no impact on protein and carbohydrate metabolism or hepatic protein synthesis due to the extract.

Effects of *Verbena hastata* Extracts on Gastric pH of Indomethacin-Induced Ulcer Rats.

Figure 3 shows the result of the effects of *Verbena hastata* extracts on the gastric pH of indomethacin-induced ulcer rats. The result shows that gastric pH is significant lower ($p < 0.05$) in the negative control group when compared with the positive control. The gastric pH is significant highest ($p < 0.05$) in the omeprazole treated control. The effects of BFVA and RFVA extracts of *verbena hastata* on the gastric pH of indomethacin-induced ulcer rats are significantly comparable ($p > 0.05$). There is no significant different ($p < 0.05$) in the gastric pH of positive control and EAFVA extracts treated rats.

Effects of *Verbena hastata* Extracts on Mucin Contents of Indomethacin-Induced Ulcer Rats.

The result of mucin content of indomethacin-induced ulcer rats is displayed in figure 4. The Mucin content is significantly lowest ($p < 0.05$) in negative control group (untreated). There are no significant differences ($p < 0.05$) in the mucin contents of the positive control, and omeprazole, EAFVA, BFVA and RFVA treated indomethacin-induced ulcer rats.

Effects of *Verbena hastata* Extracts on the Antioxidant's Activities of Indomethacin-Induced Ulcer Rats

The result of the effects of *verbena hastata* extracts on the antioxidant's activities of indomethacin-induced ulcer rats antioxidant parameters were presented in figure 5 to 13. The antioxidants- superoxide dismutase (SOD) was assayed in the serum while catalase (CAT), reduced glutathione (GSH), glutathione-S-transferase (GST), and malondialdehyde (MDA) were assayed in both the serum and in the homogenate of the stomach as markers of oxidative stress in the experimental animals. The result of the effects of *verbena hastata* extracts on activity of SOD in the serum (Figure 5) showed that there were no significant differences ($p < 0.05$) in the SOD activity of the positive control, and omeprazole, EAFVA and RFVA treated indomethacin-induced ulcer rats. The effect of NHFVA and BFVA are significant comparable ($p > 0.05$). The result of the effects of *verbena hastata* extracts on activity of CAT in the serum and stomach of indomethacin-induced ulcer rats were presented in figure 6 and 7 respectively. There were no significant differences ($p < 0.05$) in the effects of NHFVA, EAFVA and BFVA and omeprazole treated group on the activity of CAT in the serum. There effects on the activity of CAT in the serum were also significantly comparable ($p > 0.05$) to the positive control group. Similarly, the effects of the positive control, omeprazole, EAFVA, BFVA and RFVA group on the activity of CAT in the stomach do not differ significantly ($p > 0.05$). Further, figures 8 and 9 show the result of GSH activities in the serum and stomach of the indomethacin-induced ulcer rats respectively. The effect of omeprazole and EAFVA on the GSH activities of the serum of indomethacin-induced ulcer rats do not differ significant ($p < 0.05$). Whereas, the effect of NHFVA, BFVA and RFVA are significant comparable ($p > 0.05$) with the positive control. The result of the effect of the extracts on the GSH activities in the stomach of the indomethacin-induced ulcer rats showed that EAFVA and BFVA have the same significant effect ($p < 0.05$) which were significantly comparable ($p > 0.05$) with the positive control and omeprazole treated group. Figure 10 and 11 display the result of the effects of the extracts on the GST activity in the serum and stomach of the indomethacin-induced ulcer rats respectively. There were no significant differences ($p < 0.05$) in the positive control and the effects of EAFVA, BFVA, RFVA and omeprazole on the GST activity in the serum and stomach of the indomethacin-induced ulcer rats. The activity of MDA in the serum and stomach of the indomethacin-

induced ulcer rats were shown in Figures 12 and 13 respectively. The result revealed highest concentration of MDA in serum and stomach of the untreated rat group. There were no significant differences ($p < 0.05$) in the effects of EAFVA, BFVA, RFVA and omeprazole on the MDA in the serum of the indomethacin-induced ulcer rats. However, the effects of EAFVA, BFVA, RFVA on the MDA in the stomach of the indomethacin-induced ulcer rats differ significantly ($p < 0.05$) from positive control and omeprazole treated group. The significant ($p < 0.05$) antioxidant activities of *Verbena hastata* extracts on the indomethacin-induced ulcer rats may be useful in protecting the body from free radicals-induced oxidative damage to cellular biomolecules (proteins, nucleic acids, carbohydrates and lipids) and oxidative stress-associated chronic diseases (Ironi *et al.*, 2022; Imam *et al.*, 2024)

Effects of *Verbena hastata* Extracts on the Liver and Kidney Function Indices of Indomethacin-Induced Ulcer Rats

The result effects of *Verbena hastata* extracts on the liver function indices of indomethacin-induced ulcer rats are shown in table 2. The untreated group revealed the highest level of ALP, ACP, ALT and AST, but the lowest serum albumin (25.41 ± 2.10 mmol/L). The result also revealed no significant difference ($p < 0.05$) in the serum albumin concentration of the extracts (NHFVA, EAFVA, BFVA and RFVA), control and omeprazole groups. Indomethacin-induced ulcer rats treated with EAFVA showed the lowest value for ALP (80.13 ± 2.03 IU/L), ACP (77.07 ± 4.61 IU/L), ALT (24.56 ± 1.17 IU/L) and ASP (17.91 ± 1.08 IU/L) which are significantly comparable ($p > 0.05$) with the omeprazole treated and positive control groups. Further, the result effects of *Verbena hastata* extracts on the kidney function indices of indomethacin-induced ulcer rats are shown in table 3. From the result, there is no significant differences ($p < 0.05$) in serum urea and Na^+ level in all the rat groups. The effect of EAFVA and omeprazole on the serum urea electrolyte concentrations (Na^+ , K^+ , HCO_3^- and Cl^-) of indomethacin-induced peptic ulcerated rats were significant comparable ($p > 0.05$). The liver and kidney are vital organs responsible for metabolic processes, detoxification, and elimination of waste products from the body. A change in the organ body-weight ratio is a useful indicator of potential alterations or toxicity in organ size and function (Knight *et al.*, 2006).

Like total protein, serum albumen also indicates of liver function and overall protein status. Alteration in its concentration (Johnson *et al.*, 2013; Nurudeen *et al.*, 2023). The significant effect of the extracts on the serum album of indomethacin-induced ulcer rats also suggests that there may be no impact on protein metabolism or hepatic protein synthesis. ALT and AST are enzymes primarily found in liver cells, and their elevation in the bloodstream can be an indication of liver injury or damage (Pratt *et al.*, 2000). ALP is an enzyme present in various tissues, including the liver, bones, and intestines. Changes in ALP levels can also indicate liver dysfunction or bone-related issues (Kuo *et al.*, 2017).

Their elevation in the untreated rat group may suggest a possible hepatocellular injury or stress which may be due to unmanaged ulceration. However, the use of the extracts *Verbena hastata* has significant effects on these enzymes which is comparable to the positive control which may suggest they have little or no toxicological effects of the liver. Sodium ion, Phosphate ion, bicarbonate and chloride ion are electrolytes involved in various physiological processes, including acid-base regulation and fluid balance (Hoorn *et al.*, 2013; Nurudeen *et al.*, 2023). The effects of extracts on the level of these electrolytes were significantly ($p < 0.05$) with the control. This may indicate that the extracts have little or no significant impact on their metabolism or homeostasis. Also, the extracts may have little or no harmful effects in the reabsorption of metabolic products in renal tubules has observed in the level of the serum urea (Nurudeen *et al.*, 2023).

Microscopic examination of the stomach of rats

Plates 1- 6 show the microscopic picture of the stomach walls of the control, untreated, and omeprazole and *Verbena hastata* extracts treated indomethacin-induced ulcer rats. The untreated animals (plate 2) showed a great level of lesions whereas, the lesions were unnoticed in the stomach walls of the control (plate 1), and omeprazole (plate 3) and extracts treated (plate 4, 5, and 6) indomethacin-induced ulcer rats.

Histopathological examination of the stomach

Plate 7-11 shows the histological micrographs of the gastric mucosa of indomethacin-induced ulcer rats treated with the different extracts of *verbena hastata* leaves. There was however no significant alteration ($p < 0.05$) in the histological features of the surface epithelium and mucosal damage, edema, and also leucocytes.

CONCLUSION

The present study revealed that different solvent extracts (n-hexane, ethyl acetate, and n-butanol) and residual of *Verbena hastata* leaf remarkably demonstrated antiulcerogenic effect on indomethacine induced ulcer albino rats with significant ($p < 0.05$) phytochemical quantity and antioxidant activities. They also have little to no toxicity effects has showed by the biomarker indices. Therefore, the leaf extract of *V. hastata* may be of value in the development of novel agents in the treatment and management of peptic ulcers. However, further studies are warranted to understand the exact mechanism underlining the ulcer healing properties of the extracts.

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Table 1: Phytochemical Contents of the *Verbena hastata* extracts

Phytochemical	Quantity (mg/mL)	
	Crude extract	Ethyl acetate extract
Alkaloids	3.84 ± 0.10 ^c	3.49 ± 0.06 ^c
Flavonoids	6.59 ± 0.05 ^c	7.15 ± 0.04 ^c
Tannins	2.15 ± 0.04 ^b	2.65 ± 0.20 ^b
Phenols	0.59 ± 0.15 ^a	0.79 ± 0.12 ^a
Saponins	4.23 ± 0.06 ^d	5.28 ± 0.05 ^d

Values are expressed as the mean ± S.E.M of replicate determinations. Values having different lowercase superscript letters along the same column differ significantly ($p < 0.05$).

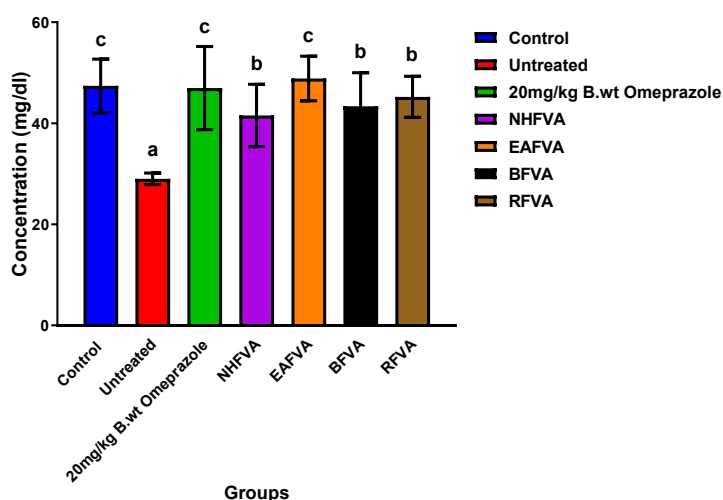
Table 2: the effect of solvent extracts on the liver function indices on indomethacin-induced ulcer rats

Parameter	Control	Untreated	20mg/kg B.wt Omeprazole	NHFVA	EAFVA	BFVA	RFVA
Serum Albumin (mmol/L)	33.54±1.01 ^a	25.41±2.10 ^c	33.42±1.41 ^a	31.26±0.67 ^a	32.28±2.48 ^a	31.41±1.27 ^a	33.19±2.58 ^a
ALP (IU/L)	82.11±1.58 ^a	107.7±3.31 ^c	82.32±1.98 ^a	82.78±3.76 ^a	80.13±2.03 ^a	83.10±8.18 ^a	95.20±0.85 ^b
ACP (IU/L)	83.97±1.79 ^a	106.5±10.07 ^c	83.67±1.10 ^a	96.14±3.82 ^b	77.07±4.61 ^a	93.34±5.66 ^b	85.25±6.82 ^a
ALT (IU/L)	25.40±0.47 ^a	35.94±1.13 ^c	24.89±2.23 ^a	28.47±0.43 ^b	24.56±1.17 ^a	27.58±0.42 ^b	27.72±0.86 ^b
AST (IU/L)	19.30±0.26 ^a	28.44±0.46 ^c	18.81±0.83 ^a	17.37±1.45 ^a	17.91±1.08 ^a	18.57±1.39 ^a	21.38±1.82 ^b

Values are expressed as the mean ± S.E.M of replicate determinations. Values having different lowercase superscript letters along the same column differ significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract; ALP: Alkaline phosphatase; ACP: Acid phosphatase ALT: Alanine Aminotransferase AST: Aspartate Aminotransferase

Table 3: The effect of solvent extracts on the kidney function indices on indomethacin-induced ulcer rats

Parameters (mmol/L)	Control	Untreated	20mg/kg B.wt Omeprazole	NHFVA	EAFVA	BFVA	RFVA
Serum Urea	327.5±17.20 ^a	353.4±22.04 ^a	334.8±21.40 ^a	357.0±44.13 ^a	373.8±21.75 ^a	363.3±8.65 ^a	364.9±15.26 ^a
Na ⁺	48.46±0.66 ^a	61.61±2.26 ^b	48.54±0.48 ^a	49.41±1.09 ^a	48.67±1.31 ^a	47.84±1.09 ^a	49.16±0.18 ^a
K ⁺	29.45±3.04 ^a	36.94±3.26 ^c	23.58±2.90 ^b	29.41±1.74 ^a	26.67±0.55 ^b	30.82±1.19 ^a	29.21±1.51 ^a
HCO ₃ ⁻	26.69±0.27 ^a	16.33±0.50 ^c	25.61±0.42 ^a	21.33±0.69 ^b	25.54±0.22 ^a	26.31±0.35 ^a	25.12±0.42 ^a
Cl ⁻	25.91±1.02 ^a	13.49±0.79 ^c	26.28±0.86 ^a	19.85±1.08 ^a	24.98±0.98 ^a	23.41±1.08 ^b	26.12±0.73 ^a



Values are expressed as the mean ± S.E.M of replicate determinations. Values having different lowercase superscript letters along the same column differ significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract; Na⁺: sodium ion; K⁺: potassium ion, HCO₃⁻: carbonium ion; Cl⁻: chloride ion.

Figure 1: Effect of solvent extracts on the total protein on indomethacin-induced ulcer in rats. Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena*

hastata extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

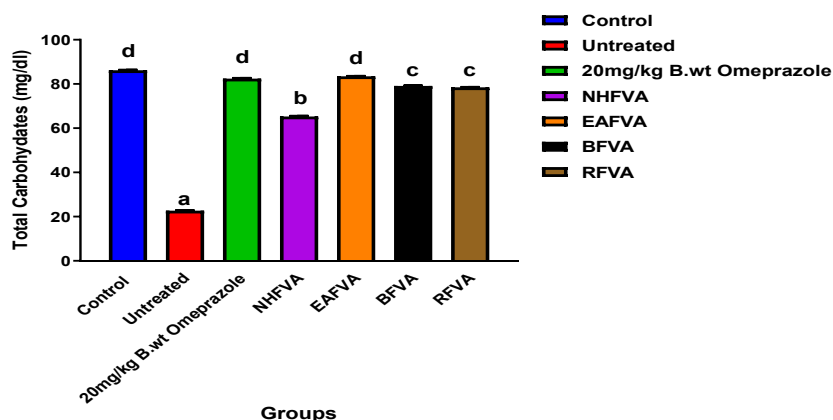


Figure 2: Effect of solvent extracts on the total carbohydrate on indomethacin-induced ulcer in rats. Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

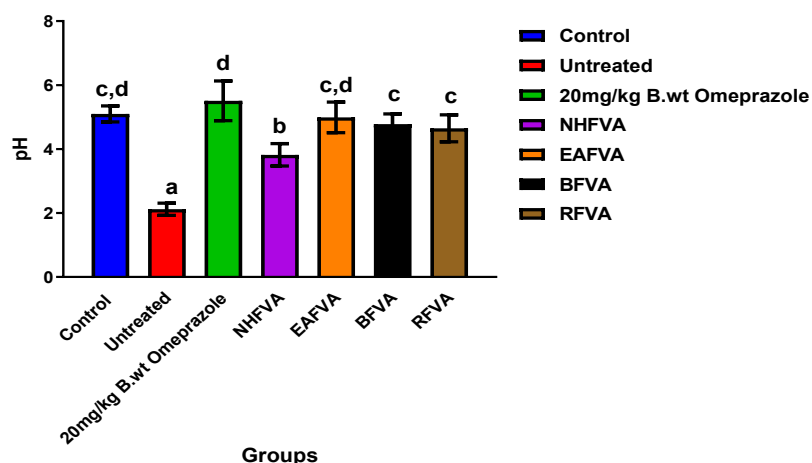


Figure 3: Effect of solvent extracts on the gastric pH on indomethacin-induced ulcer in rats. Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

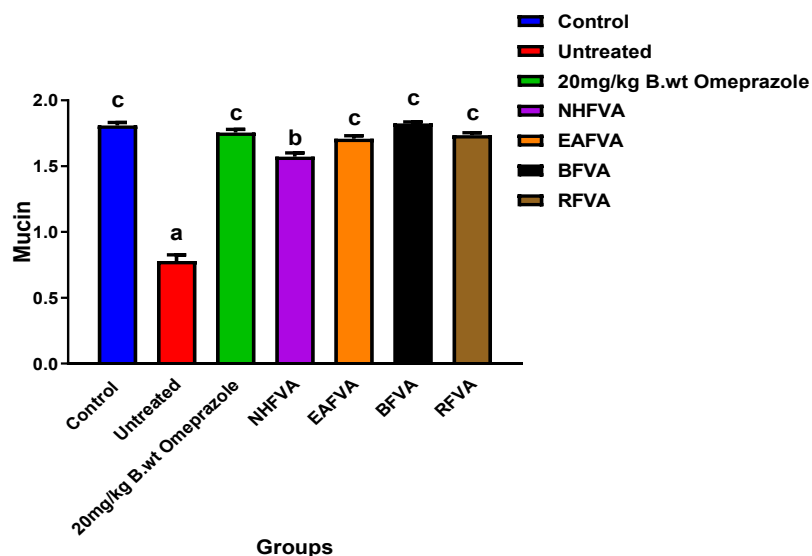


Figure 4: Effect of solvent extracts on mucin in indomethacin-induced ulcer in rats. Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

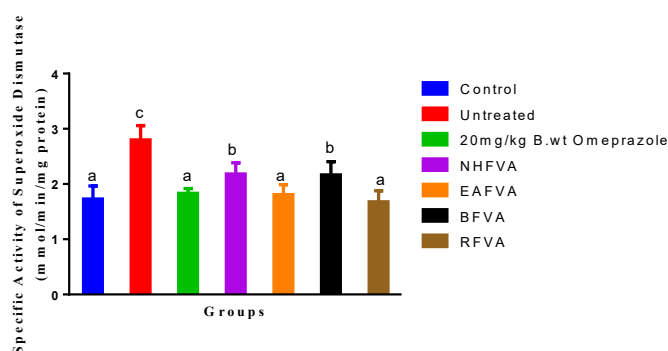


Figure 5: Effect of solvent extracts on the superoxide dismutase [in the serum] on indomethacin-induced ulcer in rats. Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

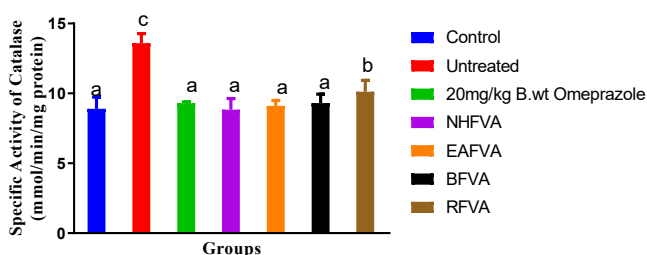


Figure 6: Effect of solvent extracts catalase in serum on indomethacin-induced ulcer in rats. Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

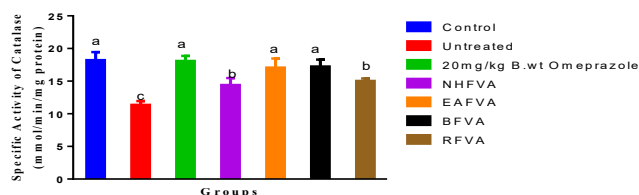


Figure 7: Effect of solvent extracts catalase in the stomach on an indomethacin-induced ulcer in rats. Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

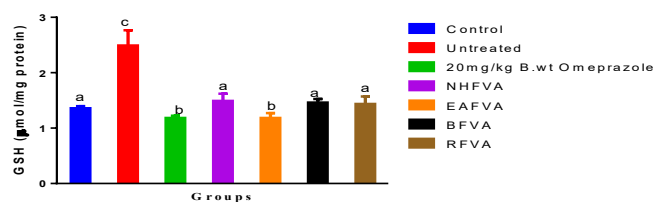


Figure 8: Effects of solvent extract on reduced glutathione concentration in the serum of rats. Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

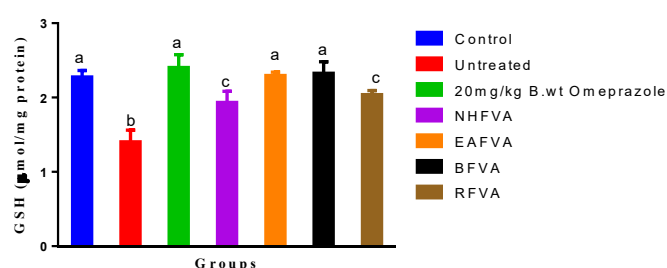


Figure 9: Effects of solvent extract on reduced glutathione concentration in the stomach of rats. Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

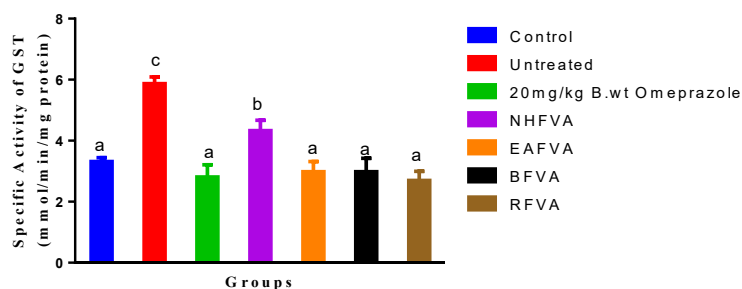


Figure 10: Effects of solvent extract on glutathione-S-transferase concentration in the serum of rats

Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

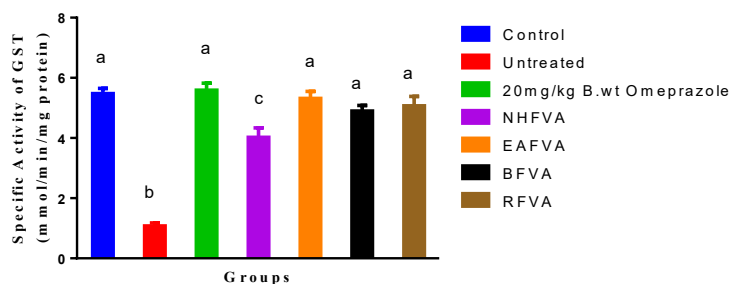


Figure 11: Effects of the solvent extract on glutathione-S-transferase concentration in the stomach of rats.

Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

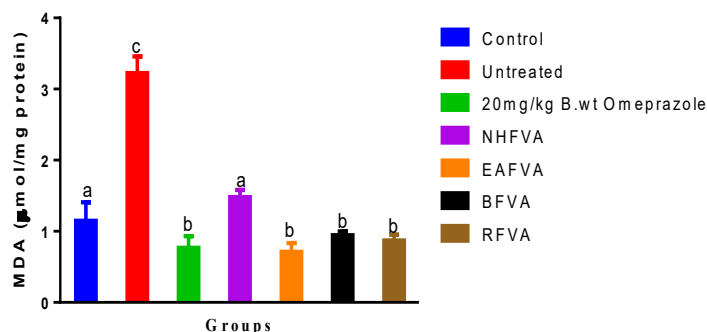


Figure 12: Effects of solvent extract on malondialdehyde concentration in the serum of rats

Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)

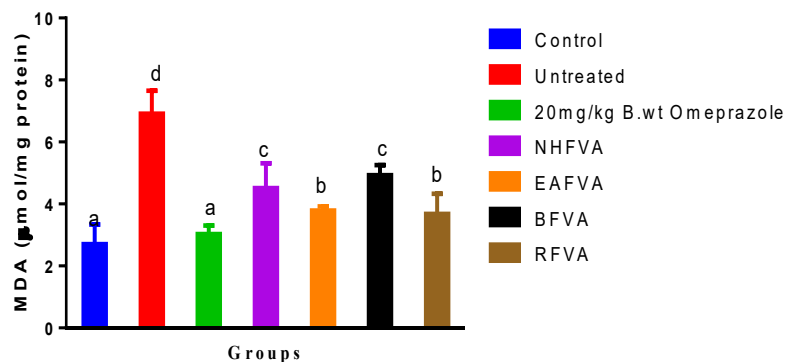


Figure 13: Effects of solvent extract on malondialdehyde concentration in the stomach of rats

Bars with different letters vary significantly ($p < 0.05$). NHFVA: n-hexane *Verbena hastata* extract; EAFVA: Ethyl acetate *Verbena hastata* extract; BFVA: n-butanol *Verbena hastata* extract; RFVA: residual *Verbena hastata* extract (Source: Findings from this study)



Plate 1: control-



plate 2: untreated rat

Plate 1: No redness, no congestion, no hemorrhagic sticks, no necrosis and no dilation of blood vessels (*Source: Findings from this study*)

Plate 2: show redness, congestion, hemorrhagic sticks, necrosis and dilation of blood vessels (*Source: Findings from this study*)



Plate 3: Omeprazole treated rat



Plate 4: 20 mg/kg ethyl acetate extract treated rat

Plate 3: No redness, no congestion, no hemorrhagic sticks, no necrosis and no dilation of blood vessels (*Source: Findings from this study*)

Plate 4: show redness, congestion, hemorrhagic sticks, necrosis and dilation of blood vessels (*Source: Findings from this study*)



Plate 5: 20 mg/kg n- hexane extract rat



Plate 6: 20 mg/kg of residual extract treated rat

Plate 5: show redness, congestion, hemorrhagic sticks, necrosis and dilation of blood vessels (*Source: Findings from this study*)

Plate 6: No redness, no congestion, no hemorrhagic sticks, no necrosis and no dilation of blood vessels (*Source: Findings from this study*)

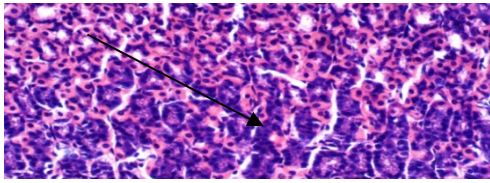


Plate 7: histological micrograph of the stomach wall of control rat (*Source: Findings from this study*)

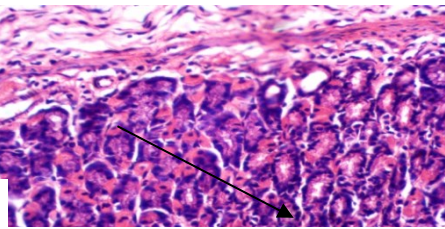


Plate 8: histological micrograph of the stomach wall of untreated rat (*Source: Findings from this study*)

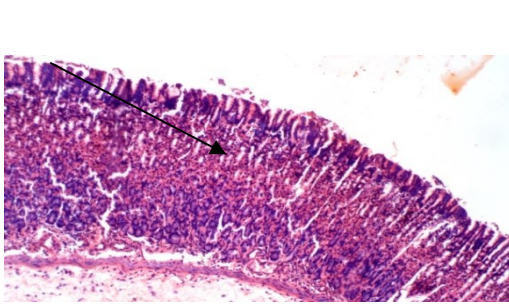


Plate 9: histological micrograph of the stomach wall of with n-Hexane extract treated rat (*Source: Findings from this study*)

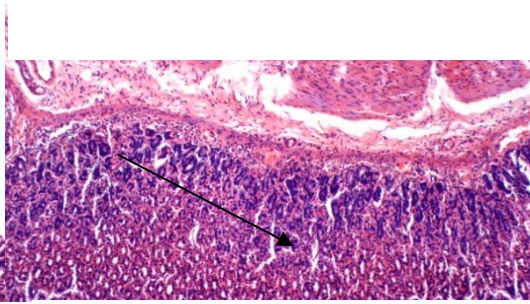


Plate 10: histological micrograph of the stomach wall of n-butanol extract treated rat (*Source: Findings from this study*)

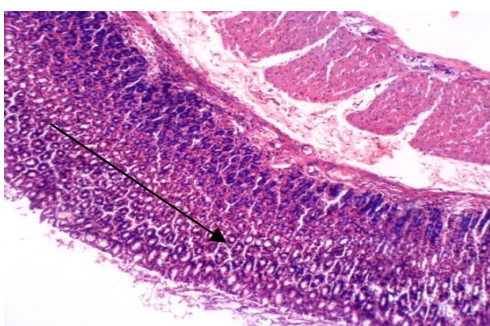


Plate 12: histological micrograph of the stomach wall of residual fraction treated rat (*Source: Findings from this study*)

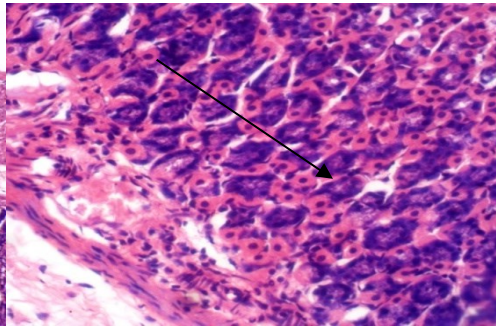


Plate 7: This image shows a normal, intact stomach wall. The epithelial lining is continuous, and the gastric glands are well-organized with minimal inflammatory cells present. The distinct layers of the stomach wall are maintained.

Plate 8 : This image depicts an ulcerated, untreated stomach wall. The epithelial lining is disrupted, and the structure of the gastric glands is lost. There is evidence of necrotic debris and a dense infiltration of inflammatory cells extending into the submucosa layer.

Plate 9: This image shows a normal, healthy stomach wall. The epithelial lining is intact, with well-organized gastric pits and glands. There are minimal inflammatory cells in the lamina propria, and the submucosa and muscularis externa layers are also intact. No signs of ulceration, necrosis, or significant inflammation are present.

Plate 10: This image shows a cross-section of the stomach wall at a higher magnification. The distinct layers are visible, including the mucosal layer with gastric pits (top), the muscularis mucosae, and the submucosa layer below it. The gastric glands appear well-organized and extend into the deeper layers of the mucosa. There is no significant inflammation or disruption of the tissue architecture observed in this section.

Plate 11: In this image, there appears a longitudinal section of the stomach wall, likely focusing on the muscularis externa layer. The smooth muscle fibers of the muscularis externa are arranged in bundles, surrounded by connective tissue. This layer is responsible for the contractile movements that aid in digestion and the movement of food through the stomach. The tissue appears well-organized and without any apparent abnormalities.

Plate 12: This image appears to be another longitudinal section of the stomach wall, potentially at a slightly higher magnification than Image E. The smooth muscle bundles of the muscularis externa are clearly visible, surrounded by connective tissue. Additionally, there are some blood vessels present, likely from the submucosal layer. The tissue architecture appears intact and well-preserved in this section.