

Diabetes & its Complications

Histological Effects of Ethanol Leaf Extract of *Vernonia amygdalina* on the Stomach of Experimentally-Induced Diabetes in Adult Male Wistar Rats

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Received: 16 Aug 2026; **Accepted:** 17 Sep 2026; **Published:** 30 Sep 2026

Citation: Arayombo Babatunde Elijah, Adewole Olarinde Stephen, Ige Mokolade, et al. Histological Effects of Ethanol Leaf Extract of *Vernonia amygdalina* on the Stomach of Experimentally-Induced Diabetes in Adult Male Wistar Rats. *Diabetes Complications*. 2026; 10(3); 1-7.

ABSTRACT

Introduction: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and oxidative stress, leading to multi-organ damage including gastric mucosal degeneration. *Vernonia amygdalina*, a widely used medicinal plant possesses antioxidant, antidiabetic, and cytoprotective properties, studies have demonstrated its antiulcerogenic and hepatoprotective activities but limited data exist on its gastric histological effects in diabetic conditions. This study investigated the histological effects of ethanol leaf extract of *Vernonia amygdalina* (ELEVA) on the stomach of experimentally-induced diabetic Wistar rats.

Methodology: Sixty adult male Wistar rats of 200 ± 20 g were used for the study. The rats were randomly assigned into six groups (I-VI) of ten rats each. Group I rats were administered citrate buffer solution (1 ml/kg) and fed on standard laboratory rat feed throughout the period of the experiment. Groups II, III, IV and VI were fed on High Fat Diet (HFD) for eight weeks and Streptozotocin administered (35 mg/kg/day) intraperitoneally for 5 days. Groups III and IV received 400 and 800 mg/kg of ELEVA for three weeks respectively, while Group VI rats were given 5 IU/kg body weight of insulin intraperitoneally for three weeks. Group V rats were fed on constituted High Fat Diet for eight weeks after which ELEVA was administered 400 mg/kg orally for three weeks before diabetes induction. The rats were sacrificed under chloroform anesthesia and the stomachs of the animals were completely excised, weighed and fixed for 24 hours and later grossed. Statistical analysis was carried out using descriptive and inferential statistics.

Results: The untreated diabetic group revealed severe mucosal erosion, glandular atrophy, and inflammatory infiltration. The extract treated groups showed restored gastric architecture, reduced mucosal erosion, and normalized glandular integrity in a dose-dependent manner. The high-dose and prophylactic groups showed near-complete restoration comparable to insulin-treated rats.

Conclusion: ELEVA demonstrated antihyperglycemic, anti inflammatory and antioxidant properties, with high doses showing superior efficacy to insulin in restoring biochemical balance in diabetic rats.

Keywords

Stomach, Wistar rats, Diabetes, *Vernonia amygdalina*, Ethanol.

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both [1]. The condition has become one of the most significant public health challenges worldwide due to its rapidly increasing prevalence and its association with substantial morbidity, premature mortality, and

economic burden [2]. Epidemiology data from the World Health Organization [3] revealed that hundreds of millions of adults are currently living with diabetes, and this number is projected to rise considerably over the coming decades, particularly in low and middle income countries where changes in lifestyle, urbanization, obesity, and population ageing continue to drive disease incidence. In Nigeria alone, a total of 2.9 million adult individuals are affected, with a prevalence of 3.0 % [4]. Beyond its metabolic consequences, diabetes imposes a significant burden on healthcare

systems through increased hospitalization, reduced quality of life, and the development of debilitating complications, underscoring the need for effective preventive and therapeutic strategies [5].

The pathogenesis of diabetes mellitus is characterized by persistent hyperglycaemia resulting from impaired insulin secretion, reduced insulin sensitivity, or a combination of both. Chronic hyperglycaemia disrupts normal carbohydrate, lipid, and protein metabolism, leading to widespread metabolic abnormalities that adversely affect the structure and function of various tissues and organs [6,7]. Sustained elevations in blood glucose promote excessive production of reactive oxygen species (ROS), resulting in oxidative stress when endogenous antioxidant defenses become overwhelmed [8]. In addition, hyperglycaemia accelerates the formation of advanced glycation end products (AGEs), activates the polyol and protein kinase C pathways, and enhances inflammatory signaling, all of which contribute to endothelial dysfunction, mitochondrial impairment, cellular apoptosis, and tissue injury [9,10]. These interconnected mechanisms underlie the development of diabetic complications by promoting chronic inflammation, microvascular damage, and progressive degeneration of susceptible organs, including those of the gastrointestinal tract [11,12].

Diabetes mellitus exerts profound effects on the gastrointestinal tract, with the stomach being one of the organs frequently affected by chronic hyperglycaemia [13,14]. Gastrointestinal manifestations of diabetes are largely attributed to autonomic neuropathy, persistent oxidative stress, microvascular dysfunction, and chronic inflammation, all of which impair normal gastric physiology. One of the most common gastric complications is diabetic gastroparesis, a disorder characterized by delayed gastric emptying in the absence of mechanical obstruction [13,14]. Chronic hyperglycaemia disrupts gastric motility by damaging the vagus nerve, impairing the function of the enteric nervous system, and reducing the number and activity of interstitial cells of Cajal, which serve as the pacemaker cells of the gastrointestinal tract [13,15]. In addition to impaired gastric motility, diabetes compromises gastric mucosal defense through reduced mucosal blood flow, diminished mucus secretion, altered gastric acid regulation, and increased susceptibility to oxidative injury [16,17]. These pathological changes predispose the gastric mucosa to inflammation, erosion, ulceration, delayed tissue repair, and structural alterations that may adversely affect digestive function and overall gastrointestinal health.

The gastric wall is composed of four principal layers: the mucosa, submucosa, muscularis externa, and serosa, with the mucosa containing the surface epithelium, gastric pits, and specialized gastric glands responsible for the secretion of mucus, hydrochloric acid, digestive enzymes, and intrinsic factor. The integrity of these structures is essential for maintaining gastric homeostasis and protecting the mucosa from chemical and mechanical injury [18]. In diabetes mellitus, chronic hyperglycaemia and the associated oxidative stress, inflammatory responses, and microvascular

dysfunction compromise gastric mucosal integrity, resulting in progressive structural alterations [14]. Experimental and clinical studies have demonstrated that diabetic gastric tissue exhibits epithelial degeneration, disruption of gastric glands, inflammatory cell infiltration, vascular congestion, oedema, reduced mucosal thickness, and, in severe cases, mucosal erosion or ulceration [19,14].

Vernonia amygdalina, commonly known as bitter leaf, is a perennial shrub belonging to the family Asteraceae and is widely distributed throughout tropical Africa, where it serves both as a dietary vegetable and an important medicinal plant [20]. *V. amygdalina* is soft wooded and rapid regenerating shrub. Owing to its characteristic bitter taste, which is due to the presence of alkaloids, saponins, tanins and glycosides, it is commonly known as bitter leaf. The plant is also known by various indigenous names across different regions and ethnic groups [20,21]. In Nigeria, it is commonly referred to as Ewuro among the Yoruba, Onugbu among the Igbo, Oriwo in Edo, and Chusar doki or Fatefata in parts of Northern Nigeria [21].

In traditional African medicine, its leaves have long been used in the management of diabetes mellitus, gastrointestinal disorders, malaria, fever, helminthic infections, and inflammatory conditions. The diverse therapeutic applications of *V. amygdalina* have been attributed to its rich phytochemical composition, which includes flavonoids, phenolic compounds, saponins, alkaloids, tannins, terpenoids, sesquiterpene lactones, steroids, and glycosides [21]. These bioactive constituents possess potent antioxidant and anti-inflammatory properties that scavenge reactive oxygen species, suppress pro-inflammatory mediators, and enhance endogenous antioxidant defense systems [22]. Experimental studies have further demonstrated that *V. amygdalina* exhibits hypoglycaemic, gastroprotective, anti-ulcer, hepatoprotective, nephroprotective, and cytoprotective activities, suggesting its potential to attenuate diabetes-induced tissue injury [23,24].

Given that oxidative stress and inflammation are key mechanisms underlying diabetic gastric injury, therapeutic agents with antioxidant and cytoprotective properties, such as *Vernonia amygdalina*, may help preserve gastric integrity. Although *Vernonia amygdalina* has been extensively investigated for its antidiabetic and antioxidant effects, its protective role against diabetes-induced histomorphological alterations of the stomach remains poorly understood. Therefore, this study was designed to evaluate the effects of *Vernonia amygdalina* leaf extract on the histomorphology of the stomach in streptozotocin-induced diabetic Wistar rats.

Materials and Methods

Plant Preparation and Extraction

Newly plucked. Leaves of *Vernonia amygdalina* (VA) were collected from Esan's Garden Road 7, Ile-Ife and verified by a taxonomist at the Botany Department, Obafemi Awolowo University, Ile-Ife. The specimen was given a voucher number

(IFE 18346) and stored in the Departmental Herbarium. Seven Kilograms of leaves were separated from the stems, washed in a succession of clean water and air-dried, the weight of the leaves was measured two times weekly, until the mass became constant to ensure dryness (3.3 kg). A pulverizer was used to reduce the leaves to a powdered form. The Soxhlet method was used to extract the leaves using 70% ethanol as the solvent. The extract was filtered to remove insoluble residues using Whatman No. 1 filter paper (0.2 mm). The liquid extract was concentrated using a rotary evaporator and lyophilized. The extract was placed in a desiccator until it is ready for use.

Animal Care and Management

Sixty male Wistar rats (200 ± 20 kg) were purchased from the animal holding of the College of Health Sciences, OAU, Ile-Ife. They were kept in plastic cages and maintained under laboratory conditions of temperature, humidity and natural daylight and night cycle; supplied with standard rat chow and a composition of High Fat Diet (Sunshine Mill, Osogbo, Osun State, Nigeria) and clean drinking water.

Experimental Design

The rats were randomly allocated to different groups; six groups of ten rats each. Group I (control) was administered citrate buffer solution (1 ml/kg body weight) and fed a standard rat chow for the duration of the experiment. Groups II, III, IV and V, were fed High Fat Diets for a duration of four weeks; Groups II, III, IV and V received Streptozotocin (30 mg/kg body weight). Intraperitoneally, dissolved in 0.1M freshly prepared citrate buffer solution at pH 4.5 for 5 days, with overnight fasting each day during diabetes induction. Group II received 30mg/kg of Streptozotocin and left untreated. Groups III and IV received 300 and 600 mg/kg MLEVA for four weeks, respectively, and Group VI rats received 5 IU/kg insulin intraperitoneally for four weeks. Group V rats received four week high fat diet and then received extract of *Vernonia amygdalina* (300 mg/kg) orally for four weeks, before receiving a 5day Streptozotocin (30 mg/kg) induction, intraperitoneally. Three days after diabetes induction, all rats were subjected to a test to confirm diabetes. MLEVA and insulin were administered.

Fasting Blood Glucose Estimation

Diabetes was induced in fasted Wistar rats with a low dose of 5 day intraperitoneal 30 mg/kg of Streptozotocin administration. To prevent hypoglycemic shock, the rats in Groups (II-VI) were given 5% dextrose water overnight. A fine test glucometer was used to estimate blood glucose level. Each rat was monitored and the tail was rubbed to ensure there was enough blood at the tip of the tail. The tail was cleansed with an alcohol swab and pierced with a lancet. The first drop of the blood was discarded, and the tail was cleansed, and the next drop was collected using a compatible glucose test strip. Each reading was then recorded appropriately. Blood sample was drawn from the tail of the rats and evaluated for blood glucose levels, and if the blood glucose levels were greater than 200 mg/dL (11.1 mmol/L), the rats were considered to be diabetic.

Sacrifice and Sample Collection

At the end of the experiment, the rats were sacrificed under chloroform anesthesia. At sacrifice, blood samples were taken via cardiac puncture, for random blood glucose level estimation, which was done using a fine test glucometer with compatible glucose test strip, the reading were then recorded appropriately. The stomachs of the animals were excised, and weighed on a weighing balance. The stomachs were fixed in Neutral Buffer Formalin (NBF) by total immersion for 24 hours and later grossed in a cross-coronal section, for further fixation in NBF for histological analysis. The relative stomach weight was determined as follows:

Relative Heart Weight = $(\text{Absolute heart weight} / \text{Final body weight}) \times 100\%$.

Tissue Processing

The stomach tissue was processed through paraffin wax embedding method of Drury and Wallington. Sections of 5µm thickness were produced from the tissue blocks and stained. The Drury and Wallington method for tissue processing. Scarlet-acid fuchsin stained cytoplasm, muscle fibers, and collagen red initially. Thereafter a mixture of phosphomolybdic and phosphotungstic acid was applied.

Photomicrography

The photomicrographs of the tissue slides were scanned using Motic Easyscan scope Pro N6 in Virtual Slides SVS format. Slide loading took up to 6 slides; these were placed in the motorized tray. Then Scanning was done which Initiated automated scanning and image capture. The autofocus system was employed to adjust focal plane dynamically based on selected mode and Image Capture with High-resolution camera (up to 12MP) was used to capture detailed images. The File output was saved in formats like MDS, SVS, JPEG, DICOM, TIFF and data were automatically uploaded by sending scanned images to a slide management server or local storage and the scanned slides were viewed thereafter.

Statistical Analysis

Data were analyzed, using GraphPad prism 8. One-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons as post hoc. A value of $p < 0.05$ was considered statistically significant. Results were expressed as Mean $\pm$ S.E.M.

Fixation and Staining

Stomach tissues were preserved with Neutral Buffered Formalin to prevent degradation and maintain cellular structure. Thin sections of stomach tissue 5 µm thick were excised to ensure proper penetration of fixative. Primary Fixative which was 10% Neutral Buffered Formalin (NBF), standard fixative to fix for 24 hours at room temperature. Post-Fixation, tissues were rinsed in running tap water to remove excess formalin and prevent pigment formation. Dehydration was done by passing the stomach tissue through ascending grades of alcohol typically from 70% to 100% ethanol. Clearing was done when alcohol was replaced with xylene, which makes the tissue transparent in preparation for infiltration

with wax. The cleared tissue was infiltrated with molten paraffin wax and then embedded in a block, allowing thin sectioning. Thin slices were cut using a rotary microtome and placed on microscope slides.

Sections were stained with hematoxylin and eosin (H&E) to highlight different cellular components. Hematoxylin and Eosin was used for demonstration of general histoarchitecture of the stomach. Masson trichrome was used for staining collagen fibres by using Weigert's iron hematoxylin which stained cell nuclei black, then Biebrich scarlet-acid fuchsin stained cytoplasm, muscle fibers, and collagen red initially. Thereafter a mixture of phosphomolybdic and phosphotungstic acid was applied which selectively removed the red dye from collagen but retains it, in the stomach muscle and cytoplasm. Aniline blue was then applied, which binds specifically to collagen fibers, staining them green.

As shown in the table above, the Body Weight in Group II (STZ) showed weight loss compared to control that is, 202 to 190 Kg, which is consistent with diabetic cachexia. Group IV (medium dose ELEVA) increased weight (210 Kg), suggesting improved metabolic balance. Group V (high dose ELEVA) significantly restored weight (220 Kg), while insulin (Group VI) also maintained weight gain (209 Kg), indicating ELEVA may better support weight recovery.

The Inflammatory Marker (TNF- α) as shown in Group II (STZ) showed a significant elevation in value (16.82 pg/mL), confirming inflammation. Groups III–V (ELEVA) progressively reduced TNF- α , with Group V (11.60 pg/mL) approaching normal control, whereas Insulin (Group VI) also lowered TNF- α (12.82 pg/mL), but less effectively than high-dose ELEVA. The Glycemic Control (RBS) in this study revealed Group II (STZ) to show severe hyperglycemia as shown in the above table (26.81 mmol/L), while ELEVA treatment reduced RBS in dose-dependent manner, the Insulin (Group VI) normalized RBS, comparable with high-dose ELEVA.

It was observed that the Antioxidant status in Group II (STZ) showed marked depletion in both GSH and SOD activities, meanwhile ELEVA restored antioxidants dose-dependently. The Insulin (Group VI) improved antioxidants' activities of both GSH and SOD, but less effective when compared with high-dose ELEVA.

Group I showed balanced biochemical profile, low TNF- α , normal glucose, high antioxidants, stable weight, while Group II (STZ) revealed deviation from normal profile with high TNF- α and RBS, low GSH and SOD with weight loss significantly different from normal control and also from treated groups. Group III (Low dose ELEVA) displayed partial improvement, the TNF- α and RBS tending towards normal but still elevated, the antioxidants were modestly restored. Group IV (Medium dose ELEVA) showed clear improvement, that is RBS and TNF- α lowered, antioxidants increased, weight gain above control. In Group V (High dose ELEVA): Near-normalization of TNF- α , RBS, GSH, and SOD levels significantly improved, showing strong therapeutic effect. Group VI (Insulin) was found to be Effective in glycemic control and inflammation reduction, but antioxidant recovery less pronounced compared to high-dose ELEVA.

STZ induction (Group II) produced classic diabetic pathology: hyperglycemia, inflammation, oxidative stress, and weight loss, while ELEVA extract showed dose-dependent efficacy: the Low dose offer partial correction, medium dose proffer improved metabolic and antioxidant balance, the High dose displayed near-normalization, outperforming insulin in antioxidant restoration and weight stabilization. The Insulin (Group VI) effectively controlled glucose and inflammation but was less potent in restoring antioxidant defenses compared to ELEVA.

H&E Group I showed Intact epithelial lining, well-organized gastric glands, Normal mucosal architecture with no inflammation or necrosis. Group II showed disrupted epithelial cells, thinned mucosa, inflammatory infiltration, Severe mucosal damage and inflammation due to hyperglycemia-induced oxidative stress. Group III revealed Partial mucosal restoration, reduced inflammatory infiltration with noticeable healing; ELEVA therapy mitigated tissue injury but not fully restores normal structure. Group IV showed mild epithelial improvement, partial glandular preservation, early signs of recovery; epithelial integrity improvement while inflammation was still present. Group V revealed improved mucosal thickness, reduced necrosis and inflammation. Strong protective effect; gastric glands near normal, minimal inflammation were observed. Group VI showed far better mucosal preservation with minimal inflammation. This means synergistic healing effect; mucosa comparable to group I control and glands fully restored, this work corroborated the work of Adefisayo et al., [25] who posited that treatment of gastric ulcer

Biochemistry Results

Table 1:

Group	Wt (Kg)	TNF α (pg/mL)	RBS (mmol/L)	GSH (μ mol/g)	SOD (U/mL)
I (Citrate buffer)	202 $\pm$ 1.3	09.44 $\pm$ 0.37	05.0 $\pm$ 0.33	23.00 $\pm$ 2.47	14.7 $\pm$ 3.24
II (STZ)	190 $\pm$ 0.9	16.82 $\pm$ 0.52* $\circ$	26.81 $\pm$ 1.14* $\circ$	08.29 $\pm$ 0.64* $\circ$	04.6 $\pm$ 0.96* $\circ$
III (STZ + LOW DOSE ELEVA)	198 $\pm$ 1.7	14.80 $\pm$ 0.36*	17.66 $\pm$ 0.73*	09.89 $\pm$ 0.63*	8.77 $\pm$ 0.32
IV(STZ + MED. DOSE ELEVA)	210 $\pm$ 2.3	13.06 $\pm$ 0.42	14.94 $\pm$ 0.56	12.04 $\pm$ 0.36	10.46 $\pm$ 0.76#
V (STZ + HIGH DOSE ELEVA)	220 $\pm$ 2.7	11.60 $\pm$ 0.42#	08.34 $\pm$ 0.64#	17.63 $\pm$ 0.63#	11.66 $\pm$ 0.56#
VI (STZ + 5 IU Insulin)	209 $\pm$ 1.2	12.82 $\pm$ 0.34#	09.25 $\pm$ 0.73#	13.69 $\pm$ 0.93#	09.44 $\pm$ 0.43

Results presented as mean $\pm$ SEM(n=5); *:Significantly different from normal control at p <0.05, # Significantly different from toxic control at p < 0.05, $\circ$:Significantly different from III, IV, V and VI at p < 0.05

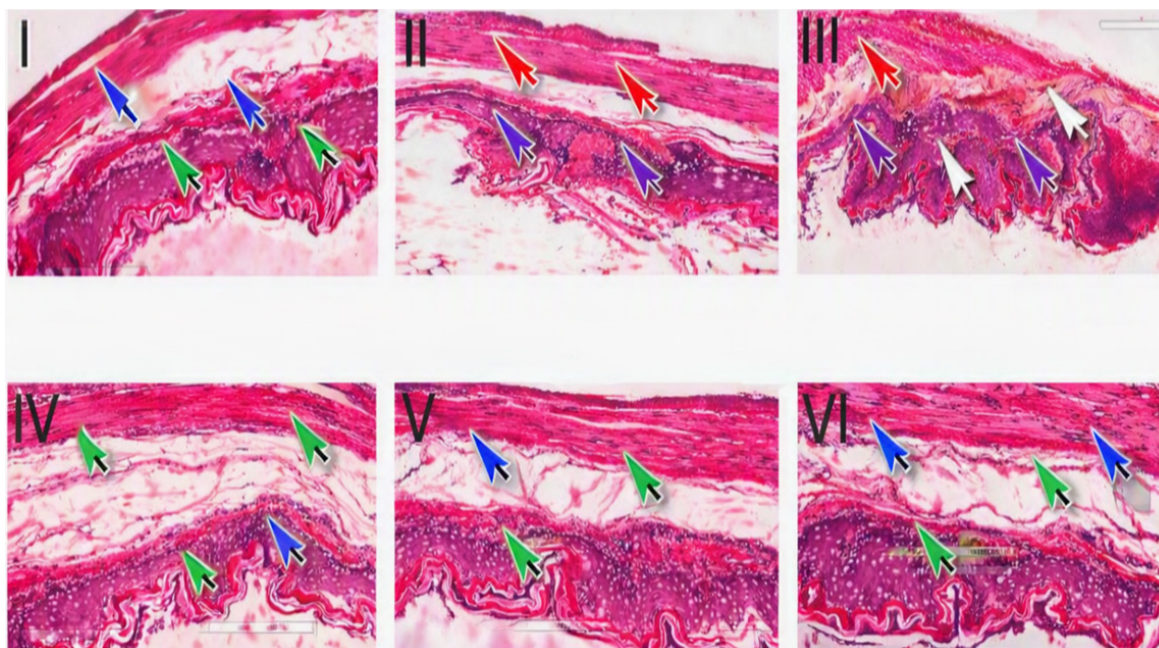


Plate 1: Representative photomicrographs of the stomach tissue of MLEVA treated streptozotocin-induced diabetic Wistar rats. H&E X400. Blue arrow (intact epithelium), Red arrow (Epithelial damage/ erosion), Green arrow (Restored gastric glands), Purple arrow (inflammatory cell infiltration), White arrow (Necrotic area).

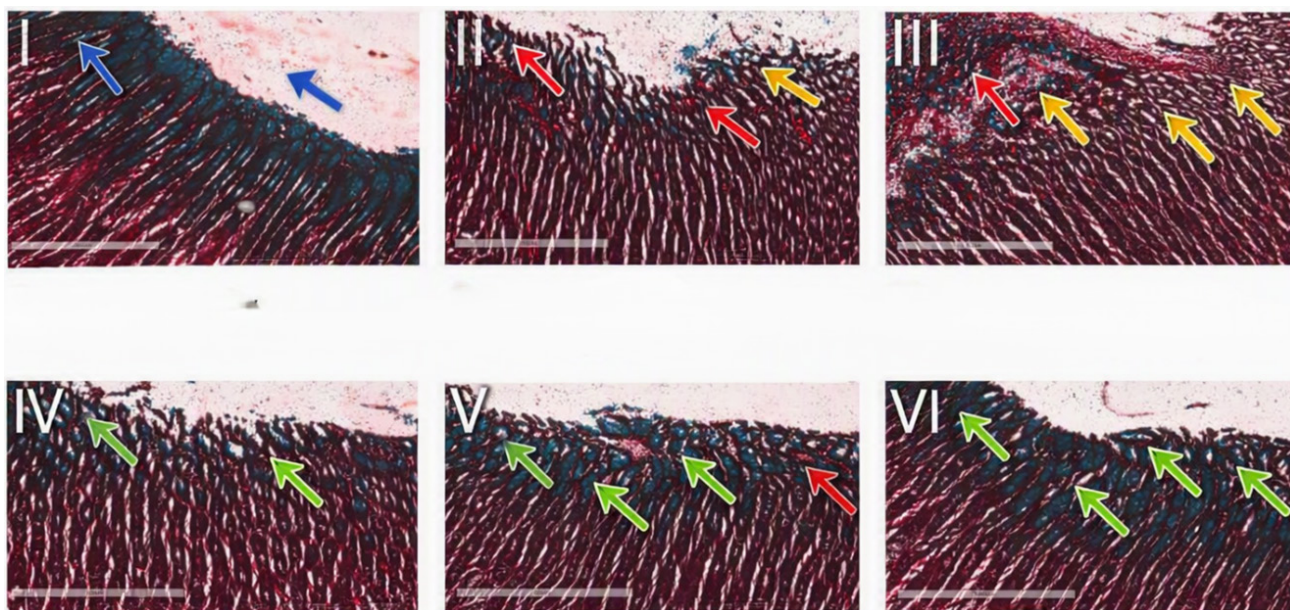


Plate 2: Representative photomicrographs of the stomach tissue of MLEVA treated streptozotocin-induced diabetic Wistar rats. VVG X400. Blue arrow (intact epithelium), Red arrow (Epithelial damage/ erosion), Yellow arrow (Collagen damage), Green arrow (Restored gastric glands), Purple arrow (inflammatory cell infiltration).

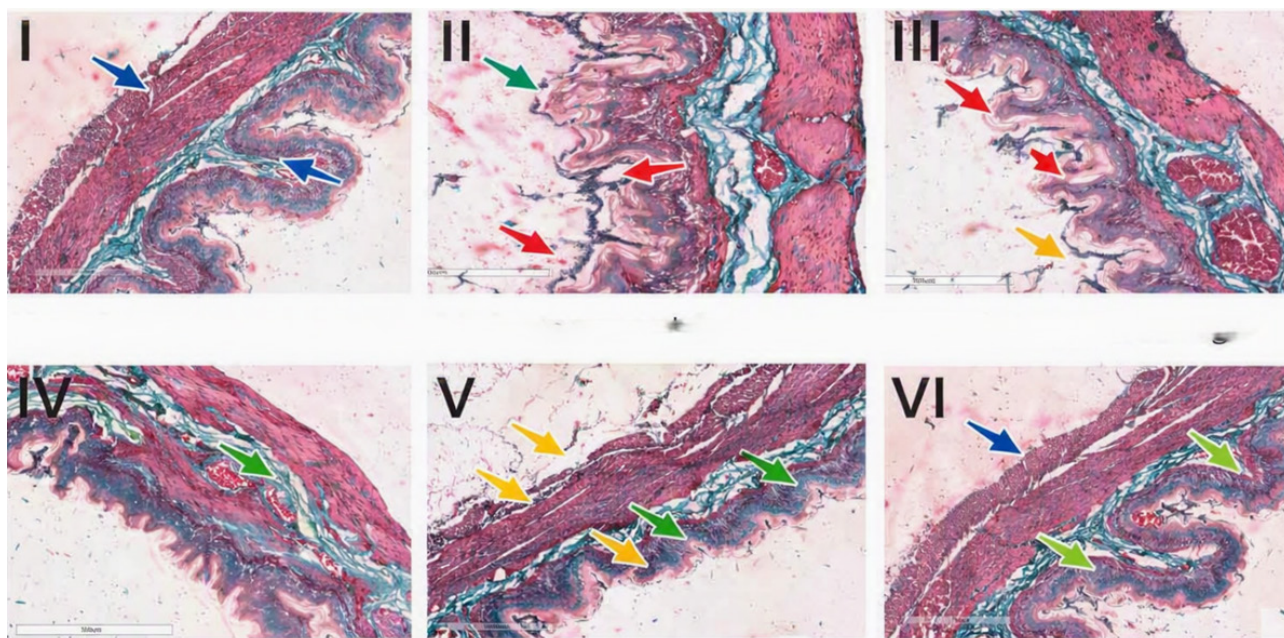


Plate 3: Representative photomicrographs of the stomach tissue of MLEVA treated streptozotocin-induced diabetic Wistar rats. MT X400. Blue arrow (intact epithelium), Red arrow (Epithelial damage/ Necrosis), Yellow arrow (Collagen damage), Green arrow (Restored Collagen).

with methanol extract of *Vernonia amygdalina* restores gastric epithelial integrity in a previously injured stomach in rats.

In the Masson Trichrome plate 2, Group I (Normal Control) showed, normal mucosa with intact epithelium and well-organized collagen fibers. While in Group II (Low Dose Extract) Moderate epithelial necrosis and collagen fragmentation with partial mucosal damage were observed. In Group III (Toxic Group), there was severe epithelial necrosis with collagen disorganization, and heavy inflammatory infiltration. Group IV (Medium Dose Extract), showed Improved collagen fibre with mild epithelial damage and moderate mucosal healing. In Group V (High Dose Extract), there were minimal epithelial damage and restored collagen fibers with significant mucosal recovery. Group VI (Insulin Group) also showed near-normal mucosa with intact epithelium and fully restored collagen this group displayed best protective effect.

There was progressive restoration of epithelial and collagen integrity across treatment groups. The high-dose extract and Insulin groups exhibited near-normal architecture, confirming dose-dependent protective and regenerative effects on gastric connective tissue in diabetic rats.

The ethanol leaf extract of *V. amygdalina* exhibited gastroprotective and antidiabetic effects, likely mediated by its flavonoids, phenolics, and antioxidant compounds. The restoration of gastric integrity suggests membrane stabilization and free radical scavenging mechanisms similar to insulin therapy. These findings align with previous reports of Odangowei et al., [26] who stated that *V. amygdalina* confers hepatoprotective and anti-ulcer properties in stomach pathology .

Conclusion

ELEVA demonstrated antihyperglycemic, anti-inflammatory and antioxidant properties, with high doses showing superior efficacy to insulin in restoring biochemical balance in diabetic rats. Moreover it significantly ameliorated stomach histopathological alterations in diabetic rats. The high-dose extract (600 mg/kg) was more effective when compared with insulin, confirming its potential as a natural adjunct in diabetes management. This suggests ELEVA could be a promising adjunct or alternative therapy for managing diabetes, inflammatory and its oxidative complications.

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