Toxicology Reports 4 (2017) 625–633

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Full length article

Gastro-protective effect of methanol extract of *Vernonia amygdalina* (del.) leaf on aspirin-induced gastric ulcer in Wistar rats

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ARTICLE INFO

Keywords:
Vernonia amygdalina
Aspirin
Gastric ulcer
Antioxidant
Rat

ABSTRACT

This study investigated the protective effects of methanol extract of *Vernonia amygdalina* leaf (MEVA) on aspirin induced gastric ulcer in rats. Thirty Wistar rats, 150–200 g were divided into six groups as follows: Group 1 (control) rats received 2 mL/kg of propylene glycol for 28 consecutive days. Group 2 (Ulcer Control) received 150 mg/kg/day of aspirin suspended in 3 mL of 1% carboxymethylcellulose in water orally for 3 consecutive days during which the rats were fasted for the induction of ulcer. Group 3 received cimetidine at 100 mg/kg/day orally for 28 consecutive days and thereafter treated as group 2. Groups 4, 5 and 6 received MEVA orally at 200, 300 and 400 mg/kg/day respectively for 28 consecutive days and thereafter were treated with aspirin as group 2. All the animals were sacrifice at the end of the study to determine the gastric pH, gastric acidity, gastric ulcer score, haematological indices, superoxide dismutase (SOD) activity, reduced glutathione (GSH) and Lipid peroxidation (LPO) levels. The result showed that aspirin significantly ($p < 0.05$) increased gastric ulcer score and index, decreased gastric pH, gastric acidity, SOD activity, GSH level as well as increased LPO level. It induced significant necrosis of the stomach tissue. Administration of MEVA significantly ($p < 0.05$) increased gastric pH, but decreased gastric acid secretion and reversed alteration of haematological parameters. It also significantly ($p < 0.05$) increased SOD activity, GSH level and decreased LPO level. The results suggest that *Vernonia amygdalina* possesses gastro-protective properties against aspirin-induced gastric ulcer.

1. Introduction

Gastric ulcer is a common gastrointestinal tract (GIT) disorder that affect about 10% of the world population [1]. It's characterized by GIT bleeding, perforation and erosion of the mucosa wall due to imbalance between aggressive (acid, pepsin and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, mucosal blood flow and growth factors) [2,3]. The incidence of this disease is multifactorial which include H. pylori, non-steroidal anti-inflammatory drugs (NSAIDs), smoking, stress, chronic alcohol, altered prostaglandin E series metabolism and bad dietary habits [4,5].

Aspirin is acetylsalicylic acid often used to treat pain, fever and inflammation [6–8]. Despite its therapeutics benefits, it's known to induce gastric ulcer in both human and animals [9,10]. The pathogenesis of aspirin-induced gastric ulceration includes the aspirin blocking the activities of the cyclooxygenase enzymes (COX-1 and COX-2) hence leading to reduced mucus and bicarbonate secretion, decreased mucosal blood flow, impaired platelet aggregation, alteration of microvascular structures leading to epithelia damage, increased leukocyte adherence and increased production of reactive oxygen species (ROS), increased lipid peroxidation and neutrophil infiltration as well as decreased antioxidant enzymes [11–13].

Treatment of gastric ulcer using a conventional therapy faces a major setback because most of the drugs currently available in the market show limited efficacy against gastric diseases and are often associated with severe side effects. However, several medicinal plants are used in folk medicine to treat gastrointestinal disorders and they have been shown to produce promising results in the treatment of these pathologies [14].

Vernonia amygdalina (VA) belong to the family of Asteraceae. It is a small shrub tree that grows in the tropical Africa [15]. VA is popularly called bitter leaves because of its bitter taste and is used as vegetables or as flavor decoction soups in some part of Africa (most especially, Nigeria). The roots and leaves decoction of VA are commonly used in

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https://doi.org/10.1016/j.toxrep.2017.11.004
Received 11 July 2017; Received in revised form 10 November 2017; Accepted 13 November 2017
Available online 13 November 2017

ethno-medicine to treat fevers, hiccups, kidney problems and stomach discomfort among other several uses [16]. It's also used in the treatment of diarrhea, dysentery, hepatitis and cough and as a laxative and fertility inducer [17]. The antibacterial, antioxidant, anti-malarial activity and wound healing properties of VA have been reported [18–20]. Also, VA has been found to be effective in the treatment of intestinal worms as well as for gastritis, enteritis and rheumatism [20]. However, its protective effects on drug-induced gastric ulcer or gastrointestinal toxicity has not yet elucidated. This study was therefore designed to investigate the gastro-protective effects of methanol extract of *Vernonia amygdalina* leaf on aspirin-induced gastric ulcers in rats.

2. Materials and methods

2.1. Drugs and chemicals

Aspirin, 300 mg tablets was purchased in SKG-PARMA LIMITED; Cimetidine, 200 mg tablet was purchased in Jiangsu Riunia Qian Pharm. Co., Ltd., China; Ketamine hydrochloride (50 mg/10 mL) injection was purchased in Popular Pharmaceuticals, Bangladesh. Propylene glycol was procured from Biovision, Milpitas, CA, USA. Methanol and carboxyl methylcellulose (CMC) were analytical graded chemicals purchased from Sigma (St. Luis, USA).

2.2. Plant extraction

The methanol extract of *Vernonia amygdalina* (VA) leaves was extracted as described by Oyedeji et al. [21]. Fresh *Vernonia amygdalina* leaves were aired-dried and pulverized using laboratory blender (DIK-2910, Daiki Rika Kogyo Co. Ltd, Tokyo-Japan). The pulverized specimen of VA (1.7 kg) was soaked in 70% methanol and shaken for 72 h using an electric shaker. The mixture was filtered with Whatmann No.1 filtered paper. The filtered extract was evaporated under reduced pressure using rotary evaporator. The resulting concentrate was freeze-dried using a lyophilizer (Ilshin Lab. Co. Ltd, Seoul, Republic of Korea) to yield final product called methanolic extract of *Vernonia amygdalina* (MEVA).

The sample obtained as a product of freeze drying was weighed to calculate for the percentage yield of the plant extract.

% yield = yield of MEVA (g) ÷ weight of pulverized leaves × 100

$$\text{Extraction yield in}\% = \frac{\text{Weight of extract of } \textit{Vernonia amygdalina}}{\text{Weight of powdered of } \textit{Vernonia amygdalina}} \times 100$$

$$= \frac{139.09\text{g}}{1700\text{g}} \times 100$$

$$= 8.18\,\%$$

2.2.1. Stock solutions of MEVA

Methanol extract of *Vernonia amygdalina* leaves (MEVA) were prepared at graded doses of 200, 300 and 400 mg. Two gramme (2 g) of MEVA was dissolved in 20 mL of propylene glycol to obtain a sample preparation (stock solution) for 200 mg/kg of MEVA. From the stock solution, the rats received 0.2 mL/100 g/day of the extract orally (*p.o*). Stock solutions for 300 and 400 mg/kg of MEVA were prepared by dissolving 3 g and 4 g of MEVA in 20 mL of propylene glycol, respectively. The control rats received 0.2 mL/100 g/day of propylene glycol.

2.3. Acute toxicity studies (LD_{50}) of MEVA

The acute toxicity of MEVA was determined by the procedure outline by Lorke's, [22]. Rats of either sex were divided into 2 phases. In the first phase of the study, 9 rats were divided into 3 groups of 3 rats each and they were treated with MEVA by gavage at the doses of 10, 100 and 1000 mg/kg. In the second phase, 8 rats were divided into 4 groups of 2 rats each and they were treated with MEVA by gavage at the doses of 850, 1700, 3400 and 6800 mg/kg. The general behavior of the animals was observed continuously for 1 h after treatment and then intermittently for 4 h, then hourly for the next 24 h. The LD_{50} was determined using the formula;

$$LD_{50} = \sqrt{a} \times b$$

Where, a = least dose that killed a rat; and b = highest dose that did not kill any rat.

2.4. Phytochemicals quantification of MEVA

Preliminary phytochemical constituents of the methanol extract of *Vernonia amygdalina* leaves were tested using methods already described for the determination of alkaloids, flavonoids, saponins, tannins, glycosides and terpenoid [23–25].

2.5. Animals

Adult Wistar rats of both sexes with body weight of 150–200 g were used for this study. They were purchased from the Animal Holding of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife. The rats were housed in the laboratory plastic cages for two weeks under normal laboratory conditions (temperature 28 ± 31 °C and relative humidity 50 ± 55% with 12 h light/dark cycle) before the commencement of the study and they were allowed to have access to rat chow and water *ad libitum*. All the animals received good care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and approved by Institutional Research Committee.

2.6. Experimental design

A total of 30 rats were divided into 6 groups (5 animals per group). Group 1 received an oral dose (2 mL/kg/day) of propylene glycol for 28 consecutive days. Group 2 received 150 mg/kg/day of aspirin suspended in 3 mL of 1% carboxymethylcellulose (CMC) in water for 3 days during which the rats were fasted for induction of ulcer. Group 3 rats received cimetidine 100 mg/kg suspended in 3 mL of 1% CMC in water orally for 28 consecutive days followed by 150 mg/kg aspirin suspended in 3 mL of 1% CMC in water for 3 days. Groups 4, 5 and 6 rats received 200, 300 and 400 mg/kg of MEVA respectively for 28 consecutive days and thereafter, they were pre-treated with 150 mg/kg of aspirin suspended in 3 mL of 1% CMC in water for 3 days. Twenty-four hours after administration of aspirin, all the animals were sacrificed under ketamine anaesthesia. The blood of each animal was collected by cardiac puncture into separate EDTA bottle for haematological studies. The stomach of the rats was removed and opened on the greater curvature and gastric content were collected into plain tubes and centrifuged for measurement of gastric secretion parameters such as gastric volume, total and free acidity and pH. Gastric mucosal were examined for evaluation of the degree of ulceration which was expressed as ulcer score, ulcer index and percentage inhibition. Part of the stomach of each rat was carefully excised, weighed and fixed in 10% formal-saline for histopathological studies, while the other stomach tissues was processed for assay of lipid peroxidation and antioxidant enzymes status.

2.7. Measurement of body weight change and food consumption

Weekly body weight of the rats for all groups were measured with the aid of a digital weighing balance (Hanson, China) to assess weekly weight gain or weight loss while the food intake was measured and calculated with the aid of the metabolic cages and digital weighing balance (Hanson China).

2.8. Measurement of gastric acid secretion

Gastric acidity was performed as earlier described by Gehan et al. [26]. Twenty-four hours after the induction of gastric ulcer, the rats were sacrificed under ketamine hydrochloride; the abdomen was opened to remove the stomach. The stomach was opened along the greater curvature and gastric content was drained into a centrifuge tube. Five ml of distilled water was added and the resultant solution was centrifuged at 3000 rpm for 10 min. The pH of gastric juice was determined using a pH meter (Microfield, pH S-25 pH meter, England). The free and total acid content of the gastric juice was determined by titrating gastric juice with 0.01 N NaOH, using topfer's reagent and phenolphthalein as indicator and was expressed as Meq/L/24 h [27,28]. A burrete was setup in the laboratory, 0.01 N NaOH was prepared and poured into the burrete. 50 mL of distilled water was added to the gastric content (aliquot) inside the plain bottles. 25 mL of gastric juice was pipetted into a beaker and three drops of Topfer's reagent added to make up for the free acid. The NaOH inside the burrette was titrated against the acidic solution in the beaker, and observed until yellow colouration was obtained. The volume of the alkali used, which correspond to the free acidity was noted.

The procedure above was repeated using phenolphthalein as an indicator. In this experiment, the colour change was from colourless to red; the total volume of alkali added was recorded for total acidity and used for the determination of concentration of gastric acid as stated below:

The following formula was used to determine the concentration of the acid; $\frac{C_A V_A}{C_A V_A} = \frac{N_A}{N_A}$ Where C_A = concentration of acid used;

V_A = Vol. of acid used;

C_B = concentration of base used;

V_B = Vol. of base used;

N_A = mol ratio of acid used;

N_B = mol ratio of base used.

The unit = mol/dl was converted to Meq/L.

2.9. Measurement of gastric ulcer scoring

Twenty-four hours after the induction of gastric ulcer, the stomach was opened along greater curvature and washed with normal saline. Gastric mucosal lesions were expressed in terms of ulcer index (U.I.) according to Peskar et al. [29] which depends on the calculation of a lesion index by using a 0–3 scoring system based on the severity of each lesion. The severity factor was defined according to the length of the lesions. Severity factor 0 = no lesions; 1 = lesions < 1 mm length; 2 = lesions 2–4 mm length and 3 = lesions > 4 mm length.

The lesions/ulcer score for each rat was calculated as the number of lesions in the rat multiplied by their respective severity factor.

The mean ulcer index (UI) was calculated by the method of Raji et al. [30].

Thus:

$$\text{Ulcer index(U.I.)} = \text{Mean degree of ulceration} \times \frac{\%\ \text{of group of ulceration}}{100}$$

The percentage inhibition (P.I.) of a given drug was calculated by the equation of Hano et al. [31].

$$\text{Percentage inhibition} = \frac{\text{U.I. of aspirin group} - \text{U.I. of pretreated/treated group}}{\text{U.I. of aspirin group}} \times 100$$

$$\%\ \text{of ulceration} = \frac{\text{number of ulcerated rat} - \text{number of non} - \text{ulcerated rat}}{\text{Number of rat in group}} \times 100$$

2.10. Measurement haematological indices

The haematocrit (HCT), haemoglobin (HB) concentration, red blood cell (RBC), mean Corpuscular volume (MCV), mean corpuscular haemoglobin concentrated (MCHC), white blood cell (WBC), granulocyte (GRAN), monocytes (MON), lymphocytes (LYMP) and platelet (PL) counts were measured using an auto-analyzer machine (SFRI blood cell Counter, H18 light, France).

2.11. Biochemical assays

The stomach samples from the rats were homogenized in a 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride, followed by centrifugation of the homogenate at 10,000 rpm for 15 min at −4 °C. The supernatant was collected to allow estimation of superoxide dismutase (SOD) activity and was assessed using the method described by Misra and Fridovich [32]. Aliquots of 2.5 mL buffer, 30 mM EDTA, and 300 µL 2 mM pyrogallol were added to 200 µL of the lysate. Any increase in absorbance was recorded at 420 nm for 3 min using a spectrophotometer. One unit of enzyme activity represents 50% inhibition of the rate of auto-oxidation of pyrogallol as determined by a change in the absorbance/min at 420 nm. The activity of SOD was expressed as µg/mg protein.

Reduced glutathione (GSH) was determined using the method described by Beutler [33]. An aliquot of 1 mL supernatant, as obtained above, was added to 0.5 mL Ellman's reagent (10 mM), and followed by addition of 2 mL phosphate buffer to the mixture. The yellow color that developed was read at 412 nm against a blank containing 3.5 mL phosphate buffer. A series of standards was also similarly prepared. The amount of GSH was expressed as µg/mg protein.

Lipid peroxidation was measured as malondialdehyde (MDA) according to the method described by Ohkawa et al. [34]. To each 0.5 mL supernatant was added 0.5 mL phosphate buffer (0.1 M, pH 8.0) and 0.5 mL 24% TCA. The resulting mixture was incubated at room temperature for 10 min, followed by centrifugation at 2000 rpm for 20 min. An aliquot of 1 mL of the resulting supernatant was mixed with 0.25 mL 0.33% TBA in 20% acetic acid and the resulting mixture boiled at 95 °C for 1 h. The resulting pink colored product was cooled and the absorbance read at 532 nm (extinction coefficient of TBARS: $\varepsilon 532 = 1.53 \times 10^5\ M^{-1}\ cm^{-1}$).

2.12. Histological analysis

The stomach tissues biopsies of the rats were fixed in 10% formalin, dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax. The tissues were then cut into 2–3 µm thick sections by a microtome, fixed on the slides and stained with hematoxylin-eosin (H&E). The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan) and photomicrographs were taken with a Leica DM 750 camera at x 100 magnification.

2.13. Statistical analysis

The results obtained were expressed as mean ± Standard Errors of Mean (SEM). Data were analyzed using One-way Analysis of Variance (ANOVA) followed by post-hoc test using Student-Newman-Keuls and *P* value less than 0.05 was considered statistically significant. The statistical analysis was performed with the aid of Graph Pad Prism 5.03.

3. Results

3.1. Acute oral toxicity test (LD_{50}) of MEVA

Administration of MEVA up to 3400 mg/kg *p.o.* did not produce any sign of toxicity in rats. There was no significant change in daily body weight or organ weight during the next 4 weeks (results not shown). In

Table 1
Acute oral toxicity test (LD_{50}) of MEVA.

No. of rats	Dose (mg/kg)	Sex	Mortality
1st phase			
3/3	10	Male	0/3
		Female	0/3
3/3	100	male	0/3
		female	0/3
3/3	1000	male	0/3
		female	0/3
2nd phase			
2/2	850	Male	0/2
		Female	0/2
2/2	1700	Male	0/2
		Female	0/2
2/2	3400	Male	0/2
		Female	1/2
2/2	6800	Male	2/2
		Female	2/2

LD_{50} of MEVA = (√6800 × 3400) mg/kg = (√18000000) mg/kg = 4808.33 mg/kg body weight. Therefore, LD_{50} of MEVA ≥ 4808.33 mg/kg body weight in adult Wistar rats. MEVA: methanol extract of Vernonia amygdalina leaves, LD_{50}: Lethal dose.

addition, no symptoms of diarrhea or abnormal behavior during this period. None of the rats died. However, oral administration of MEVA at 6400 mg/kg caused 100% mortality in rats. The oral LD_{50} of MEVA was determined to be ≥4808.33 mg/kg body weight in adult Wistar rats (Table 1).

3.2. Qualitative and quantitative screening of methanol extract of Vernonia amygdalina (del.) leaf

Phytochemical analysis of the extract showed the presence of alkaloids, flavonoids, tannins, cardiac glycoside and terpenoid (Table 2).

3.3. Body weight change and food consumption

The body weight change and food consumption are shown in Figs. 1 and 2 respectively.

There was a significantly higher ($p < 0.05$) body weight change in group 3 (cimetidine) and groups 4 and 5 (200 and 300 mg/kg) animals treated with MEVA for 4 weeks before the induction of ulcer when compared with the control. However, group 6 had no significant difference ($p > 0.05$) in body weight change when compared with the control. The ulcer control group had a significantly lower ($p < 0.05$) body weight change when compared the control, cimetidine and all groups (200, 300 and 400 mg/kg) treated with MEVA after 3 days of ulcer induction.

The food intake of rats treated with aspirin for 3 days was significantly lower ($p < 0.05$) when compared with the control and groups treated with cimetidine and MEVA. Thus, treatment with MEVA prevent the deleterious effect of aspirin on food intake after 3 days of induction of ulcer.

Table 2
Qualitative and Quantitative Screening of Methanol extract of *Vernonia amygdalina* (del.) Leaf.

Phytochemical Constituents	Percentage Composition (g/100 g)
Alkaloids	13.54 ± 0.34
Tannins	34.05 ± 1.05
Flavonoids	59.65 ± 0.83
Saponins	5.56 ± 0.03
Cardiac Glycoside	9.02 ± 0.90
terpenoids	10.05 ± 0.56

Each value (n = 3) is expressed as mean ± Standard Error of Mean.

3.4. Effects on gastric, volume pH and gastric acid secretion

There was a significantly lower ($p < 0.05$) gastric volume in cimetidine (group 3) and MEVA treated groups (groups 4 and 5) when compared with ulcer control group (group 2). However, the gastric pH of MEVA treated groups and cimetidine (Groups 3, 4 and 5 respectively) was significantly higher ($p < 0.05$) when compared with ulcer control group (group 2) (Table 3). The free and total acidity of the rats treated with MEVA and cimetidine was significantly lower ($p < 0.05$) when compared with ulcer control group. Thus, MEVA prevent or reduce the increased in gastric acid secretion induced by aspirin treatment (Table 3).

3.5. Effects on gastric ulcer scoring

As shown in Table 3, MEVA treated groups had a significantly lower ($p < 0.05$) ulcer scores in the treated rats when compared with the ulcer control (Table 3). The percentage ulcer inhibition in the extract treated rats was similar to that of the standard drug (cimetidine) used in this study (Table 3). Thus, the increase in ulcer score and index was prevented by MEVA in this study.

3.6. Hematological parameters

The mean values of hematological parameters in rats treated with graded doses of MEVA are presented in Table 4.

Administration of MEVA significantly lower ($p < 0.05$) white blood cell (WBC) count and granulocytes (GRAN) count when compared with ulcer control group. MEVA treated group had no significant difference ($p > 0.05$) in WBC and GRAN counts when compared with normal control group. However, aspirin treatment significantly lower ($p < 0.05$) red blood cell (RBC) count and hemoglobin (Hb) concentration when compared with MEVA treated groups. MEVA treated groups had no significant difference ($p > 0.05$) in RBC count and Hb concentration when compared with normal control group.

3.7. Ulcer-induced stomach oxidative stress

The induction of ulcer by aspirin was accompanied with significant depletion of gastric mucosa antioxidant system in rats. The SOD activity and GSH level in the stomach tissue decreased significantly in the rats treated with aspirin (ulcer control group) compared with normal control animals (Figs. 3 and 4). MEVA or cimetidine treatment prevented the decrease in the SOD activity and GSH level and maintained their normalcy in aspirin-exposed rats (Figs. 3 and 4). Aspirin (ulcer control group) treatment resulted in a significantly elevated mucosa MDA level compared with the control group (Fig. 5). However, treatment with MEVA or cimetidine significantly lower or reversed the increase in MDA level to normalcy (Fig. 5).

3.8. Histological observations

The representative photomicrographs of stomach sections of control and treated rats are shown in Fig. 6. The histology of the stomach sections of control rats was structurally normal having the normal epithelial architecture and laminal propria, submucosa and muscularis propria. In contrast, treatment with aspirin alone resulted in severe epithelial erosion, necrotic and distorted glands accompanied by marked cellular infiltration by mononuclear cells, degenerative changes in forestomach and fundic regions of the stomach. The morphological characteristics of the stomach of rats treated with MEVA or cimetidine plus aspirin showed significant amelioration of aspirin-induced ulcer and were comparable to those in control group.

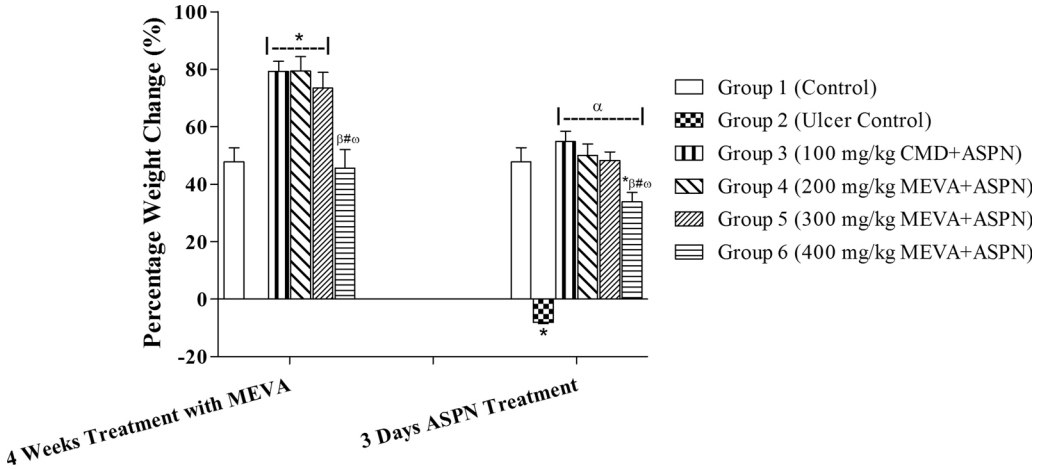

Fig. 1. Effect of methanol extract of *Vernonia amygdalina* leaf on percentage body weight on aspirin induced ulcer in rats. Each value represents Mean ± S.E.M (n = 5); * = significantly different from control ($p < 0.05$); α = significantly different from ulcer control group (aspirin alone) ($p < 0.05$); β = significantly different from 100 mg/kg CMD ($p < 0.05$); # = significantly different from 200 mg/kg MEVA ($p < 0.05$); ω = significantly different from 300 mg/kg MEVA ($p < 0.05$).

4. Discussion

Food consumption and energy expenditure are the determinant for weight gain or loss [35]. Previous studies have shown that non-steroidal anti-inflammatory drugs (NSAIDs) decreased nutrient digestion and absorption through there direct effect on the gastric and intestinal mucosal cells [36,37]. However, the direct effect of NSAIDs on gastric and intestinal mucosal cells is as a result of inhibitory effects of NSAIDs on constitutive cyclooxygenase in the gastrointestinal cells [38]. The significant decrease in food consumption and body weight change that were observed in aspirin (ulcer control) treated group can be attributed to the anorexia that was occurred during aspirin treatment or inhibition of prostaglandins synthesis in the stomach. An increase in food consumption and body weight were observed in the MEVA treated groups when compared with the rats that were treated with aspirin only. The gain in body weight could be attributed to the stimulation of feeding center in the lateral hypothalamus [35], or mobilization of endogenous prostaglandins in the gastric mucosa of rats treated with MEVA.

Aspirin has been reported to reduce the gastric juice pH and increase the volume of gastric juice [28], or decrease the volume of gastric juice and its acid output [27]. In this study, the volume of gastric juice and acid output of rats treated with aspirin were significantly increased when compared with the control. The free and total acidity of gastric juice were significantly increased after treatment with aspirin. However, the gastric pH of rats treated with aspirin was significantly decreased when compared with the control rats. An increased in gastric pH and significant decrease in gastric volume and acidity was observed in cimetidine and MEVA treated groups when compared with aspirin group. Also, the ulcer counts and ulcer index were decrease in groups that received cimetidine and MEVA when compared with aspirin treated ulcerated rats. The photomicrograph of the groups that received cimetidine and MEVA showed ameliorated effect on stomach tissues necrosis, sloughing of the mucosa with hemorrhage caused by aspirin treatment. The results of this study showed that MEVA caused an increase in gastric pH and reduction in gastric volume and free and total gastric acid concentration in rats which is similar to that produced by cimetidine. This suggests that MEVA has inhibitory effect on gastric acid secretion and its inhibitory action might mimic cimetidine effect on gastric acid secretion. The extract may act by blocking H_2 receptor and prevent histamine from binding, causing decreased in gastric acid secretion. It is established that inhibition of histamine release as a result of blockage of H_2 receptors, inhibit intracellular adenylate cyclase, Na^+-K^+ ATPase and proton pump of parietal cells, thereby reduce the gastric acid secretion [39,40].

The findings of this study also showed that MEVA has anti-ulcer effect on the development of gastric ulcer. The mechanism through which MEVA produces anti-ulcer effect on gastric ulcer by aspirin might be due to its ability to inhibit gastric acid secretion through blocking the stimulatory effect of histamine. This might also be due to eradication of aspirin inhibitory effect on prostaglandins secretion, thereby

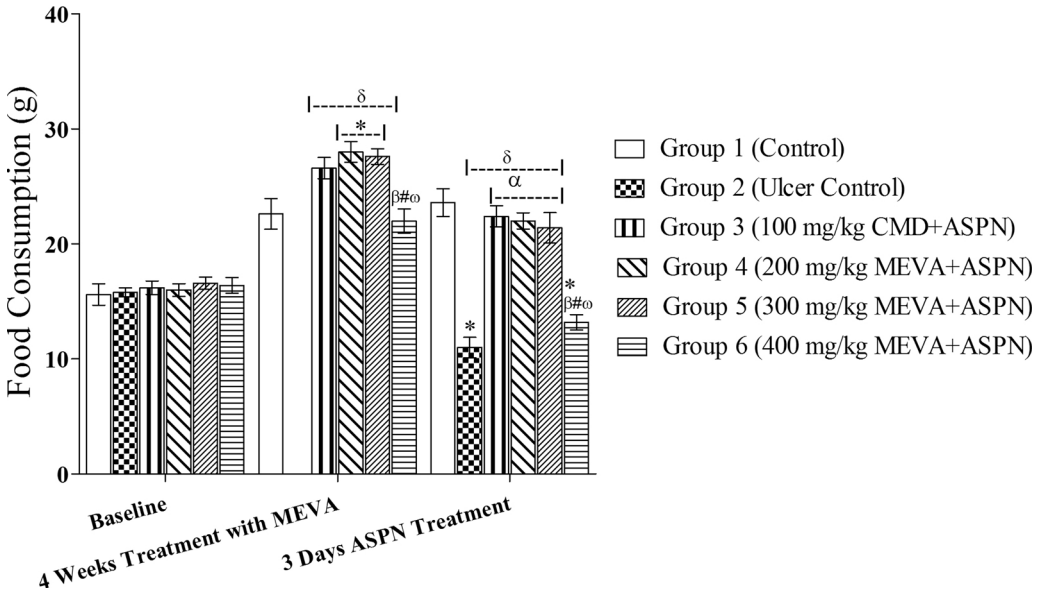

Fig. 2. Effect of methanol extract of *Vernonia amygdalina* leaf on food intake on aspirin induced ulcer in rats.
Each value represents Mean ± S.E.M (n = 5); * = significantly different from control ($p < 0.05$); α = significantly different from ulcer control group (aspirin alone) ($p < 0.05$); β = significantly different from 100 mg/kg CMD ($p < 0.05$); # = significantly different from 200 mg/kg MEVA ($p < 0.05$); ω = significantly different from 300 mg/kg MEVA ($p < 0.05$).

Table 3
Effect of methanol extract of *Vernonia amygdalina* leaf on gastric acid secretion and ulcer index on aspirin induced gastric ulcer in rats.

Groups	Gastric Volume	Gastric pH	Free acidity (meq/L/24 h)	Total Acidity (meq/L/24 h)	Ulcer Score (units)	Ulcer Index	(% Ulcer Inhibition)	% Ulceration
Group 1	2.38 ± 0.08	4.50 ± 0.30	5.40 ± 0.51	8.60 ± 0.40	–	–	–	–
Group 2 (Ulcer control)	5.24 ± 0.25*	2.43 ± 0.23*	9.60 ± 0.40*	13.60 ± 0.51*	6.20 ± 0.46	5.39	–	100%
Group 3 (100 mg/kg CMD + ASPN)	2.34 ± 0.09$^{\alpha}$	4.56 ± 0.21$^{\alpha}$	4.60 ± 0.40$^{\alpha}$	7.34 ± 0.15$^{\alpha}$	1.33 ± 0.33$^{\alpha}$	1.12	79.22	20.78%
Group 4 (200 mg/kg MEVA + ASPN)	2.54 ± 0.05$^{\alpha}$	5.27 ± 0.18$^{\alpha}$	5.20 ± 0.26$^{\alpha}$	8.10 ± 0.40$^{\alpha}$	1.50 ± 0.50$^{\alpha}$	1.26	76.62	23.38%
Group 5 (300 mg/kg MEVA + ASPN)	2.66 ± 0.09$^{\alpha\beta}$	4.05 ± 0.24$^{\alpha\#}$	7.20 ± 0.37*$^{\alpha\beta\#}$	10.80 ± 0.54*$^{\alpha\beta\#}$	2.77 ± 0.15$^{\alpha\beta\#}$	2.34	56.59	43.41%
Group 6 (400 mg/kg MEVA + ASPN)	3.76 ± 0.07*$^{\alpha\beta\#\omega}$	3.13 ± 0.11*$^{\alpha\beta\#\omega}$	7.80 ± 0.58*$^{\alpha\beta\#}$	12.06 ± 0.39*$^{\alpha\beta\#\omega}$	4.33 ± 0.33 $^{\alpha\beta\#\omega}$	3.69	31.54	68.46%

Each value represents Mean ± S.E.M (n = 5). * = significantly different from control ($p < 0.05$); α = significantly different from ulcer control group (aspirin alone) ($p < 0.05$); $^{\beta}$ = significantly different from CMD ($p < 0.05$); $^{\#}$ = significantly different from 200 mg/kg MEVA ($p < 0.05$); $^{\omega}$= significantly different from 300 mg/kg MEVA ($p < 0.05$).

increasing prostaglandin which is well known to increase gastric mucosal blood flow [41].

Methanol extract of *Vernonia amygdalina* leaf has been characterized in this study to contain alkaloids, tannin, saponins, terpenoids, glycoside and flavonoids. The reduction in gastric acid secretion seen in the group treated with *Vernonia amygdalina* might be due to action of tannin which was reported to be present in the leaf extract of *Vernonia amygdalina* [42]. Tannin tends to compete with adenosine triphosphate at the ATP hydrolysis site, thereby causing the inhibition of gastric H^+-K^+ ATPase that is necessary for gastric acid secretion [43]. It also reacts with proteins of the stomach tissues layers by precipitating micro proteins at the site of the ulcer, forming a protective pellicle (thin film) that prevent gastric mucosa from irritation [44,45]. In addition, MEVA may prevented gastric mucosal lesions through its flavonoid content [46]. Flavonoids could scavenge free radicals, inhibit lipid peroxidation, and increase prostaglandins and mucosal content of the gastric mucosa; showing cytoprotective effects [46–48]. Tangka, [49]; Oboh, [50] showed the presence of large amount of crude protein and all the essential amino acids in the leaf of *Vernonia amygdalina*. High protein diet had been shown to be associated with low incidence of gastric ulcer and was reported to have buffering effects on gastric acidity [51,52]. This finding is also corroborated by the photomicrograph of the stomach of groups that received cimetidine and MEVA shows improvement in stomach histoarchitecture compared to aspirin alone (ulcer control) group.

Hematological indices within the normal range are very important indicators of good health [53]. Aspirin is known to cause gastrointestinal tract erosion resulting in occult bleeding; it is also reported to reduce iron uptake from the stomach which result in iron deficiency [54,55]. This effect coupled with acute or chronic blood loss due to gastrointestinal tract erosion induced by aspirin is believed to cause iron deficiency anemia in humans [56,55]. In the present study, aspirin treatment decreased red blood cells (RBCs) and hemoglobin (Hb) count when compared with the control group. The significant decrease in RBCs and Hb values caused by aspirin could indicate induction of anemia and decrease in oxygen-carrying capacity of the blood as well as the amount of oxygen delivered to the tissues respectively. MEVA treatment improved RBCs and Hb count toward control value. This suggests that extract leaf of *Vernonia amygdalina* have the potential of enhancing hemoglobin contents through increasing/restoring RBCs count or iron absorption in the stomach thereby, suggesting the protective effect of MEVA against aspirin-induced hematotoxicity.

There was a significantly increased in white blood cell count in aspirin treated groups when compared with the control group. The increase in WBC count that was observed in rats treated with aspirin when compared with the control group are in conformity with the report of Merchant and Modi [57]. Moreover, the granulocytes count in rats treated with aspirin showed higher values when compared with the control and other experimental rats, while lymphocytes and monocytes count showed no significant difference when compared with the control. The significant increase in granulocytes count caused by aspirin probably indicates that the ability of the body to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) has been compromised. The non-significant change in lymphocyte count suggests that the acquired immune response of the body has not been compromised by aspirin; while no significant difference observed in monocyte count probably indicates that the phagocytic function of the body has not been compromised by aspirin. Also, the insignificant

Table 4
Effects of methanol extract of *Vernonia amygdalina* Leaf on haematological indices on aspirin induced ulcer in rats.

Parameters	Group 1 (Control)	Group 2 (Ulcer Control)	Group 3 (100 mg/kg CMD + ASPN)	Group 4 (200 mg/kg MEVA + ASPN)	Group 5 (300 mg/kg MEVA + ASPN)	Group 6 (400 mg/kg MEVA + ASPN)
WBC ($x10^3$/μL)	4.44 ± 0.38	7.50 ± 0.70*	5.36 ± 0.27$^{\alpha}$	3.28 ± 0.14$^{\alpha\beta}$	3.66 ± 1.07$^{\alpha\beta}$	3.38 ± 0.38$^{\alpha\beta}$
LYM ($x10^3$/μL)	3.86 ± 0.51	3.34 ± 0.59	3.98 ± 0.24	2.88 ± 0.15	2.52 ± 0.36	2.96 ± 0.51
MON ($x10^3$/μL)	0.42 ± 0.05	0.42 ± 0.05	0.82 ± 0.09*$^{\alpha}$	0.38 ± 0.20$^{\beta}$	0.30 ± 0.32$^{\beta}$	0.34 ± 0.07$^{\beta}$
GRAN ($x10^3$/μL)	0.76 ± 0.22	1.40 ± 0.54*	1.00 ± 0.17$^{\alpha}$	1.02 ± 0.14$^{\alpha}$	1.04 ± 0.13$^{\alpha}$	0.92 ± 0.14$^{\alpha}$
RBC ($x10^6$/μL)	6.66 ± 0.39	5.27 ± 0.15*	7.06 ± 0.17$^{\alpha}$	7.00 ± 0.37$^{\alpha}$	6.74 ± 0.28$^{\alpha}$	6.77 ± 0.17$^{\alpha}$
HGB (g/dl)	15.06 ± 0.08	13.04 ± 0.09*	14.82 ± 0.43$^{\alpha}$	14.76 ± 0.43$^{\alpha}$	14.24 ± 0.30$^{\alpha}$	14.04 ± 0.38$^{\alpha}$
HCT (%)	43.40 ± 1.46	40.68 ± 0.59	40.36 ± 0.99	41.12 ± 0.84	41.24 ± 1.00	40.34 ± 0.74
MCV (fl)	59.12 ± 0.51	59.26 ± 1.03	57.24 ± 1.11	65.60 ± 1.76*$^{\alpha\beta}$	62.30 ± 0.82$^{\beta\#}$	61.02 ± 0.34$^{\#}$
MCH (pg)	20.68 ± 0.34	20.68 ± 0.36	20.92 ± 0.24	22.06 ± 0.49*$^{\alpha\beta}$	21.50 ± 0.31	21.74 ± 0.31
MCHC (g/dl)	35.04 ± 0.15	34.96 ± 0.10	36.68 ± 0.55	35.60 ± 0.27	35.84 ± 0.37	35.72 ± 0.43
PLT ($x10^3$/μL)	672.20 ± 29.77	520.80 ± 17.80	527.20 ± 49.09	812.40 ± 146.04$^{\alpha\beta}$	606.00 ± 114.99	609.20 ± 66.21

Values are given as Mean ± SEM (n = 5). * = significantly different from control ($p < 0.05$); α= significantly different from ulcer control group (aspirin alone) ($p < 0.05$); $^{\beta}$= significantly different from 100 mg/kg CMD ($p < 0.05$); $^{\#}$ = significantly different from 200 mg/kg MEVA ($p < 0.05$); WBC = White blood cell, LYM = lymphocyte, MON = Monocyte, GRAN = Granulocyte, HGB = Haemoglobin, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, HCT = Haematocrit, PLT = Platelet Count.

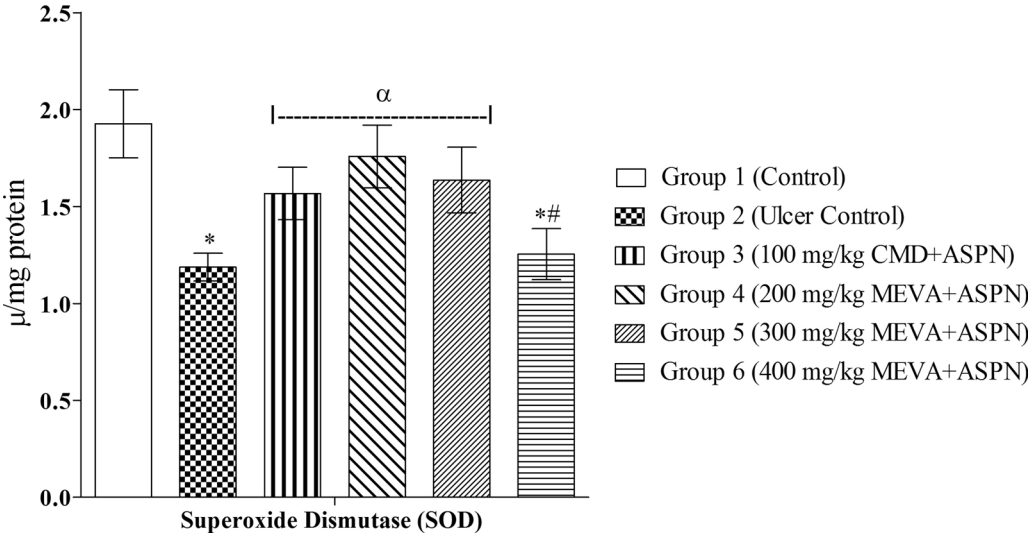

Fig. 3. Effect of methanol extract of *Vernonia amygdalina* leaf on SOD activity on aspirin induced ulcer in rats.
Each value represents Mean ± S.E.M (n = 5); * = significantly different from control ($p < 0.05$); α = significantly different from ulcer control group (aspirin alone) ($p < 0.05$); # = significantly different from 200 mg/kg MEVA ($p < 0.05$).

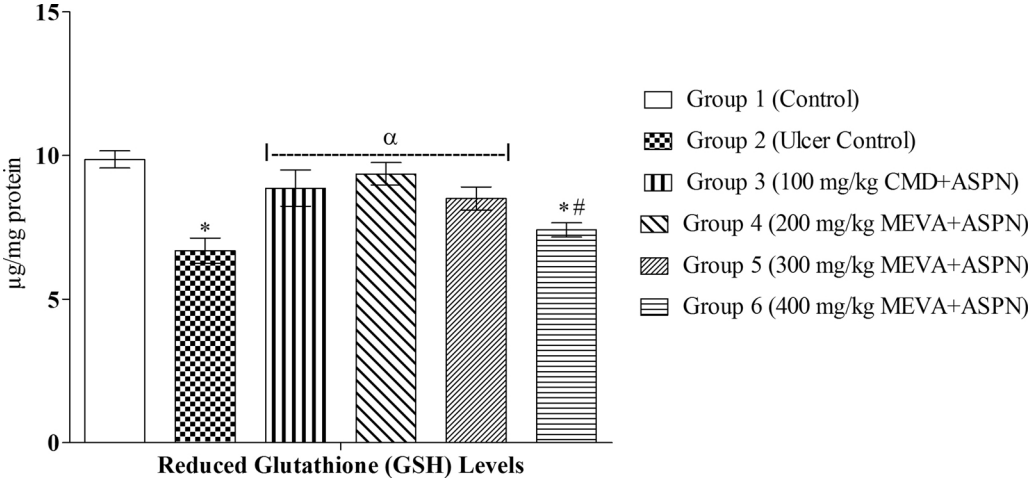

Fig. 4. Effect of methanol extract of *Vernonia amygdalina* leaf on GSH level on aspirin induced ulcer in rats.
Each value represents Mean ± S.E.M (n = 5); * = significantly different from control ($p < 0.05$); α = significantly different from ulcer control group (aspirin alone) ($p < 0.05$); # = significantly different from 200 mg/kg MEVA ($p < 0.05$).

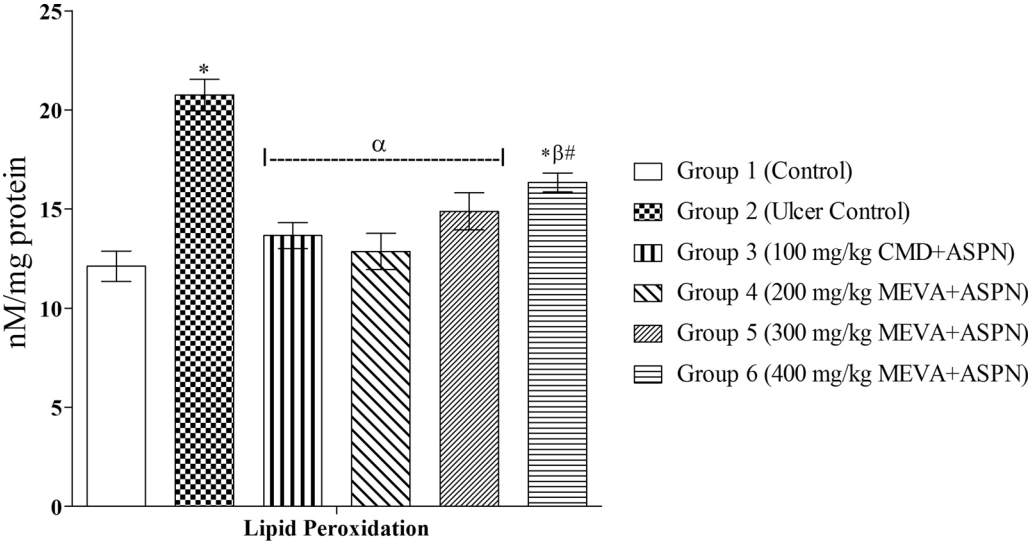

Fig. 5. Effect of methanol extract of *Vernonia amygdalina* leaf on LPO level on aspirin induced ulcer in rats.
Each value represents Mean ± S.E.M (n = 5); * = significantly different from control ($p < 0.05$); α = significantly different from ulcer control group (aspirin alone) ($p < 0.05$); β = significantly different from 100 mg/kg CMD ($p < 0.05$); # = significantly different from 200 mg/kg MEVA ($p < 0.05$).

change in the platelet count caused by aspirin could be an indication that it does not has the potential to stimulate thrombopoietin production with the hemostatic capability of the blood maintaining the status since platelets mediate in the blood clotting mechanism. Treatment with MEVA restored the granulocyte, lymphocyte and monocyte counts toward normal and this may be attributed to the enhancement of the host's immunity.

Cells or tissues are in a stable state if the rates of free radical formation and scavenging capacity are essentially constant and in equilibrium. However, an imbalance between them results in oxidative stress

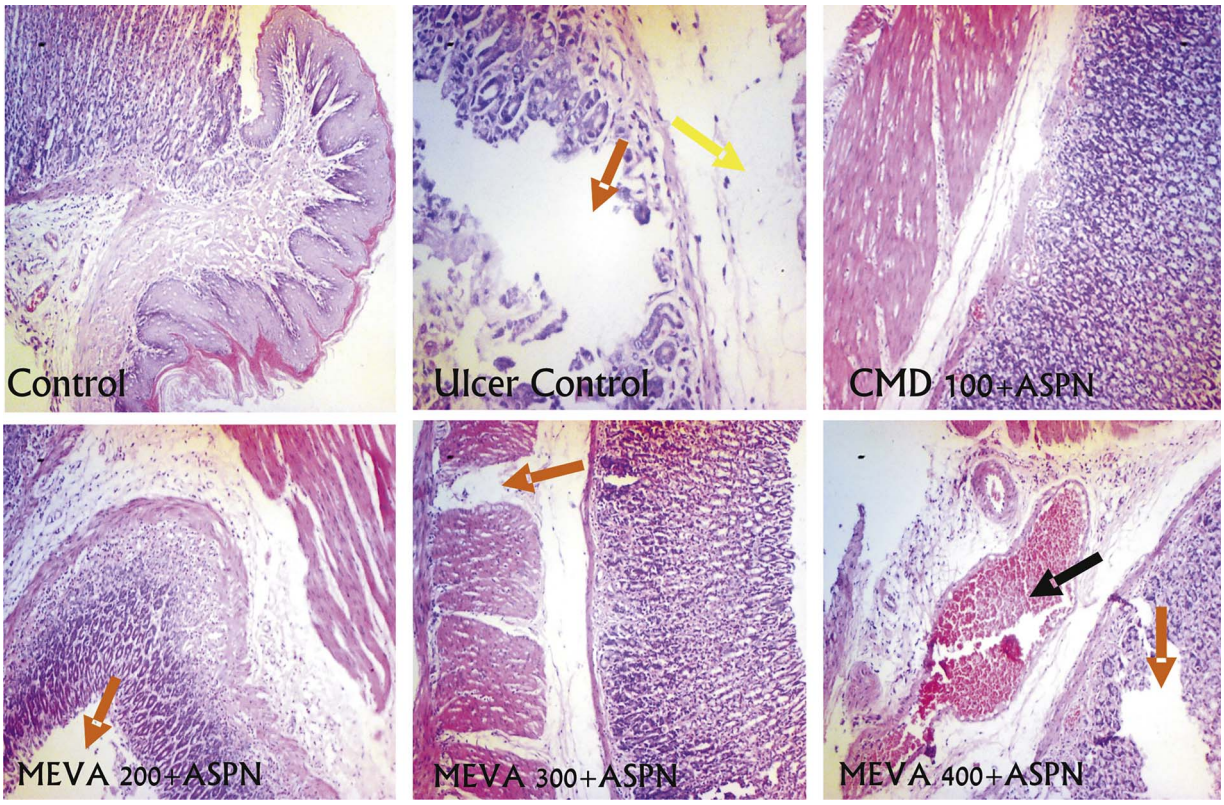

Fig. 6. Photomicrograph of stomach of Control (CN), ulcer control, MEVA 200 mg/kg + ASPN, MEVA 300 mg/kg + ASPN, CMD 100 mg/kg + ASPN and MEVA 400 mg/kg + ASPN. CN shows intact epithelium, laminal propria, submucosa and muscularis propria. Ulcer control showed degenerative changes in forestomach and fundic regions of the stomach. There is also marked ulceration with loss of cellular constituents (brown and yellow arrow) in forestomach. Yellow arrow indicates development of subepithelial space and brown arrowhead indicates distortion of mucosal architecture. There was intact architecture of the stomach in MEVA 200 mg/kg + ASPN, MEVA 300 mg/kg + ASPN and CMD 100 mg/kg + ASPN. However, MEVA 400 mg/kg + ASPN showed sign of hemorrhage (black arrow), sloughing of necrotic submucosa and mucosa layer. Sections of stomach tissues of rats were stained with hematoxylin-eosin (Magnification: ×100). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

which further deregulates cellular functions leading to different pathological conditions [58]. In this study, the increased in MDA levels as well as decreased activities of SOD and GSH levels in the stomach of aspirin-ulcerated rats is a manifestation of facilitated lipid peroxidation and over production of free radicals resulting in mucosal damage. Free radicals decreased antioxidant enzymes activities and initiate lipid peroxidation which is an important event in the toxicity mechanism of aspirin [59]. Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin have previously been reported to decrease antioxidant enzymes activity in rat stomach thereby causing gastric ulceration [60,61]. This is associated with overpowering of the cellular antioxidant defense systems by free radicals ravaging influence that subsequently results in stomach oxidative injury. However, the significantly decreased of MDA levels coupled with marked increase in the activities of SOD and GSH levels in rats post-treated with MEVA is an obvious indication of anti-oxidative potentials of MEVA. Thus, this observation is in consistent with other studies, using different plant species, showing their anti-oxidative and anti-inflammatory properties against NSAIDs-induced gastric ulceration [62,63].

The anti-oxidative properties of *Vernonia amygdalina* leaf extracts had earlier been reported [64,65]. This anti-oxidative effect of MEVA on gastric mucosa of aspirin ulcerated rats, indicate its gastro-protective effect against aspirin-induced gastric ulcer. The different mechanisms of MEVA in this study against aspirin-induced ulcer might be associated with it bioactive constituents. Taken together, these findings suggested that *Vernonia amygdalina* leaf extract favorably prevented gastric mucosal injury through inhibition of lipid peroxidation and activation of antioxidant enzymes. Last but not least, the active compounds present in *Vernonia amygdalina* leaf extract are still not entirely clear and we could not exclude the possibility that more than one compound may be responsible for its inhibitory effect on aspirin-induced gastric ulcer. Therefore, elucidation of the precise mechanism(s) underlying the preventive effect on gastric ulcer of *Vernonia amygdalina* leaf extract and the active constituent(s) responsible for this beneficial property would require further study.

5. Conclusion

The present study indicates that methanolic extract of *Vernonia amygdalina* leaves administration attenuated aspirin-induced gastric ulcer, normalized hematological indices and histopathologic changes in rat's stomach. Its gastroprotective activity may be attributed to its antioxidant constituents. Overall, the results obtained supported the beneficial effects of MEVA in preventing the development of gastric ulcer in experimental ulcerated rats, thus opening the possibility of its usage as an alternative therapy to gastric ulcer.

Conflict of interest

The authors of this manuscript declare no conflict of interest.

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