International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (4): 92-103
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved

Methanol Extract of *Gomphrena celosioides* Leaves Affects Inflammatory Markers in Acidified Ethanol-Induced Gastric Ulcer Healing in Male Wistar Rats

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Received: 02 Jul 2019 / Accepted: 9 Aug 2019 / Published online: 1 Oct 2019
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Abstract

Neutrophil infiltrations and free radical mediated processes have been reported to delay gastric ulcer healing in ethanol-induced gastric ulcer. This study investigated the potential of the methanol extract of Gomphrena celosioides (MEGC) leaves in the healing of ethanol-induced gastric ulcer in male Wistar rats. Thirty-six rats were divided into six groups of six rats each and treated orally. Group 1 rats served as control group and received 1 ml/kg body weight of 1% gum acacia solution; groups' 2-6 rats were given acidified ethanol to induce gastric ulcer. Groups 3, 4 and 5 rats were treated with 200, 400 and 800 mg/kg body weight of MEGC leaves while group 6 rats were treated with 50 mg/kg body weight of cimetidine (CIM) for fourteen days after ulcer induction. Ulcerogenic parameters, inflammatory markers and the levels of lipid peroxidation and protein carbonyl were determined.

Acidified ethanol caused severe gastric mucosa damage as revealed by the ulcerogenic parameters with increased activities and concentration of the inflammatory markers and concomitant increase in the levels of lipid peroxidation and protein carbonyl in the ulcerated untreated group, but healing were accompanied by a significant decrease in these observations with different doses of MEGC or CIM for 14-days. MEGC significantly heal the ulcer with percentage ulcer healing of 34.79%, 39.63%, and 53.81% for different at doses and CIM 58.49% respectively. Our findings thus revealed that administration of MEGC leaves promote gastric ulcer healing by suppressing the inflammatory markers could be relevant in the pathologies involving gastrointestinal disorder.

Keywords
Gomphrena celosioides, gastric ulcer, oxidative stress, neutrophil infiltrations

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I. INTRODUCTION

Oral administration of ethanol has been reported to be associated with delayed gastric ulcer healing in rats as a result of oxidative stress due to continuous generation of reactive oxygen species (ROS) [1]. Studies have also shown that ROS initiate inflammatory process in ethanol-induced gastric ulcer development with recruitment and metabolic activation of neutrophils and macrophages, releasing pro-inflammatory cytokines (TNF-α and IL-1β) [2,3]. Mounting evidences have also indicated that pro-inflammatory cytokines, oxidative stress and apoptosis play crucial roles in pathogenesis [4] and healing of gastric ulcer. Activation of neutrophils is associated with an upregulated inflammatory response with increased gastric expression of nuclear factor kappa B (NF-κB) which controls the generation of pro-inflammatory cytokines (TNF-α and IL-1β) [3,5]. These events delay gastric ulcer healing. Inflammation is a local complicated vascular and cellular reaction of an individual to an injury/irritant in an attempt to minimize the effect of the irritant or injury and heal the damaged tissue and restore the affected animal to normal health

Ulcer healing is a complex process, in which the tissue repairs itself after injury, attempting a restitution towards integrity. It has been proposed that such a process can be distinguished in sequential, partly overlapping, phases: hemostasis, inflammation, proliferation and remodeling [6].

Medicinal plants are herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, and other physical or biological processes, which may be produced for immediate consumption or as a basis of herbal drugs [7]. The therapeutic properties of medicinal plants are conditioned by the presence in their organs of active substances, such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarin compounds, which physiologically affect the bodies of humans and animals. Aromatic plants are a source of fragrances, flavors, cosmeceuticals, health beverages and chemical terpenes [8]. With the onset of scientific research in medicinal herbs, it is becoming clearer that medicinal herbs have the potential in today's synthetic era as numbers of phytochemical constituents of medicine plants are used as template for developing new drugs [9].

Gomphrena celosioides (GC) also commonly called Soft khaki weed or White-eye is a short-lived perennial plant [10]. It belongs to the Amaranthaceae family, and over 120 species of the family are found in America, Australia and Indo-Malaysia, while 46 species occur in Brazil. Few species occur in the East and West of Africa [11]. *Gomphrena celosioides* is used in ethnomedicinal practice in Nigeria for treatment of various skin diseases, bronchial infection, diarrhea and has been reported to possess antifungal, antibacterial, anti-inflammatory, analgesic and anthelmintic activity [13,14,15,16]. In South America the plant is utilized as an abortifacient [17].

The phytochemical constituents include alkaloids, saponins, steroids, amino acids, glycosides, non-reducing sugars, phenols, and flavonoids [12,18,19].

MATERIALS AND METHODS

Plant material

Gomphrena celosioides (*G. celosioides*) leaves were obtained from Iwo, Osun state, identified and authenticated at the Botany Department of Bowen University, Iwo, Nigeria with the voucher specimen (BUH-097) deposited at the University herbarium.

Preparation of *Gomphrena celosioides* Leaf Extract

Gomphrena celosioides leaves were picked, air dried at room temperature and pulverized into coarse powders. 1000 g of the pulverized leaves of the plant were then subjected to maceration extraction using 5 liters of methanol (99%) with intermittent shaking for 72 hours. The mixture was filtered, and the filtrate evaporated to dryness using rotatory evaporator under reduced pressure at 45°C to obtain a solid mass of *Gomphrena celosioides* referred to as MEGC used in this study.

Experimental Animal

Forty-eight adult male Wistar rats weighing (150 ± 20 g) were purchased from the Central Animal House, Faculty of Basic Medical Science, College of Medicine, University of Ibadan, Ibadan, Nigeria. Animals were kept in a temperature-controlled room (25 ± 2°C) with 12 h light and 12 h dark cycle. The rats were kept under standard laboratory conditions and were fed with standard rat's pellet (Ladokun Feeds, Nigeria) with freshwater *ad libitum*. They were acclimatized for 14 days, after which they were randomly divided into 6 groups. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and use of animals [20] and as approved by the Research Ethical Committee, Bowen University Iwo, Osun State, Nigeria. The "Principle of Laboratory Animal Care" (NIH publication No. 85-23) guidelines and procedures were considered in this study (NIH publication revised, 1985) [21]. The rats were deprived of food for 24 hours but had free access to clean water prior to the commencement of the experiment.

Ulcer model and experimental design.

Cimetidine (CIM) obtained from the pharmacy of Bowen University Iwo, Osun State was used as a reference anti-ulcer drug. The drug the extract was dissolved in a 1% w/v solution of gum acacia and administered orally using feeding tube.

The experiments were conducted using a slight modification of a previously reported protocol [22]. Gastric ulcer was induced using acidified ethanol containing 0.15N HCl in 70% v/v ethanol (HCl/EtOH). Forty-eight healthy adult male Wistar rats were randomly divided into 6 groups of 8 rats per group. Rats in group 1 served as the control group and received 1 ml/kg body weight of 1% gum acacia solution, rats in groups 2-6 were given 1 ml/kg body weight of acidified ethanol according to their body weights to induce gastric ulcer. Rats in groups 3, 4, and 5 were post-treated four hours after ulcer induction for fourteen days with 200 mg/kg, 400 mg/kg and 800 mg/kg body weight of MEGC and rats in group 6 were also post treated for fourteen days with 50 mg/kg body weight of CIM. Twenty-four hour after the last dose of MEGC or CIM, all rats were anaesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) and sacrificed by cervical dislocation. The selected dose of MEGC was based on preliminary experiments

Isolation of stomach and gastric juice collection

After all the rats were sacrificed, the stomach of each rat was excised, rinsed with cold saline to remove blood clots and opened along the greater curvature and the gastric content from each rat was collected separately into centrifuge tubes, the stomach of each rat was then washed with sterile distilled water and blotted dry. Each stomach was examined to locate the lesion using 2x hand lens. Section of the stomach of each rat was cut and preserved for histological assessment.

Evaluation of Gastric tissue damage

Stomachs were opened along greater curvature, rinsed in normal saline and examined using magnifying lens (Hi-Tech Zone, Ningbo, Zhejiang, China). The degree of gastric damage was scored according to [23] as follows; normal colored stomach = 0; red coloration = 0.5; spot ulcer = 1; hemorrhagic streak = 1.5; deep ulcers = 2; perforation = 3. The overall total scores divided by the number of rat in each group was designated the ulcer index. The percentage ulcer inhibition was calculated as described by [24] using the formula:

Percentage ulcer inhibition (%UI) = Control mean ulcer index – test mean ulcer index)
(Control mean ulcer index) ×100

Measurement of Gastric pH, Gastric juice volume and Acid output

The gastric contents of each rat were collected separately and centrifuged (Heraeuslabofuge 400, Heraeus, Germany) at 3000 rpm for 10 minutes. The volume of the supernatant was measured precisely and expressed as the volume of gastric juice (ml). The pH of each supernatant was measured with a digital pH meter [25] and acidities were determined by metric titration using a 0.1 N NaOH solution and phenolphthalein as indicator. Acid output for each rat were calculated by the equation:

Acid output (µEq/hr) =
Acidity (mEq/l) X Volume of gastric juice (ml)/4(hr)

Determination of gastric wall mucus secretion

The gastric wall mucus was determined according to [26]. Segment glandular portion of stomach from each was weighed and transferred immediately to 10 ml of 0.1% w/v Alcian blue solution (0.16 M sucrose solution buffered with 0.5 ml of sodium acetate at pH 5). The tissue was kept in Alcian blue for 2 hours, and the excess colour was eliminated by consecutively rinsing it twice with 10 ml of 0.25 M sucrose. Dye combined with gastric wall mucus was extracted with 10 ml of 0.5M magnesium chloride, which was irregularly shaken for 1 minute at 30-minute interval for 2 hours. Four millilitres of blue concentrate was then shaken vigorously with an equivalent volume of diethyl ether. The resulting emulsion was centrifuged at 3000 ×g for 10 minutes, and the absorbance of the aqueous layer was recorded at 580 nm. The amount of Alcian blue extracted for every gram of wet glandular tissue was extrapolated from the standard curve.

Gastric homogenate preparation.

The stomachs of all rats were collected immediately after sacrifice separately and about 500 mg was cut from each stomach for the preparation of a gastric tissue homogenate [27]. Each stomach portion was homogenized in phosphate buffer (pH 7.4) using a Teflon homogenizer (Polytron, Heidolph RZR 1, Germany), after which the homogenate was centrifuged at 3,000 *g* for 15 minutes at 4 ^{0}C and the supernatant collected for each rat was stored at -20 ^{0}C for the biochemical analyses.

Protein content determination

The protein content of the stomach supernatant was determined following Folin Ciocalteu method [28]. The absorbance was read at 690 nm and the protein content of each sample was calculated from a standard curve.

Assessment of Lipid peroxidation level

To estimate the degree of lipid peroxidation caused by the acidified ethanol (HCl/EtOH), the MDA contents of the stomach tissue supernatant were determined by means of the thiobarbituric acid (TBARS) assay, which was conducted according to the method of [29]. Briefly, 1 ml of each supernatant was treated with TCA/TBA/HCl (2 mL, 15% TCA, 0.375% TBA, 0.25N HCl) containing 0.01% BHT, heated on a boiling water bath for 15 min, cooled, and centrifuged at 3000×g for 5min. The amount of TBARS was calculated from the absorbance of the supernatant at 535nm ($\acute{\varepsilon}$ = 1.56 × $10^5 M^{-1}$ cm^{-1}).

Measurement of Glutathione (GSH) Level

The GSH level was determined according to the method of [30]. Briefly, fundic stomach homogenate was made in 0.2 M Tris, pH 8.2, containing 20 mM EDTA and centrifuged. An aliquot of 1.0 ml of homogenate was precipitated with 10% trichloroacetic acid and centrifuged. The supernatant (1.0 ml) was added to 2.0 ml of 0.8 M Tris-HCl, pH 9.0, containing 20 mM EDTA (28) and mixed with 0.1 ml of 10 mM 5,5-dithiobis-2-nitrobenzoic acid. The intense yellow color of nitromercaptobenzoate was read at 412 nm. For calibration, a standard curve was prepared treating varied concentrations of reduced glutathione with 5,5-dithiobis-2-nitrobenzoic acid under identical conditions and the values were expressed as µg/mg protein.

Assessment of Serum Myeloperoxidase (MPO)

Myeloperoxidase activity was measured following the method of [31]. The assay system was prepared by mixing the homogenate with 50mM sodium acetate buffer (pH 5.25), 30 µM TNB, and150 mM NaCl. The reaction was initiated by the addition of 0.3 mM H_2O_2, and the decrease in absorbance at 412 nm due to oxidation of TNB (ε = 27,000 M^{-1} cm^{-1}) was measured for one min. The activity of the peroxidase was expressed as n moles of TNB oxidized/min/mg protein 13,000 M^{-1} cm^{-1}.

Assessment of Nitric oxide level

Tissue nitrite (NO_2^-) and nitrate (NO_3^-) were estimated as an index of NO production. Nitric oxide levels in the stomachs were determined according to the method of [32]. One ml each of the supernatant and Griess reagent (containing 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulphanilamide in 2.5% phosphoric acid) were mixed together and incubated for 10 minutes 37°C. The absorbance was read spectrophotometrically at 540 nm. The nitrate and nitrite concentration in the gastric tissues was quantitated in units of µmol/g tissue based on a nitrate standard curve plotted at 540 nm. Results were expressed as nmol/g tissue.

Measurement of Protein Carbonyl Content (PCO)

Protein oxidation was measured as carbonyl content in the supernatant of the fundic stomach homogenate [33]. 1.0 ml of the supernatant were precipitated with 10% trichloroacetic acid and allowed to react with 0.5 ml of 10 mM 2,4-dinitrophenylhydrazine for 1 h. After precipitation with 20% trichloroacetic acid, the protein was washed thrice with a mixture of ethanol-ethyl acetate (1:1), dissolved in 1.0 ml of a solution containing 6 M guanidine HCl in 20 mM potassium phosphate adjusted to pH 2.3 with trifluroacetic acid and centrifuged, and the supernatant was read for carbonyl content at 362 nm (e = 22,000 M^{-1} cm^{-1}).

Determination of Xanthine Oxidase (XO) activity

Xanthine oxidase activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm, according to the method of [34]. Stomach supernatant from each rat was incubated (50 µl) for 30 minutes at 37 ◦C in 2.85 mL of medium containing phosphate buffer (pH7.5, 50 mM) and xanthine (0.067mmol final concentration in each tubes). The reaction was terminated by addition of 0.1 ml 100% (w/v) TCA and the mixture was centrifuged at 5000 × g for 15 min. The absorption at 293 nm of the resultant clear supernatant was measured against blank. One unit of activity was defined as1µmol of uric acid formed per minute at 37 ◦C, pH 7.5, and expressed in U/g protein.

Histopathological Evaluation

Stomach sections from each rat of all the groups were fixed in 10% buffered formalin, dehydrated in graded alcohol and embedded in paraffin. Fine (4-5 µm) sections were obtained, stained with hematoxylin-eosin (H & E) and slides were mounted for observation under a light Olympus microscope (BX41, Hamburg, Germany) and photographed using a digital camera [35].

Statistical Analysis

Results were expressed as the mean ± standard error of the mean (SEM.). Statistical analysis was carried out using Statistical package for Social Scientist (SPSS version 18. 0, Chicago, IL, USA) comparisons were conducted by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Graph Pad Prism (Graph Pad Software, San Diego, CA, USA) was used to plot the graphs. Differences were considered to be statistically significant when p<0.05.

II. RESULTS

Figure 1 shows the influence of MEGC administration on the healing of acidified ethanol-induced gastric ulcers in rats. Oral administration of acidified ethanol

severely caused gastric ulceration in rats as evident in ulcer score and ulcer index (Figure 1A and 1B) of rats given acidified ethanol alone. Treatments with different dose of MEGC after ulcer induction at the dose of 200, 400 and 800 mg/kg body weight accelerated the healing of acidified ethanol-induced gastric ulcer significantly ($p < 0.05$) in a dose dependent manner when compared to the acidified ethanol alone group. Also rats post treated with cimetidine; the reference drug showed significant ($p < 0.05$) level of healing.

Gastric juice volume (Figure 1C) of the acidified ethanol alone group was high when compared to the normal control group, but post treatment with different doses of MEGC significantly ($p < 0.05$) decreased the gastric juice volume particularly at 400 mg/kg, and the reference drug, cimetidine also decreased the gastric juice volume significantly ($p < 0.05$) when compared to the acidified ethanol alone group. The pH of the acidified ethanol alone group was decreased, but treatment with varying doses of MEGC significantly ($p < 0.05$) increase the pH value and the value compared favorably with the value of the normal control group especially at 400 mg/kg. Cimetidine also significantly ($p < 0.05$) increased the pH value (Figure 1D).

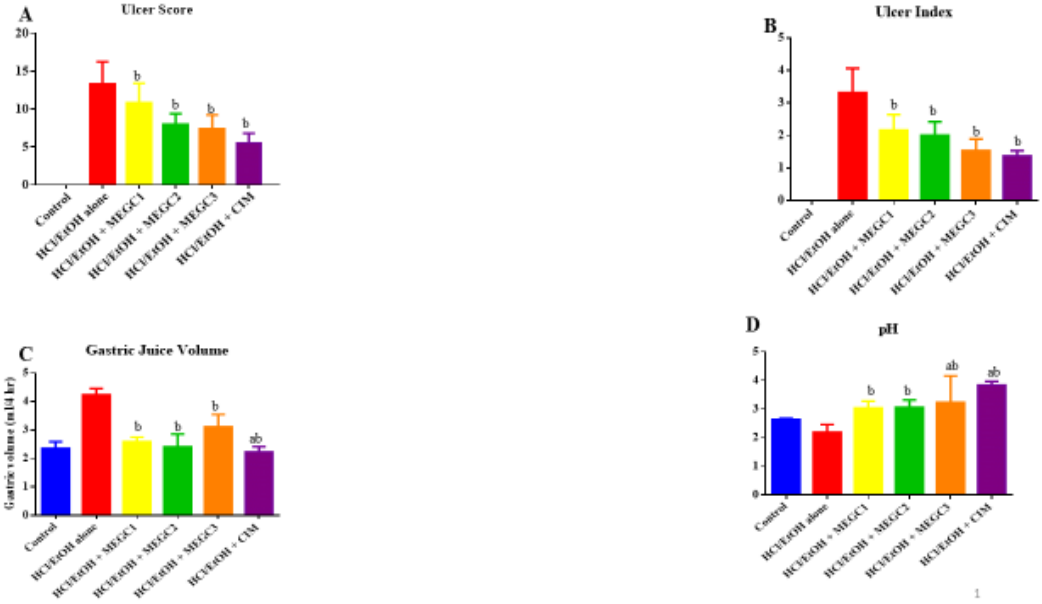

Figure 1: Effects of methanol extract of *Gomphrena celosioides* leaves on: (A) ulcer score; (B) ulcer index; (C) gastric juice volume and (D) pH in acidified ethanol-induced gastric ulcer healing in rats, (n = 8, ± SEM). Bars with different superscripts for each parameter are significantly different *($p<0.05$).* [a] significantly different from the control group ($p<0.05$); [b] significantly different from the HCl/EtOH group ($p<0.05$). HCl/EtOH: acidified ethanol (1 ml/kg b. w.), MEGC1: 200 mg/kg methanol extract of *Gomphrena celosioides*, MEGC2: 400 mg/kg methanol extract of *Gomphrena celosioides*, MEGC3: 800 mg/kg methanol extract of *Gomphrena celosioides*, and CIM: 50 mg/kg cimetidine.

Acid output in group of rats that were given acidified ethanol alone was high when compared to the normal group of rats, but post treatment with different dose of MEGC significantly ($p < 0.05$) decreased when compared to the acidified ethanol alone group, also group post treated with cimetidine decreased the acid output (Figure 2A). Pepsin activity was increased in the acidified ethanol alone group compared to the normal control group (Figure 2B), but post treatment with MEGC significantly ($p < 0.05$) decreased this observation in a dose dependent manner. Cimetidine also decreased the peptic activity significantly. However, acidified ethanol alone group revealed decreased in the mucus content when compared with the normal control group, but treatment after gastric ulceration with different doses of MECG increased the mucus content significantly ($p < 0.05$) and the increase observed were almost the same with the normal control group particularly at 400 and 800 mg/kg. Cimetidine also increased the mucus content significantly ($p < 0.05$) but not as much as does by the MEGC at 400 and 800 mg/kg respectively (Figure 2C). Figure 2D shows the healing percentage of the different doses of MEGC and cimetidine respectively.

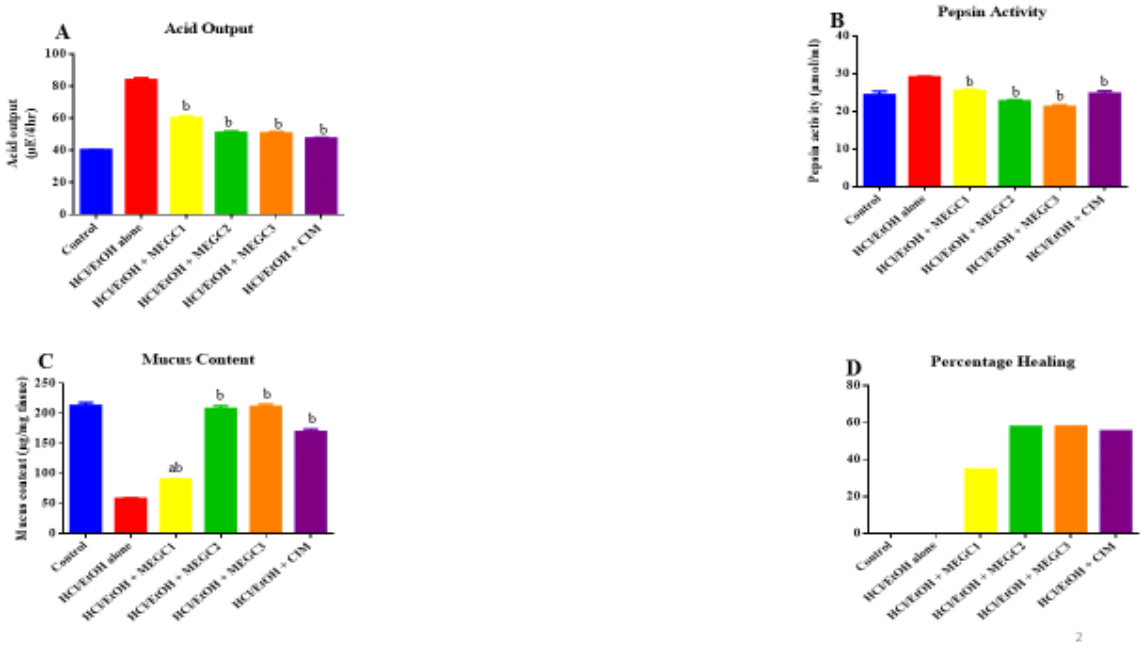

Figure 2: Effects of methanol extract of *Gomphrena celosioides* leaves on: (A) acid output; (B) pepsin activity; (C) mucus content and (D) percentage healing in acidified ethanol-induced gastric ulcer healing in rats, (n = 8, ± SEM). Bars with different superscripts for each parameter are significantly different *(p<0.05).* [a] significantly different from the control group (p<0.05); [b] significantly different from the HCl/EtOH group (p<0.05). HCl/EtOH: acidified ethanol (1 ml/kg b. w.), MEGC1: 200 mg/kg methanol extract of *Gomphrena celosioides*, MEGC2: 400 mg/kg methanol extract of *Gomphrena celosioides*, MEGC3: 800 mg/kg methanol extract of *Gomphrena celosioides*, and CIM: 50 mg/kg cimetidine.

Figure 3A showed MPO activity, the MPO activity of the acidified ethanol alone group was high compared to the activity found in the normal control group. Treatment with different doses of MEGC significantly (p < 0.05) decreased the MPO activity compared to that of the acidified ethanol alone group in a dose dependent manner. Cimetidine also decreased the MPO activity significantly (p < 0.05) compared to that of the acidified ethanol alone group and the activity obtained compared favorably with the activity obtained for 800 mg/kg MEGC. Figure 3B showed an elevated activity of xanthine oxidase in the acidified ethanol alone group, but post treatment with different doses of MEGC significantly (p < 0.05) decreased this observation. Also, cimetidine decreased the xanthine oxidase activity significantly (p < 0.05) when compared to the acidified ethanol alone group. Lipid peroxidation level was increased in the acidified ethanol alone group, but post treatment with MEGC decreased the LPO level significantly (p < 0.05) in a dose dependent manner. Level of LPO in the cimetidine post treated group was also decreased significantly (p < 0.05) compared to that of the acidified ethanol alone group (Figure 3C). The level of GSH in the acidified ethanol alone group decreased when compared to the normal control group, but treatment with MEGC at different doses significantly (p < 0.05) increased the GSH level, this was also observed in the group treated with cimetidine (Figure 3D).

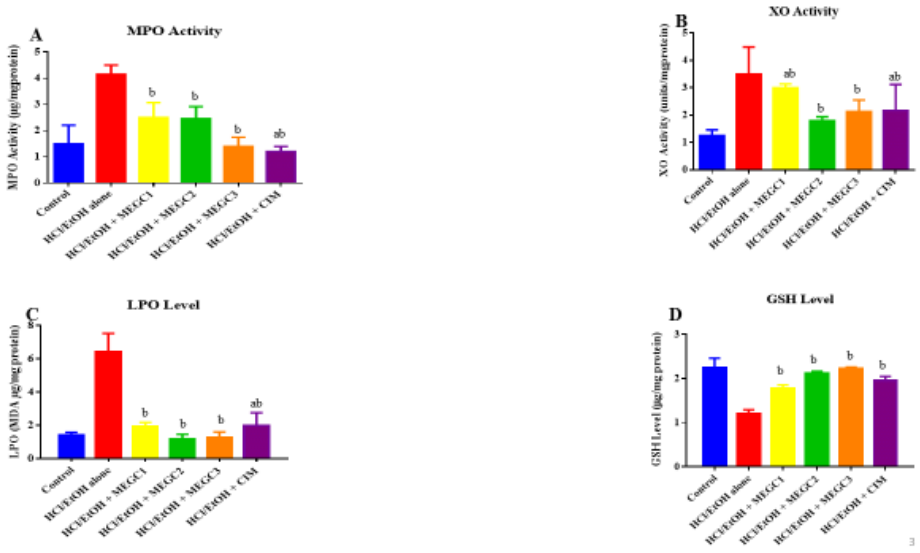

Figure 3: Effects of methanol extract of *Gomphrena celosioides* leaves on activities of: (A) myeloperoxidase; (B) xanthine oxidase; (C) lipid peroxidation level and (D) GSH level in acidified ethanol-induced gastric ulcer healing in rats, (n = 8, ± SEM). Bars with different superscripts for each parameter are significantly different *(p*<0.05*).* [a] significantly different from the control group (*p*<0.05); [b] significantly different from the HCl/EtOH group (*p*<0.05). HCl/EtOH: acidified ethanol (1 ml/kg b. w.), MEGC1: 200 mg/kg methanol extract of *Gomphrena celosioides*, MEGC2: 400 mg/kg methanol extract of *Gomphrena celosioides*, MEGC3: 800 mg/kg methanol extract of *Gomphrena celosioides*, and CIM: 50 mg/kg cimetidine.

The levels of protein carbonyl and nitric oxide were presented in figure 4. Protein carbonyl level was elevated in the acidified ethanol alone group, but treatment with MEGC after ulceration decreased the level of protein carbonyl significantly ($p < 0.05$), also cimetidine decreased the level of protein carbonyl significantly, though not as much as the MEGC (Figure 4A). The nitric oxide concentration in the acidified ethanol alone group was also elevated and this was also decreased by the MEGC significantly($p < 0.05$). Cimetidine also decreased the concentration of NO and the value obtained compared favourably with the value obtained for MEGC at 400 mg/kg (Figure 4B).

Figure 4: Effects of methanol extract of *Gomphrena celosioides* leaves on concentrations of: (A) protein carbonyl and (B) nitric oxide in acidified ethanol-induced gastric ulcer healing in rats, (n = 8, ± SEM). Bars with different superscripts for each parameter are significantly different *(p*<0.05*).* [a] significantly different from the control group (*p*<0.05); [b] significantly different from the HCl/EtOH group (*p*<0.05). HCl/EtOH: acidified ethanol (1 ml/kg b. w.), MEGC1: 200 mg/kg methanol extract of *Gomphrena celosioides*, MEGC2: 400 mg/kg methanol extract of *Gomphrena celosioides*, MEGC3: 800 mg/kg methanol extract of *Gomphrena celosioides*, and CIM: 50 mg/kg cimetidine.

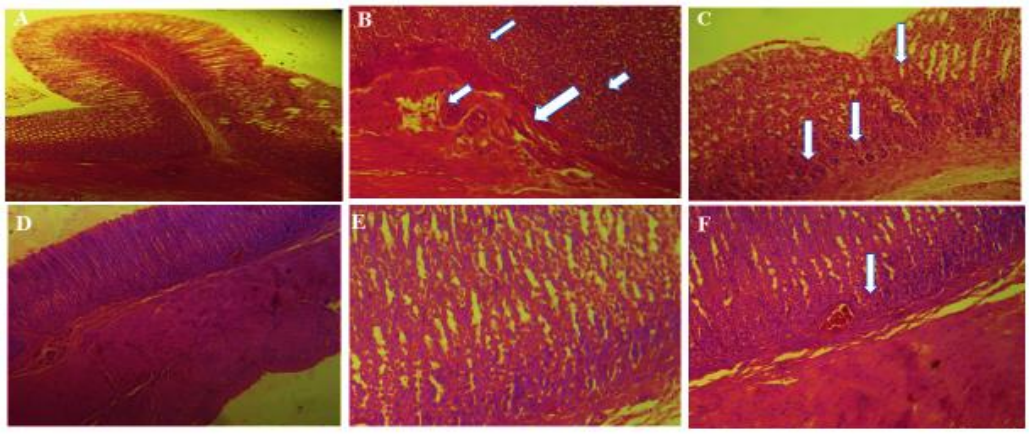

Figure 5: Histological view of the healing effect of MEGC on acidified ethanol-induced gastric mucosa damage (ulcer) in Wistar Rats. (A) Control stomach architecture appears intact; (B) Treatment with 1 ml/kg bwt of HCl/EtOH showing disruption of the epithelia cells, marked congestion in the mucosa, infiltration of the mucosa by inflammatory cells, cluster of hyperplastic cells some of whose nuclei appear vesicular while others appear normal. The cells also exhibit slight pleiomorphisms; (C) 1 ml/kg bwt of HCl/EtOH + MEGC1 (200mg/kg bwt) showing mild disruption of the surface epithelium with necrosis, infiltration of the mucosa by inflammatory cells; (D) 1 ml/kg bwt of HCl/EtOH + MEGC2 (400mg/kg bwt) showing regeneration and restoration of the mucosa cells and increased muscularis thickness; (E) 1 ml/kg bwt of HCl/EtOH + MEGC3 (800mg/kg bwt) showing regeneration of the mucosa cells and muscularis thickness; (F) 1 ml/kg bwt of HCl/EtOH + CIM (50mg/kg bwt) showing partial regeneration of the surface epithelia cells. (H & E, X 40)

III. DISCUSSION

Mixture of HCl/ethanol used to induce gastric ulcer is a fast and convenient method to showcase the cytoprotective properties of the medicinal plant extracts. Ethanol drops the transmucosal potential difference and thus weakens the lining. HCl on the other hand, accelerates the ulcerogenic process while enhancing lesions and reducing mucosal protection. In this present study, acidified ethanol severely damaged the gastric mucosal as evident by the ulcer score and ulcer index obtained. These results were consistent with the report of [36,37], however, methanol extract of *Gomphrena celosioides* inhibited the gastric damaged induced by HCl/EtOH a dose dependent manner as observed in the percentage gastric ulcer healing. This could be due to a reduction of acid secretion and the presence of the phytochemicals especially alkaloids that are involved in the reduction of acidity [12, 38].

Biochemical analysis of gastric secretions and mucosal integrity of stomach by determining the pH, gastric juice volume, bicarbonate and peptic activity is usually employed to ascertain its status following exposure to pharmacological agents [39]. The pH gives an idea of the level of acidity and volume of gastric secretions. Low pH value is a manifestation of increased hydrogen ion concentration in gastric juice. This has been linked to pathogenesis of gastric ulceration and delayed healing in experimental animals [40]. Our results revealed that oral administration of HCl/EtOH increased the gastric juice volume, acid output and peptic activity with concomitant decrease in the gastric pH, but administration of MEGC reversed these observations probably due to the presence of the phytochemical constituents of the extract. Cimetidine also produced similar effects on these parameters.

The primary function of the mucus layer is structural, creating a stable layer that supports surface contact with acid and acting as a protective physical barrier against luminal pepsin. Secretion of bicarbonate (HCO_3^-) adhered to the mucus layer (stable and adherent gel) creates a nearly neutral pH gradient on the epithelial surface of the stomach and duodenum, providing the first line of defense against luminal acid and pepsin by forming a pre-epithelial mucus-buffer barrier along with phospholipids [41, 42]. Besides providing significant buffering capacity for the neutralization of luminal acid, the mucus also offers protection against both endogenous aggressors and exogenous gastro toxic agents such as HCl/EtOH, thereby enhancing the rate of local healing process.[47] In this study, the increased pepsin activity coupled with decrease in mucus secretion in the HCl/EtOH-ulcerated rats reduced protective ability of the mucosal membrane against hemorrhage, thus,

resulting in tissue damage. In this study, MEGC decreased pepsin activity and elevated mucus contents in the gastric mucosa. This in turn encouraged speedy wound healing of the ulcerated areas of the mucosal epithelia and shielded the gastric membrane, thus abrogating the catastrophic influence of HCl/EtOH in the ulcerated rats. This is indicative of enhanced mucus secretory potential of the MEGC and suggestive of their significant role in ulcer healing process. CIM also increased the mucus contents in the gastric tissue but to a lesser extent.

Acidified ethanol administration inflicts gastric injury via direct effects including disruption of mucosal cellular membranes, dehydration, hemorrhagic gastric lesions characterized by mucosal friability, cellular exfoliation, extensive submucosal edema and inflammatory cell infiltration and cytotoxic effects with consequent propagation of the inflammatory cascade [37,43-45] Meanwhile, alcohol causes indirect damaging effects via the recruitment of leukocytes which drives inflammatory responses, oxidative stress and apoptosis.

Myeloperoxidase, a biomarker for neutrophil-dependent inflammation, is mainly released from neutrophils, and therefore is also an essential marker for normal neutrophil function. MPO and other tissue-damaging substances including reactive oxygen metabolites and cytotoxic proteins are released into the extracellular space when neutrophils are stimulated [46]. Acidified ethanol administration causes an increase of mucosal MPO activity, which indicates that the level of activated neutrophils is increased in the gastric mucosal [47,48]. In the present study, acidified ethanol induced an increase of MPO activity in the HCl/EtOH rats compared with the normal control, this conformed to the report of [49,50]. Post treatment with MEGC extracts after ulcer induction by HCl/EtOH suppressed the release of MPO, compared with HCl/EtOH control, which may indicate that the degree of inflammation induced by neutrophils was also inhibited particularly at 800 mg kg^{-1} and this value was similar to that obtained cimetidine treated group.

Xanthine oxidase (XO) is a major source of reactive oxygen species generation in the pathogenesis of various biological systems including the gastrointestinal tract [51,52]. It catalyses the conversion reactions of hypoxanthine to xanthine and xanthine to uric acid with byproduct of toxic superoxide radical. In this regard it is a key enzyme between purine and free radical metabolism [53]. In healthy tissue, XO is synthesized as xanthine dehydrogenase (XD), but under conditions like ischemia and reperfusion, XD is proteolytically converted to XO. Xanthine degradation by XO leads to the formation of hydrogen peroxide and superoxide radicals and this is responsible for the disturbance of cell membranes by lipid peroxidation and leads to tissue and/or organ damage. In our study, the elevated activity of XO in HCl/EtOH alone group compared to the normal control group may be attributed to the generation of ROS. Furthermore, treatment with MEGC at different doses decreased the activity of XO especially at 400 mg kg^{-1} and cimetidine was also observed to attenuate the elevated activity of XO.

Lipid peroxidation is triggered by the process of free radical interaction with cell membrane and produce lipid-derived free radicals. MDA is the cytotoxic product of lipid peroxidation, which leads to the cross-linking polymerization of proteins, nucleic acid and other living macromolecules, and arouses the injury of mitochondria and lysosomes [54,55]. GSH, a tripeptide with a reactive thiol group, is a reductive non-enzymatic antioxidant that maintains the intracellular redox status against ROS generated by oxidative stress, protect and maintaining the integrity of the gastric mucosal from oxidative damage and participates in the production of mucus and binding to free radicals formed during inflammation or exposure to harmful agents [56]. In our present study, acidified ethanol induced a marked oxidative stress, as evidenced by elevated MDA levels with concomitant decreased GSH levels in the gastric homogenate. Increased level of lipid peroxidation HCl/EtOH alone group in our study agreed with reports of [57-59]. However, oral administration of MEGC produced a dose dependent decrease in the LPO level and increase in the GSH level, indicating that MEGC might have therapeutic effects against acidified ethanol-induced oxidative stress. The same trend was observed in group treated with cimetidine. Reactive oxygen species not only stimulate lipid peroxidation but also cause protein oxidation to produce protein carbonyl, elevation of the protein carbonyl content in the HCl/EtOH alone treated group compared with the normal control group is an indication of generation of ROS following HCl/ EtOH administration to rats, these observation is reinforced by the finding that the activities of xanthine oxidase and myeloperoxidase were increased and the level of glutathione was decreased, but treatment with MEGC for 14 day decreased this observation and cimetidine also decreased the level of protein carbonyl.

NO, when serving as a potent vasodilator, increases blood flow in the gastric mucosa, inhibits the secretion of gastric acid and potentiates the secretion of mucus and bicarbonate, which thus protect the gastric mucosa against damage induced by a variety of corrosive substances and noxious agents [60]. On the other hand, during inflammation and pathological conditions like gastric ulcer [61], there is continuous production of NO and superoxide anion ($O_2^{\cdot-}$). NO is a weak oxidant, but when it combines with $O_2^{\cdot-}$ to produce peroxynitrite ($OONO^-$), it becomes a potent ROS, and disrupts the function of important biological molecules such as DNA, RNA, protein and lipids.[62,63] . In our experiments, we observed abundant mucosal lesions, accompanied by the rise of NO in HCl/EtOH alone treated rats. Post-treatment with MEGC remarkably inhibited the expression of NO in gastric tissues after HCl/EtOH challenge, particularly at the dose of 400 mg kg^{-1}. This observation was similar to what was obtained with the group post treated with cimetidine.

Furthermore, MEGC significantly attenuated the oxidation of protein and xanthine oxidase activity and reduced oxidative stress biomarker (MDA level), indicating an increase in the antioxidative capacity. These findings suggest that MEGC may exert therapeutic effects via anti-inflammatory and antioxidative mechanisms accompanied with upregulation of gastroprotective factor mucus contents.

Histological examination of the gastric tissue reveals glandular destruction in the gastric mucosa and inflammatory cells infiltration in acidified ethanol alone group. This is in agreement with previous studies [50,64]. Healing of gastric mucosa damage involves rapid mucosal restitution and reepithelization by migration of surrounding epithelial cells from the ulcer margin to cover the denuded area [65], which is subsequently enhanced by an increase in mucosal cell proliferation. Administration of MEGC once daily for 14 days after the induction of gastric injury with acidified ethanol accelerated the healing process through mucosal and submucosal re-epithelialization, as shown by the histological analysis.

References

[1]. Maria-Ferreira D, da Silva LM, Mendes DAGB, Cabrini DdA, Nascimento AM, et al. Rhamnogalacturonan from *Acmella oleracea* (L.) R.K. Jansen: Gastroprotective and Ulcer Healing Properties in Rats. PLoS ONE 2014;9(1): es84762. doi: 10.1371/journal.pone.0084762

[2]. Naito Y, Yoshikawa T, Matsuyama K, Yagi N, Arai M, et al. Effects of oxygen radical scavengers on the quality of gastric ulcer healing in rats. J Clin Gastroenterol. 1995; 21:1S82–86

[3]. Palileo C, Kaunitz JD. Gastrointestinal defense mechanisms. Curr Opin Gastroenterol. 2011; 27:543–548.

[4]. Al Batran R, Al-Bayaty F, Jamil Al-Obaidi MM, Abdualkader AM, Hadi HA, Ali HM, et al. In vivo antioxidant and antiulcer activity of Parkia speciosa ethanolic leaf extract against ethanol-induced gastric ulcer in rats. PLoS One. 2013; 8(5):e64751. doi: 10.1371/journal.pone.0064751. pmid:23724090

[5]. Sangiovanni E, Vrhovsek U, Rossoni G, Colombo E, Brunelli C, Brembati L, et al. Ellagitannins from Rubus berries for the control of gastric inflammation: in vitro and in vivo studies. PLoS One. 2013;8(8):e71762. doi: 10.1371/journal.pone.0071762. pmid:23940786

[6]. Stadelmann, W., et al. Physiology and healing dynamics of chronic cutaneous wounds. Am J Surgery 1998;176 (2A):26S-38S

[7]. Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal plants. Trop Hortic. 2001; 2:449-632.

[8]. Handa SS. An overview of extraction techniques for medicinal and aromatic plants. In: Handa SS, Khanuja SP, Longo G, Rakesh DD, editors. Extraction Technologies for Medicinal and Aromatic plants. Trieste, Italy: ICS-UNIDO; 1997;1:21-52.

[9]. Kurekci C, Bishop-Hurley SL, Vercoe PE, Durmic Z, Al Jassim RA, McSweeney CS. Screening of Australian plants for antimicrobial activity against Campylobacter jejuni. Phytother Res 2012; 26:186-90.

[10]. Allison MC, Howastson AG, Torrance CJ, Lee FD, Russel RI. The medical benefits of *Gomphrena celosioides*. N Engl J Med 1992; 327:749-54

[11]. Vieira CCJ, Mercier H, Chu EP, Figueiredo-Ribeiro RCL. Gomphrena species (globe amaranth): In vitro culture and production of secondary metabolites. In Bajaj YPS (ed): Biotechnology in agriculture and forestry. Berlin: SpringerVerlag, 1994; pp 257-70.

[12]. Dosumu OO, Onocha PA, Ajaiyeoba EA and Ekundayo O. Phytochemical Screening and Biological Activities of *Gomphrena celosioides* (C. Mart) Extracts. Nigerian Society for Experimental Biology. 2005;5(2):61-67.

[13]. Dosumu OO, Idowu PA, Onocha PA, Ekundayo O. Isolation of 3-(4-hydroxyphenyl) methylpropenoate and bioactivity evaluation of *Gomphrena celosioides* extracts. EXCLI Journal. 2010; 9:173-180.

[14]. Sharma N, Vijayrergia R. Study of primary metabolites and antimicrobial activity of *Gomphrena celosioides* Mart. Intern J of Pharma and Bioscience. 2011;2(4):581-586.

[15]. Abalaka ME, Damisa D. Antifungal activity of *Gomphrena celosioides* (soft khaki weed) on selected fungal isolates. J of Current research in science 2013;1(2):66-70.

[16]. Oladele GM, Abatan MO, Olukunle JO, Okediran BS. Anti-inflammatory and analgesic effects of Aqueous leaf extracts of *Gomphrena celosioides* and

Momordica charantia. J. of Natural Sciences, Engineering and Technology 2009;8(2):1-8.
[17]. Burkill HM. The useful plants of West Tropical Africa. Kew: Royal Botanical Gardens. 1984; 1:61-62; 2:337-338.
[18]. Botha S, Gerritsma-Van der Vijver LM. Pharmacochemical study of *Gomphrena celosioides* (Amaranthaceae). Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie 1986;5(1):40-45.
[19]. De Moura XRM, Pereira PS, Januario AH, Franca S, Dias DA. Antimicrobial screening and quantitative determination of benzoic acid derivatives of *Gomphrena celosioides* by TLC-densitometry. Chem Pharm Bull. 2004; 52:1342-4.
[20]. US Department of Health and Human Services. Human Health Research Act; 1985. P.H.S (Public Health Service). Public health service policy on humane care and the use of laboratory animals. Washington, DC: US Department of Health and Human Services. 1996;158.
[21]. World Medical Association, American Physiological Society. Guiding principles for research involving animals and human beings, Am J Physiol. Regul. Integr. Comp. Physiol. 2002;283: R281-R283.
[22]. Mizui, T. & Doteuchi, M. Effect of polyamines on acidified ethanol-induced gastric lesions in rats. Jpn J Pharmacol. 1983;3(**3**)**:**939–945.
[23]. Praveen PS, Pardhasaradhi P. Preliminary phytochemical investigation and anti-ulcer activity of aerial parts of Ruellia tuberosa (Acanthaceae) in male Wistar rats. Int J Phar and Biomed Res. 2013;4(3):145-148.
[24]. Anosike, C. A. and Ofoegbua, R.E. Anti-ulcerogenic activity of the methanol extract of Ceiba pentandra stem bark on indomethacin and Ethanol-induced ulcers in rats. Int journal of Pharma Sciences. 2013;3(3):223-228.
[25]. Ketuly, K. A. *et al.* Acute toxicity and gastroprotection studies with a newly synthesized steroid. Plos One 8. 2013; e59296–e59296
[26]. Corne S, Morrissey S, Woods R. Proceedings: A method for the quantitative estimation of gastric barrier mucus. J physiol. 1974; 242:116P–117P. PMID: 4142046
[27]. Yildirim A, Sahin YN, Suleyman H, Yilmaz A, Yildirim S. The role of Prednisolone and epinephrine on gastric. J Physiol Pharmacol. 2007; 58:105–116.
[28]. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 83:265-275.
[29]. Del Rio D, Stewart, AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr, Metab Cardiov Dis. 2005;15**;**316–328.
[30]. Sedlak J, Lindsay RH. Estimation of total, proteinbound, and nonprotein sulfhydryl groups in Tissue with Ellman's reagent. Anal Biochem. 1968; 25:192–205.
[31]. Slungaard A, Mahoney JR. Thiocyanate is the major substrate for eosinophil peroxidase in physiologic fluids. Implications for cytotoxicity. J Biol Chem. 1991; 266:4903–4910. http://www.ncbi.nlm.nih.gov/pubmed/2002037
[32]. Cortas NK, Wakid NW: Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem. 1990;36(8 Pt 1):1440–1443,
[33]. Biswas K, Bandyopadhyay U, Chattopadhyay I, Varadaraj A, Ali E, Banerjee RK. J. Biol. Chem. 2003; 278:10993–11001.
[34]. Prajda N, Weber G. Malignant transformation-linked imbalance: decreased xanthine oxidase activity in hepatomas. FEBS Lett.1975;59(2):245–249.
[35]. Hajrezaie, M. *et al.* Acute toxicity and gastroprotection studies of a new schiff base derived copper (II) complex against ethanol-induced acute gastric lesions in rats. *Plos One* **7,** e51537–e51547 (2012).
[36]. Esther Oluwatoyin Agbaje, Yewa Peace Doe. Gastric and duodenal antiulcer effects of aqueous bark extract of *Dialium guineense* Wild. (Fabaceae) and the possible mechanisms in laboratory models. The Journal of Phytopharmacology. 2015;4(5):268-275.
[37]. Yoo JH, Lee JS, Lee YS, Ku S, Lee HJ. Protective effect of bovine milk against HCl and ethanol-induced gastric ulcer in mice. J Dairy Sci. 2018; 101:3758–3770.
[38]. N'Guessan K, Kadja B, Ziritti GN, Traoré D, Ake-Assi L. Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou. Sc Nat. 2009;6(1):1–15.
[39]. Biplab A., Sudhir K.Y., Kshama R., Sandip K.B., Subrata C. Black tea and theaflavins assist healing of indomethacin-induced gastric ulceration in mice by antioxidative action. Evid Based Complem Alt Med. 2011; 11:11–22.
[40]. Lüllmann H., Mohr K., Ziegler A., Bieger D. 2nd ed. Thieme Stuttgart; New York: 2000. Color atlas of pharmacology; p. 166.
[41]. Yandrapu H, Sarosiek J. Protective factors of the gastric and duodenal mucosa: an overview. Curr. Gastroenterol. Rep. 2015; 17:1–8.
[42]. Hajrezaie M, Salehen N, Karimian H, Zahedifard M, Shams K, Al Batran R, et al. Biochanin A gastroprotective effects in ethanol-induced gastric mucosal ulceration in rats. PloS One. 2015;10:e0121529.
[43]. Li W, Huang H, Niu, X, Fan T, Mu Q, Li H. Protective effect of tetrahydrocoptisine against ethanol-induced gastric ulcer in mice. Toxicol Appl Pharmacol. 2013; 272:21–29.
[44]. Li WF, Hao DJ, Fan T, Huang HM, Yao H, Niu XF. Protective effect of chelerythrine against ethanol-induced gastric ulcer in mice. Chem Biol Interact. 2014; 208:18–27.
[45]. Park SW, Oh TY, Kim YS, Sim H, Park SJ, Jang EJ, et al. Artemisia asiatica extracts protect against ethanol-induced injury in gastric mucosa of rats. J Gastroenterol Hepatol. 2008;23(6):976–984. doi:

10.1111/j.1440-1746.2008.05333. x. pmid:18444990.

[46]. Nartey ET, Ofosuhene M, Agbale CM: Anti-ulcerogenic activity of the root bark extract of the African laburnum 'Cassia sieberiana' and its effect on the anti-oxidant defence system in rats. BMC Complement Altern Med. 2012; 12:247.

[47]. Chen T, Jiang W, Zhang H, You X, Liu M, Wang L, Xiang P, Xu L, Zheng D, Zhang X, Ji H, Hao K, Yan T, RSC Adv. 2016;6:20081–20088.

[48]. Santos Cerqueira G, dos Santos Silva G, Rios Vasconcelos E, Fragoso de Freitas AP, Arcanjo Moura B, Silveira Macedo D, Lopes Souto A, Barbosa Filho JM, de Almeida Leal LK, de Castro Brito GA, *et al*: Effects of hecogenin and its possible mechanism of action on experimental models of gastric ulcer in mice. Eur J Pharmacol. 2012;683: 260-269.

[49]. Xing J, Sun J, Sun J, Hu S, Guo C, Wang M, Dong Y. Protective effects of shikimic acid in acetic acid induced colitis in rats. Journal of Medicinal plants Research. 2012;6(10):2011-2018.

[50]. Al - Asmari A, Al - Shahrani H, Al - Masri N, Al - Faraidi A, Elfaki I, Arshaduddin M. Vanillin abrogates ethanol induced gastric injury in rats via modulation of gastric secretion, oxidative stress and inflammation. Toxicology reports. 2016; 3:105-113.

[51]. Huh K, Kwon TH, Shin US, Kim WB, Ahn BO, Oh TY, Kim JA. Inhibitory effects of DA-9601 on ethanol-induced gastrohemorrhagic lesions and gastric xanthine oxidase activity in rats. J Ethnopharmacol.. 2003**;** 88:269-273.

[53]. Lou LX, Geng B, Du JB, Tang CS. Hydrogen Sulphide-Induced Hypothermia Attenuates Stress-Related Ulceration in Rats. Clin Exp Pharm Phys. 2008;35(2):223–228.

[54]. Gulec M, Akin H, Yuce H H, Ergin E, Elyas H, Yalcin O, Akyol O: Adenosine deaminase and xanthine oxidase activities in bladder washing fluid from patients with bladder cancer: a preliminary study. Clin Biochem. 2003;36(3): 193–196.

[55]. Zheng H, Chen Y, Zhang J, Wang L, Jin Z, Huang H, Man S, Gao W. Chem.-Biol. Interact. 2016; 250:68–77.

[56]. Chen P, Shen Y, Shi H, Ma X, Lin B, Xiao T, Wu F, Zhu J, Li Z, Xiao J, Li X, Zhang H, Geng F. Chem.-Biol. Interact. 2016;75–83.

[57]. Chen H, Liao H, Liu Y, Zheng Y, Wu X, Su Z, Zhang X, Lai Z, Lai X, Lin Z, et al. Protective effects of pogostone from Pogostemonis Herba against ethanol-induced gastric ulcer in rats. Fitoterapia 2015; 100:110–117.

[58]. Salga MS, Ali HM, Abdulla MA, Abdelwahab SI. Gastroprotective activity and mechanism of novel dichlorido-zinc (II)-4-(2-(5-methoxy benzylidene amino) ethyl) piperazin-1-iumphenolate complex on ethanol-induced gastric ulceration. Chemico-Biological iInteractions. 2012; 195:144-53.

[59]. Rouhollahi E, Moghadamtousi SZ, Hamdi OAA, Fadaeinasab M, Hajrezaie M, Awang K, et al. Evaluation of acute toxicity and gastroprotective activity of *curcuma purpurascens* BI. rhizome against ethanol-induced gastric mucosal injury in rats. BMC Complementary and Alternative medicine. 2014; 14:1.

[60]. Hajrezaie M, Salehen N, Karimian H, Zahedifard M, Shams K, Al Batran R, et al. Biochanin A gastroprotective effects in ethanol-induced gastric mucosal ulceration in rats. PloS One. 2015;10(3): e0121529.doi:10.1371/journal. pone.0121529.

[61]. Szlachcic A, Krzysiek-Maczka G, Pajdo R, Targosz A, Magierowski M, Jasnos K, Drozdowicz D, Kwiecien S and Brzozowski T. The impact of asymmetric dimethylarginine (ADAMA), the endogenous nitric oxide (NO) synthase inhibitor, to the pathogenesis of gastric mucosal damage. Curr Pharm Des. 2013;19: 90-97.

[62]. Yu C, Mei XT, Zheng YP, Xu DH. Eur. J. Pharmacol, 2014;740:329–336.

[63]. Beckman JS, Koppenol WH. Nitric oxide, superoxide and peroxynitrite: The good, the badand the ugly. Am. J.Physiol. Cell. Physiol. 1996;271:C1424-C1437.

[64]. Medeiros JV, Gadelha GG, Lima SJ, Garcia JA, Soares PM, Santos AA, Brito GA, Ribeiro RA, Souza MH. Br J Pharmacol. 2008; 153:721–727.

[65]. Ige SF, Akhigbe RE, Olaleye SB, Adeyemi JW. Gastroprotective potentials of the methanolic extract of *Garcinia kola* in rats. Inter J Medi and Biomed Res. 2012;1(3):172-178.

[66]. Lacy ER. Epithelial restitution in the gastrointestinal tract. J Clin Gastroenterol. 1988;10 (1): S72-S77.