

EFFECTS OF AQUEOUS EXTRACT OF *VERNONIA KOTSCHYANA* SCH. BIP. EX WALP ROOTS ON EXPERIMENTAL GASTRIC ULCER IN MICE

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Abstract

The present study investigated the antiulcer/gastro-protective effects of aqueous extract obtained from *Vernonia kotschyana* roots on two animal models of gastric ulcer induced by indomethacin and, respectively, absolute ethanol. The extract proved to have weak *in vitro* antioxidant activity on scavenging free radicals (ABTS radical cation, superoxide and nitric oxide) and weak ability of ferrous ions chelating. FT-IR analysis showed the presence of carbohydrates (polysaccharides) and saponins in the aqueous extract. The results of antiulcer activity study showed that *Vernonia* aqueous extract is efficient on indomethacin-induced ulcer model ($ED_{50} = 557.12 \pm 38.29$ mg/kg b.w.) and has gastro-protective activity on absolute ethanol-induced ulcer model ($ED_{50} = 439.85 \pm 5.67$ mg/kg b.w.). Further studies are needed to elucidate other mechanisms through which the aqueous extract exerts antiulcer/gastro-protective effects. Sub-fractionation of the extract may reveal the presence of novel compounds with antiulcer/gastro-protective activity.

Rezumat

Prezentul studiu a investigat efectele antiulceroase/gastro-protectoare ale extractului apos obținut din rădăcinile speciei *Vernonia kotschyana* pe două modele animale de ulcer gastric indus de indometacin și, respectiv, de etanol absolut. Extractul a demonstrat slabe proprietăți antioxidante *in vitro*, de *scavenger* a radicalilor liberi (radicalul cation ABTS, superoxid și oxid nitric) și o slabă capacitate de chelatare a ionilor feroși. În urma analizei FT-IR, în extractul apos au fost identificată prezența carbohidraților (polizaharide) și a saponinelor. Rezultatele studiului activității antiulceroase au arătat că extractul apos din *Vernonia* are proprietăți antiulceroase în cadrul modelului de ulceratie indusă de indometacin ($DE_{50} = 557,12 \pm 38,29$ mg/kg corp) și proprietăți gastro-protectoare în cadrul modelului de ulceratie indusă de etanol ($ED_{50} = 439,85 \pm 5,67$ mg/kg corp). Studii ulterioare sunt necesare pentru a elucidate alte mecanisme prin care extractul apos exercită acțiune antiulceroasă/gastro-protectoare. Sub-fracționarea extractului poate dezvălui prezența unor noi compuși cu activitate antiulceroasă/gastro-protectoare.

Keywords: *Vernonia kotschyana*, antiulcer, gastro-protective effect, indomethacin, absolute ethanol, ED_{50}

Introduction

Gastric ulcer is one of the most disease affection throughout the world and is considered to be a global health problem [9]. It is estimated that 14.5 million people worldwide are affected by gastric ulcers with a mortality rate of 4.08 million *per year* [31]. The aetiology of the gastric ulcer disease involves the imbalance between the aggressive and the protective factors of the gastric mucosa [22]. The gastric mucosa is subjected constantly to the aggression of noxious agents, such as gastric acid, pepsin secretion, biliary acids, different ingredients from alimentation, chronic alcohol consumption, infection with *Helicobacter pylori*, chronic treatment with non-steroidal anti-

inflammatory drugs (including aspirin, diclofenac, indomethacin) and reactive oxygen species; these agents stimulate the secretion of acid and pepsin, inhibit the cytoprotective prostaglandins synthesis, decrease the blood flow at gastric level and gastric motility [2, 3]. The protective factors of gastric mucosa are: mucus [19], prostaglandins [39, 48], bicarbonate secretion [21, 44], nitric oxide [20] and growth factors [10, 22].

Treatment strategy for gastric ulcer includes available drugs such as antacids, H_2 receptor antagonists, proton pump inhibitors, muscarinic M_1 and M_3 receptor antagonists, antagonists targeting gastrin receptors, somatostatin analogues, drugs that restore and

regenerate damaged mucosa, antibacterial drugs against *Helicobacter pylori*. Unfortunately, the therapeutical benefits of these drugs is accompanied by high incidence of side effects and drug interactions [9, 38].

Plants have been used for centuries in traditional medicine by native healers in the prophylaxis and treatment of gastric ulcer. In the last decades, ethnopharmacological studies have been focused on agents in alternative medicine with promising anti-ulcer activity, with decreased relapse and minimal side effects [22, 29]. In order to justify the traditional use, these plants were intensively studied, in order to clarify the mechanism of anti-ulcerous action [38].

Vernonia kotschyana Sch. Bip. ex Walp (*Asteraceae* family) is an annually tropical African plant [15]. Several ailments such as stomach-ache, tuberculosis, gonorrhoea, gingivitis, arthritis or gastro intestinal disorders are treated in some African countries (Mali, Nigeria, Kenya) by local traditional healers using various parts of the plant [14].

The roots of *Vernonia* are used in Mali for the treatment of gastritis, stomach ulcers and wounds. Traditional healers recommend the use of dried and powdered roots in gastric dysfunction treatment as a decoction. Starting from these usages, the researchers of the Department of Traditional Medicine in Bamako, Mali obtained and conditioned an Improved Traditional Medicine (Gastrosédal[®]) that is on the National List of Essential Drugs [32, 46]. It's efficacy was evaluated in two clinical trials, which confirmed the anti-ulcerous effect of the preparation [1]. Germano *et al.* investigated the preventive and/or curative anti-ulcerous activity of the aqueous extract obtained in maceration of plant roots using different ulcerogenic models [15].

On the basis of the use of this plant in folk medicine of Mali, this study was aimed to evaluate the graded dose-response relationship of an aqueous extract obtained from *Vernonia kotschyana* in order to establish the median effective dose value (ED₅₀) of the anti-ulcerous and gastroprotective activity.

Materials and Methods

Plant material and preparation of extract

The roots of *Vernonia kotschyana* Sch. Bip ex Walp. were provided by the Department of Traditional Medicine, Bamako, Mali. The identity of the plant material was confirmed by experts from the same department. The vegetal material was dried, pulverized and stored in a cool and dry place, away from light until use. A voucher specimen of the plant product is deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" U.M.F. Iași, Romania.

The aqueous extract (VA) was obtained following a similar method to the one used in traditional medicine. Thus, the plant material was subjected to infusion,

continued by 8 h of prolonged maceration at room temperature. The obtained extract was filtered, concentrated at reduced pressure, lyophilized and stored at -20°C until use.

Drugs and reagents

Ranitidine, indomethacin, absolute ethanol, sodium carboxymethyl cellulose (CMC) were purchased from Sigma Aldrich (Germany). Physiological saline (0.9%) was obtained from B. Braun Melsungen (Germany). Distilled water was obtained from Zentiva (Czech Republic).

Phytochemical study

Quantification of total phenolic content

The total phenolic content was estimated using Folin-Ciocalteu reagent following the method of Li *et al.* [24, 37]. The results were expressed as g of gallic acid equivalents *per* 100 g of extract. The measurements were performed using an ABLE-JASCO V550 UV-VIS spectrophotometer.

Quantification of total saponin content

The total saponin content was estimated using the method described by Li *et al.*, slightly modified [27]. The content of total saponin compounds were expressed as g of betulinic acid equivalents *per* 100 g of extract. The measurements were performed using an ABLE-JASCO V550 UV-VIS spectrophotometer.

FT-IR spectroscopic analysis

FT-IR spectrum was recorded on ABB MB3000 FT-IR spectrometer from the scan range of 4000 - 650 cm⁻¹ with a resolution of 4 cm⁻¹ and with a total accumulation of 16 scans. Spectra processing was carried out using the Horizon MB software [16, 36, 43].

In vitro antioxidant assay

ABTS radical cation scavenging assay. ABTS assay was performed according to the method developed by Re *et al.* [7, 33]. Glutathione was the positive control.

Superoxide anion radical scavenging assay. The assay was performed as described by Wang and Luo [45]. Glutathione was used as positive control.

Nitric oxide radical scavenging assay. The assay was performed as reported by Tsai *et al.* with minor changes [40]. L-Ascorbic acid was the positive control.

Ferrous ion chelating ability assay. The assay was done according to Dinis *et al.* [11]. EDTA was used as positive control.

Experimental animals

Male Swiss albino mice, weighing 25 - 30 g, provided from "Cantacuzino" Institute, Bucharest, Romania, were used. They were kept in polyethylene cages and maintained under standard housing conditions (temperature of 21.00 ± 2.00°C, well-ventilated space, 12 h light/12 h dark cycle) in the Laboratory of Experimental Pharmacodynamics, Faculty of Pharmacy, "Grigore T. Popa" U.M.F. Iași, Romania. The animals were allowed for acclimatization two weeks before starting the studies with free access to standard

commercial food (provided from “Cantacuzino” Institute, Bucharest) and water *ad libitum*. 20 h prior to each experiment the animals were restricted to food, with free access to water.

The experiments were designed and conducted in accordance with the international bioethical norms of the study on laboratory animals and the specific rules of the Research Ethics Committee of the “Grigore T. Popa” University of Medicine and Pharmacy [12].

Antiulcerous activity study

Indomethacin-induced gastric ulcer

The experiment was conducted according to the method described by Chattopadhyay *et al.*, with slight modifications [8, 35]. Mice were randomized into 8 groups, each consisting of 8 - 10 animals, as follows: negative control group (AUG1), positive control group (AUG3), indomethacin-induced gastric ulcer group (AUG2) and the study groups (AUG4-AUG8). The extract/substances were suspended in 0.1% aqueous CMC solution immediately before use and administered orally.

The groups received the following substances: AUG1 - 0.1% aqueous CMC solution (20 mL/kg b.w.), AUG2 - indomethacin (18 mg/kg b.w.), AUG3 - indomethacin (18 mg/kg b.w.) + ranitidine (100 mg/kg b.w.), AUG4 - indomethacin (18 mg/kg b.w.) + VA (200 mg/kg b.w.), AUG5 - indomethacin (18 mg/kg b.w.) + VA (300 mg/kg b.w.), AUG6 - indomethacin (18 mg/kg b.w.) + VA (400 mg/kg b.w.), AUG7 - indomethacin (18 mg/kg b.w.) + VA (500 mg/kg b.w.), AUG8 - indomethacin (18 mg/kg b.w.) + VA (800 mg/kg b.w.). Indomethacin was administered in single dose 30 minutes after the administration of extract/positive control. Four hours later, the animals were sacrificed and the stomachs were removed, opened along the greater curvature, rinsed with physiological saline and lesions were viewed using a magnifying lens.

The degree of ulceration was graded according to the following scale, based on the severity of hyperaemia and haemorrhagic erosions: 0 = normal mucosa; 0.5 = hyperaemia; 1 = 1 - 2 lesions; 2 = 2 severe lesions; 3 = very severe lesions; 4 = mucosa with many lesions [13]. The ulcer inhibition was calculated for each group using the following formula:

$$\% \text{ Inhibition} = 100 - \frac{T}{M} \times 100,$$

where: M = inhibition degree in control group; T = inhibition degree in treated group [25, 35, 47].

The total number of ulcer spots divided by the number of animals gives the ulcer index for each group [8].

Absolute ethanol-induced gastric ulcer

The experiment was conducted according to the method described by Li *et al.*, with slight modifications [26]. Mice were randomly divided into 6 groups, each consisting of 8 - 10 animals, including the

following: negative control group (PGG1), positive control group (PGG3), absolute ethanol-induced gastric ulcer group (PGG2) and the study groups (PGG4 - PGG6). The extract/ranitidine were suspended in 0.1% CMC-Na mucilage immediately before use and administered orally. Absolute ethanol was administered orally.

The groups received the following substances: PGG1 - 0.1% aqueous CMC solution (20 mL/kg b.w.), PGG2 - absolute ethanol (0.2 mL/animal), PGG3 - absolute ethanol (0.2 mL/animal) + ranitidine (100 mg/kg b.w.), PGG4 - absolute ethanol (0.2 mL/animal) + VA (300 mg/kg b.w.), PGG5 - absolute ethanol (0.2 mL/animal) + VA (400 mg/kg b.w.), PGG6 - absolute ethanol (0.2 mL/animal) + VA (500 mg/kg b.w.). The aqueous extract, the suspending vehicle and gastro-protective agent were administered daily for 4 days. In the fourth day, 0.2 mL absolute ethanol (ulcerogenic agent) was administered orally to all groups, except for the negative control group. The animals were sacrificed and followed the same steps as described above.

The degree of ulceration was graded according to the scale presented above at indomethacin-induced gastric ulcer model. The ulcer inhibition and the ulcer index were calculated as described above.

Statistical analysis

The regression analysis of dose-effect graded relationship was performed to determine ED₅₀ values. The parametric data were analysed by ANOVA. A p value of < 0.05 was considered statistically significant difference between compared groups.

Results and Discussion

Low content of phenolic compounds (1.18% ± 0.04%), but high content of total saponins (40.77% ± 10.16%) were found in VA extract. FT-IR analysis showed the presence of carbohydrates (polysaccharides) and saponins in VA extract (Figure 1). Thus, the absorption bands that are characteristic for saponins are present at 3282 - 3274 cm⁻¹ (O-H group), 2929 cm⁻¹ (methyl group), 1620 cm⁻¹ (C=C group) and 1026 - 985 cm⁻¹ [49, 50]. Absorption bands at approximately 1000 cm⁻¹ (1026 and 985 cm⁻¹) are characteristic for carbohydrates (polysaccharides). Absorption bands at 1120 - 933 cm⁻¹ are assigned to C-O and C-C groups, while those between 1456 - 1218 cm⁻¹ are due to O-C-H, C-C-H and C-O-H groups of the carbohydrates [23]. Spectral data are consistent with previous studies that reported that the *Vernonia kotschyana* roots contain stigmastane and androstane-type steroid saponin glycosides (called vernoniosides) and carbohydrates (pectic polysaccharides, inulin-type fructans) [1, 32, 34].

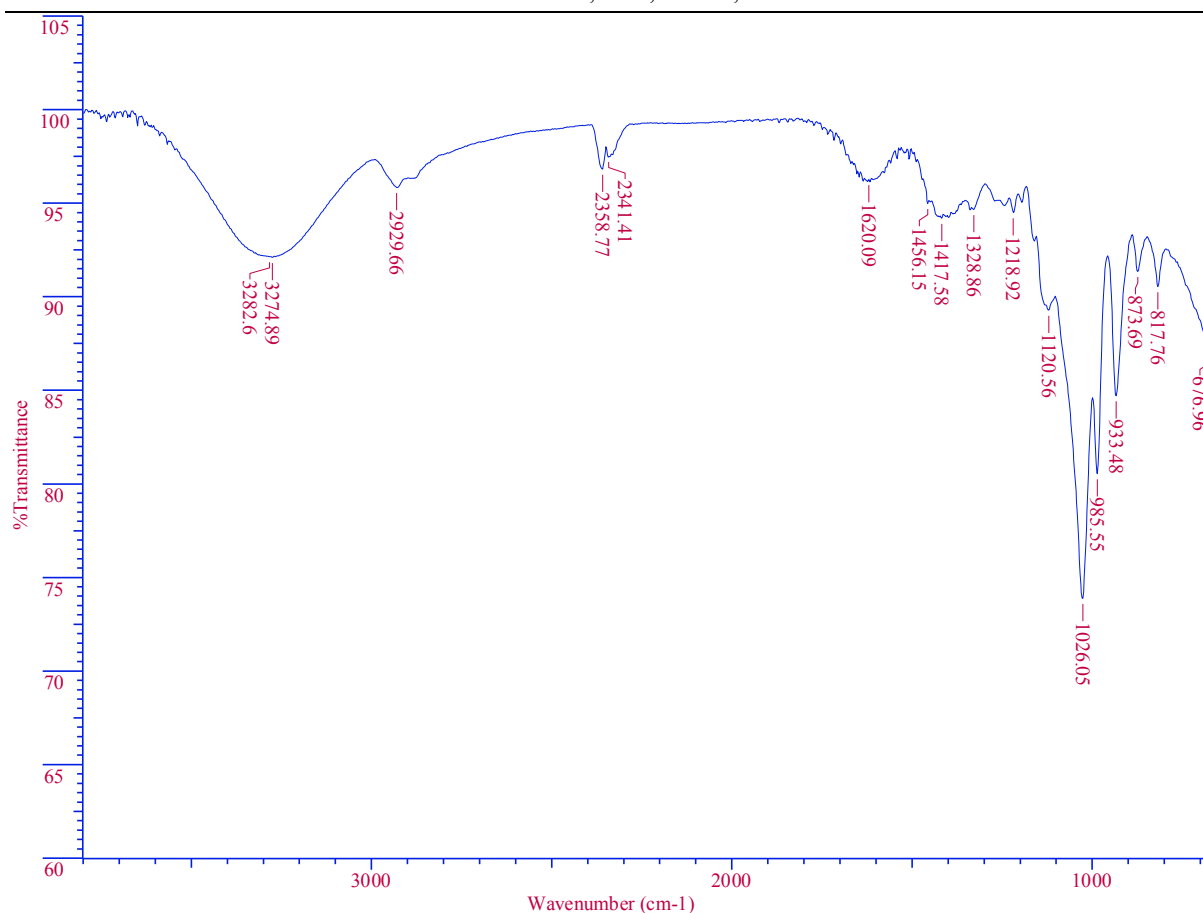


Figure 1.
FT-IR spectra of VA extract

In vitro antioxidant assay

This study was carried out to determine the *in vitro* antioxidant activity of *Vernonia kotschyana* aqueous extract. This aspect is important because it is well known that reactive oxygen species (ROS) and, to a lesser extent, reactive nitrogen species (RNS) are involved in the generation of gastric ulcerations [43]. Antioxidants may help to protect the gastric mucosa against cell damage caused by oxidative stress [5]. The free radical scavenging effects of *V. kotschyana* aqueous extract were initially evaluated against the synthetic nitrogen-centered 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation [33, 43]. The aqueous extract, tested at a concentration of 40 mg/mL, exhibited a weak scavenging effect after 1, 4 and 6 minutes of reaction: $19.41 \pm 0.58\%$, $21.43 \pm 0.45\%$ and, respectively, $22.28 \pm 0.62\%$. Further, the scavenging effects against superoxide and nitric oxide radicals [40, 43, 45] and the ferrous ion chelating activity were evaluated [11, 43]. At the same concentration (40 mg/mL), the extract showed weak effects of scavenging the superoxide anion and nitric oxide radicals and also weak ability of ferrous ions chelating ($1.35 \pm 0.26\%$, $8.85 \pm 0.47\%$ and respectively, $13.19 \pm 0.19\%$).

Antiulcerous activity study

The next part of the study was based on a recent research on acute toxicity of some *Vernonia kotschyana* extracts in mice published by Vasincu *et al.* It was found that VA extract was not toxic for dose ≤ 3200 mg/kg b.w. [42]. This aspect allowed further investigations concerning the antiulcerous/gastro-protective activity of VA extract.

Indomethacin-induced gastric ulcer

Indomethacin-induced gastric ulcer model was used to test antiulcer activity of VA extract. Indomethacin belongs to the non-selective nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit both COX enzymes and produce damage to the gastric tissue. This effect is related to their ability to suppress cytoprotective prostaglandins synthesis (PGE_2 , PGF_2 , PGI_2) and increase acid secretion. Prostaglandins have an important protective effect on the gastric mucosa. They inhibit gastric acid secretion (PGI_2), stimulate bicarbonate and mucus secretion (PGE_2 , PGF_2) and thus maintain an optimal blood flow at gastric mucosal level, regulating mucosal cell turnover and repair [17]. These drugs are widely used in therapy due to their antipyretic, analgesic and anti-inflammatory effective properties in a broad spectrum

of disorders, ranging from common cold to rheumatoid arthritis [28].

The animals of study groups (AUG4 - AUG8) received VA extract using a dose sequence in geometric progression (200 - 800 mg/kg b.w.). Preliminary data required the use of a dose sequence in arithmetic

progression for the studied dose sequence. The obtained data allowed the evaluation of graded dose-effect relationship and led to the determination of ED₅₀ value for antiulcer action. Table I shows the results obtained from macroscopic assessment of indomethacin-gastric ulcerations.

Table I

The influence of *Vernonia kotschyana* aqueous extract on indomethacin-induced gastric ulcer

No	Group	Lesion evaluation	Ulcer index (IU)	% Inhibition (EMP%)
1	AUG1	0	0	-
2	AUG2	6.6 ± 3.1	10	-
3	AUG3	1.6 ± 1.2	4	75.75
4	AUG4	7.5 ± 3.6	8	pro-ulcerogenic
5	AUG5	5.85 ± 2.3	7	11.09
6	AUG6	4.2 ± 1.8	4	36.40
7	AUG7	3.5 ± 1.5	4	46.96
8	AUG8	2.2 ± 1.2	3	64.50
Statistical parameters of regression analysis for 50% activity level				
ED ₅₀ = 557.12 ± 38.29 mg/kg b.w. TCL (439.52, 1096.2) Inhibition % = 39.73 Total IU = 5			Y = -283.40 + 121.09 * X, R = 0.974 (F _{tab} = 18.51; F _{calc} = 36.746)	

Absolute ethanol-induced gastric ulcer

Absolute ethanol-induced gastric ulcer model was used to test gastro-protective activity of VA extract. The damaging effects of ethanol have been applied to develop the ethanol model of peptic ulcers. The model is independent of gastric acid secretion and resembles acute peptic ulcers in humans [6]. Oral absolute ethanol ingestion in mice model determines acute gastric mucosal lesions through reduction of gastric defensive mechanisms. These are associated with the increased of ROS level [5] and the generation of pro-inflammatory mediators (such as TNF-α, IL-6, IL-1β) that provide an inflammatory circumstance to facilitate the development of acute gastric mucosal lesions [18].

The animals of the study groups (PGG4 - PGG6) received the extract using a dose sequence in arithmetic progression (300 - 500 mg/kg b.w.). The administration of absolute ethanol caused damage to the gastric mucosa with severe erosions in PGG2 group. Oral administration of VA extract (300, 400 and 500 mg/kg b.w.) and ranitidine (100 mg/kg b.w.) significantly inhibited gastric lesions induced by ethanol, compared to the PGG2 group. The obtained data allowed the evaluation of graded dose-effect relationship and led to the determination of ED₅₀ value for gastro-protective action. Table II shows the results obtained from macroscopic assessment of absolute ethanol-gastric ulcerations.

Table II

The influence of *Vernonia kotschyana* aqueous extract on absolute ethanol-induced gastric ulcer

No	Group	Lesion evaluation	Ulcer index (IU)	% Inhibition (EMP%)
1	PGG1	0	0	-
2	PGG2	14.14 ± 2.29	22	-
3	PGG3	4.71 ± 1.17	6	67.29
4	PGG4	10.64 ± 1.89	11.5	26.11
5	PGG5	7.5 ± 2.15	9	42.95
6	PGG6	6.16 ± 3.1	8	58.72
Statistical parameters of regression analysis for 50% activity level				
ED ₅₀ = 439.85 ± 5.67 mg/kg b.w. TCL (367.80, 511.9) Inhibition % = 42.59 Total IU = 9.5			Y = -337.02 + 146.42 * X, R = 0.994 (F _{tab} = 161.4; F _{calc} = 343.9)	

In vitro antioxidant study of VA extract revealed its weak antioxidant properties. It can be noted that the observed gastro-protective effect of VA extract on absolute ethanol-induced gastric ulcer is not probably due to oxidative stress inhibition. The findings of the present antiulcer study and of the *in vitro* antioxidant

study could explain the antiulcer and gastro-protective activity of VA extract. This may be due to the cyto-protective capacity of the aqueous extract of *Vernonia kotschyana*.

Steroid saponins and polysaccharides were determined in VA extract within the current phytochemical study.

These constituents play an important role in the anti-ulcerous activity. Antiulcerous effects for steroid saponins or extracts containing steroid saponins have already been reported in literature. One of the most active steroid saponin is hecogenin that is isolated from *Agave sisalana*; its gastroprotective effect is due to reduction of lipid peroxidation and NO⁻ levels, normalisation of the glutathione level in gastric mucosa, reduction of COX-2 expression and activation of ATP-dependent K⁺-channels. The latter plays an important role in gastric blood flow regulation, acid secretion and contractility. Saponins activate a number of factors involved in gastric mucosa protection [35]. Polysaccharides, especially peptic ones, provide mechanical protection for the gastric mucosa; in addition, they stimulate mucus production, decrease gastric acidity (hydrochloric acid and pepsinogen) [4], inactivate reactive oxygen species [30] and reduce local production of proinflammatory cytokines (TNF- α , IL-6) [41].

Conclusions

The results of the present study demonstrated that aqueous extract of *Vernonia kotschyana* roots has antiulcer activity in indomethacin-induced ulcer model (ED₅₀ = 557.12 ± 38.29 mg/kg b.w.) and gastro-protective activity in absolute ethanol-induced ulcer model (ED₅₀ = 439.85 ± 5.67 mg/kg b.w.). Because of the weak *in vitro* antioxidant activity, the observed gastro-protective effect of the extract on absolute ethanol-induced gastric ulcer is not probably due to oxidative stress inhibition.

Further studies, including histological evaluation of the effects of the extract on gastric mucosa, are needed in order to elucidate other mechanisms through which the aqueous extract exerts antiulcerous/gastro-protective effects. Sub-fractionation of the extract may reveal the presence of novel compounds with antiulcerous/gastro-protective activity.

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