ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Evaluation of anti-ulcer potential of *Vernonia arborea* Buch.-Ham. against Ethanol-HCl induced ulceration in rats

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Manuscript Info

Manuscript History:

Received: 11 November 2013
Final Accepted: 29 November 2013
Published Online: December 2013

Key words:
Vernonia arborea, gastroprotective, ethanol-HCl, anti-ulcer, ranitidine, ulcer index and gastric acidity.

Abstract

Objective: To study and evaluate the gastroprotective ability of the ethanol leaf extract of *Vernonia arborea* (ELEVA) in ethanol-HCl induced ulceration in experimental animals. **Methods:** Animals were administered 1ml of ethanol-HCl mixture (1:1 ratio) and the anti-ulcer activity of ELEVA was evaluated at dose levels of 100, 200 and 300mg/kg bw. respectively. Ranitidine (2.5mg/kg bw.) was used as the standard drug. Acute toxicity studies, effect of ELEVA on the levels of ulcer index, gastric acidity, anti-oxidant enzymes and lipid peroxidase were studied. **Results:** There was a significant decrease ($P< 0.05$) in the levels of ulcer index and gastric acidity in experimental animals pretreated with ELEVA. The anti-oxidant enzyme levels were also restored to normalcy in rats pretreated with ELEVA. These effects of ELEVA were well comparable to the effect of the standard drug ranitidine. **Conclusions:** The results obtained in this study are indicative of the gastroprotective potential of the plant *Vernonia arborea*.

Introduction

Ulcer is a crater like lesion in a membrane. Ulcers that develop in areas of the GI tract exposed to acidic gastric juice are called peptic ulcers (Tortora and Derrickson, 2006). Peptic ulcer is due to the exposure of stomach and duodenum to pepsin and gastric acid and is characterized by an imbalance between aggressive factors like acid, pepsin, *Helicobacter pylori* and defensive factors such as gastric mucus, bicarbonate ions, and prostaglandins along with innate resistance of mucosal cells (Saif et al., 2005). The majority of gastric ulcers can be attributed to either *H. pylori* or NSAID induced mucosal damage with other causes associated with genetic predisposition, psychological stress, cigarette smoking and diet.

The treatment of peptic ulcer disease has undergone a revolution in the past decade based on advances in cellular biology, pharmacology and health care delivery, has changed forever the treatment of this major disease. An improved understanding of the regulation and cellular mechanisms of gastric acid secretion has resulted in the development of specific and potent drugs for the treatment of peptic ulcer like H_2 blockers, proton pump inhibitors, anti muscarinic agents, sucralfate, colloidal bismuth subcitrate and prostaglandin analogues. Even though these agents are effective in healing of gastric ulcers, continued use is required to prevent recurrences which in turn lead to a plethora of side effects (McQuaid, 2007). This has prompted a resurgence of interest for natural drugs as the scientific fraternity believes that phytomedicine research has a good chance of contributing new strategies through the development of new and better drugs for evidence based and rational phytotherapy.

V. arborea Buch.-Ham. is a medium sized tree species belonging to the genus *vernonia* and has a wide array of medicinal activities. The bark juice of this plant is used for treating worms and chewed on the first signs of sprue in southern Sumatra. The plant is found to exhibit very good antifungal activity due to the presence of a sesquiterpene Zaluzanin D (Pradhan et al., 2009). *V. arborea* was found to possess wound healing activity (Pradhan et al., 2009) and has also been reported to possess hepatoprotective activity against carbon tetrachloride induced hepatic damage in rats (Manjunatha et al., 2006). Even though many pharmacological activities of this plant has

been analyzed and documented, literature evidences on its anti-ulcer potential has been far and few. Hence this study aims to investigate and evaluate the anti-ulcer activity of the ethanolic leaf extract of *V. arborea* against ethanol-HCl induced ulceration in albino wistar rats.

Materials and Methods

Plant material:

Fresh leaves of *V. arborea* were collected from Kolli Hills, Eastern Ghats, identified with the help of Prof. Dr. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai, Tamilnadu and authenticated with the specimens deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's college, Tiruchirappalli.

Preparation of plant extract

The plant material under study was shade dried and coarsely powdered. About 500 gms of plant material was soaked in ethanol for 48hrs. After 48 hrs of soaking the solvent was distilled off under reduced pressure at 50°C and dried in vacuum.

Experimental animals

Wistar strain of male albino rats weighing 120g-150g were obtained from Tamilnadu Veterinary and Animal Sciences University, Chennai. They were fed with standard rat chow pellet obtained form Sai Durga Food and Feeds, Bangalore, India and water *ad libitum*. Animals were maintained in a standard animal house in a controlled environment (temperature 25+2°C and 12hr dark/light cycle). The study was conducted after obtaining the necessary clearance from Institutional Animal Ethical Committee. CPCSEA approval no: 790/03/ac/CPCSEA.

Acute Toxicity Studies

Oral acute toxicity study was conducted as per the guidelines of Organization for Economic Co-operation and Development (OECD) (OECD, 2001). The ethanolic leaf extract of *V.arborea* (ELEVA) was prepared and subjected to toxicity studies in 5 different groups with each group consisting of 6 animals. A single dose of 1, 2, 3, 4, 5 g/ Kg (bw.) were given to five different groups of animals and observed continuously for 21 days.

Ulcer induction

Experimental Design

Group I : Normal albino wistar rats.

Group II : Disease control (Normal saline and 0.5ml HCl – 0.5ml ethanol mixture (1:1ratio).

Group III : Animals treated with the ethanolic leaf extract of *V.arborea* (ELEVA) (100mg/Kg bw.) orally for 10 days + 0.5ml HCl – 0.5ml ethanol mixture (single dose on 11^{th} day).

Group IV : Animals treated with the ethanolic leaf extract of *V.arborea* (ELEVA) (200mg/Kg bw.) orally for 10 days + 0.5ml HCl – 0.5ml ethanol mixture (single dose on 11^{th} day).

Group V : Animals treated with the ethanolic leaf extract of *V.arborea* (ELEVA) (300mg/Kg bw.) orally for 10 days + 0.5ml HCl – 0.5ml ethanol mixture (single dose on 11^{th} day).

Group VI : Animals treated with ranitidine (2.5mg/ kg bw.)

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Blood was collected and used for various biochemical estimations.

Determination of ulcer index in gastric tissue (Das et al., 2009)

The stomach was removed and opened along the greater curvature and washed it slowly under the running water. Placed it on the glass slid and observed under microscope (10x) for ulcers. Mean ulcer score for each animal in expressed as ulcer index and it was calculated as

$$\text{Ulcer index} = \frac{10}{x}$$

Where x = total mucosal area / total ulcerated area

Biochemical investigations

Total acidity in gastric fluid was determined (Kulkarni, 2005). The levels of enzymatic antioxidants and lipid peroxide (LPO) were assessed (Dandekar et al., 2002).

Statistical analysis

All the results were expressed as mean ± S.E.M. The data were statistically analyzed by one – way analysis of variance (ANOVA). P values <0.05 were considered as significant.

Results

Acute toxicity studies

Acute toxicity studies showed that the plant extract did not show any toxicity and mortality up to a dose of 5g/Kg bw. in experimental animals. Three submaximal doses (100, 200 & 300mg/kg.bw. which were found to be safe in rats were employed for further pharmacological and biochemical investigations.

Effect of ELEVA on Ulcer index and Gastric acidity

Increased mucosal lesions and gastric acidity were noticed in experimental animals orally administered with ethanol-HCl solution when compared with the normal control group. However there was a decrease in mucosal lesions in animals pretreated with ELEVA. Both the ulcer index and the amount of gastric acidity were significantly reduced (P< 0.05) in experimental animals pretreated with ELEVA. This gastroprotective effect of ELEVA was well comparable to the standard drug treated group (Table 1, Graph 1 & 2).

Effect of ELEVA on Anti-oxidant levels

The results reveal that the Group II animals registered a collective decrease in the levels of reduced glutathione, superoxide dismutase and glutathione peroxidase with increased levels of lipid peroxide suggestive of tissue damage. In contrast pretreatment of experimental animals with ELEVA brought down the levels of lipid peroxide significantly (P< 0.05) and simultaneously increased the levels of anti-oxidant enzymes (Table 2, 3 & Graph 3, 4, 5, 6).

Table 1: Effect of ELEVA on Ulcer index and Gastric acidity

Groups	Ulcer index (mm^2	Total acidity mEq/L
Group I	$1.21 \pm 0.34^{*}$	$101.31 \pm 1.78^{*}$
Group II	$122.52 \pm 1.11^{*,**, a}$	$141.64 \pm 1.04^{*,**, a}$
Group III	100.13 ± 1.58	129.88 ± 0.75
Group IV	61.25 ± 1.30	118.83 ± 0.60
Group V	$30.07 \pm 1.58^{**, a, b}$	$105.08 \pm 0.83^{**, a, b}$
Group VI	$3.51 \pm 1.04^{a, b}$	$100.92 \pm 1.46^{a, b}$

* - Significant when compared between Group 1 and Group 2 (p≤0.05, n=6)
** - Significant when compared between Group 2 and Group 5 (p≤ 0.05, n=6)
a - Significant when compared between Group2, Group 5 and Group 6 (p≤0.05, n=6)
b - Non significant when compared between Group 5 and Group 6 (p≤ 0.01, n=6)

Table 2: Effect of ELEVA on GR, SOD AND GPx

Groups	Reduced Glutathione µg/ mg tissue	Superoxide dismutase mM of epinephrine oxidized/min/g tissue	Glutathione peroxidase µm of glutathione oxidized/ min/g tissue
Group I	$16.28 \pm 0.33^{*}$	$25.61 \pm 0.56^{*}$	$717.62 \pm 1.81^{*}$
Group II	$6.24 \pm 0.26^{*,**, a}$	$10.48 \pm 0.41^{*,**, a}$	$265.65 \pm 1.67^{*,**, a}$
Group III	8.92 ± 0.17	14.56 ± 0.53	395.23 ± 2.58
Group IV	11.76 ± 0.38	20.02 ± 0.65	525.67 ± 3.78
Group V	$15.36 \pm 0.29^{**, a, b}$	$24.28 \pm 0.35^{**, a, b}$	$696.73 \pm 3.80^{**, a, b}$
Group VI	$15.92 \pm 0.22^{a, b}$	$25.76 \pm 0.82^{a, b}$	$707.43 \pm 2.30^{a, b}$

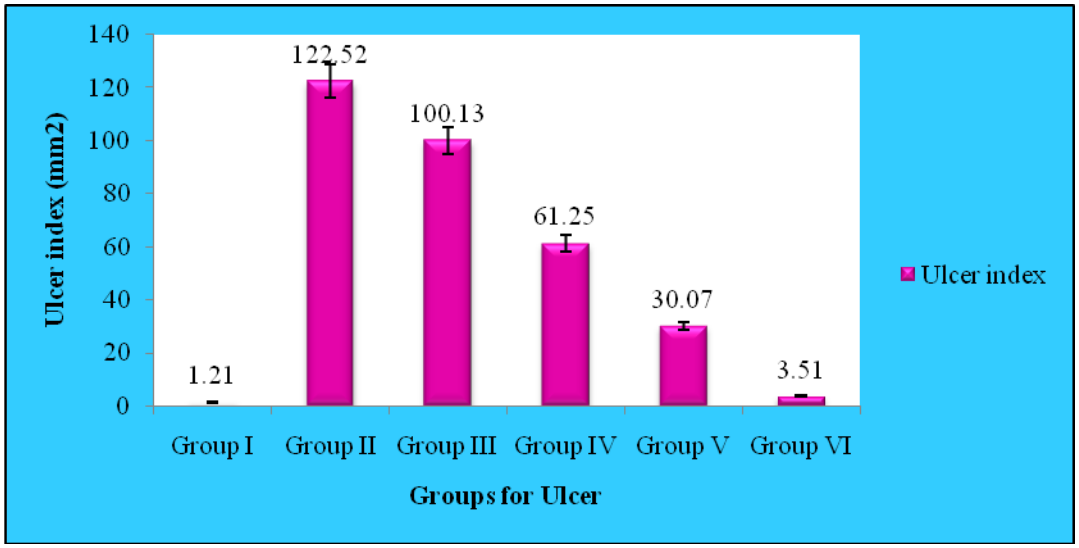
* - Significant when compared between Group 1 and Group 2 (p≤0.05, n=6)
** - Significant when compared between Group 2 and Group 5 (p≤ 0.05, n=6)
a - Significant when compared between Group2, Group 5 and Group 6 (p≤0.05, n=6)
b - Non significant when compared between Group 5 and Group 6 (p≤ 0.01, n=6)

Table 3: Effect of ELEVA on LPO

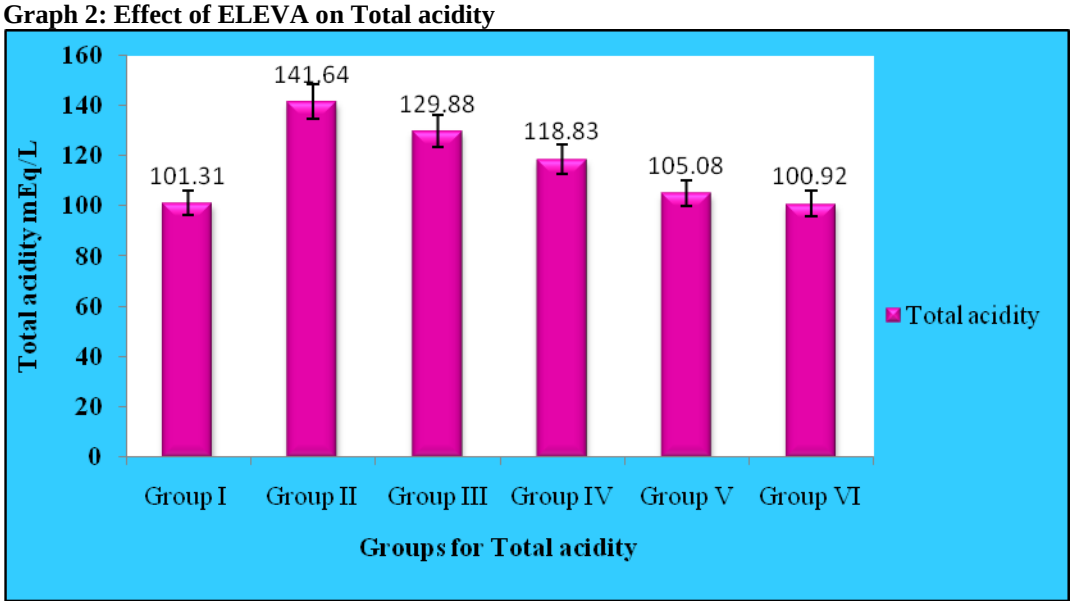
Groups	Lipid Peroxide Mmoles MDA / mg protein
Group I	122.76 ± 1.11*
Group II	335.38 ± 1.98*,**,a
Group III	297.82 ± 1.16
Group IV	210.81 ± 0.67
Group V	138.76 ± 1.97**, a, b
Group VI	122.45 ± 1.64 a, b

* - Significant when compared between Group 1 and Group 2 (p≤0.05, n=6)
** - Significant when compared between Group 2 and Group 5 (p≤ 0.05, n=6)
a - Significant when compared between Group2, Group 5 and Group 6 (p≤0.05, n=6)
b - Non significant when compared between Group 5 and Group 6 (p≤ 0.01, n=6)

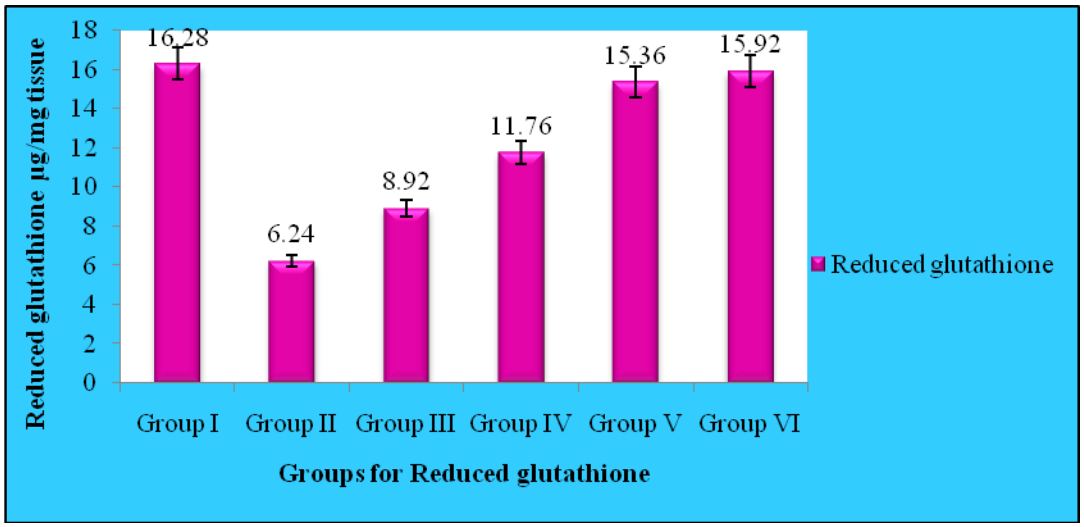
Graph 1: Effect of ELEVA on Ulcer index



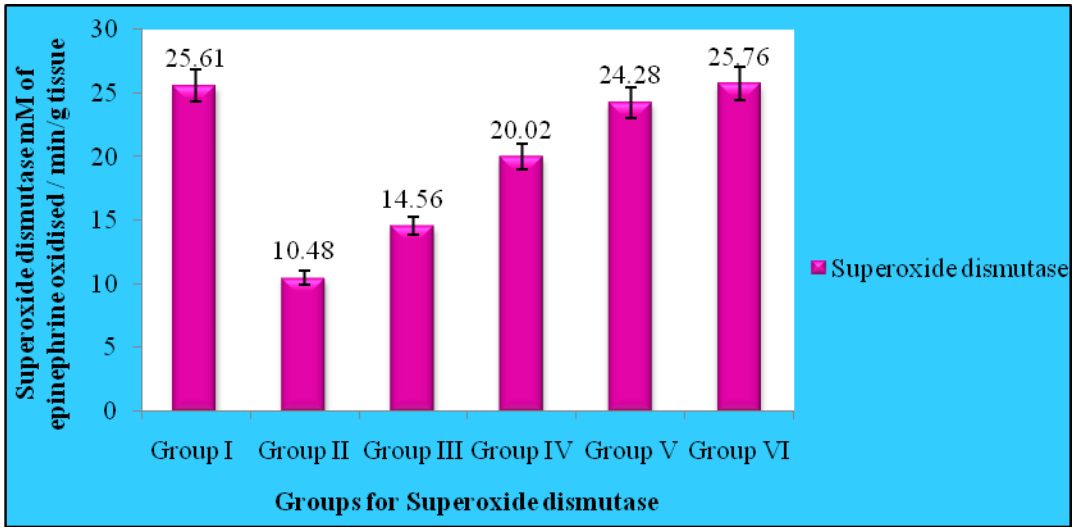
Graph 2: Effect of ELEVA on Total acidity



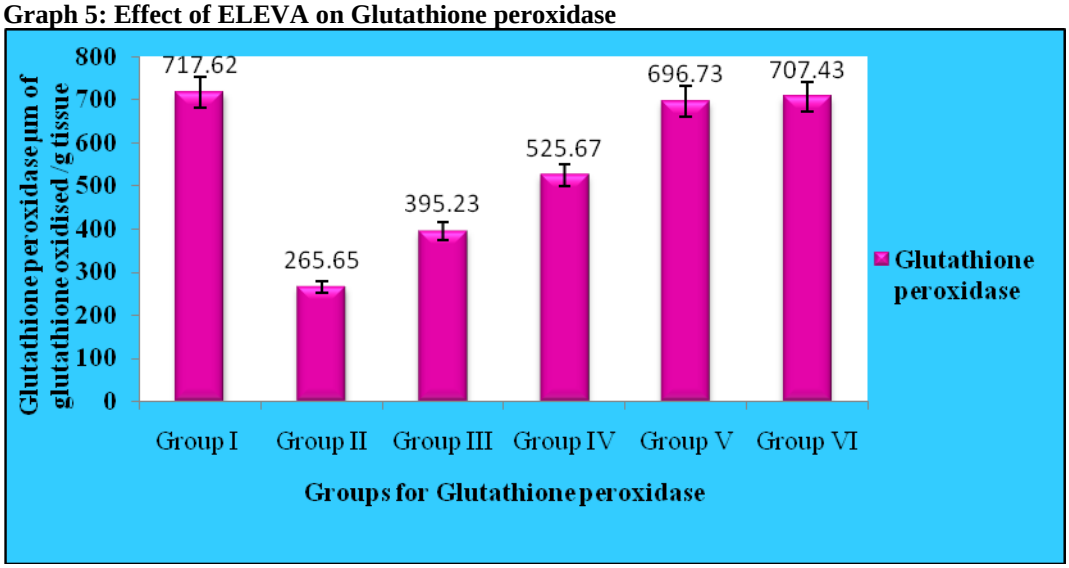
Graph 3: Effect of ELEVA on Reduced glutathione



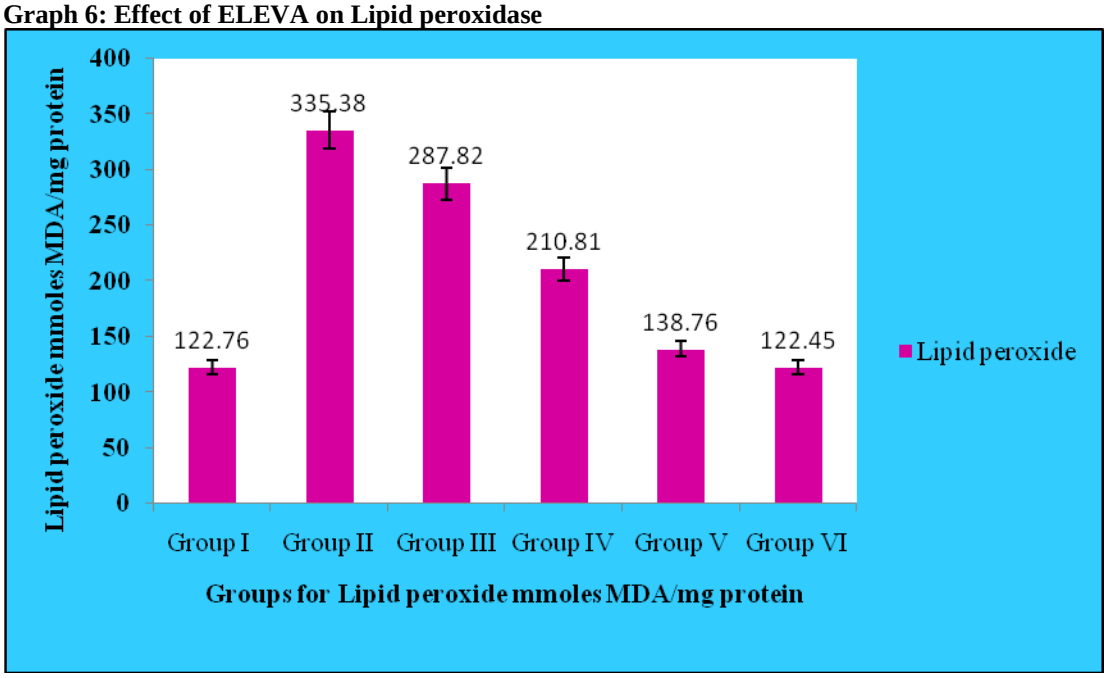
Graph 4: Effect of ELEVA on Superoxide dismutase



Graph 5: Effect of ELEVA on Glutathione peroxidase



Graph 6: Effect of ELEVA on Lipid peroxidase



Discussion

Ethanol-HCl method of ulcer induction is being widely used and is a convenient way of assessing anti-ulcer activity of a drug (Tan et al., 2000). Ethanol-HCl induced ulceration is characterized by microvascular and macroscopic gastric mucosal lesions which may be due to the inhibition of prostaglandin synthesis which results in increased production of leukotrienes and other products of the 5-lipoxygenase pathway (Nasuti et al., 2006). These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H^+ and Na^+ ions reducing the transmucosal potential difference and induce formation of gastric acidity, erosions and ulcers (Ashoka Shenoy et al., 2011). However results of the present study show that there was a notable decrease in gastric acidity and ulcer index in rats pretreated with ELEVA which may due to the positive effect of ELEVA on prostaglandin synthesis.

Anti-oxidant enzymes like SOD, GR and GPx act as a battery of defense mechanisms against oxidative tissue damage. Increased SOD activity can protect cells against threat of reactive free radicals while GR and GPx are regarded as crucial enzymes which catalyze the reduction of hydrogen peroxide. Reactive oxygen species have been

proposed to mediate cell damage via a number of independent mechanisms including the inactivation of antioxidant enzymes. Free radicals could play an important role in the degenerative or pathological processes of various serious diseases (Huang et al., 2006). In the present study experimental animals ulcerated with ethanol-HCl showed depleted levels of SOD, GR and GPx and increased LPO levels indicating lipid peroxidation. Lipid peroxidation not only serves as a marker of cellular damage *in vivo* but also has been recognized to be the inducer of various pathological disorders (Huang et al., 2011a). However pretreatment with ELEVA resulted in significant reduction of tissue lipid peroxidation and a simultaneous increase in intracellular antioxidant enzyme levels indicating that ELEVA may possess prominent free radical scavenging activity.

Conclusion

It is evident from the results of the present study that the ethanolic leaf extract of *Vernonia arborea* possesses prominent anti-ulcer activity against ethanol-HCl induced gastric ulceration. This gastroprotective ability of the plant may be due to its anti-secretory property, positive effect on prostaglandin synthesis and effective free radical scavenging activity.

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