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To Explore the Ulceroprotective and Antioxidant Potential of *Hyssopus officinalis* in Ethanol-Induced Gastric Ulcers in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. AS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. RS managed the analyses of the study and the literature searches. Both the authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: To explore the ulceroprotective and antioxidant potential of *Hyssopus officinalis* in ethanol-induced gastric ulcer in rats.
Study Design: Administration of plant extract and evaluation of antiulcer activity.
Place and Duration of Study: Department of Pharmacology, Rayat Institute of Pharmacy, Railmajra, District SBS Nagar. Performed between August 2011- June 2012.
Methodology: In the present study 1 ml of ethanol was administered to the overnight fasted rats which were sacrificed after 1 hour**.** Ethanolic extract of *Hyssopus officinalis (EEHO)* at the dose of 100 and 125 mg/kg was administered to albino rats 1 hour before the administration of ethanol. Animals were there then sacrificed and tissue homogenate was used for various biochemical parameters in order to explore the ulceroprotective and antioxidant potential of the plant.
Results: Administration of 1 ml of ethanol to overnight fasted rats resulted in increased ulcer index, total acidity and decreased pH. Further, it has been observed that in ethanol administered rats there was increased generation of reactive oxygen species estimated by increased level of TBARS and attenuated levels of glutathione, superoxide dismutase and nitric oxide along with decreased secretion of mucin. Further, ethanol administration too has a detrimental effect on the integrity of stomach. Pre-treatment with EEHO showed a great antiulcer and antioxidant potential depicted by decreased generation of ROS,

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improved the integrity of stomach, and increased the nitric oxide level. Most importantly, EEHO significantly improved the mucus secretion estimated by gastric adhesion mucus content.

Conclusion: The findings of the study indicate that pre-treatment with EEHO has a significant ulceroprotective and antioxidant activity in ethanol-induced ulcers, which supports its traditional use in folk medicine. This may open vista to explore various other therapeutic implications of this plant.

Keywords: Hyssopus officinalis; reactive oxygen species; mucin; TBARS; superoxide dismutase.

1. INTRODUCTION

Gastric and duodenal ulcer is the most prevalent gastrointestinal disorder (Onasanwo et al., 2011) that aggravate due to imbalance between gastric offensive factors (pepsin, lipid Peroxidation, nitric oxide (NO)) and defensive factors (mucin secretion, glycoprotein and glutathione) are responsible for ulceration (Oh et al., 2008). Various factors are implicated in the pathogenesis of ulcerations such as sedentary life style, alcohol intake, spicy food, NSAID and various bacterial infections such as *H. pylori* (Gisbert et al., 2004; Dharmani et al., 2003). Further, various evidences Literature review revealed numerous mediators such as cytokines such as interlukin-1β (IL-1β), Matrix metalloprotinases (MMP) and tumour necrosis factor-α (TNF-α) are too involved in the pathogenesis of gastric ulcer (Tomita et al., 2009; Tulassay and Herszenyi, 2010). Experimental and clinical studies suggested that the reactive oxygen species (ROS) and the reactive nitrogen species (RNS) play a crucial role in the aetio-pathogenesis of the inflammation and ulceration of the digestive tract (Tandon et al., 2004; Adriana et al., 2008). Nitric oxide (NO) generated from the endothelial nitric oxide synthase (e-NOS) plays an important role in ulcer healing by promoting angiogensis regulates gastric mucosal blood flow and stimulates gastric mucus secretion (Nishida et al., 1997; Wallace and Miller, 2002; Pan et al., 2005). Further, Growth factors are too implicated in ulcer healing (Milani and Calabro, 2001). Growth factors such as Expression of growth factors such as EGF (Epidermal Growth factor), transforming growth factor (TGF-α) and hepatocyte growth factor (HGF) are found tobe activated in response to tissue injury (Jones et al., 1999a, b; Milani and Calabro, 2001; Cho et al., 2006; Sharma et al., 2012). Moreover, neuropeptides such as cholecystokinin (CCK), calcitonin gene related peptide (CGRP) (Gray et al., 1994) are too responsible for gastric ulceration. Prostaglandins (PG), another important factor which play role in the ulcer healing (Cryer, 2001) has been found to modulate number of mucosal defences mechanism and downregulated the release of various inflammatory mediators (Takeuchi et al., 2008). Various experimental methods are available for the induction of ulcer which includes ethanol-induced ulcers, pylorus ligation-induced ulcers, stress-induced ulcers, stress- induced, acetic acid-induced ulcers and reserpine-induced gastric ulcers (Jain and Surana, 2009; Srinivas and Baboo, 2011; Khan et al., 2011). Ethanol serves as a most common ulcerogenic agent and has been shown to increase the risk of ulcer in humans and has been evident to produce potent ulceration in rats (Ukwe et al., 2010; Ibrahim et al., 2012; Lin et al., 2012). Clinically, various synthetic drugs are available for the management of ulceration but these drugs possess various adverse drug reactions (ADR) (Kumar et al., 2011). In addition to this various polyherbal formulations are available in the market but standardization of these polyherbal formulation in order to get a standard product repeatedly is not an easy task (Bafna and Balaraman,

2005; Shirwaikar et al., 2006; Darbar et al., 2010). *Hyssopus officinalis (H. officinalis)* plant leaves are traditionally used to treat various pathological ailments such as cough, diabetes, ulcers and bacterial infections (Khazaie et al., 2008). But still no clinical study has been conducted to explore its antiulcer activity of *H. officinalis*. Thus the present study has been intended to explore ulceroprotective and antioxidant activity of *H. officinalis* in ethanol-induced ulcer in rats.

2. MATERIALS AND METHODS

The experimental protocol used in present study was approved by Institutional Animal Ethical Committee (IAEC). Age matched young wistar rats weighing 200-250 g were employed in the study. The animals were fed on standard chow diet and water *ad libitum*. They were acclimatized in the animal house of our institute and exposed to natural light and dark cycle.

2.1 Drugs and Chemicals

Ethanolic extract of H. officinalis (EEHO) was obtained as gift sample from Herbal Bio Solutions. DTNB was purchased from Sanjay biological Amritsar, India. O-dianisidine hydrogen peroxide reagent Hexadecyltrimethyl ammonium bromide was obtained from sigma Aldrich Chemical Co. N-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) and Thiobarbituric acid (TBA) were purchased from (SDFCL) SD Fine-Chem Ltd, Mumbai. 5, 5'-Dithio-bis (2-nitrobenzoic acid (DTNB) and diethylenetriamine pentaacetic acid (DTPA) was obtained from sanjay biological museum, Amritsar. All other reagents used in the present study were of analytical grade.

2.2. Experimental Protocol

The study comprised of 5groups with 6 rats in each group. Group I (Normal Contro*l):* Rats were maintained on standard food and water and no treatment was given. *Group II* (*Ethanol-induced ulcer*): 1 ml of absolute ethanol (99.9 %) was administered to overnight fasted rats. Group III: (Standard drug treated group): Rats were treated with omeprazole (20 mg/kg/p.o) 1 hour before they were subjected to ethanol treatment. Group IV and V (*H. officinalis* 100 and 125 mg/kg, p.o treated rats) are treated with ethanolic extract of *H. officinalis*. Ethanol (1ml) was administered after one hour for inducing ulcers. Animals were sacrificed after 1h following the administration of absolute ethanol.

2.3 Antiulcer Activity

Antiulcer activity was assessed using Ethanol-induced gastric ulcer. Ulcers were induced by administering 1 ml absolute ethanol (99%; p.o) to each rat (Ukwe et al., 2010). After One hour all the rats were sacrificed by cervical dislocation, stomach was cut opened along the greater curvature and gently rinsed under tap water and gastric content was collected. Then stomach was opened along the greater curvature. The stomachs were stretched on a corkboard and the ulcer index was obtained according to scoring method of Suzuki as follows: Score 1: maximal diameter of 1mm; Score 2: maximal diameter of 1-2mm Score 3: maximal diameter of 2-3mm; Score 4: maximal diameter of 3-4mm; Score 5: maximal diameter of 4-5mm;Score 10: an ulcer over 5mm in diameter; Score 25: a perforated ulcer (Suzuki et al. (1976).

Mean score of each animal was expressed as ulcer index and ulcer protection was calculated by the given formula:

$$\% \text{ Protection} = (\text{UI control} - \text{UI treated}) \times 100 / (\text{UI control});$$

Where, UI stands for ulcer index.

2.4 Assessment of Oxidative Stress in Tissue

2.4.1 Preparation of tissue homogenate

The stomach was weighed and homogenized in chilled phosphate buffer (pH 7.4) at a concentration of 10% (w/v). The homogenate was then centrifuged at 10,000 x for 20 min. The clear supernatant was used for the assays of various parameters.

2.4.2 Estimation of reduced glutathione (GSH)

GSH was determined by the method of Jollow et al., 1974. 1ml of homogenate was precipitated with 1ml of 4% sulfosalicilic acid (SSA). The samples were then incubated at 4ºC for one hour followed with centrifugation at 1200 g for 20 min at 4ºC. The assay mixture contained 0.2 ml supernatant, 2.6 ml sodium phosphate buffer (0.1M, pH 7.4) and 0.2 ml DTNB (100 mM) in a total volume of 3.0 ml. Absorbance was studied at 412 nm immediately after the appearance of yellow colour on a spectrophotometer.

2.4.3 Estimation of superoxide dismutase (SOD) activity

SOD was estimated in terms of reduced nitroblue tetrazolium (NBT) using method of Wang et al. (1998). The tissue was minced and homogenized in a mixture of 0.1 N sodium hydroxide (NaOH) and 0.1% sodium dodecyl sulphate (SDS) in water containing 40 mg/L of DTPA. The mixture was centrifuged at 20,000 g for 20 min and the resultant pellets were suspended in 1.5 ml of pyridine and kept at 80ºC for 1.5 hours to extract formazan, an adduct formed after reaction of reduced NBT with superoxide anions. The mixture was again centrifuged at 10,000 g for 10 min and the absorbance of formazan was determined spectrophotometrically at 540 nm. The amount of reduced NBT was calculated using the following formula: Amount of reduced NBT = $A \times V/T \times Wt \times \varepsilon \times L$. Where A is absorbance, V is volume of solution (1.5 ml), T is time for which the tissue was incubated with NBT (90 min), Wt is blotted wet weight of tissue, ε is extinction coefficient (0.72 L/mmol/mm) and L is length of light path (10 mm).

2.4.4 Estimation of lipid peroxidation

Lipid peroxidation was estimated using thiobarbituric acid reactive substances (TBARs) by the method of Ohkawa et al., 1979. The reaction mixture contained 0.1 ml of sample, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution and 1.5 ml of 0.8% aqueous solution of TBA. The pH (above 3) of 20% acetic acid solution was adjusted with Sodium hydroxide (NaOH) and the volume was finally made up to 4.0 ml with distilled water (DW) and heated at 95ºC for 60 min. After cooling 1.0 ml of DW and 5.0 ml of the mixture of n-butanol and pyridine (15: 1, v/v) were added, and the mixture was shaken vigorously. The samples were then centrifuged at 4000 rpm for 10 min and absorbance of the organic layer was measured at 532 nm.

2.4.5 Estimation of nitrite/nitrate

The estimation of nitrite in the supernatant was determined using a colorimetric assay with the Griess reagent as described by Green et al., 1982. Equal volumes of supernatant and the Griess reagent (0.1% NEDA, 1% sulfanilamide and 2.5% phosphoric acid) were mixed. Then, the mixture was incubated for 10 min at room temperature in the dark, and the absorbance was measured at 540 nm (Green et al., 1982; Kumar and Kumar, 2008).

2.4.6 Estimation of gastric adhesion mucus content

Gastric wall mucus was determined according to the procedure of (Corne et al., 1974). The glandular segments from stomachs which had been opened along their greater curvature were removed and weighed. Each segment was transferred immediately to 10 ml of 0.1% w/v Alcian blue solution (in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate pH 5). After immersion for 2 h, excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose, first for 15 and then for 45 min. Dye complexed with the gastric wall mucus was extracted with 10 ml of 0.5 M magnesium chloride ($Mgcl_2$) by shaking intermittently for 1 min at 30 min intervals for 2 h. 4 mL of blue extract were then shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of net glandular tissue was then calculated.

2.4.7 Assay of myeloperoxidase activity (MPO)

MPO activity was measured by the method of (Qiu et al., 1996). The gastric mucosa was scrapped and then the scrapings were homogenized in ice-cold phosphate buffer. Hexadecyltrimethyl ammonium bromide (0.5% HTAB) was added to this phosphate buffer (50 mM, pH 6.0) to release MPO from the primary granules of neutrophils. Homogenates were then centrifuged and the supernatants were aspirated and mixed with o-dianisidine hydrogen peroxide reagent and absorbance at 460 nm was measured with a spectrophotometer. One unit of MPO activity was defined as that degrading 1mmol of peroxide per minute at 25 per g protein of gastric mucosa.

2.5 Histopathological Assessment

The stomach was excised and immediately immersed in 10% buffered formalin. They were then dehydrated in the graded concentrations of ethanol, immersed in xylene, and then embedded in paraffin. From the paraffin blocks, 4-mm thin sections were cut, and staining is done using with haematoxylin (0.6% w/v) for 15 min followed by counterstaining with eosin (1% w/v) for 2 min. They were then examined using light microscopy to analyze integrity of stomach, using an image analysis program (NIH Scion image analyzer).

2.6 Estimation of Protein Content

Protein content was estimated by Biuret method using protein estimation kit.

3. STATISTICAL ANALYSIS

All the results were expressed as Mean ± SEM. Data analysis was performed using Graph Pad Prism Version 5.0 software. Statistical comparisons were made between drug treated groups and disease control rats. Data of biochemical parameters were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple range test. *P value=0.05* was considered to be statistically significant.

4. ACUTE TOXICITY STUDY

The LD_{50} of the *H. officinalis* was reported to be safe till 850-1560 mg/kg/*p.o.* Thus the studies were carried out by using two selected doses of ethanolic extract of *H. officinalis* (EEHO) (100mg/kg and 125mg/kg). No death and side effects were found at both selected doses of plant (OECD, 2002).

5. RESULTS

Pre-treatment with EEHO (100mg/kg and 125mg/kg) did not produce any marked effect on various parameters performed in the present study.

5.1 Effect of Ethanolic Extract of *H. officinalis* (EEHO) on Ethanol-Induced Ulcers

5.1.1 Antiulcer effect of EEHO on ulcer index in ethanol administered rats

Results depicted that pretreatment with EEHO (100mg/kg and 125mg/kg) and Omeprazole produced significant decrease in the ulcer index as compare to the diseased control as shown in Fig. 1. However, marked attenuation in ulcer index was obtained with 100mg/kg of EEHO.

5.1.2 Effect of EEHO on superoxide dismutase (SOD) and glutathione in ethanol-induced ulcer

The mucosal SOD and GSH level were significantly decreased in ethanol administered rats. Treatment with the EEHO (100mg/kg and125mg/kg) and omeprazole significantly increases the SOD and GSH level in comparison with diseases control. Significant effect was obtained with EEHO at a dose of 100mg/kg (Fig. 2 and Fig. 3).

5.1.3 Gastroprotective potential of EEHO on gastric adhesion mucus content in ethanol-induced ulcers

As shown in (Fig. 4) the level of gastric adhesion mucus content was significantly decreased after ethanol administration (*p=0.05*) in comparison to the control group. However, rats treated with EEHO (100mg/kg and 125mg/kg) and omeprazole depicted a marked increase in the mucus content (Fig. 4).

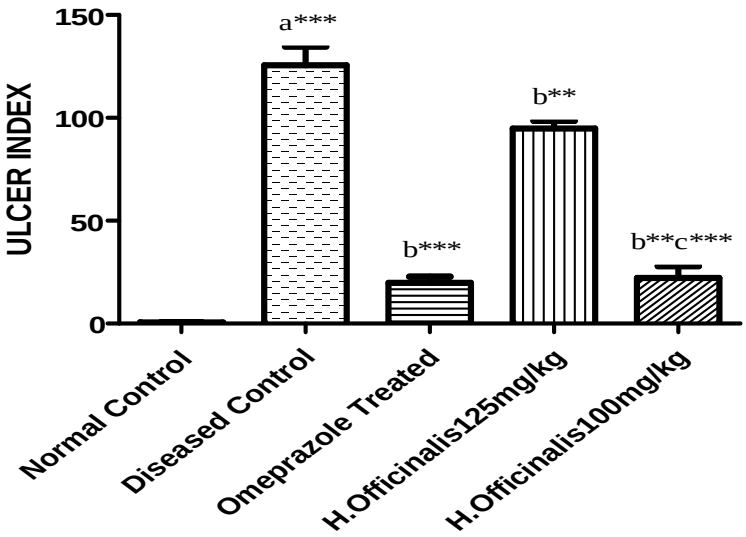

Fig. 1. Effect of EEHO (100mg/kg and 125mg/kg) on ulcer index.

All values are expressed as Mean±SEM and p=0.05 is considered to be significant. Where a=P=0.05 vs normal control; b=P=0.05vs diseases control; c=P=0.05 vs H.officinalis 125mg/kg

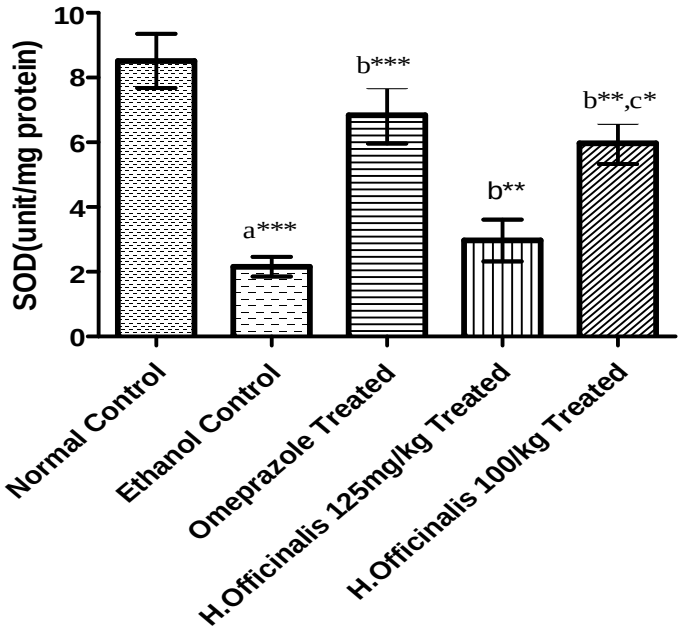

Fig. 2. Effect of EEHO (100mg/kg and 125mg/kg) on SOD.

All values are expressed as Mean ± SEM and P=0.05 is considered to be significant. Where a=P=0.05 vs normal control; b=P=0.05 vs diseases control; c=P=0.05 vs H.officinalis 125mg/kg

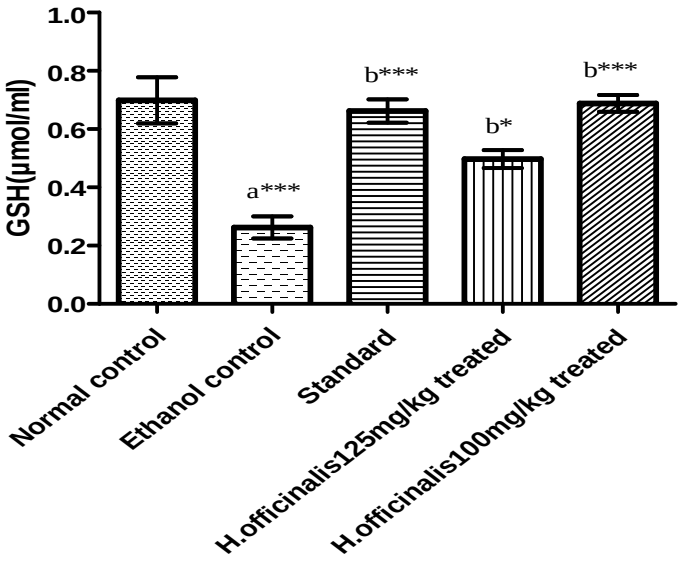

Fig. 3. Effect of EEHO (100mg/kg and 125mg/kg) on glutathione (GSH).
All values are expressed as Mean ± SEM and P=0.05 is considered to be significant. Where a=P=0.05 vs normal control; b=P=0.05 vs diseases control

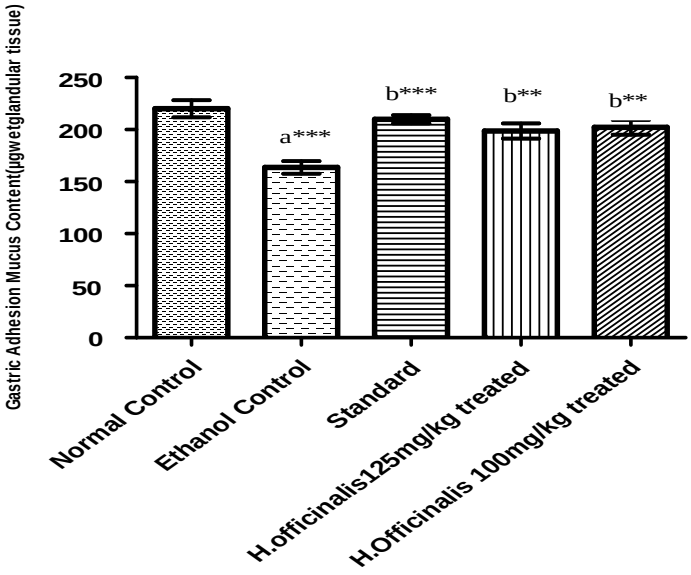

Fig. 4. Effect of *H. officinalis* (100mg/kg and 125mg/kg) on gastric adhesion mucus content.
All values are expressed as Mean ± SEM and P=0.05 is considered to be significant. Where a=P=0.05 vs normal control; b=P=0.05 vs diseases control

5.1.4 Antiinflammatory effect of EEHO in ethanol-induced ulcers estimated by myeloperoxidase (MPO) and nitric oxide

Administration of ethanol significantly elevated the MPO level in disease control in comparison to the normal control. EEHO (100mg/kg and 125mg/kg) and omerpazole markedly alleviated the gastric MPO level in comparison to rats administered ethanol (Fig. 5). Moreover, rats administered ethanol produced marked decreased in the level of NO in comparison to normal control. Pre-treatment with EEHO (100mg/kg) and omeperazole significantly increased the level of NO in ethanol administered rats. However, significant increase was produced with EEHO (100mg/kg) (Fig. 6).

5.1.5 Effect of *H. officinalis* on lipid peroxidation in ethanol-adminstered rats

Ethanol administration resulted in marked lipid peroxidation estimated by increased level of TBARs. However, treatment with EEHO (100mg/kg) and omeprazole markedly attenuated the TBARS level in comparison to 125mg/kg EEHO (Fig. 7).

5.1.6 Histopathological evaluation of gastric lesion

Microscopic observations illustrated that severe damage in the gastric mucosa characterized by edema and leukocyte infiltration in mucosal layer of ethanol administered rats whereas Rats treated with 100mg/kg of EEHO produced mild mucosal damage and mild leukocyte infiltration whereas EEHO (125mg/kg) resulted in moderate edema, leukocyte infilteration and mucosal injury (Fig. 8).

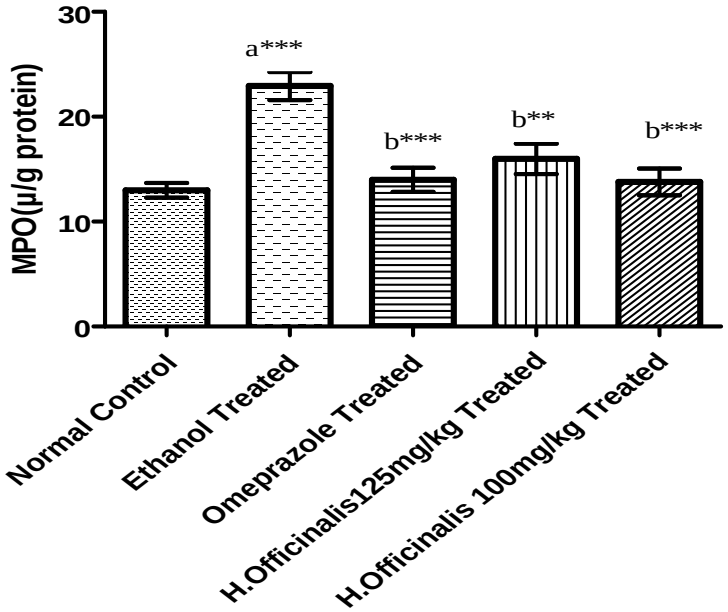

Fig. 5. Effect of EEHO (100mg/kg and 125mg/kg) on MPO.

All values are expressed as Mean ± SEM and P=0.05 is considered to be significant. Where a=P=0.05 vs normal control; b=P=0.05vs disease control.

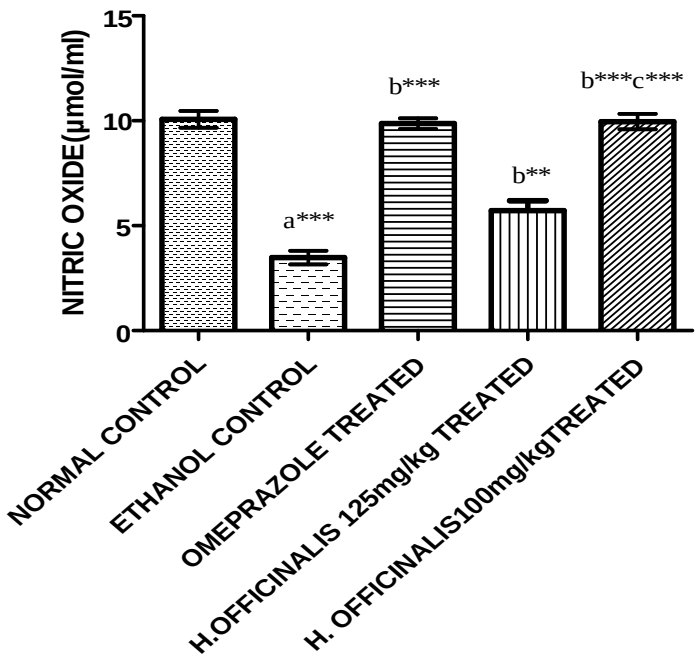

Fig. 6. Effect *of H. officinalis* (100mg/kg and 125mg/kg) on NO.

All values are expressed as Mean ± SEM and P=0.05 is considered to be significant. Where a=P=0.05 vs normal control; b=P=0.05 vs diseases control; c=P=0.05 vs H. officinalis 125mg/kg.

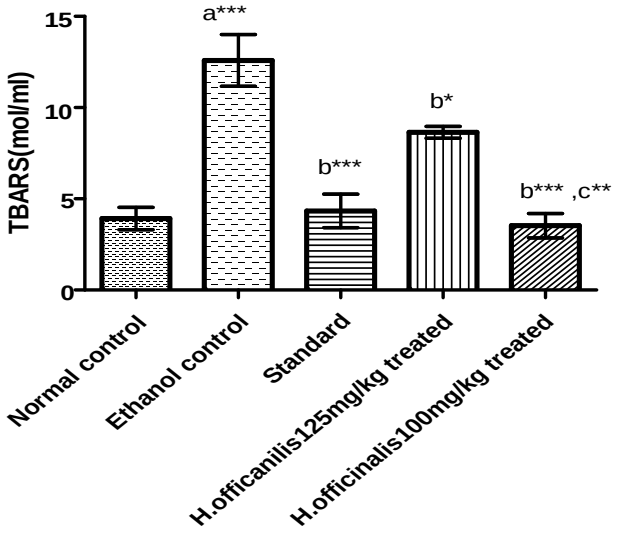

Fig. 7. Effect of EEHO (100mg/kg and 125mg/kg) on lipid peroxidation estimated by TBARs.

All values are expressed as Mean ± SEM and P=0.05 is considered to be significant. Where a=P=0.05 vs normal control; b=P=0.05vs diseases control; c=P=0.05 vs H. officinalis 125mg/kg.

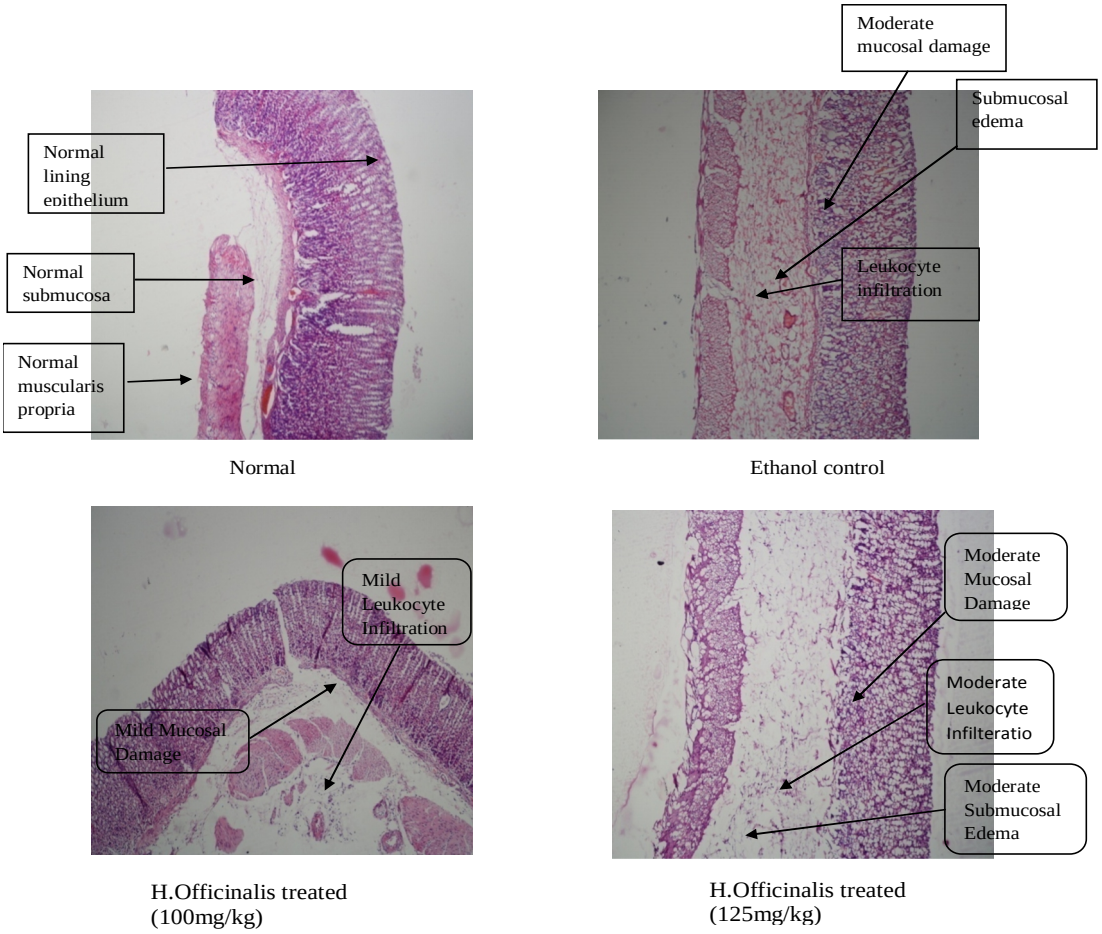

Fig. 8. Effect *of H. officinalis* on gastric mucosal injury integrity in Ethanol-induced ulcers

6. DISCUSSION

Gastric mucosa can withstand exposure to highly concentrated HCL (Wallace and Miller, 2001). Disruption of the balance between the local release of vasodilator and vasoconstrictor mediators could therefore be involved in the pathogenesis of mucosal injury (Demir et al., 2003). Various synthetic drugs such as H_2 blockers and Proton pump inhibitors are employed in the management of gastric ulcer but they possess serious adverse effects (Muchandi and Chandrashekhar, 2011). This has been the major stimulus for the development of new antiulcer drugs and anti-inflammatory for novel molecules has been extended to herbal drugs that offer better protection and decreased relapse (Wahida et al., 2007). Herbal medicines are triumph of popular therapeutic diversity and are now emerging as an alternative treatment to available synthetic drugs (Goel and Sai, 2002; Ubaka et al., 2010). Herbal plants play a vital role in the management of various diseases such as Diabetes (Modak et al., 2007), Neurodegenerative disorders (Chen et al., 2007; Kim and Oh, 2012), Various herbal plants such as *Aspilia africana* (Ubaka et al., 2010), *Ficus arnottiana* (Khan et al., 2011) and *Morinda citrifolia* (Srinkanth and Murlidharan, 2009) have found to possess antiulcer activity with high efficacy and no side effects.

H. officinalis has been used traditionally in folk medicine but still no clinical research and data is available for its antiulcer potential. *Hyssopus officinalis* Linn (Hyssop, Family: Lamiaceae), a perennial herb with a long history of medicinal use, is one of the endemic Iranian species of the genus Hyssopus (Fathiazad and Hamedeyazdan, 2011). Traditionally, *H. officinalis* named Zufa in Iran, have been used as a carminative, antispasmodic stomachic, antiseptic, expectorant and cough reliever (Khazaie et al., 2008). The essential oils isolated from hyssop are popularly used as food and drink additives as well as cosmetic materials (Murakami et al., 1998; Kizil et al., 2010). *H. officinalis* has been found to contain several polyphenolic compounds, primarily the flavonoids such as apigenin, quercetin, diosmin, luteolin and their glycosides followed by other phenolic compounds chlorogenic, protocatechuic, ferulic, syringic, p-hydroxybenzoic and caffeic acids (Fathiazad and Hamedeyazdan, 2011) which have been found to be free radical scavenger and exhibits their antioxidant property (Miyazaki et al., 2003). Research has reported that binge drinking or long-term drinking can cause acute or chronic gastric mucosal injury (Ning et al., 2012). Alcohol has been found to rapidly penetrate the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intracellular membrane permeability to sodium and water (Gupta et al., 2012). Ethanol, an ulcerogenic agent has been found to produce intense damage (Ukwe et al., 2010; Ibrahim et al., 2012; Lin et al., 2012). Our results too revealed that ethanol in the present study caused a marked increase in the acid secretion which is estimated by increase in the ulcer index.

Oxidative stress has been reported to play an important role in the progression of ulcer (Tandon et al., 2004; Srivastava et al., 2011). Generation of these ROS plays a major role in the development of multiple pathologies, such as gastritis, peptic ulcerations or gastric adenocarcinoma (Chakraborty et al., 2012; Uduak et al., 2012). Increased oxidative stress results in the generation of ROS that resulted in imbalance in the endogenous antioxidants and cellular damage in ethanol and -induced ulceration (Qader et al., 2012; Suleyman et al., 2001; Alrashdi et al., 2012; Kumar et al., 2011). This contension is supported in our results with increased level of TBARS along with marked attenuation in the level of endogenous antioxidants (GSH and SOD). Moreover, various evidences indicated that activated neutrophils are the major source of ROS (Pan et al., 2008) that play a vital role in the development of gastric damage by their aggregation and release of tissue-disrupting substance, such as oxygen free radicals and proteases (Kobayashi et al., 2001).The neutrophil infiltration into the gastric mucosal tissues is assessed by MPO as well as NOS (Coskun et al., 1996; Takeuchi et al., 1998). In the present study, significant increase in MPO activity was obtained following ethanol administration which confirmed that role of neutrophils in the gastric mucosa. Literature review indicated that Ethanol too cause severe microvascular injury resulting in increased vascular permeabiltity, edema formation and epithelium lifting (Vidya et al., 2012; Alrashdi et al., 2012). Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and narcotic aspects of tissue injury (Swapna et al., 2011). This contention is supported by results obtained from histopathology of rat administered 1ml of ethanol. NO has been recognized as one of the important mediators for the regulation of gastric mucosal microcirculation, repair and integrity (Tepperman and Soper, 1994; Qiu et al., 1996; Nishida et al., 1997; Oda et al., 1998; Catalayud et al., 2001; Ma et al., 2001; Pan et al., 2005). Inhibition of NO synthesis has been shown to produce acute gastric mucosal damage (Martin et al., 2001; Tariq et al., 2007). NO expressed in gastric mucosa and showed to increase gastric blood flow, mucus secretion and reduce neutrophil adhesion (Nabavizadeh et al., 2011; Alrashdi et al., 2012; Lin et al., 2012) which is too supported in our study. Gastric adhesion mucus content acts as a barrier against various offensive factors (Kalra et al., 2011; Geetha and Sarnaya, 2012). According to Hiruma-Lima et al. 2006 gastric mucus

is a viscous, elastic, adherent and transparent gel formed by water and glycoproteins covering the entire gastrointestinal mucosa. Moreover, mucus is capable of acting as antioxidant and thus can reduce mucosal damage mediated by oxygen free radicals (Borra et al., 2011). Our study too revealed that in ethanol administered rats there is marked decrease in the level of gastric mucus content.

In the present study we have investigated the role of *H. officinalis* in the management of ulcers. Treatment with *H. officinlais* significantly attenuated the level of ulcer index which indicated antiulcer potential of the plant. Further, it has been established that treatment with *H. officinalis* (100mg/kg and 125mg/kg) produced significant decrease in TBARs level and marked attenuation in the level of natural endogeneous antioxidants GSH and SOD. Moreover, *H. officinalis* (100mg/kg and 125mg/kg) significantly attenuated the level of MPO, marker of lipid peroxidation and significantly increased the level of NO that plays an important role in ulcer healing. In addition, a significant increase was found in the mucus content when the rats subjected to ethanol were treated with *H. officinalis* extract which showed the gastro protective property of the plant. In addition, Histopathological results of our studies revealed that treatment with *H. officinalis* (100mg/kg) resulted in the maintenance of mucosal integrity and mild mucosal ulceration.

7. CONCLUSION

Thus, it may be concluded that *H. officinalis* may possess gastro protective property assessed by significant increase in the level of mucus with marked attenuation in the level of MPO and ulcer index. Further, it may possess antioxidant activity as it significantly attenuated the level of TBARS and elevated levels of GSH and SOD. Further, the extract of the plant was also found to be beneficial in maintaining the level of NO.

ETHICAL APPROVAL

Experiments have been examined and approved by the appropriate by Institutional Animal Ethical Committee (IAEC) and have therefore been performed in accordance with the ethical standards.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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