Ulcer Protective Activity of *Jatropha gossypiifolia* Linn. in Wistar Rats

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ABSTRACT

Background: Several synthetic drugs are useful in the treatment of peptic ulcer, but almost of these drugs are used in prolonging time, it may cause several adverse reactions. However, the herbal medicines are more potent to the treatment and minimize the side effects. **Objective:** To evaluate the methanol extract of *Jatropha gossypiifolia* Linn. (MEJG) for gastro protective activity against Wistar rats. **Materials and Methods:** Anti-ulcer potency of MEJG (100 and 200 mg/kg, b.w.) was assessed using aspirin (200 mg/kg, p.o.) plus pylorus ligation ulcer model and the parameters studied were ulcer index (UI), gastric juice volume, pH, total acidity, and total acid output. Same extract was studied by ethanol-induced (80%, 5 mL/kg, intragastrically) ulcer model, and the UI and biochemical parameters were studied. **Results:** The oral administration of MEJG (100 and 200 mg/kg) significantly ($P < 0.001$) attenuated the ulcer score and anti-secretary parameters (such as the volume of gastric content, free acidity, total acidity, and total acid output) in the aspirin plus pylorus ligation rats. The extract also significantly attenuated ($P < 0.001$) ulcer score in ethanol-induced ulcer model and lipid peroxidation level and significantly increased the level of glutathione peroxides, catalase, and superoxide dismutase activity. The MEJG may possess active constituents such as alkaloids, glycosides, flavonoids, and terpenes, which may play a major role in gastroprotective effect in Wistar rats. **Conclusion:** The present study provides scientific support for the anti-ulcer activities of extracts of JG and also claimed that antioxidant potential of the extracts. However, substantiates the traditional claims for the usage of this drug in the treatment of gastric ulcer.

Key words: Antioxidant enzymes, anti-ulcer, aspirin, ethanol, *jatropha gossypiifolia,* ranitidine, wistar rats

SUMMARY

- The methanolic extract of *jatropha gossypiifolia* Linn. for gastro protective activity against aspirin plus pyloric ligation and ethanol induced ulcer models was studied in Wistar rats. JG shows significantly attenuated the ulcer score in both models. And also attenuated in anti-secretory parameters in aspirin induced ulcer model. MEJG may possess active constituents such as alkaloids, glycosides, flavonoids and terpenes, which may play a major role in gastroprotective effect in Wistar rats.

Abbreviation Used: MEJG: Methanolic extract of jatropha gossypiifolia, mg: Milli gram, kg: Kilogram, b.w.: Body weight, p.o.: Per oral, UI: Ulcer index, pH: Concentration of H+ ion, mL: Milli litre, JG: Jatropha gossypiifolia,
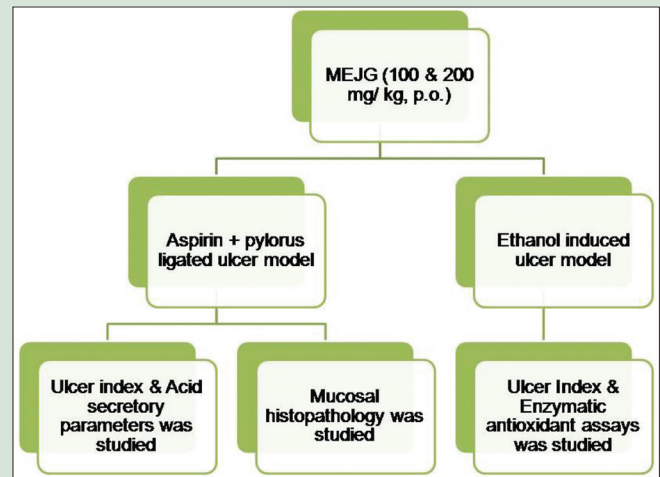
USD: United States Dollar, NSAIDs: Non steroidal anti-inflammatory drugs, v/v: Volume by volume, w/v: Weight by volume, SCMC: Sodium carboxy methyl cellulose, g: Gram, h: Hour, °C: Degree centigrade, n: Number, Rpm: Rotation per minute, Min: Minute, N: Normality, NaoH: Sodium hydroxide, mM - Millimole, TBA: Thiobarbituric acid, nmol: Nanomole, nm: Nanometer, GPx: Glutathione peroxidase, GSH: Reduced glutathione, H_2O_2: Hydrogen peroxide, SOD: Superoxide dismutase, ANOVA: Analysis of Variance, μmol: Micromole

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INTRODUCTION

Jatropha gossypiifolia (JG) (Family: Euphorbiaceae) is a native plant of Brazil, it has also been cultivated in many parts of India, Burma, and Singapore, and used in many countries as a folk medicine for various ailments.[1] The leaves of the plant are used as a drug. It has various medicinal and pesticidal properties with several constituents including jatrophenone, jatrophine, jatropholones A and B (diterpenes), jatrophatrione, cyclogessine A, a minor coumarino-lignoid, cyanidin (pentose glycoside), trihydroxy ketone, and diosphenol were reported.[2] JG is traditionally used in an emmenagog, belly aches, venereal diseases, piles, cancer, leprosy, cuts, wounds, eczema, carbuncles, itches, and as an emetic, purgative, hypotensive, vasorelaxant, and antimicrobial treatment.[3] Peptic ulcer is an almost common disease that affects the daily life of humans. Every year, approximately, 4 million people are affected by peptic ulcer disease around the world.[4] The total costs of this

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disease in the USA, both direct and indirect due to loss of work output has been estimated to reach approximately 6.46 billion USD per year.[5,6]

Peptic ulcer mainly occurs attributed to the imbalance between aggressive (acid and pepsin) and defensive (mucus and gastric mucosal barrier) factors of gastric mucosa. Diet, stress, age, genetics, individual behavior, and external stressors such as chemicals, spicy, deep-fried food, environmental pollutants, high fat diets, contaminated water, and medications cause disruption of this balance. These factors can play a limited role; it is now known that the leading cause of peptic ulcers is a kind of bacteria called *Helicobacter pylori*. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen are the second almost common causes of peptic ulcers in patients. These drugs inhibit prostaglandin biosynthesis; it inhibits the release of mucus. Mucus, mucosal blood flow, cell renewal, and bicarbonate are helpful to maintain the mucus layer integrity. Ethanol is known to promote oxygen free radicals and reduces the gastric mucosal nonprotein sulfhydryl levels, and it causes necrosis of superficial epithelial cells on gastric mucosa and erosion. Antioxidants are compounds that act as inhibitors of the oxidative process. They are quite large in number and diverse in nature that opposes the process of oxidation largely by neutralizing free radicals. Enzymatic antioxidants play a major role in the protection of mucosal damage in the rat stomach.[7]

Several synthetic drugs are useful in the treatment of peptic ulcer, but almost of these drugs are used in prolonging time, it may cause several adverse reactions such as gynecomastia, loss of libido, bradycardia, dizziness, bowel upset, dry mouth, nausea, and may alter the biochemical mechanism of the human body. Hence, herbal medicines are generally used in such cases, it produces low side effects.[8] Flavonoids are phenolic compound, present in several medicinal plants, which inhibits lipid peroxidation and in the presence of free metal ion which indicate the protection of cell from free radicals. Natural flavonoids are known for their significant free radical scavenging properties in *in vivo* and *in vitro*, affecting various steps in the arachidonic acid cascade via cyclooxygenase or lipoxygenase enzyme pathway.[9] Euphorbiaceous family has been shown to be a rich source of gastroprotective diterpenes. Pentacyclic triterpenes such as anti-ulcer, anti-tumor, anti-inflammatory, and anti-nociceptive activities have been already well documented.[10]

Therefore, we make an attempt to evaluate the anti-ulcer efficacy of methanol extract of JG (MEJG) in aspirin and ethanol-induced (80%) ulcer in experimental animals and also antioxidant enzymes study in ethanol-induced ulcer model.

MATERIALS AND METHODS

Plant collection and authentication

The fresh plant parts of JG were collected in the month of May from around Chennai, Tamil Nadu, India. The plant was identified and authenticated by Dr. Sasikala Ethirajulu, Research Officer (Pharmacognosy), Captain Srinivasa Murti Drug Research Institute for Ayurveda and Siddha, Ministry of Health and Family Welfare, Government of India, Chennai-106. The voucher specimen (CLBMCP/PCOG/0085) of the plant was dispatched with the Department of Pharmacognosy, CL Baid Metha College of Pharmacy, Chennai, India.

Preparation of methanol extract

The authenticated plant parts were dried in shade and coarsely powdered. The powder was subjected to continuous hot percolation in a Soxhlet apparatus with petroleum ether (60% v/v). The methanol (90% v/v) extract was prepared using the same marc by the continuous hot percolation. The extracts were evaporated under reduced pressure using a rota flask evaporator until all the solvents have been removed to give an extract sample with the yield of 5.7% w/v and 8.2% w/v for petroleum

ether and methanol extract, respectively. The methanol extract was used preliminary to phytochemical test and pharmacological screening. The extract/drug was administered to the animals by suspending each time in freshly prepared 1% sodium carboxymethyl cellulose (SCMC).

Phytochemical screening of *Jatropha gossypiifolia*

MEJG was subjected by qualitative chemical analysis for phytoconstituents using standard methods.[11,12]

Chemicals

Aspirin obtained from Arora Pharmaceutical Pvt. Ltd., Delhi, ethanol obtained from E. Merck (India) Ltd., and all other analytical grade chemicals were obtained from S. D. Fine Chemical Ltd., Mumbai, India.

Experimental animals

Adult Wistar rats of either sex weighing 180–250 g were used in the pharmacological study and kept in the animal house of CL Baid Metha College of Pharmacy, Thuraipakkam, Chennai, India. The animals were maintained in well-ventilated room, the temperature was maintained at 23°C ± 2°C with humidity at 55% ± 5% and 12 h light and 12 h dark cycles. Animals were fed balanced rodent pellet (Poultry Research Station, Nandanam, Chennai, India) with water *ad libitum* throughout the experimental period. The animals were housed for 1 week, before the experiments to acclimatize to laboratory conditions. All the experimental protocols were followed according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India. All the experimental research procedures were approved by the standing Institutional Animal Ethics Committee of the CL Baid Metha College of Pharmacy, Chennai, India.

Acute toxicity study

Acute oral toxicity study of methanolic extract of *Jatropha gossypiifolia* (Linn.) was carried out according to the Organization for Economic Co-operation and Development guidelines 423.[13]

Experimental gastric ulcer[14]

Animals were divided into five groups of six animals each ($n = 6$). The food was withdrawn 24 h before the experiment, but the animals were allowed for water *ad libitum*. Coprophagy (wire-based cages) was used to prevent the animals eating any other materials during the fasting.

- Group I received 1% SCMC (10 mL/kg, p.o.) and served as sham control
- Group II received aspirin (200 mg/kg, p.o.) and served as negative control
- Group III treated with MEJG 100 mg/kg (p.o.)
- Group IV treated with MEJG 200 mg/kg (p.o.) and
- Group V received standard drug ranitidine (50 mg/kg, p.o.) as positive control.

All the drugs/extracts were suspended in freshly prepared 1% SCMC. Aspirin was administered in nonfasted rats once daily for 5 consecutive days. Ranitidine (50 mg/kg) and MEJG (100 and 200 mg/kg, p.o.) were administered orally to the respective treatment groups 30 min before each aspirin treatment, whereas the sham group received only 1% SCMC and animals were kept fasting for 36 h. On day 6, immediately after aspirin treatment, pylorus ligation was done under the anesthetized condition. After 4 h pylorus ligation, the animals were sacrificed by giving overdoses of sodium pentobarbitone. The stomach was dissected out after tying the esophageal end, cut open along the greater curvature, and the content was drained into small

beaker. The content was centrifuged and the supernatant was subjected to analysis for acid secretary and biochemical parameter. The mucosa was flushed with normal saline and the stomach was examined for the gastric ulcer index (UI) by microscopically (10/x, where 'x' is a total mucosal area/total ulcerated area) under a dissecting microscope. Many mucosal lesions and their severity per stomach were determined. For histopathological evaluation, a portion of stomach was fixed in 10% formalin from each experimental group, dehydrated, and embedded in paraffin. The specimens were stained with hematoxylin and eosin and were examined under light microscope.

Gastric volume

After sacrificing the rats, the stomach portion was removed, the gastric contents were transferred into the centrifuge tube, and centrifuged at 1000 rpm for 10 min, the supernatant liquid was transferred into a measuring cylinder and the gastric volume was measured in the described method by Goswami *et al.*[15]

Free acidity and total acidity

Free and total acidity was measured by the method of Hawk *et al.*[16] One milliliter of gastric juice was pipetted into a 100 mL conical flask, 2–3 drops of Topfer's reagent was added and titrated with 0.01 N sodium hydroxide (which was previously standardized with 0.01 N of oxalic acid) until all traces of the red color disappeared and the color of the solution was yellowish orange. The volume of alkali added was noted and this volume corresponds to free acid. Again 2 or 3 drops of phenolphthalein solution were added and titration was continued until a definite red tinge reappears. Again, the total volume of alkali added was noted and this volume corresponds to the total acidity. Acidity was calculated using the following formula:

Acidity = (Volume of NaOH × actual normality of NaOH × 100) /0.1 mEq/L.

Ethanol-induced ulcer

Animals were divided into five groups, each group consist of six Wistar rats ($n = 6$).

- Group I received 1% SCMC (10 mL/kg, p.o.) and served as sham control,
- Group II received 80% ethanol (5mL/kg, p.o.) and served as negative control,
- Group III treated with MEJG 100mg/kg (p.o.),
- Group IV treated with MEJG 200 mg/kg (p.o.), and
- Group V received sucralfate (100 mg/kg, p.o.) and served as positive control.

Acute gastric ulceration was induced by 80% ethanol (5 mL/kg) administered intragastrically (i.g.) after 1 h administration of sucralfate and MEJG (100 and 200 mg/kg). Two hours after the administration of the ethanol, stomach was cut open along the greater curvature and the UI was measured as described by Robert.[17] The percentage inhibition of UI was calculated by the following formula:

% inhibition = [($UI_{control}$ − $UI_{treated}$)/$UI_{control}$] ×100

After ulcer scoring, the stomach was weighed and homogenized in cold tris buffer (10 mM, pH-7.4) at a concentration of 10% w/v. The clear supernatant liquid was used in the following enzymatic antioxidant assay.

Biochemical effects on ethanol induced ulcer
Lipid peroxidation

The lipid peroxidation assay was done according to the method of Ohkawa *et al.*[18] The reaction mixture containing 0.2 mL of stomach homogenate, 1.5 mL of thiobarbituric acid (TBA), 0.2 mL of sodium dodecyl sulfate, 1.5 mL of acetic acid, and 0.8 mL of distilled water. The above reaction mixture is kept in boiling water bath at 90°C for 1 h and cooled with tap water. After cooling, 1 mL of distilled water and 5 mL of a mixture of n-butanol and pyridine (15:1 v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance was read at 532 nm. The lipid peroxide concentration was expressed as nmol of malondialdehyde liberated/min/mg protein in stomach homogenate.

Glutathione peroxidase

Glutathione peroxidase (GPx) of stomach homogenate was assayed by method reported by Ncheles *et al.*[19] The final incubation mixture consisted of 0.2 mL stomach homogenates, 1 mL of phosphate buffer, 0.5 mL of reduced glutathione (GSH), 0.5 mL sodium azide, 0.5 mL ethylenediaminetetraacetic acid, and 2 mL of distilled water. The solution was incubated at 37°C for 5 min and the reaction was started by the addition of 1 mL of H_2O_2, 1 mL of samples were taken exactly at 0 min and 1 min after the addition of H_2O_2 and to arrest the reaction, 2 mL of 10% trichloroacetic acid was added. Nonenzymatic oxidation of GSH was measured in a blank containing the above reagents with buffer substituted for the enzyme source. Under these conditions, the nonenzymatic oxidation was minimal. The residual GSH was then measured by the reaction with 1 mL of 5,5'-dithiobis (2-nitrobenzoic acid) at 412 nm in a ultraviolet-visible spectrophotometer. The activity of GPx was expressed as nm of GSH oxidized/min/mg protein in stomach homogenate.

Superoxide dismutase

Superoxide dismutase (SOD) was assayed by Misra and Fridovich.[20] Stomach homogenate (0.05 mL) was added to 1.5 mL of buffer. The reaction was initiated by the addition of 0.4 mL of epinephrine and change in the optical density per min was measured at 480 nm. One unit of SOD activity is the amount of enzyme required to give 50% inhibition of epinephrine auto-oxidation.

Catalase

Catalase reaction was assayed by Colowick *et al.*[21] Stomach homogenate (0.1 mL) was added to 1.2 mL of phosphate buffer. The enzyme reaction was started by the addition of 1 mL of H_2O_2 solution. The change in the optical density was measured at 240 nm for 3 min at 30 s interval. Catalase activity is expressed as µmol of H_2O_2 utilized/min/mg protein in stomach homogenate.

Statistical analysis

The data were expressed as mean ± standard deviation. Results were analyzed statistically using one-way ANOVA followed by Dunnett's test. $P < 0.05$ was consider to be statistically significant.

RESULTS
Phytochemical study

The preliminary phytochemical test with the MEJG showed the presence of alkaloids, glycosides, flavonoids, terpenes, carbohydrates, proteins, phenol, and saponin.

Acute toxicity study

There was no mortality sign for up to 24 h and animal behavioral changes and any sign of toxicity was observed. MEJG extract was safe up to a 2000 mg/kg bodyweight of animals. Therefore, the biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.

Effect on aspirin plus pylorus ligated ulcer model

Oral pretreatment of MEJG to the animals at the doses of 100 and 200 mg/kg significantly (*P* < 0.001) attenuated the ulcer scores of 8.25 ± 1.33 and 6.50 ± 0.71, respectively, when compared, animals received only aspirin (27.25 ± 1.94). MEJG high dose also significantly attenuated the antisecretary parameters such as gastric volume (2.60 ± 0.62), total acidity (61.78 ± 6.11), and total acid output (202.42 ± 15.68) as compared to control animals (*P* < 0.001) [Table 1]. In normal rats, pylorus ligation caused a marked attenuation in the ulcer score (3.17 ± 0.41) and pH (4.13 ± 0.97) as compared to aspirin-treated animals. The reference compound included in this study also caused significant (*P* < 0.001) attenuation of gastric mucosal damage (4.17 ± 0.98) in a pylorus ligated model of gastric ulcer, which indicates that it possesses antisecretory mechanism.

Histopathological study

Aspirin plus pylorus ligated animal shows an ulcerated mucosa with hemorrhage and the discontinuity of the lining of epithelium. The pretreatment with MEJG animals protected the mucosal epithelium with mild hyperplasia and normal mucosa was seen standard treated animals [Figure 1].

Effect on ethanol induced ulcer

Intragastric administration of 80% ethanol has produced multiple, elongated, reddish bands of lesions in the corpus mucosa along the long axis of the stomach. The extract at the dose of 100 and 200 mg/kg significantly (*P* < 0.001) attenuated the damage of mucosal epithelium with 74.26% and 85.26% inhibition, respectively, against ethanol-treated rats [Table 2].

Biochemical effects of ethanol-induced ulcer
Effect of lipid peroxide

Figure 2 shows TBA reactive substance in the gastric mucosa, used as an index of lipid peroxidation, increased gradually after administration of ethanol from the basal concentration of 3.42 ± 1.36 to 6.50 ± 2.09 nmol/mg protein (*P* < 0.001). This increase in TBA reactants was significantly (*P* < 0.001) got attenuated when the extract was administered to animals and the base values were approached at the dose of 200 mg/kg (3.48 ± 1.19 nmol/mg protein).

Effect of glutathione peroxide

GSH peroxide levels attenuated in the gastric mucosa after 80% ethanol treatment from 5.48 ± 1.35 to 2.85 ± 1.13 nmol/min/mg protein and treatment with the extract (100 and 200 mg/kg) resulted in a significant (*P* < 0.001) dose-depended increase in levels from 3.87 ± 1.06 to 4.20 ± 1.23 [Figure 3].

Effect of superoxide dismutase

The inhibition of epinephrine auto-oxidation for stomach homogenate showed a marked increase in treatments with extract at the level of 4.15 ± 0.90 and 4.60 ± 1.05 units/mL protein and significantly (*P* < 0.001) attenuated after the administration of ethanol-treated animals (3.17 ± 0.70 units/mL protein) [Figure 4].

Effect of catalase

Hydrogen peroxide levels consumed by the stomach homogenate after treatment to extract (100 and 200 mg/kg) resulted in a significant dose-depended increase in enzymatic activity at the level of 5.90 ± 1.02 and 6.32 ± 1.24 µmol/mg protein per min [Figure 5].

DISCUSSION

The present study shows the protective effect of MEJG against aspirin plus pylorus ligated and ethanol-induced (80%) gastric lesions in the rats. Gastric-ulcer induced by NSAIDs such as aspirin, is known to be correlated with the inhibition of cyclooxygenase pathway, which prevents prostaglandin biosynthesis, and it inhibits the release of mucus, a defensive factor of gastric mucosa is a well-known phenomenon.[22] The present results showed that JG at a dose of 200 mg/kg has a protective effect in significantly (*P* < 0.001) reduced gastric lesions produced by NSAIDs. It reveals that JG possesses the healing of gastric lesions against the aspirin-induced ulcer in rats. Mucin is a viscous glycoprotein,
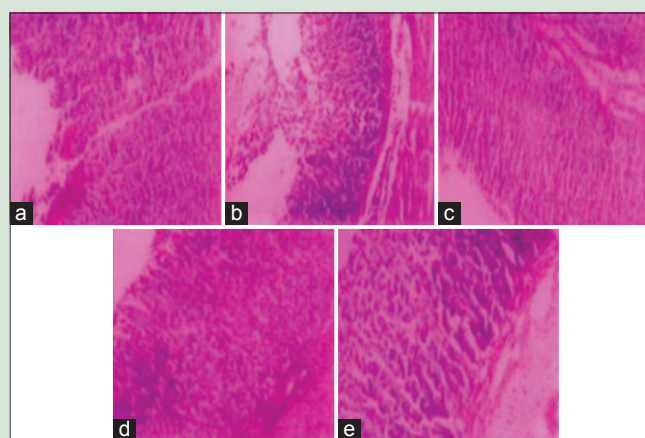

Figure 1: *Jatropha gossypiifolia* ameliorate aspirin plus pylorus ligated induced ulcer model: Photomicrograph of rat stomach mucosa section was showing (a) normal mucosal epithelium in control group; (b) ulcerated mucosa with discontinue of the lining of mucosal epithelium in aspirin treated animal; (c) normal mucosal epithelium with hyperplasia in methanol extract of *Jatropha gossypiifolia* 100 mg/kg treated animal; (d) normal mucosal epithelium with mild hyperplasia in methanol extract of *Jatropha gossypiifolia* 200 mg/kg treated animal and (e) normal mucosal epithelium with no ulcer in ranitidine treated animal

Table 1: Effect of MEJG on gastric secretion in aspirin plus pylorus-ligated Wistar rats

Treatment	*N*	Ulcer Score	Gastric volume (ml /100 g)	pH	Total acidity	Acid output (mEq/ 100g)
Vehicle	06	3.17 ± 0.41	3.18 ± 0.89	4.13 ± 0.97	59.04 ± 7.27	189.67 ±13.09
Aspirin (200mg/kg)	06	27.25 ± 1.94a*	5.37 ± 0.88a*	1.85 ± 0.52a*	84.59 ± 8.98a*	489.91 ± 23.79a*
MEJG 100 mg/kg	06	8.25 ±1.33b*	3.42 ± 0.82b*	3.13 ± 0.48b*	64.65 ± 6.46b	257.46 ± 20.15b*
MEJG 200 mg/kg	06	6.50 ± 0.29b*	2.60 ± 0.62b*	3.87 ± 0.52b*	61.78 ± 6.11b*	202.42 ± 15.68b*
Ranitidine (50 mg/kg)	06	4.17 ± 0.98b*	2.33 ± 0.42b*	4.00 ± 0.51b*	60.64 ± 4.81b*	196.00 ± 11.31b*

All values are expressed as mean ± SD of 6 animals (*n* = 6) in each group. Comparisons were made between: a-Group I vs II, b-Group II vs III, IV & V. If *P* < 0.001 statistically significant
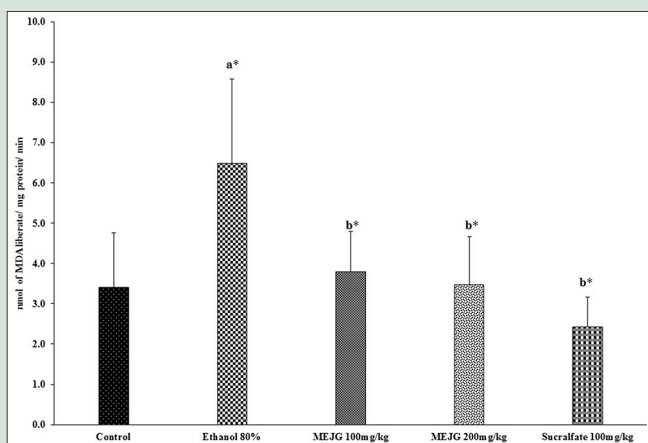
Figure 2: Effect of lipid peroxidation on ethanol induced gastric ulcer. Comparisons were made between: 'a' - Group I versus II, 'b' - Group II versus III, IV and V: If *P < 0.001 statistically significant
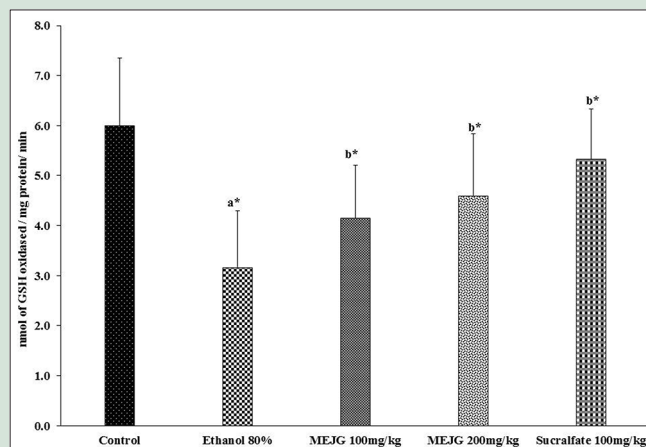

Figure 3: Effect of glutathione peroxidase on ethanol induced gastric ulcer. Comparisons were made between: 'a' - Group I versus II, 'b' - Group II versus III, IV and V: If *P < 0.001 statistically significant

Figure 4: Effect of superoxide dismutase on ethanol induced gastric ulcer. Comparisons were made between: 'a' - Group I versus II, 'b' - Group II versus III, IV and V: If *P < 0.001 statistically significant
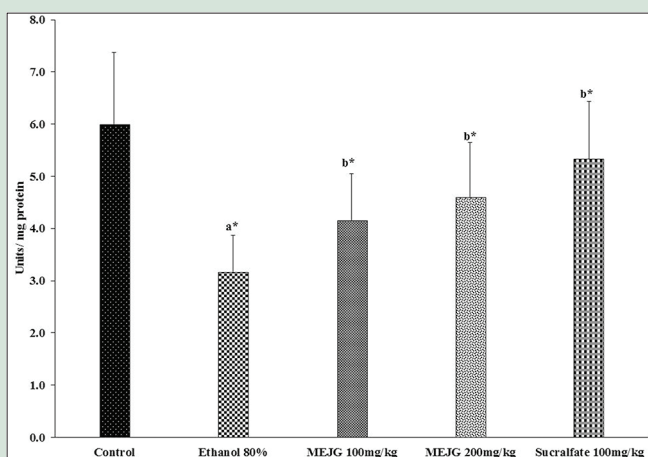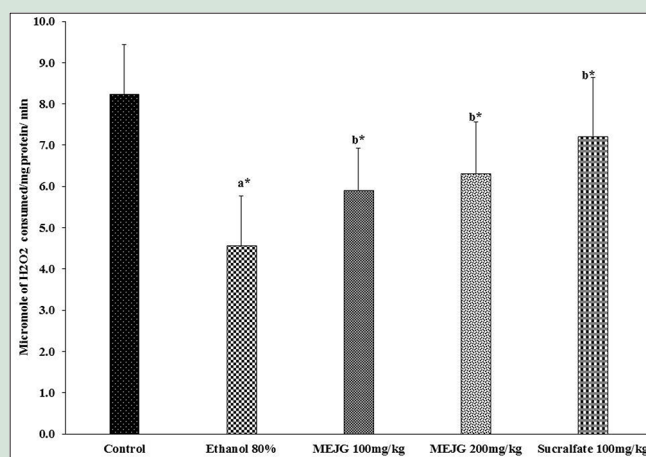

Figure 5: Effect of catalase on ethanol induced gastric ulcer. Comparisons were made between: 'a' - Group I versus II, 'b' - Group II versus III, IV and V. If *P < 0.001 statistically significant

Table 2: Effect of MEJG on gastric secretion in ethanol induced Wistar rats

Treatment	N	Ulcer Score	% inhibition
Vehicle	06	-	-
Ethanol (80%)	06	32.83 ± 2.79a*	-
MEJG (100 mg/kg)	06	8.00 ± 0.52b*	75.6
MEJG (200 mg/kg)	06	4.50 ± 0.22b*	86.3
Sucralfate (100 mg/kg)	06	3.17 ± 0.31b*	90.3

All values are expressed as mean ± SD of 6 animals (n = 6) in each group. Comparisons were made between: a-Group I vs II, b-Group II vs III, IV & V. If *P < 0.001 statistically significant

an important preepithelial factor that acts as the first line of defense mechanism against ulcerogens. Increased mucin secretion by the gastric mucosal cells can prevent the gastric lesions by several mechanisms, including lessening stomach wall friction during peristalsis as well as acting as an effective barrier to the back diffusion of hydrogen ions.[23] In aspirin-induced ulcer model, JG showed significant reduction in mucosal lesion which indicated that it may be increasing the secretion level of mucin in stomach due to the presence of terpenes and flavonoids.

Pylorus ligation model proposed that the digestive effects of accumulation of gastric juice and interference in gastric blood circulation are responsible for the induction of gastric ulcers.[24] Aspirin plus pylorus ligated group showed significant (P < 0.001) increase in the ulcer score and acid secretary parameters such as the volume of gastric content, total acidity, and total acid output, which indicated that JG possesses the significant gastroprotective effect in the stomach. The circulating neutrophils play a critical role in the pathogenesis of the mucosal lesion provoked by NSAIDs and ethanol.[25] Histopathological studies of the MEJG treated rat exhibit normal architecture of stomach tissues.

Ethanol is known to promote oxygen-free radicals and reduces the gastric mucosal nonprotein sulfhydryl (thiol) levels and it causes necrosis of superficial epithelial cells on gastric mucosa and erosion.[26] JG prevents gastric damage caused by ethanol, the commonly employed tests in the evaluation of cytoprotective activity. It is suggested that oxygen radicals may contribute to the formation of ethanol-induced gastric mucosal lesions. High dose of JG showed 85.26% protection against the ethanol-induced gastric ulcer, which indicated that JG may increase the

secretion levels of mucin in the stomach. However, depending on the dose levels, it attenuates the gastric lesion in rat stomach.

Anti-ulcer activity of JG against the gastric damage might be due to cytoprotection against leukotrienes or 5-lipooxygenase pathway. Prostaglandin synthesis has protected the cytoprotection in gastric mucosal lesion and antisecretory agent such as ranitidine is not inhibited by ethanol-induced ulcer, a well-known mechanism; hence, the agent such as sucralfate is enhancing the defense mechanism in gastric mucosa. GPx level was decreased by ethanol-induced gastric mucosal injury due to accelerated accumulation of hydrogen peroxide (H_2O_2) and lipid hydroperoxide, generated during the lipid peroxidation to water using reduced GSH as substrate. Reactive oxygen species are involved in the pathogenesis of ethanol-induced gastric mucosal injury. Ethanol has been shown to deplete the levels of nonprotein sulfhydryl content, especially GSH in stomach tissues and restoration of it appear to be important in gastroprotection, since they provide a substrate for hydroxylated and other free radicals to replenish GSH stores.

In the present study, JG at either dose level could restore the ethanol-associated depletion of nonprotein sulfhydryls. The depletion of GSH results in enhanced lipid peroxidation and excess lipid peroxidation cause increased GSH consumption. In our experimental conditions, the significant protective effect of the high dose of JG was significantly increased in GSH levels. However, the results revealed a significantly reduced levels of lipid peroxidation in a dose-depended manner. Lipid peroxidation is a free radical mediated process, which has been implicated in a variety of disease status. It involves the formation and propagation of lipid radicals, the uptake of oxygen, and arrangement of double bonds in unsaturated lipids, which eventually results in destruction of membrane lipids. Biological membrane is often rich in unsaturated fatty acids and bathed in oxygen rich metal containing fluid. Therefore, it is not surprising that membrane lipids are susceptible to peroxidative attack. The results show that treatment with JG at the dose of 200 mg/kg significantly decrease the levels of lipid peroxidation in gastric tissue compared to ethanol alone treated rats. SOD and catalase are shown to be extremely effective in ameliorating the increase in vascular permeability and morphologic changes found during intestinal ischemia or reperfusion.[27,28] Lipid peroxidation was attenuated and increases in free radical scavenging activity in the extract-treated animals compared to ulcerated group, suggest that the extract has the ability to protect the gastric mucosa against free radical mediated tissue injury. The protective action may be, via the increase and maintenance of near activity of SOD, which is preventing neutrophil-induced damage.[29]

CONCLUSION

The MEJG has a significant gastro-protective action at 200 mg/kg against the aspirin- and ethanol-induced ulcer models in rats. And also, antioxidant enzymes study is supportive to cure the ulcer study. The present study provides scientific support for the anti-ulcer activities of extracts of JG and substantiates the traditional claims for the usage of this drug in the treatment of chemicals induced ulcer. Since the presence of flavonoids and terpenes in JG may be considered as a natural source in both traditional ayurvedic and modern drug development areas for its versatile medicinal uses, further work is needed for a proper understanding of the mechanism of action with chemically identified active substances.

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Conflicts of interest
There are no conflicts of interest.

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