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Research Article
Evaluation of Antiulcer Activity of *Lawsonia inermis* and *Murraya koenigii* Seed Extract in Ethanol-induced Gastric Mucosal Damage in Rats

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Abstract

Background and Objective: Ulcer is the common gastrointestinal damage resulting from an inadequate gastric mucosal defense. Many synthetic drugs are available in the market to treat and these drugs produce side effects. The present research aims to evaluate the anti-ulcer activity of ethanolic extract of *Lawsonia inermis* and *Murraya koenigii* seeds. **Materials and Methods:** Ulcer was induced by administration of 95% ethanol (1 mL/200 g p.o.) in rats. Animals were 7 days pre-treated with *Lawsonia* (200 mg kg^{-1} p.o.) and *Murraya* (200 mg kg^{-1} p.o.) and their combination (200 mg kg^{-1} p.o.), respectively. **Results:** After treatment with extracts at 100 and 200 mg kg^{-1} significantly ($p<0.001$) shows the ulcer protective action. **Conclusion:** The selected plant extracts showed significant anti-ulcer activity.

Key words: Peptic ulcer, *Murraya koenigii*, *Lawsonia inermis*, ethanol

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Peptic ulcer is a chronic disease, characterized by an inequity between the factors that damages the mucosa and those for its defense, resulting in an abrasion of the lining of the upper digestive tract[1]. It has been one of the most prevalent diseases in the world and some of its complications have been the major causes of morbidity and mortality[2]. The prevalence differs in the world population between the duodenal and gastric ulcers and the mean age of people with the disease is between 30 and 60 years, but it can happen in any age[3]. Oxidative stress plays an important role in the pathogenesis of more than 100 diseases. Experimental studies have demonstrated that oxygen-generated free radicals (ROS) and lipid peroxidation are involved in the pathogenesis of acute gastric lesions induced by ethanol, non-steroidal anti-inflammatory drugs or *Helicobacter pylori*[4]. Ethanol stimulates gastric juice production, even when food is not present and as a result, its consumption will stimulate acidic secretions normally intended to digest protein molecules. Consequently, the excess acidity may harm the inner lining of the stomach[5]. Endogenous glutathione and prostaglandin (PG) levels are also lowered by ethanol while the release of histamine, influx of calcium ions and generation of free radicals and production of leukotriene's are all increased[6]. Ethanol is metabolized in the body and releases superoxide anion and hydroperoxy free radicals. It has been found that oxygen derived free radicals are implicated in the mechanism of acute and chronic ulceration in the stomach. Furthermore, gastric acid secretion now accepted to play an important role in the formation of gastric ulcer[6].

Lawsonia inermis Linn. (Lythraceae) or henna, a traditional plant with religious associations has been widely used over centuries for medication and cosmetics in some regions of the world especially in the Middle East, Africa and Asia[7]. Henna plant has several ethnobotanical uses. Henna leaves are used to cure jaundice, skin diseases, venereal diseases, smallpox, spermatorrhoea, rheumatoid arthritis, headache, ulcers, diarrhea, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and coloring[8,9].

Murraya koenigii (L.) spreng popularly known as curry leaf plant is a familiar spice used in India for its characteristic flavor and aroma. The traditional Indian medicine as analgesic, febrifuge, stomachic, carminative and for the treatment of dysentery, skin eruptions, anti-diarrhoeal, anti-microbial, hepatoprotective, radical-scavenging, hypoglycemic and immunomodulatory properties[10]. Phytochemical analysis of leaves, bark and root showed the presence of several bioactive constituents like carbazole alkaloids, carotene, polyphenols, terpenoids and coumarins[11].

No reports are available so far on the evaluation of the seeds of this plant for possible anti-oxidant and gastroprotective activities. Therefore, to justify the traditional claim, the objective of the study to assess the gastroprotective and anti-oxidant effect of ethanolic extract of seeds of leaves of *Lawsonia inermis* and *Murraya koenigii* in laboratory animals.

MATERIALS AND METHODS

Collection and extraction of plant material: The seeds of *Lawsonia inermis* and *Murraya koenigii* were collected from Hanamkonda, Telangana, India during the month of June-September, 2016. The seeds were authenticated by Prof. Ajmeera Ragan, Department of Botany, Kakatiya University, Warangal and Telangana, India. The research work was carried out in the advanced pharmacology and medicinal chemistry laboratories of Vaagdevi College of Pharmacy and Department of Biotechnology and Department of Pharmaceutical Chemistry, K L College of Pharmacy for a period of 6 months.

About 50 g of each *Lawsonia inermis* and *Murraya koenigii* seeds was dried under shade and crushed in electric blender to form coarse powder and extracted with ethanol by soaking coarse powder and allowed to stand for 7 days with occasional shaking and stirring. When the solvent become concentrated, the liquid alcohol content was filtered through cotton and then through Whatman filter paper # 1. Then the solvent were allowed to evaporate using rotary evaporator at temperature 40-45°C. Thus the highly concentrated crude extracts were obtained. Then obtained extract was preserved and used for further studies.

Experimental animals: Wistar rats weighing 150-200 g were used in the present study which was purchased from Mahaveer Enterprises, Medipally and Hyderabad. They had free access to food and water *ad labitum* they were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. They were acclimatized to laboratory conditions for one week before experimental studies. The experimental protocol was approved by the Institution Animal Ethical Committee (IAEC), Vaagdevi College of Pharmacy, Warangal (TS) and India (1047/PO/Re/S/07/CPCSEA: IAEC No: 7/ IAEC/VCOP/2016).

Acute toxicity study: The acute toxicity study was carried out as per the procedure given in Organization for Economic Co-operation and Development (OECD) Guideline[12] No. 420. The male Wistar rats (1500-200 g) were used in the study. After the sighting study (allow selection of the appropriate starting

concentration for the main study), *Lawsonia inermis* and *Murraya koenigii* at the dose of 2000 mg kg^{-1} b.wt., was given to 5 animals. The animals were continuously observed for 14 days for mortality and general behavior.

Induction of ulcer: Selected animals were randomly divided into six groups (n = 6). Gastric lesions were induced through ethanol (95%) at a dose of 0.2 mL/animal[13]. Group I served as control group received 1% sodium CMC orally. Group II animals served as ulcerogenic group received ethanol orally. Group III was orally administered 10 mg kg^{-1} (i.p) lansoprazole as a standard drug. Group IV animals received ethanolic extract of EELI at a dose of 200 mg kg^{-1} orally. Group V animals received ethanolic extract of EEMK at a dose of 200 mg kg^{-1} orally. Group VI animals received ethanolic extract of EELI+EEMK at a dose of 200 mg kg^{-1} orally. Forty-five minutes after treatment with plant extract and standard drug, each animal was given orally 0.2 mL of ethanol (95%) were sacrificed 30 min later. After 30 min, the rats were sacrificed and the stomach was removed. The gastric content was collected and centrifuge for 5 min at 2000 rpm and the supernatant was separated. The ulcer index, ulcer scores, pH, free, total acidity, liver function test (LFT), kidney function test (RFT) and lipid profiles were determined.

Ulcer index:

$$UI = UN+US+UP \times 10^{-1}$$

Where:

UI = Ulcer index
UN = Average of number of ulcer per animal
US = Average of severity score
UP = Percentage of animal with ulcer[14,15]

Ulcer scores: The 0 is the normal coloured stomach, 0.5 is the red colouration, 1.0 is the spot ulcers, 1.5 is the haemorrhagic streaks, 2.0 is the deep ulcers (ulcers $\geq 3 \leq 5$) and 3.0 is the perforations (ulcer>5)[14,15].

Percentage protection was calculated by using the equation:

$$\text{Protection (\%)} = \frac{\text{Ulcer index control-Ulcer index test}}{\text{Ulcer index control}} \times 100$$

Estimation of gastric pH: A volume of 1 mL gastric juice was diluted with 1 mL of distilled water and pH of the solution was measured using a pH meter[16].

Estimation of free acidity and total acidity: About 1 mL of gastric juice was pipette into a 100 mL of conical flask, 2 or 3 drops of methyl orange reagent was added and titrated with 0.01 N sodium hydroxide until the colour of the solution became yellowish. The volume of alkali added was noted. This volume corresponds to free acidity. Then, 2 or 3 drops of phenolphthalein solution was added and the solution was titrated until a definite pink colour appears. The total volume of sodium hydroxide (NaOH) added was noted and this corresponds to total acidity[16,17]:

$$\text{Acidity} = \text{Volume of NaOH} \times \text{Normality of NaOH} \times 100/0.1 \text{ m.eq/lit}$$

Serum biochemical analysis: The collected blood samples were centrifuged at 2500 rpm for 10 min, which was stored at -8°C before use for biochemical analysis. Serum samples were analyzed to evaluate possible changes in serum biochemical parameters like liver function test (LFT), kidney function test (RFT) and lipid profile[18].

Estimation of lipid peroxides, reduced glutathione and antioxidant enzymes: The excised stomach tissue was mixed with 5 mL of 0.1 M Tris-HCl buffer pH 7.4, homogenized on ice using Potter-Elvehjem glass homogenizer for 15 min. The homogenate was used for the estimation of thiobarbituric acid reacting substances (TBARS), glutathione (GSH) and antioxidants. Lipid peroxides (LPO) in terms of TBARS were estimated using 1, 1', 3, 3'-tetra methoxypropane as the standard and expressed as nM mg^{-1} protein[19].

Glutathione (GSH) was determined by the method of Moron *et al.*[20]. Aliquots of homogenate were mixed with equal volume of ice cold 5% TCA and the precipitated proteins were removed by centrifugation. The supernatant was added to equal volume of 0.2 M phosphate buffer, pH 8.0 and measured at 412 nm. The GSH was used as a reference standard. Glutathione peroxidase (GPx) was assayed by the method of Flohe and Gunzler[21]. The activity of GPx was expressed as nM GSH oxidized min^{-1} mg^{-1} protein. Superoxide dismutase (SOD) activity was measured according to the method of Kakkar *et al.*[22]. The inhibition of reduction of nitroblue tetrazolium to blue colored formazan in the presence of phenazine methosulfate and NADH was measured at 560 nm using n-butanol as blank. The enzyme activity was expressed as units mg^{-1} protein. Decomposition of H_2O_2 in the presence of catalase (CAT) was kinetically measured[23] at 240 nm. CAT activity was defined as the amount of enzyme required to decompose 1 μM of H_2O_2 min^{-1}. The enzyme activity was expressed as μM of H_2O_2 consumed min^{-1} mg^{-1} protein.

Statistical analysis: Results were expressed as Mean±SD; statistical significance was calculated by applying one way ANOVA. The $p<0.05$ was considered as significant (Dunnett's test).

RESULTS

Acute toxicity studies: The acute toxicity studies confirmed that ethanolic seed extract of *Lawsonia inermis* and *Murraya koenigii* does not shows any toxic sign or mortality up to the dose of 2000 mg kg^{-1} b.wt. The rats were observed for behavior of animals an interval of every 8 h during 72 h. From the results, test drug doses of 200 mg kg^{-1} b.wt., were chosen for the efficacy studies.

Effect on ulcer index and percentage protection: The combination of EELI and EEMK possess remarkable reduction in ulcer index (3.28±0.05) and increase the percentage of gastroprotection (74.1%) when compared with disease control. The results were summarized in Table 1.

Effect on free acidity, total acidity and pH: The present study showed free acidity, total acidity and pH in ulcer rats as compared with control shows in Table 2. The treatment with EELI+EEMK were showed more significant reduction in total acidity (68.83±5.49), free acidity (86.30±1.63) and increased in gastric pH (3.71±0.18) observed when compared with disease control.

Estimation of serum biochemical parameters: Table 3 elucidates the serum biochemical parameters like SGOT, SGPT and creatinine. The treatment with EELI+EEMK at 200 mg kg^{-1} significantly ($p<0.001$) brought to the normal values when compared with disease control.

Effects of EELI and EEMK on serum lipid profile: The treatment with EELI+EEMK at 200 mg kg^{-1} remarkably ($p<0.001$) reduced in total TGs, TC, LDL and VLDL when compared to diabetic control group, while the HDL levels were almost near to normal values when compared with disease control group (Table 4).

Estimation of lipid peroxides, reduced glutathione and antioxidant enzyme: The combination of EELI+EEMK (200 mg kg^{-1}) were more significantly ($p<0.001$) increase the enzyme levels in relation to respective disease control group in Table 5.

Table 1: Effect of EELI and EEMK treatment on ulcer index and percentage protection

Groups	Dose (mg kg^{-1})	Ulcer index	Protection (%)
I	-	-	-
II	-	12.68±0.19	-
III	10	1.25±0.08***	93.1
IV	200	7.32±0.07***	42.2
V	200	5.60±0.08***	55.8
VI	100+100	3.28±0.05***	74.1

Mean±SD (n = 6), ***$p<0.001$ compared to disease control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test, EELI: Ether extract of *Lawsonia inermis*, EEMK: Ether extract of *Murraya koenigii*

Table 2: Effect of EELI and EEMK treatment on free acidity, total acidity and pH

Groups	Dose (mg kg^{-1})	Free acidity (m.eq L^{-1})	Total acidity (m.eq L^{-1})	pH
I	-	53.50±1.04	71.50±1.04	4.95±0.10
II	-	98.16±7.13	130.16±1.72	1.71±0.14
III	10	57.10±3.03***	75.66±1.21***	4.61±0.11***
IV	200	85.16±6.91**	109.83±1.47**	2.21±0.42**
V	200	75.66±7.84***	95.83±0.75***	3.03±0.13***
VI	100+100	68.83±5.49***	86.30±1.63***	3.71±0.18***

All values are expressed as Mean±SD (n = 6), **$p<0.01$, ***$p<0.001$ compared to disease control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test, EELI: Ether extract of *Lawsonia inermis*, EEMK: Ether extract of *Murraya koenigii*

Table 3: Effect of EELI and EEMK treatment on liver enzymes

Groups	Dose (mg kg^{-1})	SGOT (IU L^{-1})	SGPT (IU L^{-1})	Creatinine (mg dL^{-1})
I	-	33.50±1.04	41.33±1.21	0.41±0.07
II	-	68.66±1.21	80.33±1.63	1.45±0.18
III	10	36.83±1.47***	44.16±1.16***	0.45±0.10***
IV	200	59.33±1.63***	70.32±1.47***	0.82±0.08***
V	200	50.83±1.16***	61.50±1.04***	0.61±0.07***
VI	100+100	43.66±1.21***	53.33±1.75***	0.53±0.12***

All values are expressed as Mean±SD (n = 6), ***$p<0.001$ compared to disease control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test, EELI: Ether extract of *Lawsonia inermis*, EEMK: Ether extract of *Murraya koenigii*, SGOT: Serum glutamic oxaloacetic transaminase, SGPT: Serum glutamic pyruvic transaminase

Table 4: Effect of EELI and EEMK treatment on lipid profile

Groups	Dose (mg kg^{-1})	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
I	-	72.66±1.21	79.83±1.16	41.33±1.03	15.96±1.02	42.36±1.06
II	-	115.51±1.04***	125.33±1.63***	16.55±1.04***	25.06±1.53***	73.93±1.26***
III	10	75.83±0.75***	82.16±1.47***	39.00±0.89***	16.43±0.92***	20.42±1.44***
IV	200	103.66±1.03***	111.33±1.96***	23.16±0.75***	22.26±0.84***	58.24±1.32***
V	200	93.66±1.36***	98.52±1.04***	29.66±1.21***	19.70±1.34***	44.00±1.47***
VI	100+100	84.16±0.98***	89.66±1.21***	34.83±1.47***	17.93±1.52***	31.39±1.38***

All values are expressed as Mean±SD (n = 6). *$p<0.05$, compared to disease control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test, EELI: Ether extract of *Lawsonia inermis*, EEMK: Ether extract of *Murraya koenigii*, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein

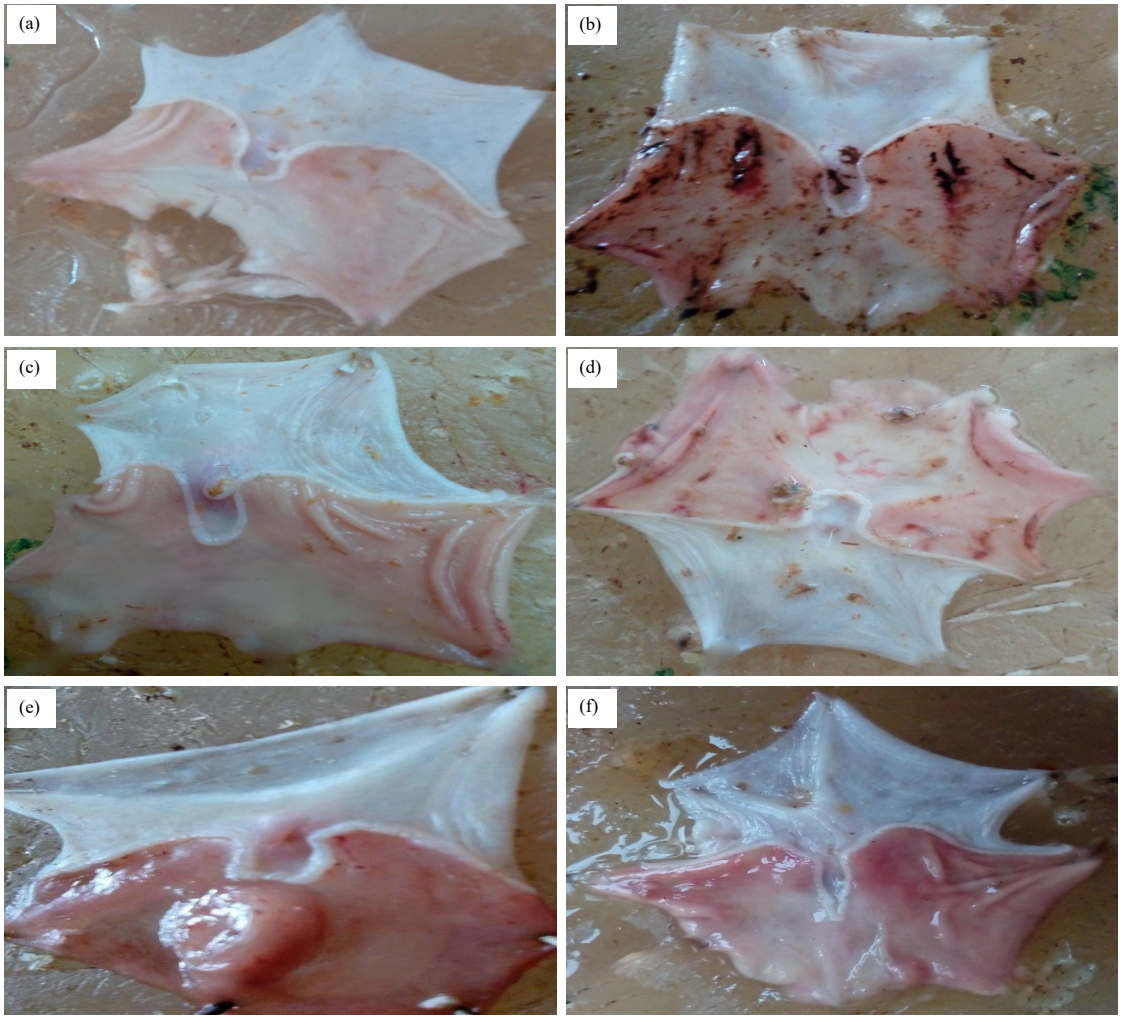

Fig. 1(a-f): (a) Normal, (b) Diseased, (c) Standard, (d) EELI treated, (e) EEMK treated and (f) EELI+EEMK treated rat stomach

Table 5: Estimation of lipid peroxides, reduced glutathione and anti-oxidant enzyme

Groups	Dose (mg kg^{-1})	TBARS (nM mg^{-1} protein)	GSH (nM mg^{-1} protein)	GPx (nM GSH oxidized min^{-1} mg^{-1} protein)	SOD (U mg^{-1} protein)	CAT (μM H_2O_2 consumed min^{-1} mg^{-1} protein)
I	-	1.06±0.15	6.52±0.56	218.12±21.53	45.32±5.56***	4.04±0.51***
II	-	2.52±0.41***	3.61±0.48***	103.45±12.56***	38.23±4.52***	2.28±0.32***
III	10	0.99±0.05***	6.30±0.66***	210.23±21.34***	44.84±5.60***	3.92±0.45***
IV	200	1.34±0.11***	6.43±0.72***	200.50±20.42***	42.63±4.78***	3.78±0.64***
V	200	1.30±0.16***	6.40±0.55***	203.34±19.56***	42.81±5.20***	3.81±0.73***
VI	100+100	1.26±0.09***	6.34±0.60***	207.76±20.39***	43.56±5.46***	3.85±0.42***

All values are expressed as Mean±SD (n = 6), ***p<0.001 compared to disease control, analyzed by one-way ANOVA followed by Dunnett's multiple comparison test

Histopathological studies: Histopathological evaluation of gastric lesions in control indicated that surface epithelium was not disrupted, while in diseased showed extensive gastric mucosal damage with necrotic lesions penetrating deeply into the mucosa accompanied by extensive edema and leucocyte infiltration of the sub-mucosal layer. In Lansoprazole treated rats no disruption of the surface epithelium, without sub-mucosal edema and leucocyte infiltration were observed. The EELI and EEMK treated rats exhibited mild disruption of the surface epithelium, with edema and leucocyte infiltration of the sub-mucosal layer. EELI+EEMK treated rats showed no disruption of the surface epithelium without edema and leucocyte infiltration of the sub mucosal layer. These results demonstrated that the plant extracts exerted cytoprotective effects in a dose-dependent manner (Fig. 1).

DISCUSSION

The disturbances in gastric production, injury to the mucosa, changes in the permeability, gastric mucus reduction and free radical generation in ethanol induced model. The continuous releases of superoxide anion and hydroperoxy free radicals take place during metabolism of ethanol. The gastric ulceration may be developed due to stability in gastric blood flow which contributes to the progress of the hemorrhage and necrotic tissue damages. Alcohol has capacity to enter the gastric mucosa and initiating the cellular damage which raises the penetrability to sodium and water. In additional, the acceleration of intracellular calcium affects the pathogenesis of gastric damage that causes cell death and exfoliation of surface epithelium[24]. In the current study shown significant antiulcer activity of ethanolic extract of EELI, EEMK and EELI+EEMK seeds in ethanol induced gastric ulcer by cytoprotective action through antioxidant properties. From the outcomes of this study establish that EELI and EEMK proven a cytoprotective action against ethanol induced cellular damage in the gastric mucosa of rats. Cytoprotection of anti-ulcer drugs has been known due to the production of prostaglandins[17]. The defense latent of mucus edge of gastric mucosa be contingent upon a mild balance between the methods affecting the synthesis and emission of mucin components. EELI and EEMK stopped the mucosal injuries persuaded by alcohol[25]. The new approach towards a powerful antiulcer agent contains a slight balance of governing the synthesis, secretion and metabolism of proteins, glycoproteins and lipids, so as to support the mucosal integrity[26]. Numerous scientific studies exposed that the phytoconstituents like flavonoids, tannins, terpenoids and saponin were accountable for gastroprotective agents[27]. Tannins have an anti-ulcer property by its astringency property and vasoconstriction effects. By precipitating the micro proteins on the ulcer site, a protective layer was formed which delays gut secretions and care for the mucosa from toxins and other irritants. Previous studies have recommended that these above active compounds had ability to stimulate mucus, bicarbonate and prostaglandin secretion and neutralize with the deteriorating effects of reactive oxidants in gastrointestinal lumen[28]. Therefore, EELI and EEMK possess antiulcer activity, may be due to presence of tannins, flavonoids and terpenoids. Gastric tissue homogenate prepared from the groups that were pretreated with plant extract exhibited significant anti-oxidant activity, with decreased levels of MDA and elevated levels of GSH and SOD, in response to oxidative stress due to ethanol treatment. SOD converts superoxide to hydrogen peroxide (H_2O_2), which is transformed into water by catalase in the lysosomes or by glutathione peroxidase in the mitochondria[29]. The MDA is the final product of lipid peroxidation and is used to determine lipid peroxidation levels[30]. Lipid peroxidation causes a loss of membrane fluidity, impaired ion transport and membrane integrity and ultimately a loss of cellular function. The mechanisms underlying the protective action of the extract against ethanol induced gastric lesions are unclear. Further studies using more specific methods are required to explore the compounds responsible for the protective effect and the mechanism of this activity.

CONCLUSION

This research indicated a significant anti-ulcer activity of combination of ethanolic extract of *Lawsonia inermis* and *Murraya koenigii* seeds by preventing the development of gastric ulcers in ethanol induced rats. It also showed significant protection compared to standard. Extract of combination of *Lawsonia* and *Murraya* are very effective herbal alternative for the treatment of ulcers. This study will help the researchers to uncover the critical areas of gastro intestinal tract that many researchers were not able to explore. Thus a new theory on anti-ulcer activity may be arrived at.

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