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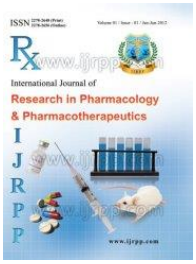

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Ulceroprotective effect of ethanolic extract of aegle marmelos leaves in wistar rats

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ABSTRACT

Title
Ulceroprotective effect of ethanolic extract of Aegle marmelos leaves in wistar rats..

Objectives
To evaluate the Ulceroprotective effect of ethanolic extract of Aegle marmelos leaves in wistar albino rats.

Materials & Methods
The study was conducted in 24 male adult wistar albino rats weighing 150-200g. 24 rats were randomized into 4 groups and ulceroprotective property of the extract was studied employing indomethacin induced ulcer model. Groups 1 & 2 were the normal and ulcer control respectively .Groups 3 and 4 were pretreated with misoprostol and extract (200mg/kg) respectively for 5 days followed by ulcer induction in groups 2, 3 and 4. Ulceroprotective activity was expressed as Percentage inhibition (PI). Statistical analysis was performed by one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. $p < 0.05$ was considered to indicate statistical significance.

Results
The result data indicated that, mean ulcer index was significantly lower in the leaf extract pretreated group [Group 4 (5.175±0.05)] compared to control group with a PI of 50%. Histopathological examination confirmed the ulceroprotective activity of the extract evident from the reduction in number and severity of gastric ulcers

Conclusion
Based on the above findings it can be inferred that ethanolic extract of Aegle marmelos leaf extract has ulceroprotective property and pretreatment of the extract is beneficial in prevention of gastric ulcer.

Keywords: Ulceroprotective, Aegle marmelos, NSAID, Misoprostol.

INTRODUCTION

Gastric ulcer is one of the most common gastrointestinal disease with a high prevalence rate around 10 %. [1] Peptic ulcer, a term that refers to both duodenal and gastric ulcers is a multifactorial disease characterised by mucosal erosions that occurs mainly due to an imbalance between the mucosal defensive and damaging factors. [2] Recurrence, gastric mucosal bleeding and perforation are the long term complications in untreated patients.

Gastric mucosal blood flow, gastric mucus secretion ,bicarbonate synthesis and prostaglandins (PGE2, I2) have been identified as the protective or mucosal defensive factors against the formation of gastric and duodenal ulcers .[3]Gastric acid, pepsin, bile are the aggressive or damaging factors which lead to ulcers by overpowering the mucosal protective factors. [4] An imbalance between the protective and defensive factors is considered to be the important risk factor in the pathogenesis of ulcer

Gastrointestinal side effects, nephrotoxicity and hepatotoxicity are commonly encountered with use of NSAIDs. [5] Among these, the gastrointestinal side effects such as peptic ulcers are considered to be most common. [6] NSAIDs are considered to be the second most common cause of peptic ulcer following Helicobacter pylori (H.pylori) infection. [7] In India alone, the incidence of gastric and duodenal ulcers due to NSAID usage has been reported to be 10-40 % and 5- 15% respectively. [8]

Various studies have proposed that either reducing the dose of NSAID, switching to a lesser gastrotoxic alternative, avoiding the concomitant use of other ulcer inducing drugs or co administration of gastroprotective drugs can reduce the adverse effects. [9]But none of these strategies are fully effective since reducing the dose can compromise the primary activity of the drug and gastroprotective drugs such as misoprostol are costly and are also associated with adverse effects.

In the recent times, there is an increased awareness towards drugs derived from natural sources. [10] Aegle marmelos is a plant of Indian origin used as an ailment for various diseases in traditional medicine. [11] Our study aims at evaluating gastroprotective effect of the extract thereby providing scientific data to validate all the traditional claims. Roy et al., observed that the pretreatment of aqueous extract of Aegle marmelos fruit pulp effectively reduced the ulcer index in aspirin induced ulcer models. [12] The extract offered excellent gastric protection in rats and was attributed to its antioxidant property .In another study by Dhuley et al., pretreatment of unripe fruit extract of bael produced significant gastroprotective activity against hypothermic restraint stress and indomethacin induced ulcer models. [13] Sharma et al. reported that the methanolic and aqueous extract of Aegle marmelos seed had significant antiulcer activity against indomethacin, stress and pylorus ligation induced ulcer models. [14]

Though previous studies evaluating the antiulcer effect of the plant extract are available, they have been mostly conducted on the fruit and seeds. Studies evaluating the leaf extract are very few. Most of these studies were done with either aqueous or methanolic extract of the plant. Hence we decided to evaluate the antiulcer activity of ethanolic extract of Aegle marmelos leaves. We have compared the ulceroprotective effect of the leaf extract with misoprostol, since misoprostol is the drug of choice for NSAID induced ulcer. In previous studies, such comparative data with misoprostol is not available.

OBJECTIVES

Primary objective

To evaluate the Ulceroprotective effect of Aegle marmelos in Indomethacin induced gastric ulcers

Secondary objective

To evaluate the histopathological changes produced by Aegle marmelos in indomethacin induced gastric ulcers.

MATERIALS AND METHODS

24 adult male wistar albino rats weighing 180-200g and aged around 2-3 months were used for the study. After acclimatization for a period of one week, the 24 rats were randomly divided into 4 subgroups of 6 rats each (n=6) by a computer generated numbering software. Institutional Animal Ethics committee approval (IAEC) was obtained prior to the commencement of study.

Drugs, chemicals and instruments

Indomethacin manufactured by JagsonPal Pharmaceuticals and Misoprostol manufactured by

FDC limited were procured from a local chemist. Crude extract of Aegle marmelos leaves was procured from Amir Chemicals Private Limited, Madhya Pradesh, India. Ethanol was procured from Sigma Aldrich chemicals Pvt Ltd, Bengaluru. All chemicals and reagents utilized in the study were of analytical grade.

All drugs were suspended in normal saline and administered. Doses of the drugs were calculated based on the body weight and the respective volume of drugs were administered orally with help of tuberculin syringe with gavage needles.

Soxhlet Extraction apparatus used for preparation of ethanolic extract, manufactured by INCO industries, Ambala was used in the study.

METHODS

Preparation of extract

The extract of Aegle marmelos that was obtained as dry powder (40 g) was subjected to soxhletion with 95 % ethanol in order to prepare the ethanolic extract. The obtained extract was air dried and stored in a dessicator. The plant extract in the form of dark green powder was obtained. [15] The Percentage yield of the ethanolic extract was approximately 15 % (6g)

Indomethacin induced ulcer method

Ulcer induction

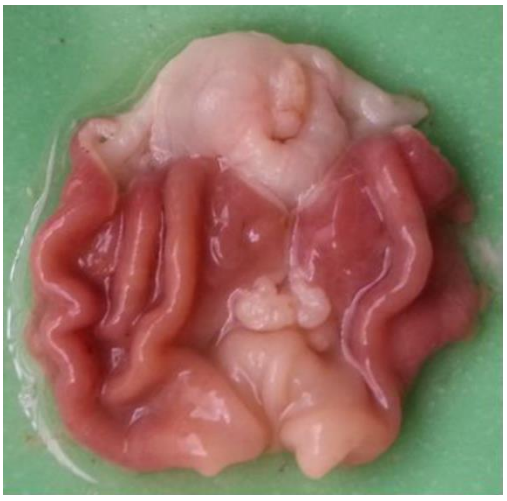
Group I was the normal control. Groups 3 and 4 were pre-treated with misoprostol and extract 200mg/kg [16] respectively for a total period of five days. All the rats were fasted but had free access to water 24 hours prior to ulcer induction. On fifth day, the last dose of standard, test drugs were administered to group 3 and group 4 respectively. [17, 18] Half an hour following drug administration, ulcers were induced in groups 2, 3 and 4 by oral administration of indomethacin. Six hours after ulcer induction, all the rats were humanely sacrificed by an overdose of sodium pentobarbital (150mg/kg i.p) and examined for gastric ulcers. [19]

Macroscopic examination of gastric ulcers

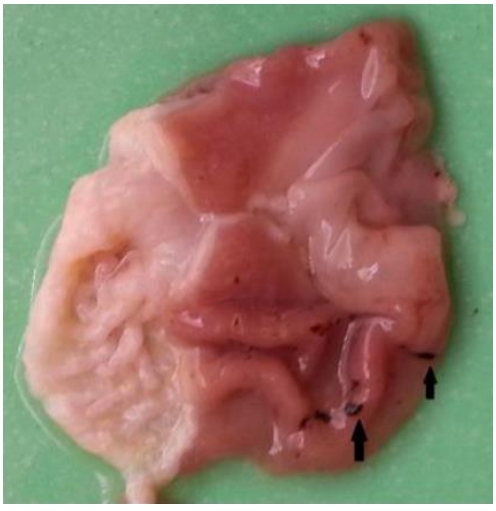
The glandular portion of the stomachs were examined for ulcers under a 3-fold magnifier by an observer unrelated to the study. Total number of ulcers present, severity of ulcer scoring and percentage of animals with ulcers were observed and noted. The identified ulcers were scored according to the method described by Ganguly et al., 1973. [20]
Ulcer index was calculated using the following formula

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

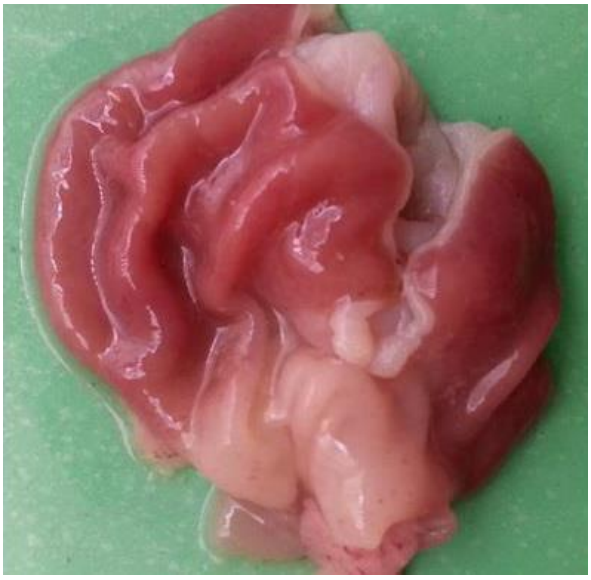
- Where UI -ulcer index; UN -average no. of ulcer per animal; US- average of severity score; UP - percentage of animal with ulcer
- Mean ulcer score for each animal were expressed as ulcer index. The percentage of ulcer inhibition were calculated by the following formula [21]
- Inhibition of ulcer (%) = control mean ulcer index − test mean ulcer index / control mean ulcer index



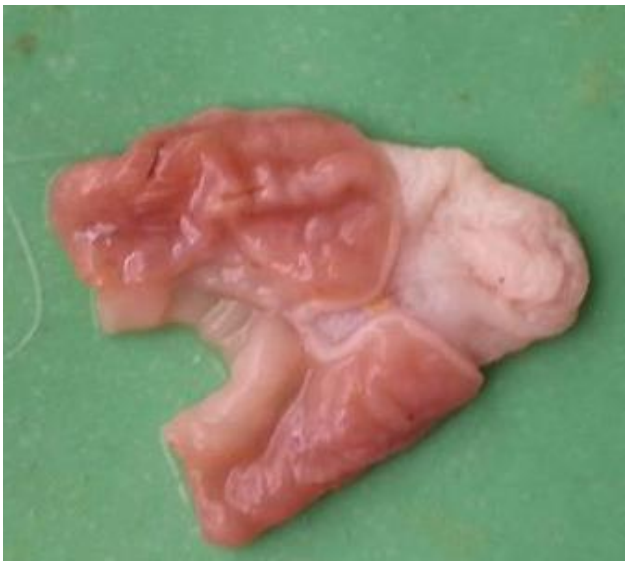
Group 1- Normal control



Group 2- Ulcer control



Group 3-Misoprostol



Group 4- Aegle marmelos extract

Figure 1: Macroscopic appearance of gastric mucosa in all groups

Group 1 showing normal gastric mucosa. Group 2 showing congested gastric mucosa and arrow marks indicate haemorrhagic streaks. Group 3 and 4 shows near normal gastric tissue with mild congestion.

Histopathological examination

Inorder to study the ulcero protective effect, the stomach of each animal were dissected and small bits of gastric tissues were fixed in 10% v/v buffered formalin for about 24 hours and processed using a tissue processor. The processed tissue was embedded in paraffin blocks and four micrometer thick sections were prepared with a rotary microtome. These sections were stained with haematoxylin and eosin using standard techniques and the stained sections were examined for histopathological changes under light microscope and photographed. [22] A minimum of eight fields for each stomach section were examined for severity of changes by an observer blinded to the treatments of the animals. Gastric damage for each histopathological section was observed and photographed

RESULTS

Table 1: Effect of Extract on Indomethacin induced ulcers

Groups	Drug administered	% of animals with ulcer	Mean ulcer index
I(Normal control)	Vehicle	-	-
II (Ulcer control)	Indomethacin	100	10.408±0.047 (0%)
III(Standard drug)	Misoprostol	33	3.433±0.040**# (67%)
1V (Test drug)	Aegle extract	50	5.175±0.05**# (50.2%)

Values are expressed as mean ±SEM for groups of six animals each.

**P<0.001 as compared to vehicle control.

# P<0.05 when Groups III and IV were compared.

DISCUSSION

Peptic ulcer is a disorder characterized by involvement of gastric and duodenal ulcers affecting around 4 million people every year and the incidence is expected to rise in the future owing to increased NSAID use ,diet and lifestyle modifications. [23]

Although the currently available antiulcer drugs are effective, adverse effects greatly limit the use of these drugs. Hence our study evaluated the ulceroprotective property of ethanolic extract of Aegle marmelos leaves by employing NSAID induced ulcer model.

Effect on ulcer index

In our study, mean ulcer index was highly increased in the indomethacin administered group (Group 2). This showed that indomethacin clearly produced gastric ulcers and haemorrhagic erosions which were more in number as well as severity. Indomethacin promoted gastric ulcer by reducing prostaglandin, bicarbonates and by increasing oxidative damage in the gastric mucosa.

Group 3 which was pretreated with misoprostol (standard drug) was protected against the ill effects of indomethacin. The mean ulcer index was significantly reduced in Group 3 when compared to Group 2. Since misoprostol is a prostaglandin analogue, it prevented ulcers by increasing mucus secretion, bicarbonate secretion and mucosal blood flow. This cytoprotective action of misoprostol effectively reduced ulcer index and prevented gastric ulceration.

The group administered with the extract of Aegle marmelos (Group 4) has shown to possess gastro protective activity against experimentally induced NSAID induced ulcer model. This was evident from the fact that the extract pre-treated group produced significant reduction in ulcer index when compared with the control. Both the number and severity of gastric lesions were found to be reduced in the extract treated group leading to a significant percentage inhibition of ulcer.

In another study [24] the antiulcer property of ethanolic extract of Aegle marmelos leaves was investigated against ethanol induced gastric damage in wistar rats. In this study, there was no pretreatment of the extract. The extract was administered half an hour prior to ethanol administration and the antiulcer property of Aegle marmelos extract was studied. The extract produced marked reduction in the ulcer formation. The findings of the study were in accordance with our study.

A drug that protects against NSAID induced gastric ulcer may act through mechanisms such as cyto protection ie by increasing prostaglandins, mucus and bicarbonate secretion or due to its antioxidant nature or due to its antisecretory action. [25] Based on the results of our study, gastroprotective action of the extract could be attributed to cytoprotection or presence of antioxidants or a combination of both. Cytoprotective action could be due to increased mucus and bicarbonate secretion. The antioxidants present in the extract such as glycosides, saponins and tannins might have reduced the oxidative mucosal damage thereby reducing gastric lesions. The Antisecretory effect of the extract could not be commented since the effect of the extract on gastric acid volume and pH was not tested in our study.

Ilavarasan et al ., studied the effect of pretreatment of aqueous extract of Aegle marmelos leaves on aspirin and pylorus ligation induced ulcer models. [26] The extract was found to significantly reduce the ulcer index. The volume of gastric juice, total gastric acidity as well as the pH was also reduced in extract pretreated group. Thus the study revealed significant antiulcer effect of the extract which was attributed to its cytoprotective as well as ant secretory effect.

The extract pretreated group has significantly reduced the mean ulcer index which inturn implies that the extract prevented the formation of gastric ulcers which could be attributed to its cytoprotective and antioxidant effect.

Effect on histopathology

The slides were examined microscopically for histopathological changes such as congestion, haemorrhage, edema, erosions and ulcers in order to assess the severity of gastric lesions in various drug treated groups. Sections taken from Group 1 (normal control) group showed normal histological structure of the gastric mucosal and submucosal layers. The gastric mucosa composed of single layer of surface

columnar epithelial cells with normal gastric mucosal glands were observed.

Exposure to indomethacin cause reduced mucus and bicarbonate secretion thereby weakening gastric mucosal defense leading to erosions and ulcer. In addition, direct topical injury of the mucosa by indomethacin can also trigger inflammation leading to cellular infiltration. Our results were consistent with these findings. Gastric mucosa of ulcer control (Group 2) in our study showed breach in the surface epithelial cells and mucosal glands, petechial hemorrhages and congested dilated blood vessels. Few sections showed fragmented and distorted glands with diffuse leucocyte infiltration. Normal gastric mucosa without edema was found in almost all the slides of misoprostol treated group (Group3) which proved its significant cytoprotective action.

Most of the slides that belonged to extract pretreated group revealed normal and intact mucosa with mild congestion. This implies that the extract has reduced gastric lesion and submucosal edema similar to misoprostol treated group. Some slides showed mucosa with few breaches and mild edematous mucosa. This could be due to lesser days of extract pretreatment or due to lower dose of the extract or both.

Therefore pre-treatment with Aegle marmelos ethanolic extract before indomethacin has maintained the normal histological structure of the glandular mucosa with a low level of leucocyte infiltration between glandular and non-glandular gastric portions. The protective effect of the extract that was revealed by macroscopic examination and quantitative assessment by ulcer index has been confirmed again by histopathological examination that has showed prevention of mucosal lesions and submucosal oedema. This has proved the gastroprotective potential of the extract against NSAID induced ulcers. Thus it could be concluded that the ethanolic extract of Aegle marmelos leaves has gastroprotective potential. The probable mechanism for the action could be mainly due to cytoprotection and antioxidant mechanisms.

CONCLUSION

In the present animal study in rats, Pretreatment of ethanolic extract of Aegle marmelos leaves at a dose of 200mg/kg exhibited significant gastroprotection in indomethacin induced ulcers as evidenced by reduction in ulcer index. Nonetheless further studies are needed to correlate the therapeutic activity with the bioactive compounds of the plant as well as studying the mode of action of those marker compounds. Further research is needed with different doses inorder to evaluate the ulceroprotective property. Measurement of antioxidant and gastric ph levels with further studies in humans could confirm the traditional claims of the plant.

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