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Evaluation of Antiulcer Activity of Ethanolic Peel Extract of *Punica granatum* in Rats

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Abstract
The present work incorporates the study of gastric antiulcer of dried *Punica granatum* (PG) peel 50% ethanol extract (PGE) in rats. PGE (200 mg/kg & 400 mg kg) was administered orally once daily to rats either before or after induction of gastric ulcers (GU) for 7 d. Antiulcer effects of PGE were seen against acute GU, induced by Pylorus Ligation (PL), Indomethacin induced, Cold Restraint Stress (CRS) in rats. Ulcer index (UI), Gastric juice volume, pH of gastric HCl, Free acidity and Total acidity were estimated. PGE showed a decrease in UI in PL method (23.77% to 36.42%) indicating both protective and healing effects. PGE showed effects on volume of gastric HCl (3.95±0.44**), pH of gastric acid (3.21±0.29**), Free acidity (59.16±5.32**), Total acidity (86.66±7.03**) in PL rats. In NSAIDs induced and cold restraint stress induced gastric ulcer models, 50% Ethanolic extract produced significant dose dependent decrease in ulcer index (34.68%) and (37.07%),P<0.01**, and it was found that maximum ulcer protective activity was shown by Ethanolic extract when given orally at a dose of 400 mg/kg body weight. Statistical analysis was carried out by one-way analysis of variance followed by Dunnet's test. The effect of Anti-ulcer activity of *Punica granatum* seems to be dose dependent and significant. The presence of Flavonoids which have astringent property could be responsible for the Anti-ulcer activity, is more likely to be involved in the reaction with the proteins of the layer tissues and thereby showing the activity.

Keywords
Punica granatum peel, Gastric ulcer, Astringent, Free radicals

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1.0. INTRODUCTION
Herbs have been used by people for longer than we have been keeping written record. Originally, they were found in the wild and used for a lot of different things. They were used to flavour food, as a source of nutrition, as medicines and for magical purposes. Herbs were used by the "wise women and men" of small bands of people very often with good, reliable results.

Medicinal plants have always been considered a healthy source of life for all people. Therapeutic properties of medicinal plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural. Nowadays people are being bombarded with thousands of

unhealthy products, the level of sensibility in front of diseases is very high and that's why the use of medicinal plants can represent the best solution. The World Health Organisation (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. (Aebi, H.E., *et.al*,1974).

India is one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. Many of the pharmaceuticals available to physicians today have a long history of use of herbal remedies such as Opium, Aspirin, Digitalis and Quinine as raw materials in the modern bio-pharmaceutical and bio-cosmetic industries. Such discoveries are made from the knowledge of traditional system of medicine. The traditional system of medicine is bourn out the fact that even today, in most of all the rural areas, people are depending on the local traditional healing system for their primary health care. (Presser, D., Fuhrman, B., *et.al* 2000).

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the body and lesser side effects. However, last few years have seen a major increase in their use in the developing world.

Medicinal plants are reservoir of drugs and lead compounds for many therapeutic agents. There are avalanche of scientific support on the efficacy of medicinal plants in the treatment of gastrointestinal disorders and in the management of ulcers of different etiologies.

The typical anti-ulcer treatment of today's mainstream medicine is triple therapy regimen; it includes two antibiotics combined with one drug from either of two other classes of drugs- one designed to inhibit acid secretion and one designed to protect the gastric mucosa from chemical attack. Now-a day, Quadruple therapy is also suggested. But this treatment doesn't work in everyone, and research from Michiagn suggests that there may be more ulcer bacteria than Helicobacter. These triple and quadruple regimens gladden the heart of pharmaceutical industry, but not of Mother Nature, who prefers natural remedies without side effects. (Banerjee, R.K.,1990).

Nature has given mankind many remedies for almost all kind of diseases, peptic ulcer is no exception. Beneficial effects of a number of drugs like Drakshagrhitham, Sitamundaram, Thriphalamundaram, Sathavareeghritham, Taramanduram etc have claimed to be effective in the treatment of hyperacidity and peptic ulcers. Good amounts of work have been done on natural antiulcer remedy which includes Shankabhasma, Tephrosia purpurea, Tea root extracts, Deglycyrrhizinated Liquorice, Rhizomes of Curcuma longa, Banana, Ginger, alpha-tocopherol, Selenium and many more.

Punica granatum has been traditionally used as antioxidant, anti-inflammatory, carcinogenesis, invasion and motility etc. Pomegranate fruit extracts exhibit scavenging activity against hydroxyl radicals and superoxide anions, which could be related to anthocyanidins.

The study is intended to evaluate "The Anti-ulcer activity of *Punica granatum* of family Punicaceae, in gastric ulcer induced models such as Pylorus ligation induced ulcers and Cold restraint induced gastric ulcers." (R.K. Gadekar, Sinaganun, *et.al* 1987).

2.0. MATERIALS AND METHODS:

2.1. Collection and authentication of plant

The seeds of *Punica granatum* belonging to family Punicaceae were collected from surroundings of Warangal and authenticated by **Dr. Vatsavaya S. Raju,** Professor, Botany Department, Kakatiya University, Warangal, Telangana. A voucher specimen was submitted at Botany Department, Kakatiya University,Warangal.

2.2. Drugs and chemicals

Ranitidine, Indomethacin and Mesoprostol (invision Medi sciences, Bangalore), Ethanol (Ranbaxy) was used in this study.

2.3. Preparation of extract and preliminary phytochemical screening

The seeds were collected, and they were shade-dried and pulverized to coarse powder and subjected to cold maceration process. The dried powdered plant of 300 gm weight was weighed and soaked in 750 ml of 50% ethanol and kept for maceration for 3 days with occasional shaking after which it was filtered, and the filtrate was concentrated. A brownish black waxy residue was obtained. The dried hydro alcoholic extract, PGE was stored in desiccators until use. During experimentation, the dried hydro alcoholic extract was always suspended in 1%CMC for administration. The extract was subjected to qualitative chemical tests for various phytoconstituents like Alkaloids, Carbohydrates, Saponins, Tannins, Proteins, Lipids, Flavonoids and Steroids.

2.4. Experimental animals

The animals were housed in the animal house, Department of Pharmacology, St.John College of Pharmacy, Yellapur, Hasanparthy, Warangal,

Telangana. The animals were housed in stainless steel cages with husk as bedding, fed with normal commercial pellet diet, given water *ad libitum* and maintained under laboratory conditions (temperature 24 - 28°C, relative humidity 60 - 70%, and 12 h light-dark cycle).

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of institute and conducted according to the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines with the approval number.

2.5. Acute toxicity study according to OECD 423

Either sex of Wister rats (150-200) were used in this study. The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic class method).

2.6. Method I

Pylorus ligation(PL) induced gastric ulcers in rats:

Group-1: Served as control
Group-2: Standard drug Ranitidine (30mg/kg/p.o)
Group-3: Plant extract (200mg/kg/ p.o)
Group-4: Plant extract (400mg/kg/p.o)

Procedure

The Wister rats of 150-200 g of either sex animals (24 numbers) were used for the study. The animals were fasted for 48hours before the operative procedure. However, they were given free access to water. To prevent cannibalism and coprophagy, the animals were housed singly in cages with raised bottoms of wide wire mesh. Under anaesthesia a one-inch midline abdominal incision was made below the xiphoid process. The pylorus was carefully lifted out with minimal handling and traction and ligated without damaging its blood supply. The stomach was replaced, and the abdominal wall closed with sutures. The test compound was administered orally for 7 days and the animals were placed in plastic cylinders.

After 17-19 hours of pyloric ligation, the animals were sacrificed, and the stomach dissected out. The contents o stomach was drained into graduated centrifuge tube and their volume of gastric content, pH of gastric acid, after centrifugation its acidity was determined by titrating with 0.1N NaOH. The stomach was opened along its greater curvature pinned on a cork plate and inner surface was examined for ulceration with binocular microscope.

Scoring of ulcers was made as follows:

Normal stomach…… (0)
Red coloration…… (0.5)
Spot ulcer…… (1)
Hemorrhagic streak…..... (1.5)
Ulcers……..... (2)
Perforation…….. (3)

Mean ulcer score for each animal as expressed as ulcer index.

The percentage inhibition was determined as follows;

$$\frac{\text{(Control mean lesion index – Test mean lesion index) x 100}}{\text{Control mean lesion index}}$$

The gastric contents were centrifuged at 1000rpm for 10min and the volume was noted, then 1ml of supernatant liquid was pipette out and diluted to 10ml with distilled water. Then the pH of this solution was noted. Followed by titration of the solution against 0.1N Sodium hydroxide using Topfer's reagent (Dimethyl-amino-azo-benzene with phenolphthalein) as indicator. Titration was carried out to the end point when the solution turned to orange colour. The volume of NaOH was noted which corresponds to the free acidity.

Titration was continued further till the solution regained pink colour and the total volume of NaOH was noted which corresponded to the total acidity.

2.7. Method II

Indomethacin Induced gastric ulcers in rats:

Group-1: Served as control
Group-2: Standard drug Mesoprostol (100mg/kg/p.o)
Group-3: Plant extract (200mg/kg/ p.o)
Group-4: Plant extract (400mg/kg/p.o)

Procedure

The gastric ulcers was induced by administering Indomethacin (5mg/kg b.w p.o) for five days to groups .After induction of ulcer, group 2 was treated with Mesoprostol (100mg/kg b.w p.o) and group 3 and 4 are treated with ethanolic extract of *Punica granatum* in a dose of 200mg/kg b.w p.o and 400mg/kg b.w p.o respectively for 7 days. The rats were sacrificed on the seventh day and ulcer index was determined.

2.8. Method III

Colds restrain stress induced ulcers in rats:

Group-1: Served as control
Group-2: Standard drug Ranitidine (30mg/kg/p.o)

Group-3: Plant extract (200mg/kg/ p.o)
Group-4: Plant extract (400mg/kg/p.o)

Procedure

The Wister rats of 150-200 g of either sex animals (24 numbers) were used for the study. The ulcer was induced by subjecting the animals to cold restraint stress. Ranitidine (30 mg/kg P.O) and the ethanolic extract of *Punica granatum* (200mg/kg & 400mg/kg p.o) was administered in groups 2, 3&4 respectively 30min prior to subjecting the rats to stress. Then the rats from all the groups were kept in a restraint cage and were placed at temperature of 2OC for 3 hours. The rats were sacrificed after three hours and the ulcer index was determined.

Statistical analysis:

The data was represented as Mean±SEM. The data of antiulcer activity of whole plant of *Punica granatum* extract was analyzed by one-way ANOVA followed by Dunnet's t-test and the whole analysis was carried out using Graph Pad Prism 5.0 version. 'P' value were considered significant **P<0.01 when the test and reference were compared with the control group.

3.0. RESULTS

3.1. Plant Extraction:

The weight of extract was 17.0 grams. Its percentage yield was found to be 6.53%.

3.2. Preliminary Phytochemical analysis:

The phytochemical analysis of ethanolic extract of peel of *Punica granatum* indicated the presence of Carbohydrates, Glycosides, Flavonoids, Alkaloids, Tannins, Phenols, Gums and Mucilages, Terpenes and Sterols.

3.3. Acute toxicity study:

The ethanolic extract of peel of *Punica granatum* was found to be safe at the maximum tested dose of 2000mg/kg body weight by oral route. After 24hrs animals was found well tolerated. There was no mortality and no signs of toxicity. General behavioral, neurological and autonomic profiles were found to be normal and all the two concentrations of extract were found to be safe. Thus 1/10th of the maximum tolerated dose i.e., 200mg/kg b.w p.o and 400mg/kg b.w p.o.

3.4.0. Pylorus ligated induced ulcers in rats

3.4.1. Volume of gastric HCl:

The volume of gastric HCl in Ranitidine treated group decreased significantly upto 2.23±0.19, compared to control group in which the value was 6.21±0.11 in groups treated with Ethanolic extract, the volume of gastric HCl was reduced significantly to 4.8±0.55 and 3.95±0.44 at doses of 200& 400 mg/kg respectively as compared to control group. The results have been tabulated in table 1 and represented by Fig no. 1.

3.4.2. pH of gastric HCl:

The pH of gastric HCl in Ranitidine treated group was significantly much more i.e., 4.91± 0.18, compared to control group in which the value was 1.53±0.08. In groups treated with Ethanolic extract, pH value was increased significantly to 2.64±0.42 and 3.21±0.29 at doses of 200 &400 mg/ kg respectively as compared to control group. The results have been tabulated in Table no. 2 and represented by Fig no. 2.

3.4.3. Free Acidity:

In groups treated with Ethanolic extract, the value of free acidity was reduced to 70±5.91 and 59.16±5.32 at doses of 200 &400mg/kg respectively when compared to control group. In Ranitidine treated group the value of free acidity was decreased significantly to 25.83±4.16. When compared with control group in which the value was 90±5.16. The results have been tabulated in Table no.3 and represented by Fig no. 3.

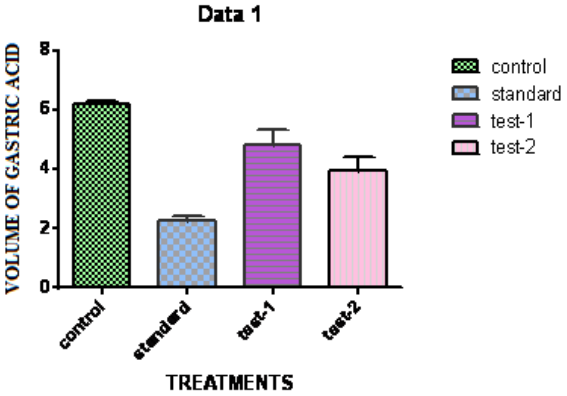
3.4.4. Total Acidity:

Total acidity in Ranitidine treated group decreased significantly upto 32.50±2.14, compared to control group in which the value was 118.33±4.21. In groups treated with Ethanolic extract, the value of total acidity was reduced significantly to 96.66±6.28 and 86.66±7.03 at doses of 200 & 400mg/kg respectively when compared to control group. The results have been tabulated in Table no. 4 and represented by Fig no. 4.

Table No. 1: Pylorus ligated ulcer model in rats
Effect of Ethanolic extract of peel of *Punica granatum* on volume of gastric acid

Treatment	Dose	Volume of gastric acid(ml) [Mean±SEM]
Control	(5ml/kg b.w)p.o	6.21±0.11
Ranitidine	(50mg/kg b.w)p.o	2.23±0.19***
Punica granatum	(200mg/kg b.w)p.o	4.80±0.55*
Punica granatum	(400 mg/kg b.w)p.o	3.95±0.44**

Fig No. 1: Pylorus Ligated ulcer model in rats
Effect of Ethanolic extract of peel of *Punica granatum* on volume of gastric acid



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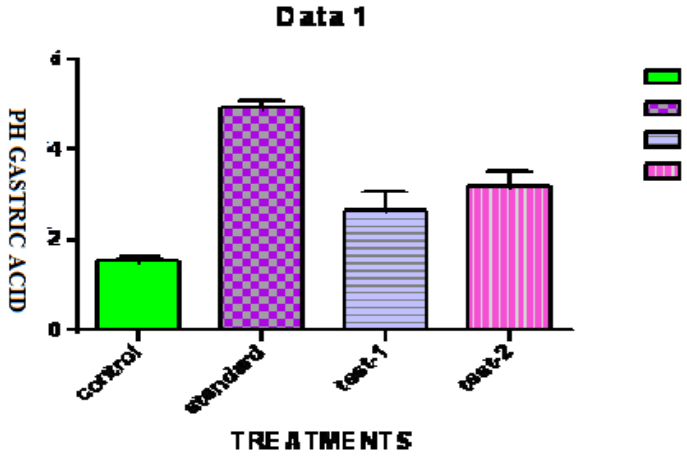
All values are expressed as Mean±(n=6)

*P<0.05, **P<0.001, ***P<0.0001 compared with control and analysed by One Way ANOVA followed by Dunnet's t-test.

Table No. 2: Pylorus Ligated ulcer model in rats
Effect of Ethanolic extract of peel of Punica granatum on pH of gastric acid

Treatment	Dose	pH [Mean±SEM]
Control	(5ml/kg b.w)p.o	1.53±0.08
Ranitidine	(50mg/kg b.w)p.o	4.91±0.18***
Punica granatum	(200mg/kg b.w)p.o	2.64±0.42*
Punica granatum	(400mg/kg b.w)p.o	3.21±0.29**

Fig No. 2: Pylorus ligated ulcer model in rats
Effect of Ethanolic extract of peel of *Punica granatum* on pH of gastric acid



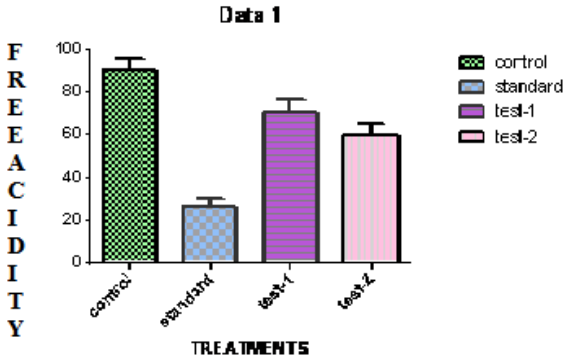
All values are expressed as Mean±SEM(n=6)

*P<0.05, **P<0.001, ***P<0.0001compared with control and analysed by One-way ANOVA followed by Dunnet's test.

Table No. 3: Pylorus ligated ulcer model in rats
Effect of Ethanolic extract of peel of *Punica granatum* on Free Acidity

Treatment	Dose	Free acidity [Mean±SEM]
Control	(5ml/kg b.w)p.o	90±5.16
Ranitidine	(50mg/kg b.w)p.o	25.8±34.16***
Punica granatum	(200mg/kg b.w)p.o	705.91*
Punica granatum	(400mg/kg b.w)p.o	59.16±5.32**

Fig No.3: Pylorus ligation ulcer model in rats
Effect of Ethanolic extract of peel of *Punica granatum* on Free Acidity

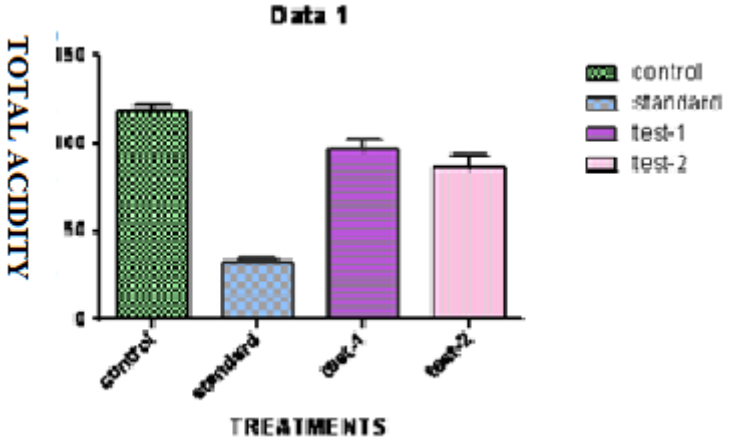


All values are expressed as Mean±SEM(n=6)
*P<0.05, **P<0.001, ***P<0.0001 compared with control and analysed by One Way ANOVA followed by Dunnet's t-test.

Table No. 4: Pylorus ligated ulcer model in rats
Effect of Ethanolic extract of *Punica granatum* on Total Acidity

Treatment	Dose	Total Acidity [Mean±SEM]
Control	(5ml/kg b.w)p.o	118.33±4.21
Ranitidine	(30mg/kg b.w)p.o	32.50±2.14***
Punica granatum	(200mg/kg b.w)p.o	96.66±6.28*
Punica granatum	(400mg/kg b.w)p.o	86.66±7.03**

Fig No.4: Pylorus Ligated ulcer model
Effect of Ethanolic extract of peel of *Punica granatum* on Total acidity



All values are expressed as Mean±SEM(n=6)
*P<0.05, **P<0.001, ***P<0.0001 compared with Control and analysed by One Way ANOVA followed by Dunnet's t-test.

3.4.5Effect of Ethanolic extract of peel of *Punica granatum* on ulcer index in Pylorus ligated ulcer model in rats:

Ulcer index in Ranitidine treated group decreased significantly up to 6.25±0.73, compared to control group in which the value was 17.16±0.42 and the percentage protection was 63.57%.

In groups treated with Ethanolic extract, the value of ulcer index was reduced significantly to 13.08±1.07 and 10.91±1.57 at doses of 200 & 400 mg/kg b.w., respectively when compared to control group whereas the percentage protection was 23.77% & 36.42% respectively. The results have been tabulated in Table No. 5 and represented by Fig no. 5.

3.5. Effect of Ethanolic extract of *Punica granatum* on ulcer index in Indomethacin induced gastric ulcers in rats:

Ulcer index decreased significantly in Misoprostol treated group upto 6.58±0.78, compared to control group in which the value was 20.41±0.81. The value of ulcer index reduced significantly in the groups treated with Ethanolic extract to 15.75±1.49 and 13.33±138 at doses of 200 & 400 mg/kg b.w., respectively when compared to control group. The result was significant at a dose of 400mg/kg b.w when compare to control group and the percentage protection was 34.68% as compared to 67.76% in Mesoprostol treated group. The results have been tabulated in Table no. 6 and represented by Fig no. 6.

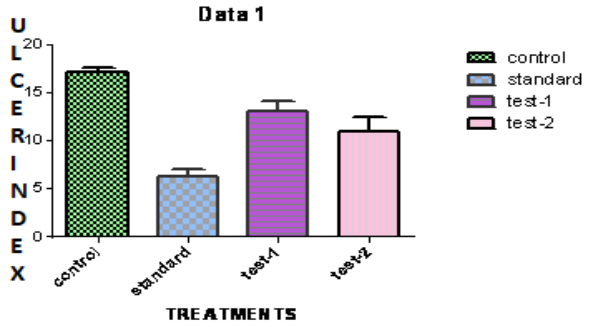
3.6. Effect of Ethanolic extract of *Punica granatum* on ulcer index on Cold restraint induced gastric ulcers in rats:

Ulcer index in Ranitidine treated group decreased significantly upto 6.66±0.7, compared to control group in which the value was 21.58±1.10. In groups treated with Ethanolic extract, the value of ulcer index was reduced significantly to 16.41±1.32 and 13.58±1.83 at doses of 200 & 400 mg/kg b.w respectively as compared to control group. The result was significant at a dose of 400mg/kg b.w when compared to control group and then percentage protection was 37.07%. The result has been tabulated in Table No. 7 and represented by Fig no. 7.

Table No. 5: Effect of Ethanolic extract of peel of *Punica granatum* on ulcer index in Pylorus ligated ulcer model in rats

Treatment	Dose	Ulcer Index [Mean±SEM]	%Protection
Control	(5ml/kg b.w)p.o	17.16±0.42	_
Ranitidine	(30mg/kg b.w)p.o	6.25±0.73***	63.57
Punica granatum	(200mg/kg b.w)p.o	13.08±1.07*	23.77
Punica granatum	(400mg/kg b.w)p.o	10.91±1.57**	36.42

Fig No.5: Effect of Ethanolic extract of peel of *Punica granatum* on ulcer index in Pylorus ligated ulcer model in rats

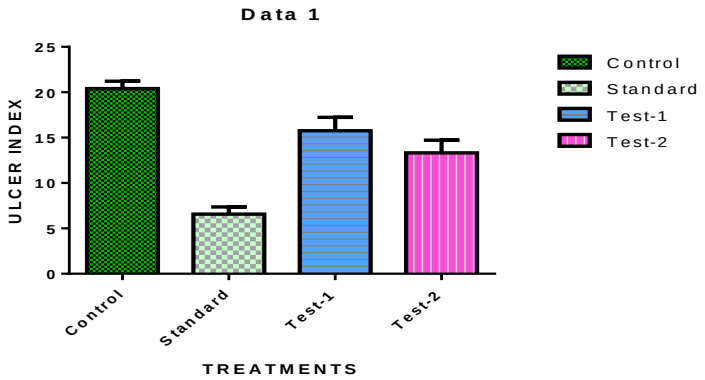


All values are expressed as Mean±SEM(n=6)
*P<0.05, **P<0.001, ***P<0.0001 compared with Control and analysed by One-way ANOVA followed by Dunnet's t-test.

Table No. 6: Effect of Ethanolic extract of peel of *Punica granatum* on ulcer index in Indomethacin induced gastric ulcers in rats

Treatment	Dose	Ulcer Index [Mean±SEM]	%Protection
Control	(5ml/kg b.w)p.o	20.41±0.81	-
Mesoprostol	(100*u*g/kg b.w)p.o	6.58±0.78***	67.76
Punica granatum	(200mg/kg b.w)p.o	15.75±1.49*	22.83
Punica granatum	(400mg/kg b.w)p.o	13.33±1.38**	34.68

Fig No. 6: Effect of Ethanolic extract of peel of *Punica granatum* on ulcer Index in Indomethacin induced gastric ulcers in rats

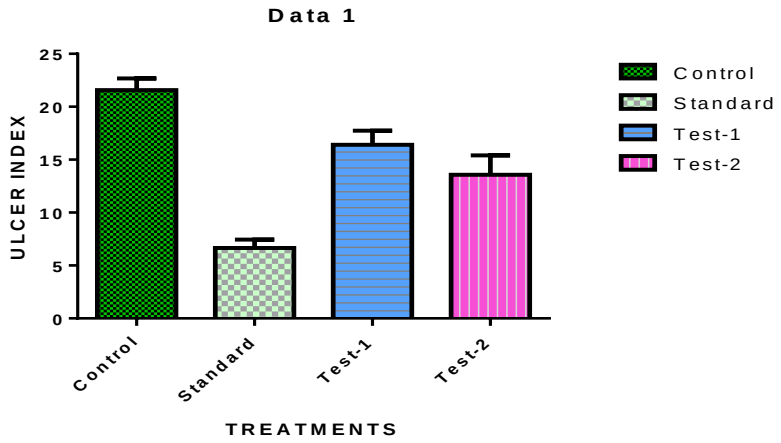


All values are expressed as Mean±SEM(n=6)
*P<0.05, **P<0.001, ***P<0.0001 compared with Control and analysed by One Way ANOVA followed by Dunnet's t-test.

Table No. 7: Effect of Ethanolic extract of peel of *Punica granatum* on ulcer index in Cold Restrain stress induced ulcers in rats

Treatment	Dose	UlcerIndex[Mean±SEM]	% Protection
Control	(5ml/kg b.w)p.o	21.58±1.10	-
Ranitidine	(30mg/kg b.w)p.o	6.66±0.78***	69.13
Punica granatum	(200mg/kg b.w)p.o	16.41±1.32*	23.95
Punica granatum	(400mg/kg b.w)p.o	13.58±1.83**	37.07

Fig No. 7: Effect of Ethanolic extract of peel of *Punica granatum* on ulcer Index in Cold Restraint stress induced ulcers in rats



All values are expressed as Mean±SEM (n=6)
*P<0.05, **P<0.001, ***P<0.0001 compared with Control and analysed by One Way ANOVA followed by Dunnet's t- test.

4. DISCUSSION

The present study was designed to evaluate the anti-ulcer activity of 50% Ethanolic extract of peel of *Punica granatum*. Three models, viz. Pylorus ligation, Indomethacin induced, and Cold restraint stress induced ulcer models were used for the evaluation of Anti-ulcer activity. Ulcer indexes, Volume of gastric HCl, pH of gastric HCl, Free and Total acidity of gastric HCl were the parameters used for the assessment of anti-ulcer activity.

Preliminary qualitative phytochemical analysis of 50% Ethanolic peel extract of *Punica granatum* showed the presence of Carbohydrates, Tannins,

Flavonoids, Glycosides, Alkaloids, Phenols Gums and Mucilages, Terpenoids, Sterols.

Acute Toxicity Studies revealed that 50% Ethanolic extract of peel of *Punica granatum* was found to be practically non-toxic upto a dose of 2000mg/kg b.w when given orally. The study was conducted using two doses i.e., 200mg/kg b.w and 400mg/kg b.w p.o.

In Pylorus ligation induced gastric ulcer model, there is an accumulation of the gastric acid in the stomach which reduces the gastric pH and thereby due to high acidity it induces gastric ulcers.

In the present study, upon pylorus ligation, there was a decrease in the volume of gastric acid after the administration of 50% Ethanolic extract of *Punica granatum* (200 and 400mg/kg b.w p.o) when compared with that of control group. The decrease in the gastric acid was found to be dose dependent.

In addition, the extract showed a significant increase in the pH of gastric acid which was also dose-dependent when compared to control group, similarly, in this pylorus ligation model there was also a reduction observed in its free acidity, total acidity and ulcer index after treatment with the extract, and an inference may be drawn that the extract may be having an Anti-ulcer activity.

It is seen that various plants from ethanopharmacological studies have proved to have Anti-ulcer activity, plants such as *Alpinia galangal* (Ethanolic extract of rhizome) 500mg/kg b.w. p.o., showed the Anti-ulcer activity in pylorus ligation ulcer model and stress induced ulcer model. There was reduction in ulcer index up to 0.16±0.00 as compared with the control in which the value was 2.0_+0.06 and it also reduced volume of gastric acid secretion up to 6.16±0.41 as compared with the control (9.41±1.09).

In the NSAIDs induced gastric ulcer model, the ulcers in rats by the administration of Indomethacin (5mg/kg b.w p.o) there will be an elimination of the prostaglandins from the stomach, which may cause ulceration, as it is known that the prostaglandins have a major role in the gastric mucous production, hence the elimination of prostaglandins reduced the protective mucous secretion and increases acid secretion, thereby leading to the ulceration.

In the present study, in gastric ulcers model induced by Indomethacin the values of ulcer index were reduced in the treated group (13.33±1.38) and there was a significant reduction in ulcer index (**P<0.001) as compared with the control group (20.41±0.81), and the reduction in ulcer index was found to be dose dependent. This is in accordance with the results of Ethanolic extract of whole plant of Swertia *chirata* against Indomethacin induced gastric ulcers where the extract reduced ulcer index to 20.33±3.23 as compared to control 34±4.05.

The ulcer can also be induced by subjecting the animals to the stress; viz in Cold restraint stress induced ulcer model, the mechanism of inducing ulcer in this model can be explained as during stress, gastrointestinal peristalsis becomes disturbed and the gastrointestinal lining may become abraded, buckled or even broken. Once this happens, the gastric acid can erode the wall behind the lining. During stress there can be an increase in gastric acidity; this may ulcerate even a normal, intact mucinoid lining.

In the present study, the results in the cold restraint stress induced ulcer model were significant and the ulcer was seen to be reduced upon increasing the dose of extract (400mg/kg b.w. p.o) in treated group (13.58±1.83) and there was a significant reduction in ulcer index (**P<0.001) when compared to the control group (21.58±1.10).

Thus, the results obtained in the present investigation is suggestive of the 50% Ethanolic extract of peel of *Punica granatum* in rats having Anti-ulcer activity which may be due to the presence of Tannins. Further qualitative studies are required for the phytochemical which is responsible for the specific observed effects.

5. CONCLUSION

Present study was conducted to evaluate the Anti-ulcer activity of 50% Ethanolic extract of peel of *Punica granatum* using Pylorus ligation model, NSAIDs induced and restraint stress induced ulcer model.

50% Ethanolic extract of peel of *Punica granatum* was found to be practically non-toxic up to a dose of 2000mg/kg b.w. when given orally

In Pylorus Ligation Method 50% Ethanolic extract of peel of *Punica granatum* (200 & 400mg/kg b.w.), produced significant dose dependent decrease in ulcer, volume of gastric HCl, pH of gastric acid, free acidity and total acidity.

In NSAIDs induced and cold restraint stress induced gastric ulcer models, 50% Ethanolic extract produced significant dose dependent decrease in ulcer index, and it was found that maximum ulcer protective activity was shown by Ethanolic extract when given orally at a dose of 400 mg/kg body weight.

The effect of Anti-ulcer activity of *Punica granatum* seems to be dose dependent and significant. The presence of Flavonoids which have astringent property could be responsible for the Anti-ulcer activity, is more likely to be involved in the reaction with the proteins of the layer tissues and thereby showing the activity.

Having confirmed the Anti-ulcer activity of *Punica granatum* on GIT, they deserve further studies to identify which exact chemical constituents of the Ethanolic extract mediated the specific observed effects and investigate their mechanism, as also the isolation and characterization of active principles responsible for Anti-ulcer activity.

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CONFLICT OF INTEREST:

We declare that there was no conflict of interest in this research work.

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