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EVALUATION OF ANTI-ULCER AND ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *ANNONA RETICULATA* LINN.

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ABSTRACT

Annona reticulata are used as source of medicine and also for industrial products. It possesses several medicinal properties such as anthelmintic, analgesic, antiulcer, antipyretic, wound healing, anti-inflammatory and cytotoxic effects. It is contains phytochemicals like tannins, flavonoids, alkaloids, phenols, glycosides and steroids. Peptic ulcer is a major disease of gastrointestinal tract, affecting about 40 lakh people each year worldwide and affects 10% of world population with different aetiologies. **Aim**: To evaluate the antiulcer and analgesic activity of methanolic extract of the leaves of *Annona reticulata* (MEAR) on albino rats. **Materials and Methods:** The animals were divided into four groups as control, standard, test 1 (extract: 100 mg/kg) and test 2 (extract: 200 mg/kg) with six rats in each group. Gastric lesions were induced by oral administration of ethanol (1ml/200g), indomethacin (20 mg/kg), pylorus ligation and water immersion stress. Standard group of animals were treated with ranitidine (50mg/kg) and test group of animals were treated with MEAR at doses of 100 and 200 mg/kg. To determine the antiulcer activity of extract, mean ulcer index, % ulcer protection, volume of gastric juice, pH, free acidity and total acidity were evaluated. To determine analgesic activity by using tail flick and hot plate method diclofenac were used as standard drug and 15 sec was considered as cut off time in both methods. **Results:** The extract was evaluated for antiulcer and analgesic activity. In acute toxicity study, MEAR was found to be safe till 2000mg/kg, So the doses of MEAR at various concentration of 100 and 200mg/kg body weight was administered orally for prevention of ulcer from various induced ulcer model. Analytical parameters like Percentage of ulcer protection was calculated based on Ulcer index and Gastric juice volume,

pH, free acid and total acidity of gastric juice. The analgesic activity of extract at both dose was found to be more significant as compare to the standard drug. **Conclusion:** The MEAR has shown significant antiulcer and analgesic activity at both 100mg/kg and 200mg/kg dose level in a dose dependent manner. Antiulcer activity of *A. reticulata* leaves may be due to cytoprotective, antisecretory and antioxidant potential of phytoconstituents present in the extract.

KEYWORDS: *Annona reticulata*, pylorus ligation, ulcer index, gastric juice, tail flick method.

INTRODUCTION

Peptic ulcer is the most common gastrointestinary disorder in clinical practice.[1] It is a defect in the lining of the stomach or the first part of the small intestine (duodenum). It becomes one of the must public health problem with high rate of morbidity and substantial mortality and has become the focus of experimental and clinical investigations, mainly due to its high prevalence in the global population.[2] Every year peptic ulcer affects nearly four million people worldwide and affects 10% of world population with different aetiologies.[3, 4] Ulcers are an open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue. A gastric ulcer can give epigastric pain during the meal, as gastric acid production is increased after the meal and food enters the stomach. Symptoms of duodenal ulcers would initially be relieved by a meal, as the pyloric sphincter closes to concentrate the stomach contents; therefore acid is not reaching the duodenum.[5]

Peptic ulcers are usually aggravated by the imbalance between the destructive and defensive factors in the stomach. The endogenous destructive factors in the stomach are HCl, biliary reflux, pepsin, lipid peroxidation and the formation of reactive oxygen species (ROS) and the exogenous factors are excessive due to excessive use of ethanol, indiscriminate use of non-steroidal anti-inflammatory drugs (NSAID), stress, smoking, and infection by *Helicobacter pylori* bacteria. The defensive factors are mucus-bicarbonate barrier, prostaglandins (PGs), mucin secretion, surface phospholipids, nitric oxide (NO), mucosal blood flow, cell renewal, growth factors, and antioxidant enzymes.[6-10]

Oxidative stress, present in the process of gastric ulceration, increases the formation of ROS that can disrupt epithelial cell integrity. An excess production of ROS metabolites may overwhelm the endogenous antioxidants.[11] Along with this, ROS accumulates neutrophils in

the tissues of the mucosa during gastric ulceration. Studies have shown that pro-inflammatory cytokines induce the activation of neutrophils and are strong contributors to the of ulcer damage.[12, 13]

Effective therapies for peptic ulcers use alternatives that control acidic hyper secretion and its direct effects on the gastric mucosa. There are two main classes of drugs used to treat acid-related disorders include proton pump inhibitors (PPI) that inhibit the hydrogen pump in the parietal cell directly, independently of any membrane receptor stimulation and histamine type 2 receptor antagonists (H2RAs), which block the histamine receptor on parietal cells thereby reducing hydrogen ion release.[14] PPI is among the most prescribed drugs in the world; however, it may lead to the development of parietal cell hyperplasia of the gastric glands.[15] Long-term use of H2RAs is associated with the development of undesirable effects such as gynecomastia and galactorrhea as well as alteration of the bacterial flora of the gastrointestinal tract.[16] In *H. pylori* colonization, there is increased gastrin responses, the increase in acid can contribute to the erosion of the mucosa and therefore ulcer formation. In Western countries the percentage of people with *Helicobacter pylori* infections roughly matches age (i.e., 20% at age 20, 30% at age 30, 80% at age 80 etc).[17]

According to the International Association for the Study of Pain (IASP), pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage".[18] Common drugs for pain relief like aspirin and morphine have been widely used in recent decades. In most instances, these analgesic drugs, particularly opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), can only relieve 50% of the pain in about 30% of patients.[19]

In addition to many of these drugs cause serious side effects. Studies have shown that opiates cause physical dependency, tolerance and addiction while NSAIDs usually cause gastrointestinal disorders.[20]

As such, research need to discover other alternatives to treat pain is crucial. Various medicinal herbs have been used for centuries for therapeutic purposes. Many of these herbs with analgesic activity had been used without any adverse effects or less adverse effect.

Annona reticulata Linn. (Bullock's heart) is one of the traditionally important plant used for the treatment of various ailments.[21] It belongs to family Annonaceae.[22] The synonyms of

plant are Ramphal, Bullock's heart and Custard apple.[23] Numerous phytoconstituents have been identified from different parts of *A. reticulata*. Stem bark contains tannins, alkaloid and phenolic compounds. Leaves contain wide range of chemicals like alkaloids, amino acids, carbohydrates, steroids, flavonoids, proteins, tannins, glycosides and phenolics. In the same way root has been identified for the content of acetogenin, alkaloid, carbohydrates, proteins, flavonoids, tannins. The plant also found to be rich in minerals such as Ca, P, K, Mg, Na, Cl, S, Mn, Zn, Fe, Cu, Se, Co, Ni and Cr.[24, 25] Traditionally the plant has been employed for the treatment of epilepsy, dysentery, cardiac problem, parasite and worm infestations, constipation, haemorrhage, bacterial infection, dysuria, fever, ulcer and as insecticide. Bark is a powerful astringent and used as a tonic whereas leaves used for helminthiasis treatment.[26, 27] Even today, most of the people traditionally use herbal medicine for primary health care in developing countries because of lesser side effects.[28, 29] The aim of the present study was to evaluate the antiulcer activity of MEAR on albino rats.

Regulation of Acid Secretion by Parietal Cells

The regulation of acid secretion by parietal cells is especially very important in the pathogenesis of peptic ulcer, and constitutes a particular target for drug action. The secretion of the parietal cells is an isotonic solution of HCl (150 m mol/l) with a pH less than 1, since the concentration of hydrogen ions is more than a million times higher than that of the plasma. The Cl- are actively transported into canaliculi in the cells which communicate with the lumen of the gastric glands and thus with the stomach itself. This Cl- secretion is accompanied by K+, which is then exchanged with H+ from within the cell by a K+/H+ ATPase and bicarbonate ions. Later on there is exchanges across the basal membrane of the parietal cell for Cl-. The principal stimuli acting on the parietal cells are.

Gastric: Gastrin which is a peptide hormone synthesized in endocrine cells of the mucosa of the gastric antrum and duodenum, and secreted into the portal blood. Its main action of this hormone is stimulation of the secretion of acid by the parietal cells. Gastrin indirectly increases pepsinogen secretion, gastric motility and stimulates blood flow. The release of this hormone is controlled both by neuronal transmitters and blood-borne mediators as well as the chemistry of the stomach contents. The amino acids and small peptides that directly stimulate the gastrin secreting cells.

Acetylcholine: Acetylcholine which is released from neurons (e.g. Vagal) and stimulates specific muscarinic receptors on the surface of the parietal cells and on the surface of histamine containing cells.

Histamine: Histamine within the stomach, mast cells (or histamine containing cells similar to mast cells) lying close to the parietal cell release a steady basal release of histamine, which is further increased by gastrin and acetylcholine. The hormone that acts on parietal cell H2 receptors, which are responsive to histamine concentrations that are below the threshold required for vascular H2 receptor activation.

Prostaglandins: Prostaglandins (mainly E2 and I2), synthesised in the gastric mucosa mainly by cyclooxygenase-1, stimulate mucus and bicarbonate secretion, decrease acid secretion and cause vasodilatation, which serve to protect the stomach against damage from the gastric secretion.[30]

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of *Annona reticulata* was collected from the forest, nearby Tirupati and authenticated by the botanist. The collected plant material (leaves) was washed thoroughly with water to remove the adhering soil, mud, and debris. All insect damage or fungus infected leaves were removed. The plant material was dried in the shade at room temperature to a constant mass. The plant material was coarsely powdered using blender. The powder was stored in an air tight container and protected from light.

Extraction and Isolation[31]

Collected *Annona reticulata* Linn. Leaves were converted to small pieces then dried under the shade at room temperature. Powder of dried leaves about 200g were prepared using grinder. Then, the methanolic extract was obtained by Soxhlet extractor using 1 L methanol for 8 h at 64^0c and sample was concentrated with the help of rotary evaporator and extract obtained were used for further study.

Experimental Animals[32]

Wister albino rats of either sexes weighing 160-180 g were used. The animals were housed in polypropylene cages with free access to food and water at 24±2^{o}C, relative humidity of 40-45% and in a 12:12 hour light and dark cycle. The rats were divided into four groups of six

animals in each and acclimatised to laboratory atmosphere for a minimum of seven days prior to the study and were used only once throughout the experiment. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) at Karnataka College of Pharmacy, Bangalore, India. Studies were performed in accordance with the CPCSEA guidelines.

Acute Toxicity[33]

Acute toxicity study was conducted for the methanolic leaves extract of *Annona reticulata* as per OECD guidelines 425 using Swiss albino mice. Swiss albino mice of either sex (five mice) was randomly selected were used for this study. Animal was kept overnight fasting with free access to water but not food, next day each animal was administered methanolic extracts by oral route. The animals were observed for any changes continuously for the first 2 h and up to 48 h for mortality and then next dose was given to other animal. There was no mortality and noticeable behavioral changes in all the groups tested up to 14 days. The extracts were found to be safe up to 2000 mg/kg body weight and dose of 5000 mg/kg was found to be toxic.

Preparation of Dose

A dose of $1/10^{th}$ and $1/20^{th}$ of 2000mg/kg were considered to be high dose and low dose prepared by dissolving in miliQ water. The doses were prepared as per the OECD guideline no. 425.

Animal Models Used in the evaluation of Antiulcer Activity

Various screening models were used for the screening of the antiulcer activity. It helps to understand the aetiology of the ulcer and screening of antiulcer agents.

Rats were divided into four groups of six animals each and all the animals were fasted for 24 hours before the study but had free access to water.

Group-1: Control, received only distilled water

Group-2: Standard, received ranitidine (50mg/kg/po)

Group-3: Test group, receives MEAR (100mg/kg/po)

Group-4: Test group, receives MEAR (200mg/kg/po)

Ethanol induced ulcer: In this method after 1 hr of treatment to different groups, gastric ulcers were induced in rats by administrating absolute ethanol (99%) (1 ml/200 g) orally to

all groups. Rats were kept in special constructed cages to prevent coprophagia during and after the experiment. The animals were anaesthetized one hour later with anaesthetic ether and stomach was incised along the greater curvature and its contents were collected into tubes for analysis of volume of gastric juice, pH, total and free acidity and ulceration was scored to find ulcer index and percentage of ulcer protection.[34]

Pylorus Ligation Induced Ulcer: After 1 hr of treatment to different groups, the animals were anaesthetized using thiopentone sodium (35 mg/kg, i.p.), the abdomen was opened and pylorus ligation was done without causing any damage in its blood supply to all groups. After 4 hr their stomachs were dissected and its contents were collected into tubes for analysis of volume of gastric juice, pH, total and free acidity and ulceration was scored to find ulcer index and percentage of ulcer protection.[35]

Water immersion stress induced ulcers: Stress induced ulcers were induced by force swimming in the glass cylinder (height 45cm, diameter 35cm) containing water up to 35cm maintained at 35^{o}c for 3 hrs. All the animals were fasted 24hrs prior to the experiment. After the drug treatment (standard/test) animals were allowed to swim for 3hrs then animal were dissected stomachs were removed. All stomachs were opened along the greater curvature and its contents were collected into tubes for analysis of volume of gastric juice, pH, total and free acidity and ulceration was scored to find ulcer index and percentage of ulcer protection.[36]

Indomethacin induced ulcers: All the animals were fasted 24 hours before administration of Indomethacin. Each rat was administered with the dose of 20 mg/kg Indomethacin orally.30 min prior to the administration of the Indomethacin, standard/test drug was administered. The rats were anaesthetized with ether 1 hour later, the stomach was incised through the greater curvature and its contents were collected into tubes for analysis of volume of gastric juice, pH, total and free acidity and ulceration was scored to find ulcer index and percentage of ulcer protection.[37]

Determination of Ulcer Index

The rats were sacrificed; abdomen was opened by making an incision. The gastric contents were collected for the determination of total acid output and the stomach was washed with water. Ulcer index was calculated with the help of an ulcer score scale.

Normal stomach without any red colouration= 0

Red colouration= 0.5

1 Ulcer spot= 1

Haemorrhagic streaks= 1.5

3-5 ulcer spots= 2

More than 5 ulcer spots= 3

Mean ulcer score for each group was calculated and recorded as Ulcer index.[38, 39]

% Ulcer protection was calculated according to;

% Ulcer protection = [(UIC-UIT)/UIC] ×100

Where, UIC-ulcer index of control.

UIT-ulcer index of test[40]

Determination of Total Gastric Output

Gastric contents of all groups of rats were collected and centrifuged at 2000 rpm for five minutes. Supernatant clear fluid was pipetted out. A 1 ml of this fluid was mixed with 9 ml of distilled water and titrated with 0.01 N sodium hydroxide, one drop phenolphthalein and two to three drop of Topfer's reagent being used as an indicator. Titre value was recorded at the end point (yellow to salmon pink/orange yellow colour) which was expressed as free acid. Titration was continued till the appearance of pink colour and the titre value was recorded again which was expressed as total acid.[41]

Acid strength (in m eq/L) = (Volume of NaOH x Normality/0.1) x 100

The comparison among control, standard and test groups was done in tabular manner.

Animal Models Used in the evaluation of Analgesic Activity

Analgesic activity of the leaves of the *Annona reticulata* was estimated by Tail Flick method and Eddy's hot plate method.

Rats were divided into four groups of six animals each and all the animals were fasted for 24 hours before the study but had free access to water.

Group-1: Control, received only distilled water

Group-2: Standard, received Diclofenac (10mg/kg/ip)

Group-3: Test group, receives MEAR (100mg/kg/po)

Group-4: Test group, receives MEAR (200mg/kg/po)

Tail-Flick Method: Antinociceptive (analgesic) activity of the extract was evaluated by the tail-flick method described.[42] About 5 cm from the distal end of the tail of each rat was immersed in warm water maintained at 50^{o}C. The reaction time (in seconds) is the time taken by the rat to flick its tail due to pain. The first reading was omitted and reaction time was taken as the average of the next two readings. The reaction time was recorded before (0min) and at 15, 30, 45 and 60 min after the administration of the treatments to different groups. The maximum reaction time was fixed at 15 sec to prevent any tail tissue injury due to worm water. If the reading exceeds 15 sec, tail should remove from water and it is called as cut off time.

Eddy's Hot Plate Method: Woolfe and MacDonald (1944) originally developed this.[43] The paws rats are very sensitive to heat at mild temperature. The end point taken is in the form of jumping or the licking of the paws.[44-48] The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5^{0}C. A period of 15˙sec was kept as cut off period to avoid damage to the paw. Type of response and reaction time were noted using a stopwatch at 0 min, 15 min, 30 min, 45 min and 60 minutes.

Statistical Analysis

The results were expressed as the mean±SEM. Statistical differences were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Results were considered to be statistically significant at $p<0.05$.

RESULTS

Phytochemical analysis

On phytochemical analysis of MEAR, the extract has shown the presence of steroids and triterpenes, alkaloids, saponins, phenolic compounds and tannins, flavonoids and carbohydrates.

Results of Acute oral toxicity

The LD50 of the leaves extract of *Annona reticulata* was found to be 2000mg/kg after performing the acute oral toxicity studies. 1/10th and 1/20th of the same dose was selected (200mg/kg and 100mg/kg respectively) and the study was carried out.

Antiulcer Activity

Effect of MEAR on ethanol induced gastric ulcer model

Table 1 and fig 1, 2 and 3 indicates the significant antiulcer activity of MEAR at the both doses. Ulcer index reduced at both doses, but the dose 200 mg/kg is significant one. Percentage of ulcer protection of 200 mg/kg is more when compared to 100 mg/kg. The volume of the gastric juice in ml is also reduced in 200 mg/kg. PH of the gastric juice at 200mg/kg was found to be more significant when compared to control group and free acid and total acidity was found to be as significant as standard. Hence, it can be said that both extracts have antiulcer activity, but at 200 mg/kg is more potent.

Table no. 1: Effect of MEAR on ethanol induced gastric ulcer.

Sl. No	Treatment	Ulcer index (mean±SEM)	% Ulcer protection	Vol. of gastric juice (ml)	pH	Free acid (meq/L/100g)	Total acidity (meq/L/100g)
1	Control	4.5±1.87	-	2.69±0.04	3.6±0.12	27.12±0.58	45.47±0.22
2	Ranitidine (50 mg/kg)	1.59±1.12***	64.67%	1.45±0.02***	5.83±0.31***	14.46±0.24***	28.17±0.34***
3	MEAR (100 mg/kg)	2.72±2.22**	39.56%	2.29±0.08**	3.76±0.34**	22.75±0.39**	36.26±0.66**
4	MEAR (200mg/kg)	1.89±0.89***	58.0 %	1.92±0.07***	5.41±0.52***	16.33±0.65***	31.23±0.56***

n = 6 animals in each group; values are Mean±SEM; ** $p < 0.05$, *** $p < 0.05$ when compared to control (By using one way ANOVA followed by Dunnett t-test)

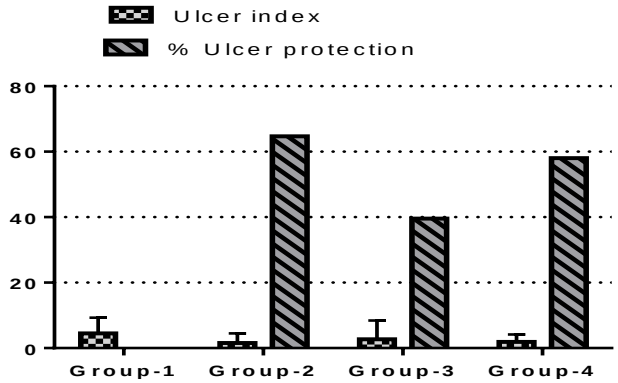

Fig no. 1: Effect on Ulcer index and % ulcer protection.

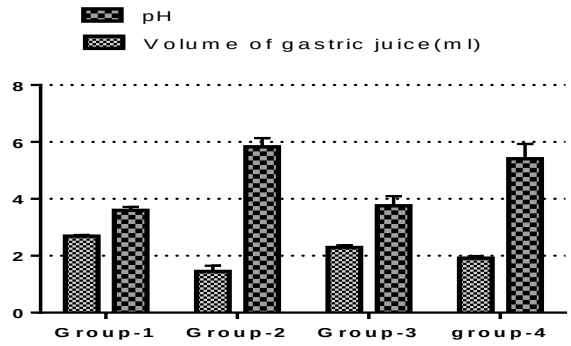

Fig no. 2: Effect on Volume of gastric juice and Ph.

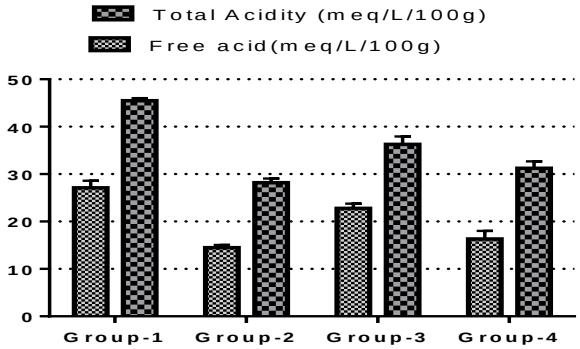

Fig no. 3: Effect on free acid and total acidity.

Effect of MEAR on Indomethacin induced gastric ulcer model

Table 2 and fig 4, 5 and 6 shows the dose dependent ulcer reduction and increase in the ulcer protection at higher dose. The volume of gastric juice also reduced at higher dose. The pH also shows significant increment and acidity of the juice was also reducing significantly at higher dose. Hence MEAR shows significant activity at 200 mg/kg on comparing to control and standard.

Table no. 2: Effect of MEAR on Indomethacin induced gastric ulcer.

Sl. No	Treatment	Ulcer index (mean±SEM)	% Ulcer protection	Vol. of gastric juice (ml)	pH	Free acid (meq/L/100g)	Total acidity (meq/L/100g)
1	Control	2.5±0.92	-	3.31±0.34	2.65±0.67	54.28±3.48	67.52±1.42
2	Ranitidine (50 mg/kg)	0.57±1.23***	77.20%	2.64±0.72***	4.48±0.45***	23.35±1.32***	27.37±0.94**
3	MEAR (100 mg/kg)	1.25±1.52**	50.00%	3.29±0.25**	3.88±0.65**	45.55±2.33**	57.72±1.67**
4	MEAR (200mg/kg)	0.93±0.34***	62.80 %	2.97±0.48***	4.29±0.55***	26.74±2.38***	35.53±1.26***

n = 6 animals in each group; values are Mean±SEM; **p<0.05, *** p < 0.05 when compared to control (By using one way ANOVA followed by Dunnett t-test)

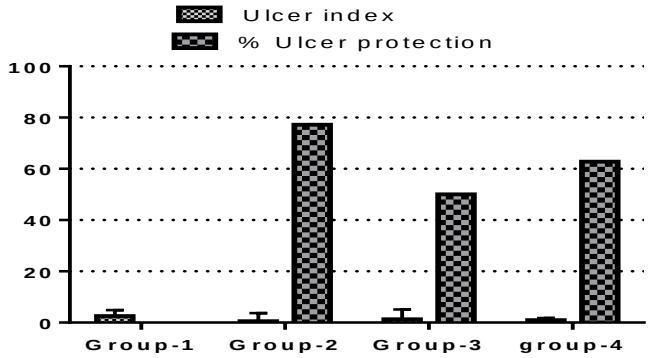

Fig no. 4: Effect on Ulcer index and % ulcer protection.

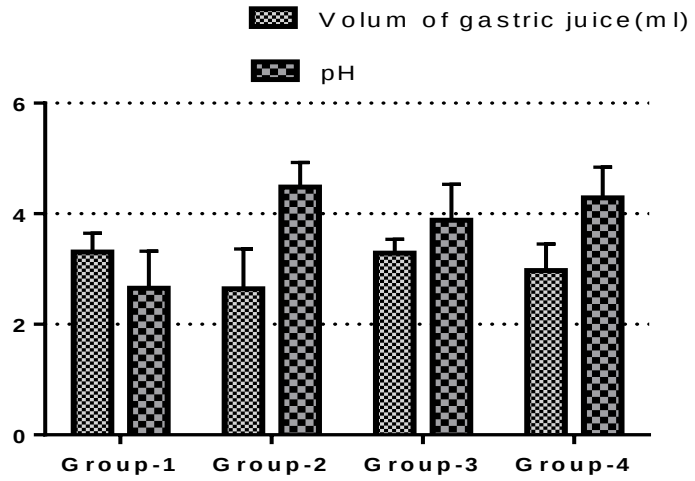

Fig no. 5: Effect on Volume of gastric juice and pH.

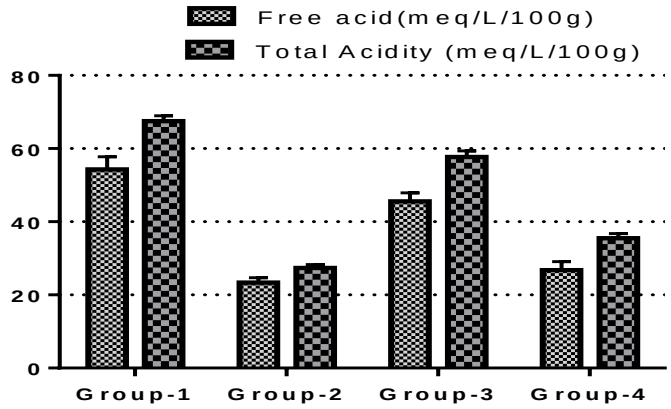

Fig no. 6: Effect on free acid and total acidity.

Effect of MEAR on Stress induced gastric ulcer model

Table 3 and fig 7, 8 and 9 show the stress induced ulcer. At the high dose of the drug show the significant reduction in the ulcer index and there is significant ulcer protection as compare

to the standard. The volume of gastric content also decreases and pH value is increase on higher dose. There is decrese in free acid and total acidity as the dose of drug increases. Thus, both dose show the antiulcer activity but higher dose show more sinificant activity.

Table no. 3: Effect of MEAR on Stress induced gastric ulcer.

Sl. No	Treatment	Ulcer index (mean±SEM)	% Ulcer protection	Vol. of gastric juice (ml)	pH	Free acid (meq/L/100g)	Total acidity (meq/L/100g)
1	Control	3.85±0.56	-	3.63±0.55	2.33±0.76	48.56±0.88	56.34±1.12
2	Ranitidine (50 mg/kg)	1.71±0.63***	55.58%	1.94±0.65***	3.68±0.65***	36.54±2.12***	45.17±0.69**
3	MEAR (100mg/kg)	2.36±1.20**	38.70%	2.89±0.60**	3.14±0.49**	46.45±1.48**	52.38±1.92**
4	MEAR (200mg/kg)	2.14±0.67***	44.42%	2.27±0.78**	3.38±0.42***	39.62±0.98***	48.53±0.96***

n = 6 animals in each group; values are Mean±SEM; **p<0.05, *** p < 0.05 when compared to control (By using one way ANOVA followed by Dunnett t-test)

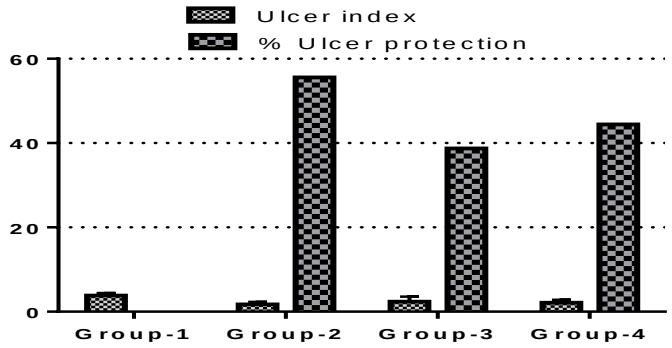

Fig no. 7: Effect on Ulcer index and % ulcer protection.

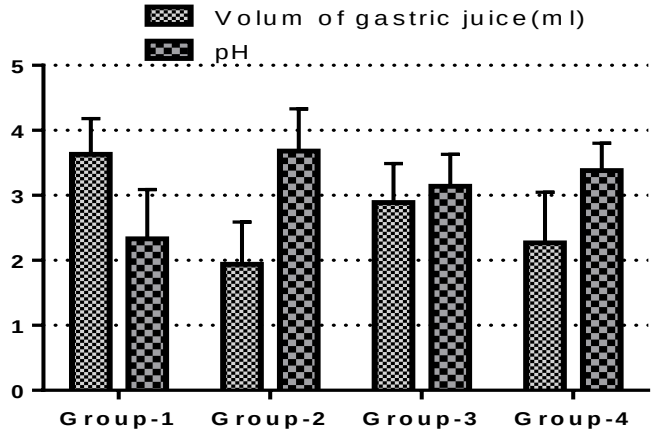

Fig no. 8: Effect on Volume of gastric juice and pH.

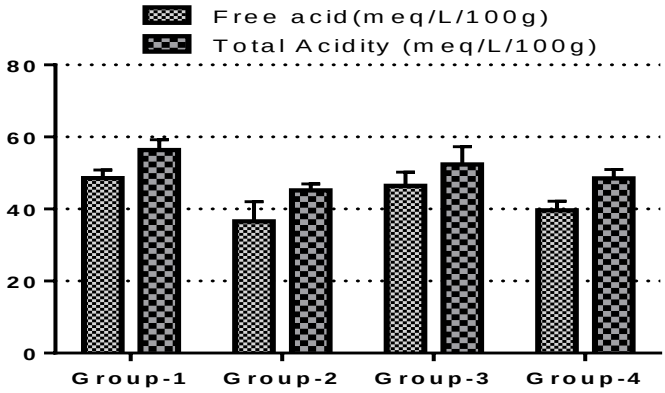

Fig no. 9: Effect on free acid and total acidity.

Effect of MEAR on pyloric ligation induced gastric ulcer model

Table 4 and fig 10, 11 and 12 show the pyloric ligation induced ulcer. A significant improvement in the level of inhibition against ulceration was observed in the extract treated animals. The extract at 200mg/kg show the better protection against the ulceration than the 100mg/kg regimen and compare to the standard drug used. Pre-treated with the extract produced significant increase in pH value coupled with decrease in gastric volume when compared with ulcerated control rats. There is also significant decrease in the free acid and total acidity in the extract treated animals as compare to the ulcerated animals.

Table no. 4: Effect of MEAR on pyloric ligation induced gastric ulcer.

Sl. No	Treatment	Ulcer index (mean±SEM)	% Ulcer protection	Vol. of gastric juice (ml)	pH	Free acid (meq/L/100g)	Total acidity (meq/L/100g)
1	Control	3.17±0.49	-	4.67±0.14	2.13±0.70	68.52±0.73	76.34±1.05
2	Ranitidine (50 mg/kg)	0.68±0.76***	78.55%	2.33±0.55***	3.37±0.65***	28.69±1.11***	35.67±0.86***
3	MEAR (100mg/kg)	2.17±0.62**	31.54%	3.59±0.63**	3.11±0.47**	56.65±0.78**	62.33±1.22**
4	MEAR (200mg/kg)	0.86±0.57***	72.87%	2.68±0.72***	3.48±0.49***	36.72±0.74***	44.63±0.88***

n = 6 animals in each group; values are Mean±SEM; ** $p < 0.05$, *** $p < 0.05$ when compared to control (By using one way ANOVA followed by Dunnett t-test)

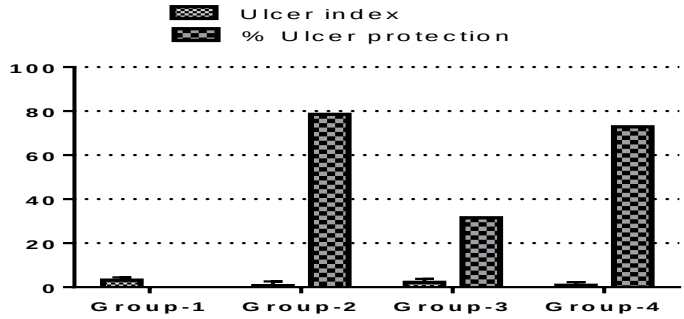

Fig no. 10: Effect on Ulcer index and % ulcer protection.

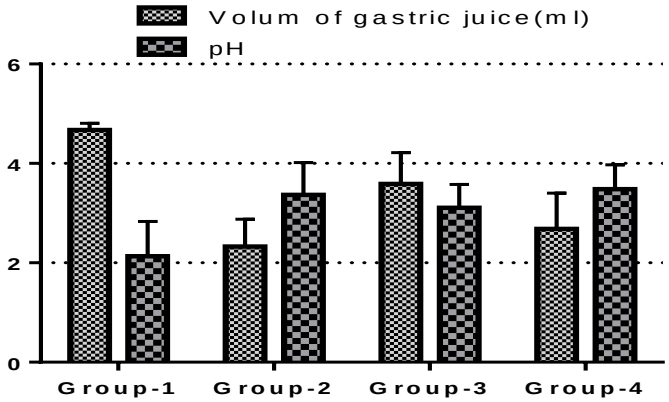

Fig no. 11: Effect on Volume of gastric juice and pH

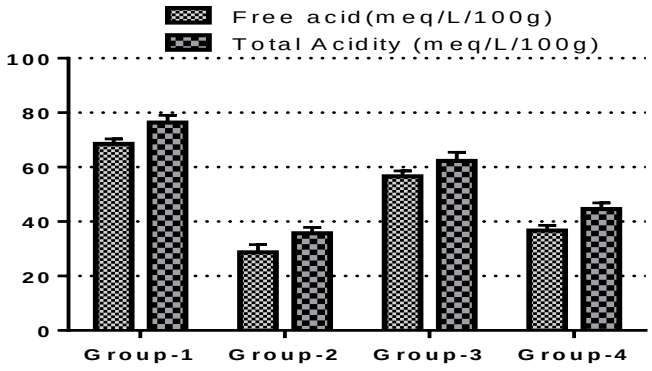

Fig no. 12: Effect on free acid and total acidity

Analgesic Activity

The phytochemical analysis revealed the presence of terpenes, steroids, alkaloids, flavonoids, tannins and glycosides. The extracts of *Annona reticulata* leaves showed significant analgesic activity at a dose of 100 mg/kg and 200mg/kg.

Analgesic effect of MEAR by tail-flick method in rats

The results of the analgesic activity of the methanol extract of the leaves of *Annona reticulata* are shown in Table 5. Rats treated with normal saline (negative control) did not show any significant difference in the reaction time on tail-flick throughout the 60 min observation. In comparison with the baseline values within the same treatment groups, the increase in reaction time at different time points significantly differed ($P < 0.05$) for all the treated groups. Duration of the reaction time in diclofenac and extract treated animals was significantly higher compared to saline treated animals. The highest reaction time for the extract treated group was 9.50 sec at 60 min for 100mg/kg, while it was 11.12 sec for 200mg/kg. On comparing all the treated group with control group it was found to be significant p<0.05 as shown in the table below and it is also found that the both dose of extract show more significant than diclofenac as shown in fig 13.

Table 5: Analgesic effect of MEAR by tail-flick method

Treatment	0 min	15 min	30 min	45 min	60 min
Control	4.75±0.85	4.50±0.68	3.58±0.87	4.32±0.47	5.89±0.54
Diclofenac (10 mg/kg)	4.50±0.64	4.75±1.25	5.64±0.95*	7.75±0.85**	8.25±1.11**
MEAR (100 mg/kg)	4.32±1.08	5.00±0.41	5.75±0.25*	8.54±1.47**	9.50±0.88**
MEAR (200mg/kg)	4.21±0.41	5.25±1.13	6.57±0.87*	8.75±2.21**	11.12±1.68**

n = 6 animals in each group; values are Mean±SEM; * p < 0.05, ** p < 0.05 when compared to control (By using one way ANOVA followed by Dunnett t- test).

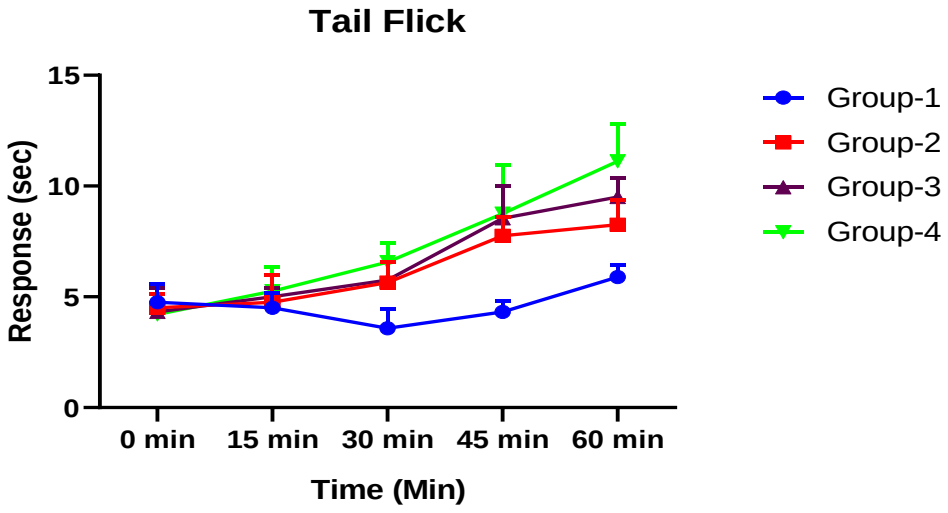

Fig no. 13: Effect of MEAR by tail-flick method in rats.

Analgesic effect of MEAR by hot plate method in rats

The results of the analgesic effect of the methanol extract of the leaves of *Annona reticulata* using hot plate method are presented in Table 6.The results showed that there was no significant difference on the thermal stimulus in rats treated with normal saline (negative control) throughout the 60 min observation. There was no increase in reaction time at all-time points compared to baseline values (0 min) within the same treatment groups. The observation in diclofenac and extract treated animals was found to be significant increase in response time as compare to the control as shown in fig 14. The reaction time was significantly different between the extract and diclofenac at 15, 30, 45 and 60 min after treatment at significant value $P < 0.05$ but there were more increase in response time in extract treated than that of diclofenac.

Table 6: Analgesic effect of MEAR by hot plate method.

Treatment	0 min	15 min	30 min	45 min	60 min
Control	4.52±0.42	4.76±0.71	5.15±0.40	4.50±0.43	5.50±0.32
Diclofenac (10 mg/kg)	4.85±0.63	6.58±0.74*	8.84±0.38*	9.32±0.27**	10.29±0.95**
MEAR (100 mg/kg)	5.84±0.42	6.98±0.49*	9.25±0.79**	11.69±0.61**	13.90±0.74***
MEAR (200mg/kg)	4.18±0.52	7.36±0.72*	9.52±0.23**	12.76±0.59***	14.63±0.83***

n = 6 animals in each group; values are Mean±SEM; * p < 0.05, ** p < 0.05, *** p < 0.05 when compared to control (By using one way ANOVA followed by Dunnett t- test).

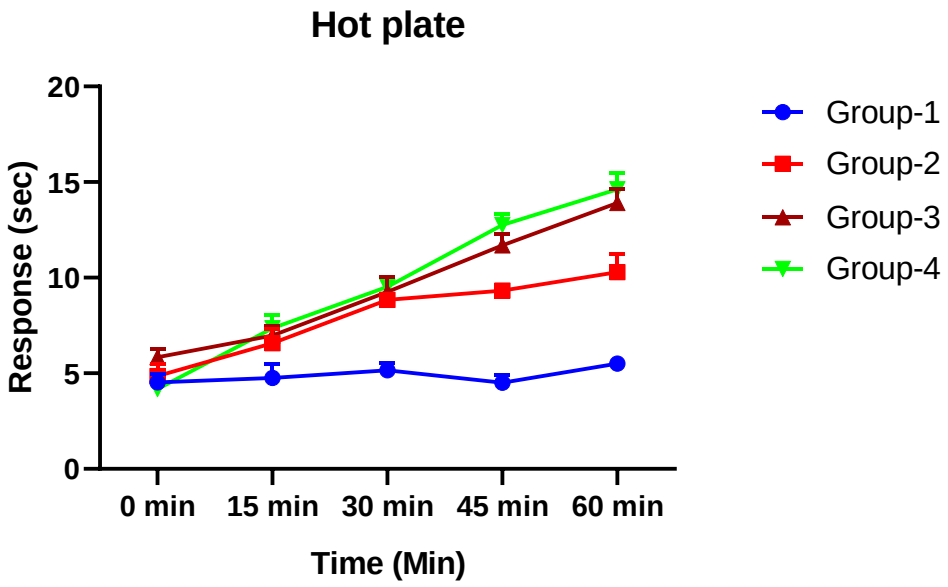

Fig no. 14: Effect of MEAR by hot plate method in rats.

DISCUSSION

The greater beneficial effects have been reported for natural products in folk medicine. Different studies on medicinal plants, have shown them to be alternative source for new compounds with potential pharmacological activity. Gastroprotective effect of MEAR was evaluated against ethanol, indomethacin, stress and pyloric ligation induced ulcers in rat model. The methanolic extracts (100 and 200 mg/kg) showed significant antiulcer activity. The percentage ulcer protection is been observed in all the Models, but the extent of percentage protection is more in indomethacin induced ulcer and pyloric ligation ulcer. The ulcer index is reduced in the higher dose. In the pre-treated group, the volume of gastric juice is reduced in all the Models and pH value is increased significantly as the dose of extract increases. It is also found that, there is significantly decrease in the free acid and total acid in all the four models.

Inhibitory action of indomethacin on prostaglandin synthesis coupled with free radicals formation has been opined as critical biochemical events in the pathogenesis of gastric ulceration.[49-51] An understanding of these events might be of utmost relevance in designing new antiulcer drugs. With the intrinsic adverse side effects and considerably high cost of synthetic drugs, exploiting natural products of plant source which are believed to be non-toxic, efficacious and affordable will be appropriate in the treatment of gastric ulcer. Phytotherapy is rapidly gaining grounds in sustaining human health and in the prevention of certain diseases like gastric ulcer resulting from drug toxicity.[52]

The results of this study revealed that the MEAR was beneficial to treat gastric ulcers by preventing oxidative stress. The mechanism of gastro protection of *Annona reticulata* leaves may be due to cytoprotective, antisecretory and antioxidant potential of phytoconstituents present in the extract.[53, 54] Prostaglandin synthesis protects the stomach from the irritation and injuries by stimulating the secretion of protective substance mucus and bicarbonates. The MEAR reduces mucosal damage compared to control. The ability of the extract to reduce gastric mucosal damage further supports cytoprotective effect and suggests the possible involvement of prostaglandins in the antiulcer effect of the extract.[55] Acute toxicity studies showed no toxic symptoms at 2000 mg/kg, which indicates that MEAR was safe.

Analgesics are the drugs that act on peripheral or central nervous system to selectively relieve pain without significantly altering consciousness.[56] Centrally acting analgesics are the drug that act by raising the threshold for pain and also altering the physiological response to pain.

On the other hand, peripherally acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain.[57] The animal models employed for evaluation of analgesic activity in this study are pain-state models using thermal stimuli which include tail-flick and hot plate methods. Both methods are useful in illustrating centrally mediated antinociceptive responses which focus generally on changes above the spinal cord level.[58] While the tail-flick method mediates a spinal reflex to a nociceptive stimulus and hot plate method involves higher brain functions and is regarded a supraspinally organized response.[59]

In tail-flick model, the methanol extract from the leaves of *Annona reticulata* exhibited significant analgesic activity by increasing the reaction time of the rats compared to control (saline treated rats) at all-time points. Diclofenac were used as reference drugs. In comparison with control, diclofenac and extract produced the significant antinociception effect during all observation times but diclofenac treated show less significant than the extract treated group.

The methanol extract from the leaves of *Annona reticulata* increase the reaction time of the rats on hot plate method in this study. The difference in the mean reaction time of the extract and the diclofenac groups was statistically significant during all observation times except for the control group. Hot plate method produces two measureable behavioural components in response to thermal pain, with regard to their reaction times such as paw licking and jumping.

Taken together, hot plate is a better method to evaluate analgesic activity compared to tail flick as significant results were observed for all treatments using hot. It was found that there were increase in response time of the extract in hot plate method than that of the tail flick method. Result of the hot plate test is suggestive of strong analgesic effect in all extracts most probably of the opioid type as the positive effect against the thermal nociceptive stimuli are indicative of opioid type analgesic effect[60] and also significant level of extract was found to be more than that of diclofenac it may be due to the extract show its activity by acting centrally (CNS) and diclofenac act peripherally.

Further detailed study on the isolation and characterisation of the compounds responsible for the antiulcer and analgesic activity in methanolic extract of *Annona reticulata* needs to be carried out.

CONCLUSION

The results obtained in this study showed that the methanolic extract of *Annona reticulata* possesses antiulcer and analgesic activity and showed the dose dependent gastroprotective effect against indomethacin induced and pylorus ligation ulcers and the analgesic effect by acting on CNS.

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CONFLICT OF INTEREST

The authors declared that there are no conflicts of interest.

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