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Anti ulcer activity of methanolic extract of *Jatropha curcas* (Linn.) on Aspirin-induced gastric lesions in Wistar strain rats

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ABSTRACT

This study has been undertaken to investigate the effect of methanolic extract of leaves of Jatropha curcas Linn. (JC) on pylorus ligation and Aspirin-induced gastric ulcers in Wistar rats. 30 albino wistar rats of either sex were selected and divided into 5 groups of 6 animals each. Gastric lesions produced by pylorus ligation (PL) plus aspirin (200mg/Kg, PO). Treatment with JC extract of (100 mg/Kg bodyweight, 200 mg /Kg body weight) and ranitidine (50 mg/ Kg body weight) for 6 days to the aspirin and PL rats were given and absorb the acid parameters.A significant dose dependant reduction ($P < 0.05$) in the acid parameters like gastric volume, pH, total acidity, total acid output, total proteins and ulcer index were observed after treatment with 100 mg, 200 mg JC extracts in PL plus aspirin induced ulcers compared to the normal PL rats. Histopathological examination of stomach mucosa showed the protective action of JC extracts against mucosal epithelial damage caused by aspirin. The present study provides a strong evidence of antiulcer activity of JC extract against gastric lesions. The antiulcer activity is recognized by a reduction in acid-secretary parameters (i.e. total and free acid), gastric volume and ulcer score suggesting that acid inhibition accelerates ulcer healing, thereby strengthening of mucosal barrier.

Keywords: Jatropha Curcas, aspirin, pylorus ligation, gastric lesions, ranitidine.

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INTRODUCTION

For at least five thousand years mankind has relied on natural products as the primary sources for medicines. However, the last two centuries have brought an explosion of understanding how these natural products are produced and how they interact with other organisms. Now at the start of a new millennium, it is estimated by the World Health Organization that, 80% of the world's inhabitants must rely on traditional medicines for health care; these traditional medicines are primarily plant-based [1]. It is estimated that 25% of all prescriptions dispensed in USA contained a plant extract or active ingredients derived from plants [2]. All of these investigations demonstrate the importance of natural products in drug discovery.

Peptic ulcer occurs due to imbalance between aggressive (acid, pepsin) and defensive (mucus gastric mucosal barrier) factors of gastric mucosa. Local mechanisms implicated in mucosal defense are mucus-alkaline secretion, mucosal hydrophobicity, rapid epithelial cell renewal and rich mucosal blood flow [3]. Prostaglandins E_2 and I_2 are the predominant prostaglandins synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate [4]. The treatment of peptic ulcer is directed against either reduction of aggressive factors or enhancement of mucosal defense of stomach and duodenum with cytoprotective agents.

Even though a range of drugs are available for the treatment of ulcer, as they produce side effects such as arrhythmias, importance and hematopoietic changes [5], I have selected this plant, which is a folk medicine and is reported to have antiulcer activity [6]. It is evident that the increased acid secretion along with decreased production of mucous cummulatively could have regulated in the formation of lesions on the gastric mucosa [7]. Studies reveal that gastrin is a major regulator of acid secretion in both normal and ulcer subjects [8].

Ulcerative lesions of GI tract are one of major side effects associated with the use of NSAIDS, alcohol, stress and ischemic reperfusion. Several herbal drugs and ayurvedic preparations have been shown to protect against the drug-induced gastric mucosal injury [9].

The aim of the study is to investigate the antiulcer effect of JC extract on aspirin + PL induced gastric ulcers.

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MATERIALS AND METHODS

Plant Material and Extraction

The leaves of *Jatropha Curcas* (fam. Euphgorbiaceae) was collected from Aringar Anna Hospital, Arumbakkam, Chennai during the month July. The plant was identified and authenticated by Dr. Sasikala, Research Officer, Aringar Anna Hospital, Arumbakkam, Chennai. The plant was cultivated throughout India in plains and in hedges. It is a large deciduous soft wooded shurb. It is commonly called as purging nut or physic nut in English and Katalamanakku in Tamil. The leaves of this plant are used as galactagogue, rubifacient, and have insecticidal properties and used in foul ulcers, tumours and scabies [6,10].

The collected leaves were shade dried and coarsely powdered with commercial blender. The air-dried powder of *Jatropha curcas* (200 g) was extracted with methanol in soxhlet apparatus (continuous hot percolation). After completion of the extraction process, the extract was concentrated by distillation under vacuum and weighed as 4.67 gms.

Animals and Treatments

Albino rats of either sex weighing between 150-200 g were used for gastric ulcer models. The animals were housed in an animal house at 27 $\pm$ 1°C and were fed with standard pellet diet and water ad libitum. The animals were divided into 5 groups of 6 animals each.

Group-I	-	PL Control
Group-II	-	Aspirin + PL Control
Group-III	-	MEJC (100mg/Kg/P.O) + Aspirin + PL
Group-IV	-	MEJC (200mg/Kg/P.O) + Aspirin + PL
Group-V	-	Ranitidine (50mg/Kg/P.O) + Aspirin + PL

MEJC - Methanolic extract of *Jatropha curcas*
PL - Pylorus ligation

Acute Oral Toxicity Study

The acute oral toxicity study was done according to the OECD GUIDELINES 423 (Acute toxic class method) [11]. A starting dose of 2000 mg/kg body weight of methanolic extract of *Jatropha curcas* was administered orally to 5 male rats, observed for three days. There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. When the experiments were repeated again with the same dose

level, 2000 mg/kg PO of Methanol extract of *Jatropha curcas* for 3 days more, and observed for 4 days. No changes were observed from first set of experiment. LD_{50} was observed as 370 mg/Kg body weight (Graph I).

Aspirin plus Pylorus Ligation Induced Gastric Ulcers

Aspirin was suspended in1% Carboxy Methyl Cellulose (CMC) solution and administered orally in the dose of 200 mg/Kg in non-fasted rats once daily for 5 days.

Methanolic extract of JC (100, 200 mg/Kg, PO) and ranitidine (50 mg/Kg, PO) were administered orally to the respective treatment groups 30 min before each aspirin treatment whereas the control group received only vehicle (1% CMC solution). On the sixth day immediately after aspirin treatment pylorus ligation was performed under ether anesthesia on 36 hrs fasted rats. Four hours after pylorus – ligation the animals were sacrificed by giving over dosage of ether. The stomachs were removed and opened along the greater curvature and the gastric lesions were observed using dissecting microscope.

ESTIMATION OF PARAMETERS

Collection of Gastric Juice

The stomach was excised carefully by keeping the oesophagus closed and opened along the greater curvature and the luminal contents were removed. The gastric contents were collected and centrifuged at 1000 rpm for 10 min; the volume of the supernatent was expressed as ml/100 gm body weight and the centrifuged samples were decanted and analyzed for gastric volume, pH and total acidity. The mucosa was flushed with saline and observed for gastric lesions using a dissecting microscope, ulcers were scored and the ulcer index was determined.

Estimation of Total and Free Acidity

It was measured by the Method of Hawk et al., [12] 1ml of supernatant liquid was pipette out and diluted to 10ml with distilled water. pH of this solution was noted with the help of pH meter. The solution was titrated against 0.01N sodium hydroxide using topfer's reagent as indicator. The end point was titrated when the solution turned to orange colour. The volume of NaOH was noted, which corresponds to free acidity. Further, it was titrated till the solution regains pink colour. The total volume of NaOH was noted, which corresponds to the total acidity.

Ulcer Index

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It was measured by method of Ganguly and Bhatnagar [13]. It was calculated from an arbitrary scale by taking into consideration, the ratio of total area of the stomach mucosa and area of ulceration.

Protein Content

It was measured by method of Lowery et al. [14]

Histopathological Examination

The stomachs were washed thoroughly with saline and collected in small bottles containing 10% formalin solution and were subjected to histopathalogical examination after stained with haematoxylin and eosin. The attained thin section was observed under magnification 100X (Figures I, II, III).

Statistical Analysis

The data were expressed as Mean ± SEM. Results were analyzed statistically by one-way ANOVA followed by DUNNETT'S TEST using SPSS software students version. The difference was considered significant if $P < 0.05$.

RESULTS

The table I shows the gastric volume, pH, acidity, protein content and ulcer index of experimental groups. All these parameters were found to be significantly increased in the untreated Aspirin + PL rats compared to the PL - control. Treatment with methanol extracts of JC (100 mg/Kg, 200 mg/Kg) and ranitidine (50 mg/Kg) for 6 days to Aspirin & PL rates caused a significant reduction ($P < 0.05$) in the above parameters.

In the present study, methanolic extracts of JC have been shown to possess anti ulcer activity against experimentally induced ulcer model (Aspirin + PL Method). Methanolic extract of JC, significantly reduced ($P < 0.05$) the acid secretary parameters i.e. Total and free acidity as well as the gastric volume and an ulcer index suggests that acid inhibition accelerates ulcer healing. The decrease in gastric volume and simultaneous decrease in acidity may be one of the causes of ulcer healing.

The dose levels of methanolic extracts of JC at (100 mg/Kg, 200 mg/Kg) caused a significant reduction in protein concentrations in gastric juice, which

indicates strengthening of the gastric mucosa, thereby restricting the entrance of the plasma proteins into gastric juice [15].

Ulcer index was significantly reduced in JC extracts and ranitidine treated groups compared to the control treatments. It is evident from results (Table I) that these drugs causes a reduction in the intensity of gastric ulcerations as observed from the reduced ulcer index in the drug treated groups.

Histopathological examination of stomach mucosa shows that pretreatment with methanolic extracts of JC (100 mg/Kg) (not shown in figure), JC (200 mg/Kg) and ranitidine (50 mg/Kg) protected the mucosal epithelium from the damage caused by aspirin. Aspirin + PL group shows the ulcerated mucosa with haemorrhage and discontinuity of lining epithelium while extract of JC (200 mg/Kg body weight) shows the normal mucosa with mild hyperplasia and mild edematous submucosa, compared to ranitidine treated group which shows the normal mucosa with no ulcer.

DISCUSSION

Ulcers are defined histologically as a breach in the mucosa of the alimentary tract that extends through the muscularis mucosa into the submucosa or deeper. Although they may occur anywhere in the alimentary tract, none are as prevalent as the peptic ulcers that occur in the duodenum and stomach. Peptic ulcers are relapsing lesions that are most seen in middle-aged to older adults, but they may first become evident in young adult life [1,2].

Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion, decreasing mucin activity and back diffusion of H^+ ions. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier [16].

The increase in the gastric volume of the untreated Aspirin + PL group is undoubtedly due to the increased production of HCL as it is evident from the total acidity of the gastric juice. The increase in protein content of the gastric juice in untreated ulcer group indicates the damage to the gastric mucosa as the result of plasma proteins leak into the gastric juice.

Histopathological study revealed that methanolic extracts of JC treated groups, the mucosa was found to be almost normal with mild muscularis mucosa. Ranitidine treated section showed the normal mucosa with no ulcer in the submucosa.

It is well known fact that gastric secretions are under vagal control. Vagal over activity appears to contribute to any stress ulcer formation. Various causes

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responsible for the development of ulcers due to PL-model, such as increased metabolism of carbohydrates, increased synthesis of nucleic acids and also exhaustion of carbohydrates and other compensatory mechanisms (Mozsik et al).

At the same time methanolic extract of JC alone (100 mg/Kg) and methanolic extract of JC alone (200 mg/Kg) treated groups did not produce any significant changes in the biochemical parameters and they preserved the normal architecture of the stomach mucosa by significantly reducing the gastric volume and ulcer index as compared to PL control animals. This further establishes the fact that the extracts have ulcer protective in nature.

Table -I
Effect of MEJC on pH, gastric volume, total acidity, free acidity, Total acidity output, total protein and ulcer score in Aspirin + PL induced ulcers

Group	pH	Gastric Volume (ml/100g)	Total Acidity (mEq/L/4h)	Free Acidity (mEq/L/4h)	Total Acid Output (mEq/L/4h)	Total protein	Ulcer index
Group I	2.85 ± 0.30	2.80 ± 0.14	134.79 ± 1.69	117.75 ± 1.68	375.05 ± 0.30	7.3 ± 0.016	2.05 ± 0.25
Group II	1.71 ± 0.12	5.34 ± 0.10	232.09 ± 1.59	190.89 ± 3.09	1243.84 ± 18.12**	10.71 ± 0.021	2.60 ± 0.25
Group III	3.49 ± 0.22	3.40 ± 0.15	160.98 ± 0.87**	97.49 ± 1.98**	541.98 ± 22.91	8.49 ± 0.007	1.40 ± 0.10**
Group IV	4.49 ± 0.22	2.79 ± 0.10**	127.28 ± 1.29	86.98 ± 2.11	360.38 ± 13.27**	7.89 ± 0.001	1.22 ± 0.20**
Group V	4.99 ± 0.25	2.45 ± 0.10	147.61 ± 1.58	96.65 ± 1.54	379.98 ± 13.27	7.51 ± 0.016	1.31 ± 0.20

PL – Pylorus ligation
MEJC – Methanolic extract of *Jatropha curcas*
Values are Mean ± SEM of 6 animals each in a group. Comparison was done by ANOVA, followed by Dunnet's test (n=6)
**P <0.05 compared with aspirin treated group

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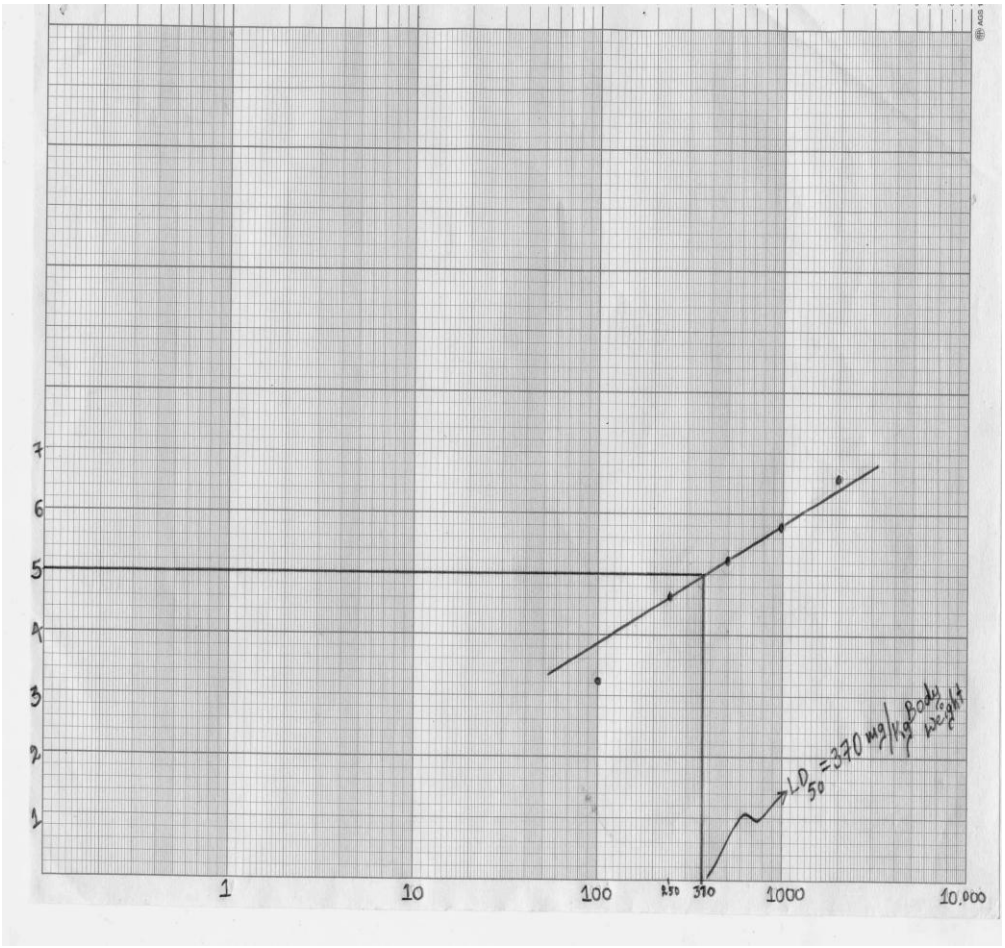
Graph I: ACUTE ORAL TOXICITY TESTING

(determination of LD_{50})



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Group II Aspirin + PL Control

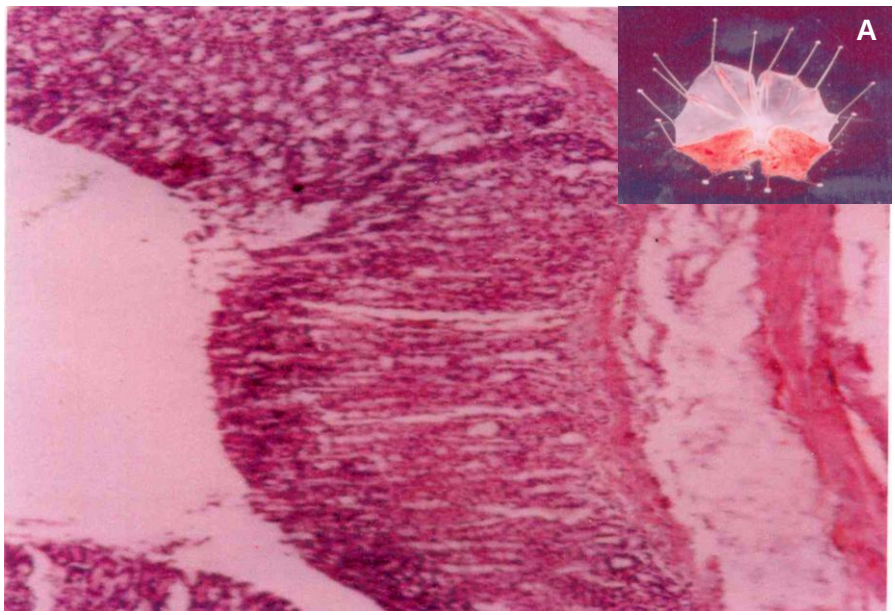

Figure I. Ulcerated mucosa showing Hemorrhage and discontinuity in the lining epithelium

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Group IV MEJC (200 mg/Kg, PO) + Aspirin + PL rats

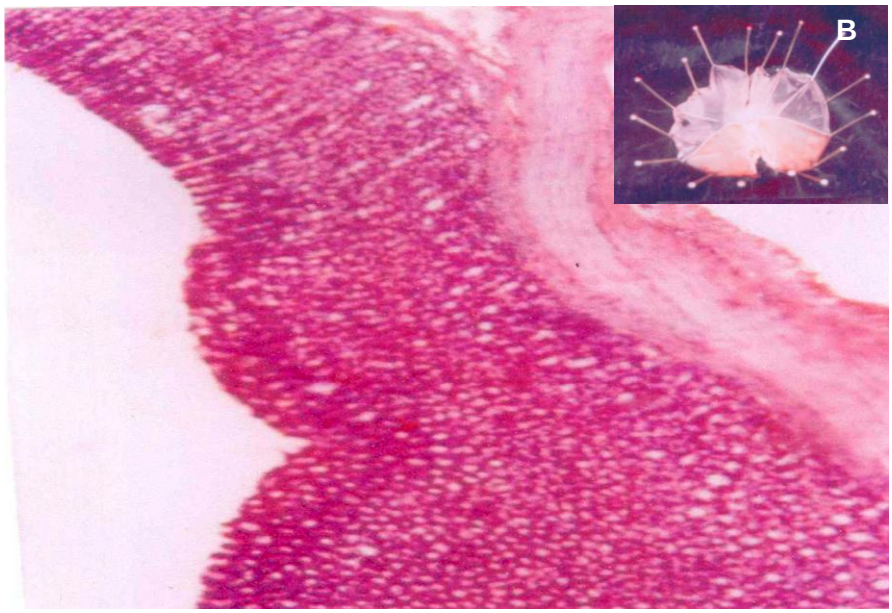

Figure II. Normal mucosa with mild hyperplasia and mild edematous submucosa

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Group V Ranitidine (50 mg/Kg, PO) + Aspirin + PL rats

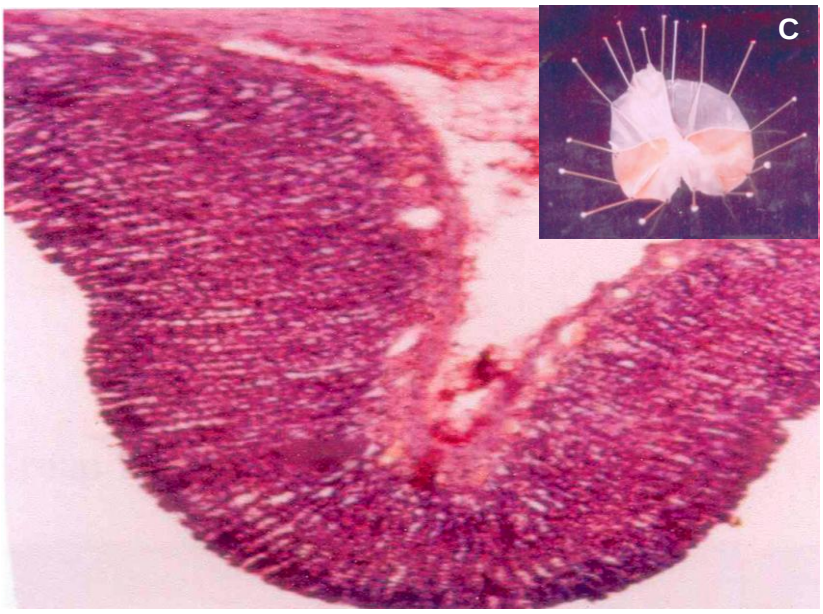

Figure III. Normal mucosa with no ulcer

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Figure I. Ulcerated mucosa showing Hemorrhage and discontinuity in the lining epithelium

Figure II. Normal mucosa with mild hyperplasia and mild edematous submucosa

Figure III. Normal mucosa with no ulcer

In the inset of figures I, II, III, the A, B, C indicates following respectively:

A Gastric mucosa of aspirin + PL treated rats

B Gastric mucosa of JC extract (200 mg/Kg body weight) + aspirin + PL treated rats

C Gastric mucosa of ranitidine (50 mg/Kg body weight) + aspirin + PL treated rats

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