ANTISECRETORY ACTIVITY OF SOLVENT EXTRACTS OF*JATROPHA CURCAS* (LINN.) ON PYROLUS LIGATED RATS

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Summary

The present study has been undertaken to investigate the antisecretory activity of the solvent extracts of stems of *Jatropha curcas Linn.* on pylorus ligated rats. The experimental groups were treated with petroleum ether (60-80^{0}C), chloroform, methanol and aqueous extract of *J. curcas* (200 and 400 mg /kg, p.o.) and ranitidine (50 mg/kg, p.o.), 1 h prior to pyloric ligation. The parameters evaluated were gastric content, pH, total acidity and ulcer index. The dose dependant significant reduction in the evaluation parameters were observed after treatment with 200 mg/kg and 400 mg/kg of methanolic and aqueous extracts as compared to the normal control group. Histopathological examination showed the protective action of *Jatropha curcas* extracts against mucosal epithelial damage caused by pylorus ligation. The present study provides a strong evidence of antisecretory activity of different extracts especially the methanolic and aqueous extracts of *J. curcas* against pylorus ligated rats.

Key words: *Jatropha curcas*, Pyloric ligation, antisecretory.

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Introduction

Peptic ulcer is one of the common diseases in human population. Its incidence is increasing due to rapid development and civilizational constraints. Although exact etiology of the disease is not known, an imbalance between defensive mechanisms and aggressive factors result in peptic ulcer.[1] The pathophysiology of this disease is attributed to the difference between aggressive factors like acid, pepsin, *H. pylori* infection and local mucosal defenses like secretion of bicarbonate, mucus and prostaglandins. [2] The common clinical features of peptic ulcers are hyperacid secretion and ulcer formation in the stomach and duodenal part of the intestine. Acute gastric ulcers can be produced in laboratory animals by use of corrosive substances such as alcohol, aspirin, indomethacin or by surgical technique like pyloric ligation or by subjecting the animals to acute stressful condition. The most commonly used technique is the pyloric ligation induced acid secretion and ulcer formation. [3.]

Jatropha curcas (Linnaeus) also called as 'Physic Nut' or 'Purging Nut', is a drought resistant shrub or tree belonging to the family Euphorbiaceae, which is cultivated in Central and South America, south-east Asia, India and Africa. [4] The roots, stems, leaves, seeds and fruits of the plant have been widely used in traditional folk medicine in many parts of West Africa. [5] Extract of the leaves showed potent cardiovascular action in guinea pigs and might be a possible source of beta-blocker agent. [6] A decoction of leaves is used against cough and as an antiseptic after birth. The curcain, a proteolytic enzyme isolated from latex has wound-healing property. [7] Branches are used as a chewing stick in Nigeria The leaves of this plant are used as galactagogue, rubifacient, and have insecticidal properties and used in, tumours and scabies. [8]

Material and methods

Collection of plant material and preparation of extract:

Stems of *Jatropha curcas* were collected from Nashik district in a month of July- August and authenticated at Agharkar Research Institute, Pune (AHMA -14141). The collected stems were shade dried and coarsely powdered and then successively extracted with Petroleum ether (60-80^{0}C), chloroform, methanol and water using Soxhlet apparatus. The extracts were concentrated under reduced pressure and refrigerated. They were labeled as PEJC, CEJC MEJC and AEJC respectively. A preliminary phytochemical test was performed for chemical constituents such as carbohydrates, alkaloids, glycosides, saponins, tannins, flavonoids and steroids.

Experimental animals:

Adult wistar rats weighing (150-200 g) of either sex were used. The animals were maintained under standard conditions (room temperature 24-27^{o}C and humidity 60-65%) with 12 h light and dark cycle. They were given a standard laboratory diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee

Experimental Procedure

Acute oral toxicity studies:
The acute oral toxicity study was done according to the OECD guidelines 425. A starting dose of 2000 mg/kg body weight of extracts of *Jatropha curcas* were administered orally to 3 male albino mice, observed for fourteen days for any behavioral changes and mortality. [9]

Antisecretory activity:
The Wistar rats were divided into 10 groups containing six animals in each. Group 1 was control administered with vehicle (1 ml), group 2 treated with standard-ranitidine (50 mg/kg p.o). Other eight groups were administered with different doses (200 and 400 mg/kg) of test drug (Petroleum ether extract, Chloroform extract, Methanolic extract and Aqueous extract. All the doses were prepared in 1%CMC. The animals were fasted for 48 h. The control vehicle, standard drug or different extracts were administered 1 h prior to pyloric ligation. Under light ether anesthesia, the abdomen was cut, open and the pylorus was ligated. The abdomen was then sutured. At the end of 4 h after ligation, the animals were sacrificed by using cervical dislocation and the stomach was dissected out. Gastric juice was collected and its volume was measured. Acidity was determined by titration with 0.01N sodium hydroxide using phenolphthalein indicator. The glandular portion was then exposed and examined for ulceration. The number of ulcers was noted and the severity recorded with the following scores:
0 = no ulcer, 0.5= spot ulcers, 1 = superficial ulcers, 2 = deep ulcers, 3 = perforation.
Mean ulcer score for each animal was expressed as ulcer index

Histological studies:
Stomach samples from each group were fixed in 10 per cent formalin for 24 h. The formalin fixed specimens were embedded in paraffin and stained with haematoxylin and eosin. The histochemical sections were evaluated by light microscopy.

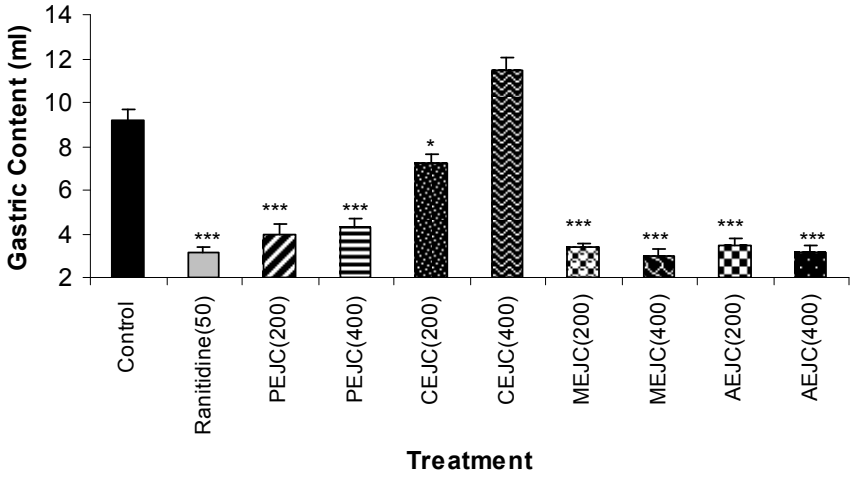
Statistical Analysis:
The data were expressed as Mean ± SEM. Results were analyzed statistically by one-way ANOVA followed by Dunnett's test.

Results

The extracts of *Jatropha curcas* were evaluated phytochemically and the results showed the presence of steroidal compounds in petroleum ether and chloroform extract and alkaloids, and alkaloids were observed in methanolic and tannins in aqueous extract. All the extracts were safe and showed a LD 50 value of 2000 mg/kg dose. In pylorus ligation induced gastric lesions in rats, the ranitidine (50 mg/kg), petroleum ether, methanol and aqueous extracts (both doses), showed significant ($P<0.001$) reduction in the gastric content (Fig 1). The chloroform extract with dose 200 mg/kg showed significant ($P<0.05$) reduction in the gastric content, but with dose 400 mg/kg does not show significant reduction in the gastric content. All the test groups showed significant ($P<0.001$) reduction in the pH (Fig 2), Ulcer index (Fig 3), Total acidity (Fig 4) as compared to control.

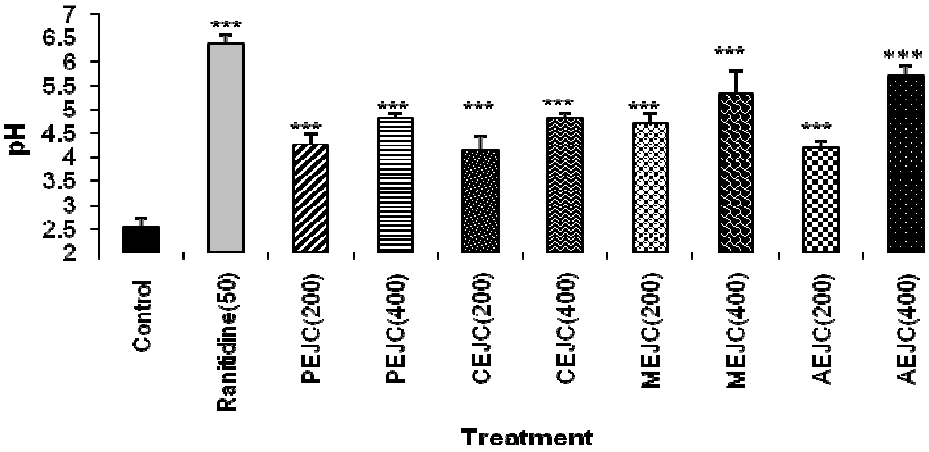
Histological examination of stomach mucosa revealed that pretreatment with Methanolic extract and Aqueous extract (both doses) protected the mucosal epithelium from the damage caused due to accumulation of acid. It was also observed that normal cyto-architecture of gastric mucosa with no pathological changes was restored. In case of control stomach mucosa, ulcer with distorted gastric glands, breach in the mucosa with inflammatory infiltration of neutrophils and lymphocytes in the lamina propria region were seen. Chloroform extract (200 and 400 mg/kg) showed superficial ulcerations in gastric mucosa and Petroleum ether extract (200 mg/kg) showed mild destruction of mucosal epithelium. In case of standard it showed intact mucosa with no ulcer, glandular organization, and maintenance of mucularis mucosa. (Fig 5).

Figure 1: Effect of different extracts of *J. curcas* on gastric content in pyloric ligation rats



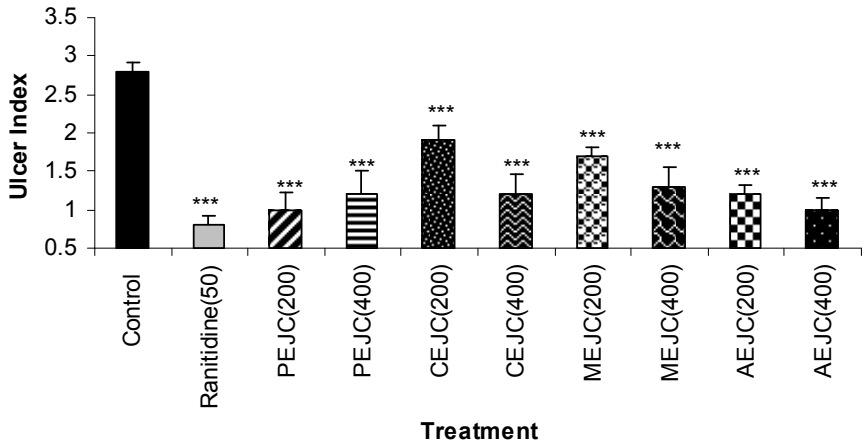
n=5, values are expressed as mean ± SEM, *P< 0.05, ***P< 0.001 considered significant compared to control and standard. (ANOVA followed by Dunnett's test). PEJC : Petroleum Ether Extract, CEJC, Chloroform extract, MEJC : Methanolic extract, AEJC : Aqueous extract.

Figure 2: Effect of different extracts of *J. curcas* on pH in pyloric ligation rats



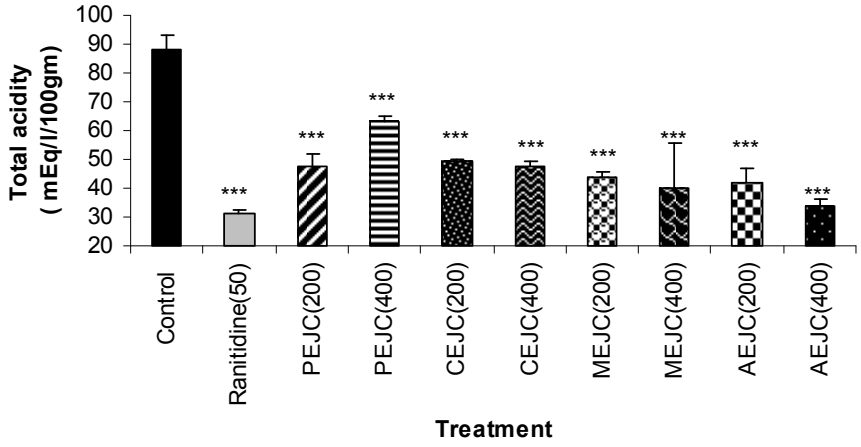
n=5, values are expressed as mean ± SEM, ***P< 0.001 considered significant compared to control and standard. (ANOVA followed by Dunnett's test). PEJC : Petroleum Ether Extract, CEJC, Chloroform extract, MEJC : Methanolic extract, AEJC : Aqueous extract.

Figure 3: Effect of different extracts of *J. curcas* on Ulcer index in pyloric ligation rats



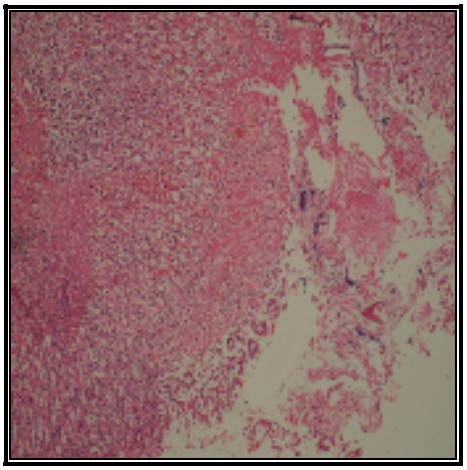
n=5, values are expressed as mean ± SEM, ***P< 0.001 considered significant compared to control and standard. (ANOVA followed by Dunnett's test). PEJC : Petroleum Ether Extract, CEJC, Chloroform extract, MEJC : Methanolic extract, AEJC : Aqueous extract.

Figure 4: Effect of different extracts of *J. curcas* on total acidity in pyloric ligation rats

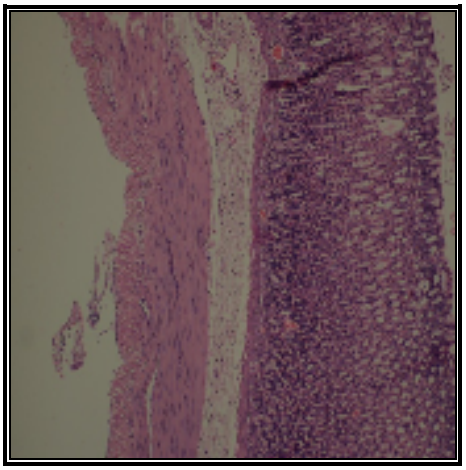


n=5, values are expressed as mean ± SEM, ***P< 0.001 considered significant compared to control and standard. (ANOVA followed by Dunnett's test). PEJC : Petroleum Ether Extract, CEJC, Chloroform extract, MEJC : Methanolic extract, AEJC : Aqueous extract.

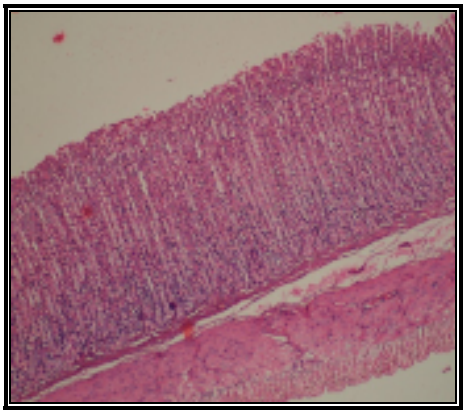
A) Group I – Control



B) Group II - Ranitidine treated



C) Group VI- MEJC 400mg/kg.p.o



D) Group VIII- AEJC 400mg/kg.p.o

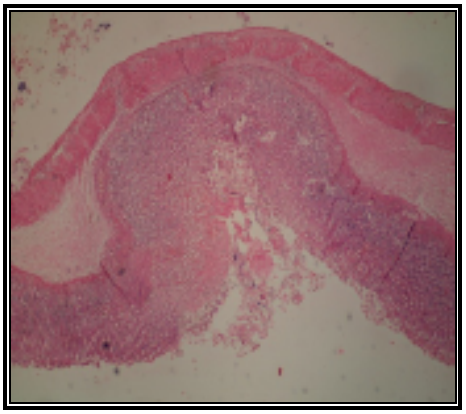


Figure. 5 : Histopathological report of stomach mucosa for four groups .

A) Control showing distorted mucosa, B) Ranitidine treated showing intact mucosa with no ulcers. C) and D) Extract treated groups showing restoration of normal cyto-architecture of gastricmucosa.

Discussion

Pylorus ligation induced ulcer is one of the most widely used methods for studying the effect of drugs on gastric secretion. Agents that decrease gastric acid secretion and increase mucus secretion are effective in preventing the ulcers induced by this method. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach, leading to the development of ulcers in the stomach. The ulcers are produced due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier.[10]Ulcer index parameter was used for the evaluation of antisecretory activity since ulcer formation is directly related to the factors such as gastric volume and total acidity. In case of vehicle control, pylorus ligation increased the acid secretion, which in turn caused increase in gastric volume, low pH, increased total acidity resulting in increase in ulcer index. Ranitidine a comparative antagonist of H_2 receptor reduces acidity and volume of gastric secretion by blocking the effect of histamine. Ranitidine exerts its antisecretory effect by inhibiting the histamine induced cyclic AMP pathway [11]

In pylorus ligation induced gastric lesions in rat model the different extracts of *J. curcas* significantly reduced the gastric content, pH, ulcer index, and total acidity as compared to control group, thus showing antisecretory mechanism. The results suggest that extract of *J. curcas* probably act by inhibiting gastric acid secretion. Since endogenous histamine formation and release in the gastric mucosa have been implicated in the pathogenesis of gastric ulcer, anti histamine agents may be useful in the prevention of such lesions [(12)]. It is possible that some of the chemical constituents *J. curcas* extract might acts as antihistamine. This study indicates that *J. curcas* extracts has a potential anti secretory activity especially the aqueous and methanolic extracts.

The preliminary phytochemical studies revealed the presence of alkaloids in aqueous and methanolic extract of *J. curcas*. So the possible mechanism of anti secretory action of *J. curcas* may be due to its alkaloid content.

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