Available online at http://www.journalijdr.com

ISSN: 2230-9926

IJDR

International Journal of
DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 3, Issue, 05, pp.049-054, May, 2013

Full Length Research Article

ANTIULCER ACTIVITY OF SEED EXTRACTS OF G*ynocardia odorata* ROXB. ON PYLORUS LIGATION AND INDOMETHACIN INDUCED GASTRIC LESIONS IN ALBINO RATS

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ARTICLE INFO

Article History:
Received 28th January, 2012
Received in revised form
23rd February, 2013
Accepted 10th April, 2013
Published online 13th May, 2013

Key words:
Chaulmoogra plant,
Ulcer,
Gynocardia odorata,
Pylorus ligation,
indomethacin.

ABSTRACT

Objective: The study was designed to evaluate anti-ulcer activity of ethanolic and aqueous seed extracts of Gynocardia odorata roxb. by using pylorus ligation and indomethacin induced ulcer models inrats.

Material and method: The authenticated Gynocardia odorata roxb. seeds were powdered coarsely and ethanolic and aqueous extracts were prepared for it. The phytochemical screening was performed to find out the active composition of the extracts. Antiulcer activity was evaluated by pylorus ligation and indomethacin induced ulcer models at two different dose levels (100 and 200 mg/kg b.w. orally) using parameters like gastric content, total acidity, ulcer index, and pH of gastric juice. Ranitidine at a dose of 20 mg/Kg body weight orally was used as standard control in both the models.

Result: The preliminary phytochemical screening of the ethanolic and aqueous extracts of the plant Gynocardia odorata showed the presence of phytoconstituents such as Flavonoids, Proteins, Fixed oils, Tannins, Proteins, Alkaloids, Carbohydrates, Glycosides, Saponin's and Triterpenoids. In both the ulcer models the aqueous extract exhibited more significant activity than the ethanolic extracts. The observed pharmacological activity reveals that the aqueous extract significantly raised the pH of gastric contents; lowered the free and total acidity and ulcer index as compared to standard drug Ranitidine significantly.

Conclusion: Both extracts of Gynocardia odorata roxb. reduced ulcer incidence, when compared to the control as evident by the decrease in ulcer scores, gastric content, total acidity, ulcer index, and pH of gastric juice in both the two models. Although this study establishes the traditional role of Gynocardia odorata roxb. in the gastric ulcers but still further exhaustive studies are required to find out and establish the main compound responsible for this activity as drug.

INTRODUCTION

Ulcer

Gastric ulcer, one of the most widespread, is believed to be due to an imbalance between aggressive and protective factors (Kofahi and Atta 1999). The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (Helicobacter pylori) and drugs (Peskar and Maricic 1998). These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility (Toma *et al*., 2005). Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor "PAF", leukotriene's, endothelins, bile or exogenous factors including (NSAIDs) or stimulating the mucosal defenses (mucus, bicarbonate, normal blood flow, prostaglandins, nitric oxide) (Borelli and Izzo 2000). The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources. The plant *Gynocardia odorata* roxb. is also known as Chaulmoogra plant, seeds of the chaulmoogra tree [Hydnocarpus (Tarakto-genos) kurzii], which is indigenous to parts of India, Malaysia and tropical countries of the world, contain fatty acids chaulmoogric acid, hydnocarpic acid. Chaulmoogra oil is an important therapeutic agent in certain medical traditions (Kirtikar and Basu 1985). The seeds of plant *Gynocardia odorata* roxb. are most commonly used (Handa

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and Kaul ?; Kokate *et al*., 2007). These are grayish, irregularly ovoid, compressed, and somewhat angular and smooth, a little over an inch long, and have an oily taste and a peculiar, nauseous odors (Ali Mohhammed 2008). The fruits are hot anthelmintic and used in bronchitis, skin diseases, small tumor's leprosy, and as an analgesic (Handa and Kaul 1998) these activities has been already reported but anti-ulcer activity is not established yet. In the present study we made an attempt to establish the anti-ulcer potential of ethanolic and aqueous seed extracts of *Gynocardia odorata*.

MATERIALS AND METHODS

Plant Material

Plant part (seeds) was collected from the Authenticated crude drug supplier in Delhi and authentication of the plant was carried in Botanical Department of Barkatullah university Bhopal (M.P.). The seeds were then shade dried and grinded and made a coarse powder and the coarse powder were used for further studies.

Preparation of Extract

Extraction was done according to standard procedures using analytical grade solvents. For ethanolic extract 250 gm. powdered seed was taken in a pouch of filter paper and kept inside the Soxhlet thistle then it was extracted with petroleum ether for 48-72 hours for defatting after that it was extracted with ethanol (99.9%) for 48-72 hours. Aqueous extract was separately prepared by maceration process. The extracts were then concentrated until dryness under reduced pressure and controlled temperature (40-50ºC). Then Preliminary Phytochemical screening was performed (Kokate *et al*., 2007). The % yield of ethanolic and aqueous extracts was found to be 9.4% and 23.33% respectively.

Preliminary Phytochemical Screening

The extracts were subjected to preliminary phytochemical qualitative screening to evaluate the presence of various primary or secondary metabolites following standard procedures (Kokate *et al*., 2007; SahuVinod 2010).

Experimental Animals

Wistar albino rats weighing 180-200g of either sex maintained under standard husbandry conditions at temp. 23±2^{o}C, relative humidity 55±10% and 12 hours light dark cycle. Animals were fed with standard laboratory food and *ad libitum*. The experiments were performed following CPCSEA (2003) after the experimental protocols approved by the institutional animal ethics committee, India 2012 under the registration no. NU/PH/M/COL/12/19

Experimental Protocol

Pylorus ligation

Animals were randomly divided into six groups of six animals each. Group I served as pylorus ligated control. The Group II received ranitidine 20 mg/Kg body weight by oral route, group III and group VI received different doses of *Gynocardia odorata* roxb. aqueous extracts at dose of 100mg/kg and 200 mg/Kg body weight and group V and VI received ethanolic extract of *Gynocardia odorata* roxb. seeds at dose of 100mg/kg and 200 mg/Kg body weight (Deshpande *et al*., 2003). Four hours after pylorus ligation, rats were sacrificed and stomach was isolated. The total severity of the ulcers was determined by recording the severity of each ulcer (Vogel 2002).

Determination of free acidity and total acidity

One ml. of gastric juice was pipetted into 100 ml conical flask, added 2 or 3 drops of topher's reagent and titrated with 0.01N sodium hydroxide until all traces of red colour disappear and the colour of the solution turn into yellowish orange. The volume of alkali added was noted. The volume corresponds to free acidity. Then 2 to 3 drops of phenolphthalein was added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted.

Indomethacin induced ulcer

Animals were randomly divided into six groups of six animals each. Group I served as indomethacin control, treated with Indomethacin (20 mg/kg body wt. p.o.), Group II treated with (ranitidine 20 mg/kg b.w.) with indomethacin (20 mg/kg b.w.) for 5 days. Group III-VI treated with aqueous and ethanolic extract (100,200 mg/kg b.wt. i.p.) of *Gynocardia odorata* roxb. for 5 days + indomethacin (20 mg/kg body wt., p.o.) Mahendran *et al.* (2002).

Procedure

Experimental procedure was started after 24 h of fasting, at the end of the experimental period (5 days) and an oral dose of indomethacin 20 mg/kg body weight was given. The rats were sacrificed and stomach was removed. The number of ulcers were noted and the severity recorded with the following scores (Sharma *et al*., 2011)

Normal coloration	0
Red coloration	0.5
Spot ulcer	1.0
Haemorrhagic stress	1.5
Ulcer≥3 but ≤5	2
Ulcer>5	3

Ulcer index (UI) was calculated using the formula (Bhalke Rasika *et al*., 2010)

UI=US+ UN+ UP×10-1

Where, US =Mean severity of ulcer score, UN =Average number of ulcers per animal, UP=Percentage of animals with ulcer incidence

Percentage protection from ulcers:

CUI-TUI/CUI

Where, C UI = Ulcer index of control groups, T UI = Ulcer index of treated groups

Statistical Analysis

The significance of difference among the control group and various treated groups were analysed by means of one-way ANNOVA followed by Dunnett's multiple comparison tests. The experimental results are represented as ± SEM (standard error mean).

Table 1. Effect of extracts of *Gynocardia odorata* roxb. on Pylorus ligation model in rats

Treatment	Volume of Gastric juice	Free acidity (mEq/liter)	Total acidity	Ulcer index	Ulcer Protection	pH
Pylorus ligated Control	3.36 ± 0.45	42.80±0.154	94.80±17.59	10.69±0.11	--	1.34±0.11
Ranitidine (20mg/kg b.w.)	1.54± 0.17**	4.00± 0.71**	10.20±1.28**	1.48±0.05***	84.50***	4.3±0.34**
GOA (100mg/kg b.w.)	1.70± 0.30**	3.20± 0.58**	6.00±0.71***	3.425±0.04***	68.38***	4.1±0.22**
GOA (200mg/kg b.w.)	1.86±0.10***	11.80±2.48**	24.80±4.38**	3.386±0.05***	68.68***	3.30±0.11**
GOE (100 mg/kg b.w.)	1.80± 0.34**	26.20± 4.10*	56.40± 8.84*	5.414±0.08***	52.79***	2.30±0.21*
GOE (200 mg/kg b.w.)	1.34± 0.12**	14.59±1.23**	20.40±2.42**	3.451±0.05***	68.38***	2.85±0.02**

Data in each column is represented as Mean ± SEM; *indicates $p<0.05$, ** indicates $p<0.01$ and ***indicates $p< 0.001$ when compared to the control group.Where, GOA is *Gynocardia odorata* roxb. aqueous extract and GOE is *Gynocardia odorata* roxb. ethanolic extract.

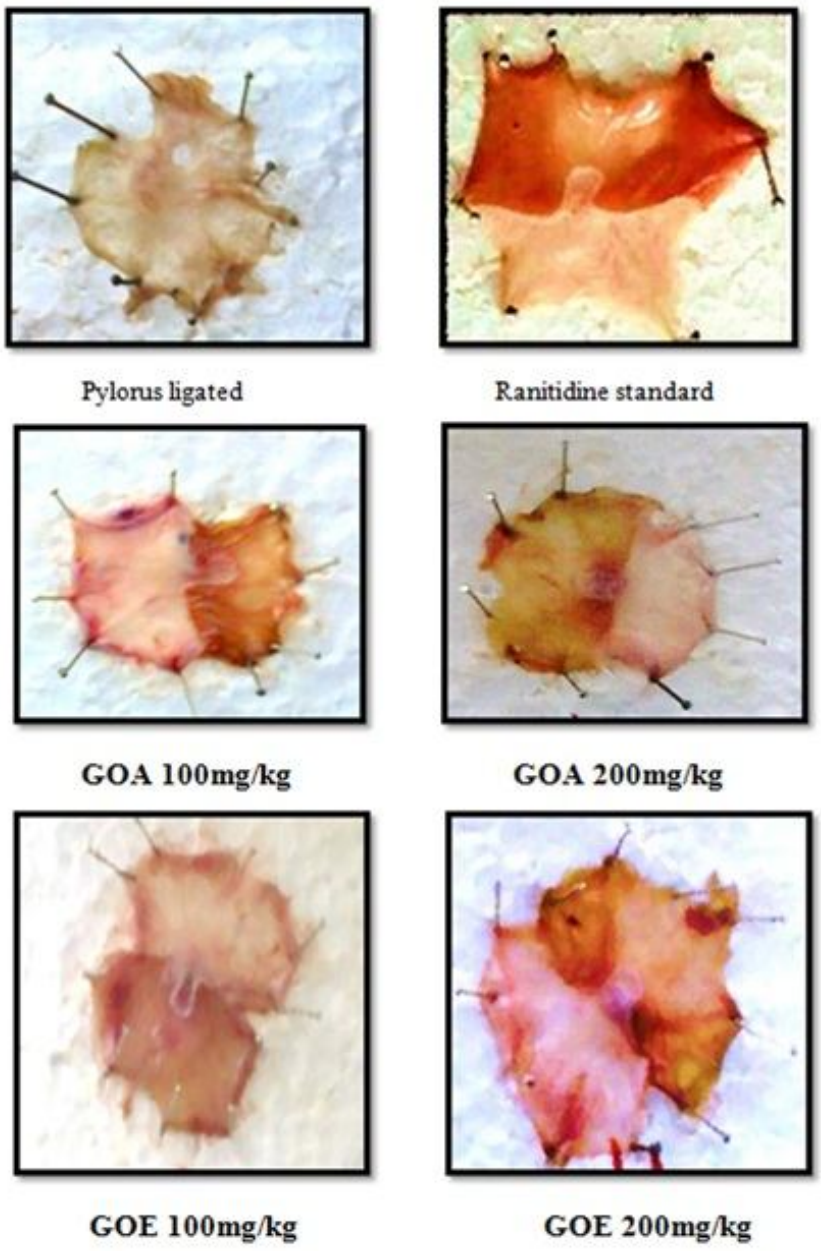

Figure 1. Effect of *Gynocardia odorata* roxb. seed extracts on pylous ligation induced ulcer

Table 2. Effect of *Gynocardia odorata* roxb. extracts on indomethacin induced ulcer in rats

Treatment	Ulcer index	Ulcer protection
Indomethacin Control	10.75±0.062	-
Ranitidine (20 mg/kg)	4.24±0.074***	70.45%
GOA(100mg/kg)	6.51±0.017***	46.63%
GOA(200mg/kg)	6.52±0.071***	52.61%
GOE(100mg/kg)	6.41±0.079***	46.63%
GOE(200mg/kg)	5.24±0.045***	41.63%

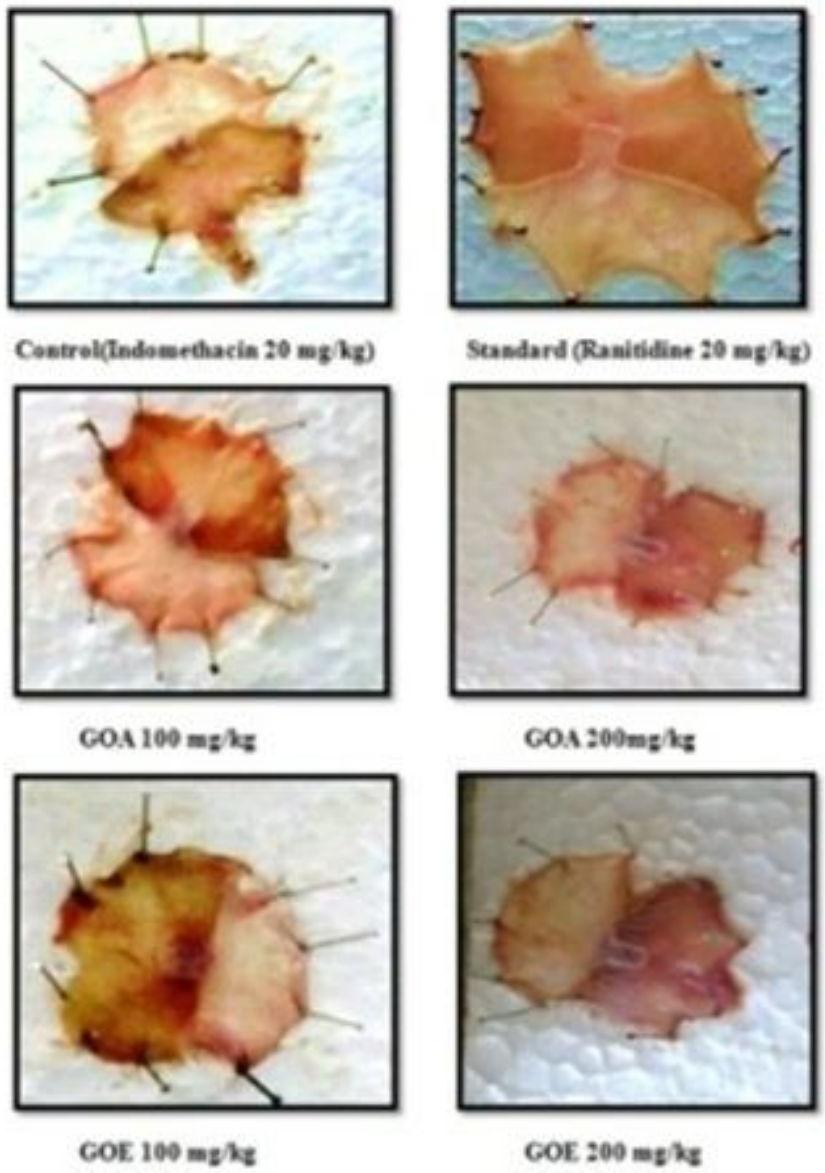

Figure 2: Effect of *Gynocardia odorata* roxb. seed extracts on indomethacin induced ulcer in rats

RESULTS

Preliminary phytochemical screening of ethanolic and aqueous extracts of *Gynocardia odorata* showed the presence of phytoconstituents such as Flavonoids, Proteins, Fixed oils, Tannins, Proteins, Alkaloids, Carbohydrates, Glycosides, Saponin's and Triterpenoids.

Pylorus ligation induced ulcer model

The ulcer index in pylorus ligated control animals was (10.69±0.11). The reduction in ulcer index observed were significant ($p<0.001$) when compared to control and compared to ranitidine (20mg/kg) (1.48±0.05), after the treatment by aqueous extract GOA at the dose of 100 mg/kg and 200 mg/kg body weight and ethanolic extracts GOE at a dose of 100 mg/kg and 200 mg/kg body weight were (3.425±0.04, 3.386±0.05, 5.414±0.08 and 3.451±0.05) respectively. (Table 1) pH of the pylorus ligated control group was (1.34±0.11) and when compared to test drug namely, aqueous extract GOA at a dose of 100 mg/kg and 200 mg/kg b.w. (4.1±0.22 and 3.30±0.11) and ethanolic extract GOE at a dose of 100mg/kg and 200 mg/kg were found to be (2.30± 0.21 and 2.85±0.02). When standard drug Ranitidine (20 mg/kg b.w.) was administered the pH increase to 4.3±0.34 which is comparable to test extracts. (Table 1) Gastric free acidity is increased to (42.80±0.154) in control animals due to pylorus-ligation which was decreased by both the extracts significantly, GOE 200mg/kg (14.59±1.23) and GOA at a dose of 200mg/kg and 100mg/kg was (11.80±2.80 and 3.20±0.58). The gastric free acidity is expressed as mEq/lt. (Table 1) Gastric total acidity is increased to (95.80±17.59) in pylorus ligated control animals. And significantly reduced by aqueous extract GOA at a dose of 100mg/kg and 200mg/kg were (6.00±0.71 and 24.80±4.38) as compared to pylorus ligated control. Results are tabulated in Table 1and shown in Figure 1. The gastric total acidity is expressed as mEq/lt. Pharmacological activity led to the conclusion that the aqueous extract exhibited more significant activity than the ethanolic extracts. The results of pharmacological activity also

concluded that the aqueous extract significantly raised the pH of gastric contents; it lowered the free and total acidity and ulcer index as compared to standard drug Ranitidine.

Indomethacin induced ulcer

The results of antiulcer activity of the seed extract on indomethacin induced ulceration in rats are shown in Table 2 and Figure 2. There was a progressive decline in ulcer index of the rats, pretreated with the extract. The decline was dose dependent and significant ($p<0.001$) compared to indomethacin control ($p<0.05$). These results showed that aqueous extract is more effective than the ethanolic extract but less effective than Ranitidine. Results of ulcer index for treated group were observed as, Ranitidine (20mg/kg b.w.) (4.24±0.074), GOA at a dose of 100 mg/kg b.w. and 200 mg/kg b.w. (6.51±0.017 and 6.53±0.071) respectively, GOE at a dose of 100 mg/kg and 200 mg/kg b.w. were (6.41±0.079 and 5.24± 0.045).These results showed that both aqueous and ethanolic extract have significant ($p<0.001$) effect but less than Ranitidine (10.75±0.062). Ulcer protection of ranitidine, GOA at a dose of 100 mg/kg and 200 mg/kg b.w. and GOE at a dose of 100mg/kg and 200 mg/kg b.w. were 70.45%, 46.63%, 52.61%,46.63% and 41.63% respectively and aqueous extract at both doses i.e. 100mg/kg and 200 mg/kg b.w. showed more significant results as compared to ethanolic extracts GOE at both dose levels of 100mg/kg and 200 mg/kg b.w. but less than standard drug ranitidine (20 mg/kg b.w.).

DISCUSSION

It is generally accepted that gastric ulcers results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defence mechanism (Harsh Mohan 2002). The role of free radicals is also reported in the indication of ulcers. Prostaglandins (PG) offer protection to duodenum through both increases in mucosal resistance as well as decrease in aggressive factors, mainly acid and Pepsin (Aly 1987). Pylorus ligation induced ulcers are due to auto digestion at the gastric mucosa and breakdown of the gastric mucosal barrier. In case of pyloric ligation, ulcer formation is mainly due to the stasis at the gastric juice and stress. Indomethacin is known to cause ulcer especially in an empty stomach and mostly on the glandular (mucosal) part of the stomach by inhibiting prostaglandin synthetase through the cycloxygenase pathway. Prostaglandin functions to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair.

Suppression of prostaglandins synthesis by indomethacin results in increase susceptibility of the stomach to mucosal injury and gastro duodenal ulceration. *Gynocardia odorata* roxb. seed extracts was observed to significantly reduce mucosal damage in the indomethacin–induced ulcer model, suggesting the possible extract mobilization and involvement of prostaglandin in the anti-ulcer effect of the extract Okokon and Nwafor (2008). *Gynocardia odorata* roxb. produced antiulcer activity in both models used in the current study. Ethanolic and aqueous extracts at both the doses, reduced ulcer incidence significantly ($P<0.01$) when compared to the control as evident by decrease in ulcer score in both models. Anti-secretory activity of the extracts was noticed in pylorus ligation induced ulcer model. There was also a decrease in gastric volume and reduction in free and total acidity in the animals treated with ethanolic and aqueous extracts. *Gynocardia odorata* roxb. is reported to contain antioxidant properties (Seal Tapan 2012). Quercetin is reported to prevent gastric mucosal lesions induced by various models (pylorus ligation, ethanol induced, cold restraint stress). Quercetin may increase the amount of natural glycoproteins, the most important proteins in the gastric mucosa, which may in turn facilitate the defence against an aggressive action. Quercetin also stimulates the synthesis of cyclooxygenase and of local prostaglandins. Other mechanism proposed includes inhibition of the gastric proton pump, lipoxygenase pathway, or inhibition of lipid peroxidation (Malairajan *et al*., 2006). *Gynocardia odorata* roxb. may have its antiulcer activity because of its active constituents like flavonoids and especially quercetin. From the phytochemical tests done on the extracts of *Gynocardia odorata*, it was confirmed that the same classes of active constituents were present. Both extract of *Gynocardia odorata* roxb. has shown significant effect in indomethacin induced ulcer model. Indomethacin causes generation of reactive oxygen metabolites (such as superoxide anion, hydrogen peroxide and hydroxyl radical), which damages the gastric tissue and causes ulcer formation. The pathogenesis of gastric mucosal lesions by indomethacin is associated with increased lipid peroxidation. Reduced glutathione in the gastric mucosa acts as the major scavenger of the oxygen-derived free radicals. It may be concluded that GOA and GOE has preventive action on indomethacin induced ulcer in rats. It is possible that the antioxidant effect of *Gynocardia odorata* roxb. seed extract might also play a role in the mechanism of antiulcer activity (Hollander *et al*., 1984; Goel and Bhattacharya 1991).

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