AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Phytochemical Screening and Ulcer Protective Activity of Ethanolic Seeds Extract of *Gynocardia odorata* In Different Ulcer Model

Shrish Kumar Srivastava*[1], Hemant kumar[2], Mrityunjoy Acharya [3]

1.Doon College of Education (Department of Pharmacy), NH-27A, Delhi-Dehradun Road, Sunderpur, Saharanpur- 247662.UP, India.

2. Saroj Institute of Technology and Management, Ahimamau, Lucknow - 226001, U.P, India

3.GRD (PG) IMT (Department of Pharmacy), 214-Rajpur Road Dehradun-248009. Uttarakhand, India.

ABSTRACT

Peptic ulcer is the areas of degeneration and necrosis of gastrointestinal mucosa exposed to acid peptic secretion. Some other factors, such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by Helicobacter pylori, may be responsible for the development of peptic ulcer. The several herbal formulations derived from Ayurveda and its additional systems of medicine, not yet to be scientifically validated that they have exhibited anti-ulcer activity. Our present study was to evaluate the ulcer protective activity of ethanolic extract of *Gynocardia odorata* (G. ordata) seeds on cold restraint and ethanol induced ulcer model. Five groups were taken (n=6). First groups serve as negative control (Without any treatment). Second groups were treated with vehicles only. Third groups were treated with some standard marketed drugs (Ranitidine and sucralfate). Fourth and fifth groups were treated with ethanolic seed extract of *G. odorata* in two increasing dose (250 and 500mg/kg body weight p.o respectively). Both the group showed significant ulcer protective activity in a dose dependent manner.

Keywords: Gynocardia odorata, Cold restrain ulcer, Ethanol induced ulcer, Flavonoids. Ulcer protective.

*Corresponding Author Email: shrish.srivastava@yahoo.com

Received 08 September 2013, Accepted 21 September 2013

Please cite this article in press as: Shrivastava S. *et al.*, Phytochemical Screening and Ulcer Protective Activity of Ethanolic Seeds Extract of *Gynocardia odorata* In Different Ulcer Model. American Journal of PharmTech Research 2014.

INTRODUCTION

For over a century, peptic ulcer disease has been one of the leading causes of gastrointestinal surgery, with high morbidity and mortality rates. As the prevalence of this disease increases over time, one would expect peptic ulcers to continue to have a significant global impact in the basic health and economic systems and in patients' life quality [1]. Recent research suggest that peptic ulcers were been caused by an imbalance between the aggressive factors and a number of known defense mechanisms. Exogenous aggressive factors such as smoke, anti-inflammatory drugs, alcohol, stress, fatty foods and *Helicobacter pylori* infections triggered tissue necrosis through mucosal ischemia, free radical generation and cessation of nutrient delivery, hydrochloric acid together with pepsin, pancreatic enzymes and bile decreased the defense mechanisms of gastrointestinal mucosa such as the intercellular junctions, local blood flow, mucus/bicarbonate secretion and cellular growth [2, 3]. With the ever growing interest in natural medicine, many plants have been screened and reported to be useful in treating and managing ulcer[4]. The plant *G.odorata* is also known as Chaulmoogra plant, seeds of the chaulmoogra tree, 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. The fruits are hot anthelmintic and used in bronchitis, skin diseases, small tumor's leprosy, and as an analgesic [5]. Recent study has been shown that hydroalcholic extract of *G. odorata* seed has shown there ulcerprotective activity in indomethacin and pylorus ligation induced ulcer[6]. In our recent investigation an attempt had been made to identify ulcerprotective activity in ethanol and cold restraint induce ulcer of *G. odorata* seed and to identify probable mechanism of actions.

MATERIAL AND METHODS

The seeds of *G. odorata were* collected and authenticated from Botanical Garden of National Botanical Research Institute, Lucknow, India. were deposited in the departmental herbarium of National Botanical Research Institute, Lucknow, India for future reference.

Preparation of extract [7]

Initially these seeds were washed with fresh water to remove adhering dirt and foreign particles and dried at 35 -40^{0}c in an oven. The dried seeds were crushed and grinded to get powder and weighed. The weighed powder was then placed with 70% ethanolic solution in a cylinder. 500g of *G. odorata powder* in 1.0 liter of ethanolic solution were macerated for 7 days. The mensturm was removed and concentrated by vacuum distillation. Again the crude material was

allowed to undergo maceration for 4 days followed by 2 days for complete extraction. The mensturm was collected and concentrated using Rotary evaporator at 50°C**.** This mixture was cooled and filtered by Buchner funnel and filter paper and then air dried in an evaporating dish till constant weight was obtained.

Preliminary Phytochemical screening

The ethanolic extracts was subjected to preliminary phytochemical qualitative screening to evaluate the presence of various primary or secondary metabolites following standard procedures[8].

Animals

Wister albino rats weighing (150-200 gm) and Swiss albino mice (weighing 20-25gm) were housed in the departmental animal house under standard conditions (26 ± 2°C and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and had free excess to water. All the experiment was approved by Institutional animal ethical committee (IAEC) as per the guideline of CPCSEA

Acute toxicity study

Four groups (n=6) of male albino mice were used to study the acute toxicity of ethanolic extract of *G.odorata* seeds (50, 500 and 2000mg/kg). The control animal receives 1% carboxy-methyl cellulose in distilled water (10ml/kg) orally. Any behavioral change had been noted before and after the administration of the dose for 24h. Treated animals were observed up to 14 days for any signs of toxicity[9].

Ethanol induced ulcer [10]

The gastric ulcers were induced in rats by administrating 99.5% ethanol (1 ml/200 gm) orally. 1h before sacrifice extract, Ranitidine and vehicle was administered to the respective group orally (p.o). The following groups (n=6) of animals were used.

I Group - Ethanol (20 mg/kg body wt,)

II GROUP- Ethanol + CMC (0.5% Suspension,)

III Group- Ethanol + Ranitidine (50 mg/kg,)

IV Group - Ethanol + EtOH Extract (250mg/kg)

V Group - Ethanol + EtOH Extract (500mg/kg)

Cold restraint induced ulcer[11]

The experiment was performed according to the method of Levine (1971). Rats were deprived of food, but not water, for about 18 h before the experiment. Than the experimental rats were

immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2 h, at temperature of 4–6 ◦C. Two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs as described above. The following groups of animals (n=6) were used and treated orally (p.o).

I Group - Cold-restraint stress (CRS)

II GROUP- Cold-restraint stress (CRS) + CMC (0.5% Suspension)

III Group- Cold-restraint stress (CRS) + Sucralfate (200 mg/kg)

IV Group - Cold-restraint stress (CRS) + EtOH Extract (250mg/kg)

V Group - Cold-restraint stress (CRS) + EtOH Extract (500mg/kg)

Statistical analysis

The values were represented as Mean ± S.E.M. and Statistical significance between treated and controlled group will be analyzed using of one way ANOVA, followed by Student- Newman-keuls test. ⁎P< 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of ethanolic and aqueous extracts of *G. odorata* showed the presence of phytoconstituents such as Flavonoids, Proteins, Fixed oils, Tannins, Proteins, Alkaloids, Carbohydrates, Glycosides, Saponin's and Triterpenoids (Table-1). Ethanolic extract of seed up to 2000mg/kg not exhibit any sign of toxicity up to 14 days in mice. Hence the ethanolic extract is considered as safe up to 2000mg/kg[12].

In Ethanolic extract with concentration of 250 mg/kg and 500 mg/kg resulted in a reduction in ulcer index in dose dependent manner with compared to control in both Ethanol and cold restrained induced ulcer model (Table-2 and Table-3). Statistical analysis revealed that ethanolic extract of seeds *G. odorata* contains antiulcer activity due to the presence of flavonoids and sterol viz. stigmasterol Peptic ulcer in that part of the gastrointestinal tract (g.i.t) which is exposed to gastric acid and pepsin, i.e., the stomach and duodenum. The etiology of peptic ulcer is not clearly known. Various mechanisms have been suggested for explaining the pathogenesis of peptic ulcer, which results due to an imbalance between the protective mechanisms and aggressive factors such as pepsin and acid. A variety of psychometric, humoral and vascular derangements have been implicated and the importance of Helicobacter pylori infection as a contributor to ulcer formation and recurrence has been recognized.

Table -1: Phytochemical analysis of ethanolic extracts of *Gynocardia odorata seeds*

Constituent	Test	EEGO
Alkaloids	Mayer's test	-
	Dragendroff' test	-
	Hager's test	+
	Wagner's test	+
Carbohydrates	Molisch's test	+
	Fehling's test	-
Glycosides	Brontrager's test	-
	Legal's test	-
Fixed oil and fats	Spot test	-
	Soap formation test	+
Tannins	$Fecl_3$	+
	Alkaline reagent	-
Protein and amino acids	Million's test	-
	Ninhydrin test	+
	Biuret test	+
Flavonoids	With NaOH	+
	With H_2SO4	-
Steroid and tri-terpenoids	Libermann's Burchard test	+
	Salkowski's test	+

Table-2 Effect of ethanolic seed extract of *Gynocardia odorata* on ulcer index on Ethanol induced gastric ulcers

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	(%) protection
Negative	Ethanol	20mg/kg	19.05±0.008	-
Control	CMC	10ml/kg	18.465±0.007	-
Standard	Ranitidine	50 mg/kg	4.07 ± 0.007*	77.94
Treated	Ethanolic extract	250 mg/kg	6.2± 0.002*	66.37
Treated	Ethanolic extract	500 mg/kg	4.9± 0.036*	73.46

Values are mean ± SEM (n=6) one way ANOVA followed by Student- Newman-keuls test, Where * represents significant at $p<0.05$, When compared to control group

Table-3 Effect of ethanolic seed extract of *Gynocardia odorata* on ulcer index of Cold Restraint Induced gastric ulcers.

Group	Treatment	Dose (mg/kg)	Ulcer index (mm^2/rat)	% Protection
Negative	CRS	-	14.33±0.016	-
Control	CMC	10 ml/kg	13.53±0.014	-
Standard	Sucralfate	200 mg/kg	3.9±0.06*	71.17
Treated	Ethanolic extract	250 mg/kg	5.46±0.076*	59.64
Treated	Ethanolic extract	500 mg/kg	4.138±0.084*	69.41

Values are mean ± SEM (n=6) one way ANOVA followed by Student- Newman-keuls test, Where * represents significant at $p<0.05$, When compared to control group.

CONCLUSION

Gastric ulceration is mainly caused by the alteration of the antioxidant enzymes of the gastric mucosa with concomitant loss of cytoprotection due to decreased activity of the PG synthesis. Ethanol-induced gastric injury is associated with significant production of oxygen-free radicals leading to increased lipid peroxidation, which causes damage to the cell and the cell membranes[13].*G.odorata* seeds significantly protects the gastric mucosa against the ethanol challenge as shown by the reduced values of the ulcer index, as compared to the solvent control group, suggesting its potent gastroprotective effect, which may be due to extract contain flavonoid because bioflavonoid have potent antioxidant activity or free radicals scavenging activity. Stress plays an important role in the causation of gastro duodenal ulceration and antistress drugs were found to be effective in stress-induced gastric mucosal damage. Disturbance of gastric mucosal microcirculation, alteration in gastric secretion and abdominal gastric motility have been considered as pathogenesis mechanisms responsible for the stress-induced gastric mucosal lesions. It can thus be concluded that acute stress generates reactive oxygen species, causing gastric injury by various mechanisms. While pretreatment with a suitable antioxidant like *G. odorata* seed extract may provide gastroprotection[14]. But to conclude this further extensive study is to be carried out.

REFERENCES

1. Yuan Y, Padol IT, Hunt RH. Peptic ulcer disease today. Nat. Clin. Pract. Gastroenterol. Hepatol. 2006, 3, 80- 89.
2. Tarnawski AS. Cellular and molecular mechanisms of gastrointestinal ulcer healing. Digest. Dis. Sci. 2005, 50, 24-33.
3. Kaunitz, JD, Akiba Y. Gastroduodenal mucosal defense: role of endogenous mediators. Curr. Opin. Gastroenterol. 2004, 20, 526-532.
4. Marslin Gregory, B. Divya, Revina Ann Mary,. M. M. Hipolith Viji, V. K. Kalaichelvan, V. Palanivel. Anti-ulcer activity of Ficus religiosa leaf ethanolic extract. Asian Pac J Trop Biomed 2013; 3(7): 554-556.
5. Handa S.K, Kaul M.K. Supplement to cultivation and utilization of medicinal plants, 727-739, 1998.
6. Antiulcer Activity of seed extracts of gynocardia odorata roxb. on pylorus ligation and indomethacin induced gastric lesions in albino rats. Int J Develop Res 2013;3(5): 049-054.

7. Rita M., Charde., Hemant J. Dhongade1., Manoj S., Charde, Kasture AV. Evaluation of antioxidant, wound healing and anti- inflammatory activity of ethanolic extract of leaves of Ficus religiosa. Int J Pharma Sci 2010; 1(5): 73-82.
8. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, Nirali Prakashan; 39thedi. Aug. 2007. 284-285.
9. Turner RA, Screening methods in pharmacology. New York: Academic Press; 1965: 302-304.
10. Szabo,S . Mechanism of mucosal injury in the stomach and duodenum: time sequence analysis of morphologic, functional, biochemical and histochemical studies. Scand. J. Gasteroenterol .22 (suppl.127), 21-28.
11. Levine RJ, A method for rapid production of stress ulcers in rats. In, peptic ulcer,. ed. by C.J. Pfeiffer, Munksgaard, Copenhagen . 1971: 92-97..
12. OECD 425. Acute Oral Toxicity – Up-and-Down Procedure. OECD Guideline for testing of chemicals. Adopted: 17th December 2001.
13. Hollander D, Tarnawski A, Gergely H, Zipser RD. Sucralfate protection of the gastric mucosa against ethanol, induced injury: A prostaglandin, mediated process. Scand J Gastroenterol, 1984; Supplement 19(101): 97- 102.
14. Shobha V. Huilgol and M.G. Jamadar. Gastroprotective role of bioflavonoid silymarin in animal model of acute cold-restraint stress induced gastric ulceration. Al Ameen J Med Sc i 2013; 6(1) :40-43.