ORIGINAL ARTICLE

Antiulcer potential of *Rubia cordifolia* Linn. in experimental animals

Pankaj Kalra, Ashok Kumar Datusalia, Sunil Sharma
Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hissar, India

Alcoholic extract of roots of *Rubia cordifolia* Linn. (AERC) has been examined to evaluate its antiulcer potential on alcohol, ibuprofen, cold-restraint stress and pyloric ligation-induced gastric lesions. AERC of 100-400 mg/kg was administered orally, once daily for 15 days, to rats of different groups. Ranitidine at a dose of 50 mg/kg was used as a standard drug for these gastric ulcer models. The gastric content was collected and the volume was measured. The ulceration index was determined by examining the inner lining of each stomach. Furthermore, the effect was assessed by free acidity, pepsin activity, total carbohydrate, protein content. The extract demonstrated significant protection against 100% alcohol-(P<0.05-0.01), ibuprofen-(P<0.05-0.01), cold-restraint stress-(P<0.01) and pylorus ligation-(P>0.01-P>0.01) compared to ranitidine (P<0.01).

Key words: Alcoholic extract, antiulcerogenic activity, *Rubia cordifolia* Linn

INTRODUCTION

Gastric ulcers, one of the most widespread disease states, are believed to be due to an imbalance between acid and pepsin along with weakness of the mucosal barriers.[1] The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products and drugs.[2] These agents have been implicated in the pathogeneses of gastric ulcer, including increased gastric acid and pepsin secretion, decreased gastric blood flow, the suppression of endogenous generation of prostaglandins, inhibition of mucosal growth and cell proliferation and alteration of gastric mobility.[3] Although there are many products used for the treatment of gastric ulcers, most of these drugs produce several adverse reactions.[4] Plant extracts, however, are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers.[5] In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders, including gastric ulcers.[6,7]

R. cordifolia Linn. (Rubiaceae) is a climber growing in the north-west Himalaya and other hilly areas of India. It has common names like *Manjistha* (Bengali), *Manjith* (Hindi), Indian madder, *Chiranji* (Telugu), etc. It is an important medicinal plant which is used for treatment of various ailments in Ayurvedic system of medicine. Various crude extracts from this plant have been evaluated for therapeutical potential in anti-inflammatory,[8] immunomodulatory,[9] anticonvulsant, anxiolytic[10] and anticancer[11] studies. Preliminary phytochemical investigation leads to identification of several active constituents such as saponins, rubiadin (Anthaquinone glycosides), 6-methoxygeniposidic (Iridoids) and triterpenoids.[12] Out of these compounds rubiadin (isolated from roots) was found to have potent antioxidant and inhibit lipid peroxidation. Roots of this plant are the most abundantly used in folkore medicine to treat the disorders including gastric ulcer,[13] but none of study has been reported to prove it experimentally.

Henceforth, the present study has been undertaken to evaluate the role of the roots of *R. cordifolia* in ulcerogenic models of rats. Further, ulcerogens have a vast mechanism to induce gastric ulcer so we had used NSAIDS-induced ulcer, cold restraint ulcer, pylorus ligation and alcohol-induced ulcer models to understand the gastro-protective mechanism. Effects on peptic activity, gastric secretion and oxidative mucosal contents were also investigated.

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MATERIALS AND METHODS

Plant Material and Preparation of Extract

The dried roots of *R. cordifolia* were purchased from local market of Hissar, Haryana, India. The crude drug

Address for correspondence: Mr. Pankaj Kalra, Department of Pharmaceutical Sciences, Guru Jambheshwar, University of Science and Technology, Hissar – 125 001, Haryana, India. E-mail: pankajchd84@gmail.com
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was authenticated by Dr. H. B. Singh, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Dried roots (500 g) were extracted successively with petroleum ether at 60–80°C (to remove fatty matter) and alcohol (95%) at 60°C using Soxhlet's extractor till the material was exhausted as indicated by a blank thin layer chromatography. Alcoholic extract of roots of Rubia cordifolia Linn. (AERC) was concentrated by slow heating on water bath and stored at a temperature below 10°C until used for the study. The yield of AERC was found to be 36.4 g. The extract was suspended in freshly prepared 1% carboxymethyl cellulose (CMC) and administered orally.

Animals

Wistar rats of either sex (weighing 150-180 g, 90 days old), obtained from Disease Free Small Animal House, CCHAU, Hissar India, were maintained at controlled room temperature (25±2°C) and 50% humidity at 12-hour light/dark with free access to food and water. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) and care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Reg. No. 0436).

Drugs and Chemicals

Ranitidine (Martin Brown, India), ibuprofen (IBU; Martin Brown, India), alkaline reagent (S.D.Fine-Chem Ltd.), folin-ciocalteu reagent (Phenol reagent; S.D.Fine-Chem Ltd.), bovine albumin (Hi-Media), trichloroacetic acid (TCA; Qualigens Fine Chemicalls), thiobarbituric acid (TBA; Hi-Media), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB; S.D.Fine-Chem Ltd.) were used. All drugs were administered in a volume of 1 ml/100 g body weight (rats) in 1% CMC.

Experiment Protocols

AERC extract (100, 200 and 400 mg/kg body weight) and standard antiulcer drugs ranitidine (50 mg/kg body weight) were prepared in 1% sodium CMC vehicle as suspension and administered orally (p.o.) once in a day at a volume of 1 ml/100 g of body weight for 15 days.

All animals were deprived of food for 18 hours but not of water before subjecting to ulcerogens and were randomly allocated into three groups, each carrying six rats:

- Experimental group treated with 100, 200 and 400 mg/kg doses of AERC extract
- Experimental group treated with ranitidine
- Control group treated with vehicle similar to that of the experimental group

Cold Restraint Stress-induced Ulcer

Animals of different experimental groups were subjected to cold stress on the 15th day of treatment after treatment of AERC extract (test drug) and ranitidine (standard drug) respectively. All the animals were immobilized in restraint cages kept at 4°C in an environmental chamber for 2 hours.[14] All animals were killed using ketamine (60 mg/kg); the stomach was removed, cut along the greater curvature, washed with saline (0.9%) and ulcer index was scored.

Alcohol-induced Ulcer

Gastric ulcers were induced by administration of absolute alcohol at a dose of 1 ml/200 g of body weight, orally on the 15th day after 45 minutes of respective treatment.[15]

Ibuprofen-induced Ulcer

Ibuprofen was given in two doses of 300 mg/kg orally on the 15th day of treatment at 15 hours interval to 24-hour-fasted animals.[16] All animals were killed 6 hours after the second dose of ibuprofen.

Pylorus Ligation-induced Ulcer

A pylorus ligature was carefully done in rats under ketamine anaesthesia.[17] Under ketamine anaesthesia, abdomen was opened by a small midline incision below the xiphoid process; the pyloric position of the stomach was slightly lifted out and ligated avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures and animals were killed at the end of 6 hours after operation. Stomachs were dissected out, and contents were drained into tubes and subjected for analysis of biochemical parameters. The stomachs were then cut along the greater curvature and the inner surface was examined for ulceration.

Measurement of Ulcer Index

Ulcer scoring was done by viewing ulcers with magnifying glass. The following a bitrary scoring system was used to grade the incidence and severity of lesions.[18]

Shedding of epithelium=10, petechial and frank haemorrhages=20, one or two ulcers=30, more than two ulcers=40, perforated ulcers=50.

Ulcer index (U_i) was calculated from scoring as -
$U_i = U_s + U_p \times 10^{-1}$
where U_s is the mean severity of the ulcer score, U_p is the percentage of animals with ulcer incidence.

The percentage protection index was calculated as
$C - T/C \times 100$ (where C = ulcer index in the control group; T = ulcer index in the treated group)

Effects on Gastric acid Secretion in Pylorus-ligated Rats

The gastric content of each stomach was obtained through an incision in the stomach and juice was filtered through glass wool. The gastric content was centrifuged at 2500×*g* for 20 minutes at 4°C and the volume of the supernatant (ml) was measured. The volume was expressed as ml/100 g body weight. An aliquot (1 ml) of each sample was titrated against 0.01 N NaOH using the phenolphthalein reagent as an indicator. The acid concentration was expressed as mEq/l.

Peptic Activity

Gastric pepsin activity was determined by using bovine serum albumin as a substrate and expressed in terms of micromoles of tyrosine/ml.[19] A mixture of gastric juice (0.1 ml) and 0.5% bovine serum albumin in 0.01 NHCl (1 ml) was incubated at 37°C for 20 minutes and the reaction was stopped by adding 10% trichloroacetic acid (2 ml). After the denaturation of protein by heating in a boiling water bath for 5 minutes, the precipitate was removed by centrifugation (9000×*g*, 10 minutes). A total of 1 ml of the supernatant was mixed with 0.4 ml of 2.5 N NaOH and 0.1 ml of Folin-Ciocalteu reagent and the volume was adjusted to 10 ml with distilled water. The absorbance was measured at 700 nm. A sample (gastric juice blank) containing no albumin was used as the control. The total protein[20] and total carbohydrates[21] were also measured from gastric juice.

Determination of Oxidative Parameters

The stomach were rapidly dissected off the animals and stored at −80°C until they were analysed. Tissues were homogenized in nine volumes of ice-cold 0.15 mol/l KCl-1 mmol/l EDTA using a microhomogeniser. The homogenates were used for the estimation of malondialdehyde (MDA), non-protein sulfhydryl (NP-SH) and total sulfhydryl groups (T-SH).

Malondialdehyde Estimation

The level of lipid peroxides was determined as malondialdehyde (μmol/100 mg wet tissue) by measurement of thiobarbituric acid (TBA)-reactive substance at 532 nm.[22]

Determination of the Non-protein Sulfhydryl and Total Sulfhydryl Groups

The T-SH and NP-SH groups were determined with some modifications using glutathione (GSH) as the standard.[23] For determination of the T-SH group, the homogenate was mixed with Tris buffer (0.2 M, pH 8.2) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and further mixture was diluted with absolute methanol. A reagent blank (without sample) and a sample blank (without DTNB) were prepared in a similar manner. After the addition of DTNB, colour was developed in the reaction mixture which was centrifuged at 3000×*g* at room temperature for 15 minutes. The absorbance of supernatant was read at 412 nm. For the determination of the NP-SH group, aliquot of the homogenate was mixed with distilled water and 50% trichloroacetic acid. Tubes were centrifuged for 15 minutes at 3000×*g*. Supernatant was mixed with Tris buffer (0.4 M, pH 8.9) and DTNB. The absorbance was read within 5 minutes at 412 nm against a reagent blank (without sample).

Statistical Analysis

All results were expressed as mean ± standard error of mean (SEM). Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. In all tests, the criterion for statistical significance was $P<0.05$.

RESULTS

The effect of AERC on various parameters of gastric ulceration and secretion and mucosal offensive and defensive factors has been summarized in Table 1 and Figures 1-4 respectively.

Effect of AERC on the Ulcer Index

AERC showed a significant protection against the experimental ulcers induced by alcohol, ibuprofen, cold-restraint stress and pylorus ligation. The percentage protection ranged from 18.18 to 57.5%, 31.6 to 45.1%, 31.0 to 57.92% and 14.88 to 40.73% respectively and was comparable with ulcer protection afforded by standard protective drug, ranitidine (50 mg/kg; percent protection 63%–72.4%) in all above models studied [Figure 1].

Effect on Gastric Secretion

The effect of AERC on offensive acid-pepsin secretion and defensive mucin content (in terms of TC: P ratio) was studied in gastric juice in the Pylorus ligation-induced ulcer (PL) model. AERC dose-dependently (100-400 mg/kg) decreased acid and pepsin secretion by 19.0% to 55.2% and 15.4 to 32.0% respectively. On the other hand, the mucin content was increased by 35.0 and 43.63% at doses of 200 and 400 mg/kg respectively. The standard drug ranitidine showed a significant decrease in free peptic activity 55.2% and 62.5% respectively compared to control but no significant increase in mucus content was observed [Table 1].

Effect on the Lipid Peroxidation, Non-protein Sulfhydryl and Total Sulfhydryl Groups in Gastric Homogenate

In lipid peroxidation, AERC showed significant reduction ($P<0.01$) at 200 and 400 mg/kg in alcohol-induced ulcer (AL), Ibuprofen-induced ulcer (IB) and Cold restraint stress-induced ulcer (CRU) models whereas ranitidine and 100 mg/kg dose were less significant ($P<0.05$). In T-SH and NP-SH, there was a significant ($P<0.01$) increase at higher doses (200, 400 mg/kg) whereas 100 mg/kg dose and ranitidine were less significant ($P<0.05$) [Figures 2-4].

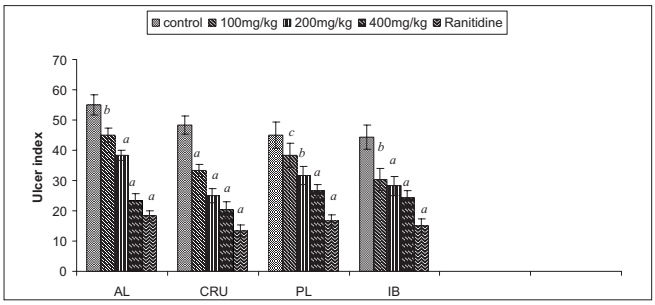

Figure 1: Effect of AERC on ethanol, ibuprofen, cold-restraint stress and pylorus ligation in rats. The values represent Mean±S.E.M. (*n*=6); [a]*P*<0.01, [b]*P*<0.05, [c]*P*>0.05 significantly different from control

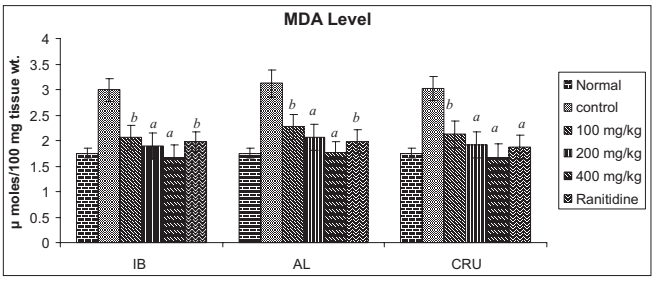

Figure 2: Effect of AERC and ranitidine on MDA levels (μmol/100 mg tissue) at different doses (100, 200, 400 and 50 mg/kg respectively) in the stomach of rats treated with alcohol, ibuprofen and cold restraint stress. The values represent mean±S.E.M. (*n*=6); [a]*P*<0.01, [b]*P*<0.05, [c]*P*>0.05, significantly different from control

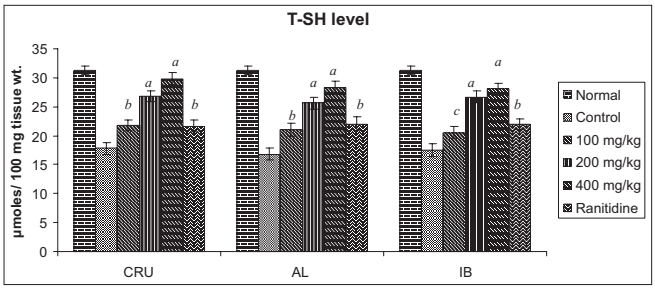

Figure 3: Effect of AERC and ranitidine on T-SH levels (μmol/100 mg tissue) at different doses (100, 200, 400 and 50 mg/kg respectively) in the stomach of rats treated with alcohol, ibuprofen and cold restraint stress. The values represent mean±S.E.M. (*n*=6); [a]*P*<0.01, [b]*P*<0.05, [c]*P*>0.05, significantly different from control

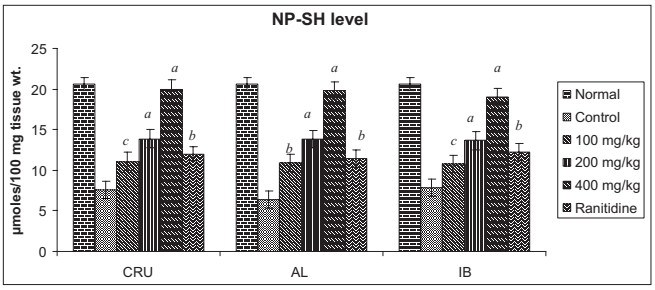

Figure 4: Effect of AERC and ranitidine NP-SH levels (μmol/100 mg tissue) at different doses (100, 200, 400 and 50 mg/kg respectively) in the stomach of rats treated with ethanol, Ibuprofen and cold restraint stress. The values represent mean±S.E.M. (*n*=6); [a]*P*<0.01, [b]*P*<0.05, [c]*P*>0.05, significantly different from control

Table 1: Effect of alcoholic extract of roots of *Rubia cordifolia* Linn. and ranitidine on biochemical parameters from pylorus ligated rats

Group (mg/kg body wt.)	Gastric vol. (ml/100 g)	Free acidity (mEq./l)	Peptic activity (U/ml)	TC (μg/ml)	P (μg/ml)	TC/P
Control (1% CMC)	2.72±0.53	190.5±16.33	42.2±3.6	524.6±35.76	423.56±44.78	1.24±0.12
AERC (100)	2.13±0.44	154.3±14.2	35.7±3.1	623.56±17.91[b]	385.98±27.71	1.61±0.16
AERC (200)	1.78±0.40	134.7±1.38[b]	30.6±3.7	684.75±18.64[a]	356.89±18.69	1.91±0.12b
AERC (400)	1.23±0.32[b]	97.6±10.2[a]	28.7±2.7[b]	723.67±14.15[a]	318.98±15.42[b]	2.2±0.17[a]
Ranitidine (50)	0.8±0.2[a]	85.3±9.7[a]	15.8±2.2[a]	599.9±18.45	378.98±18.28	1.58±0.19

The values represent mean±S.E.M. (*n*=6); [a]*P*<0.01, [b]*P*<0.05, [c]*P*>0.05, significantly different from control; AERC - Alcoholic extract of roots of *Rubia cordifolia* Linn.; TC - Total carbohydrate; P - Total Protein

DISCUSSION

The aim of this study was to evaluate the gastro-protective effect of AERC in various experimental ulcer models namely ethanol, ibuprofen, cold-restraint stress and pylorus ligation. AERC showed a dose-dependent ulcer-protective effect against alcohol, ibuprofen, cold-restraint stress and pylorus ligation and also showed the antioxidant property in all gastric ulcers models.

Gastric ulcers include imbalances between offensive and defensive mucosal factors.[24] AERC depicted significant effectiveness (*P*<0.01) in PL-induced gastric ulcers showing a 40.73% protection index at dose 400 mg/kg whereas ranitidine showed a 62.95% protection index. AERC significantly (*P*<0.05-0.01) increased the defensive mucin secretion quantified in terms of TC: P ratio of the gastric juice[25] at 200 and 400 mg/kg doses. Mucin is a viscous glycoprotein with physiochemical properties producing a relatively resistant acid barrier.[26] It makes up the major part of the mucus, an important pre-epithelial factor that acts as a first line of defence against ulcerogens.[27] The increase in mucin was due to a significant increase in individual mucopolysacharide like sialic acid and total hexose leading to a significant increase in total carbohydrates. AERC also significantly increased the glycoprotein content of mucosal cells as seen from the increase in the TC: P ratio of gastric mucosa.[28] This shows that AERC induces turnover of glycoprotein in the mucosal cells, thus increasing the quantity of cellular mucous. On the other side it also showed a significant (*P*<0.05) decrease in peptic and free acidity at higher dose (400 mg/kg) whereas a lower dose was insignificant in all offensive (free acidity and peptic activity) and defensive factors (mucus content). The second dose 200 mg/kg significantly (*P*<0.05) decreased free acidity

and increased mucus content ($P<0.05$). The ineffectiveness of lower dose may be attributed to the low effectiveness of lower doses in protecting mucosa from ulcerogens compared to the dose of 400 mg/kg.

We selected CRU based on previous reports,[29] suggesting that peripheral sympathetic activation plays an important role in induction of ulcers by restraint. In CRU, incidence of ulcers is mainly due to increased acid secretion, generation of free radicals, etc. AERC showed significantly reduction in ulcer index in this model when compared to control. Efficacy of AERC in this model shows potent anti-oxidant activity by virtue of which it decreased the susceptibility of gastric mucosa to the damaging effect of ulcerogen. In the AL model, ulcers are caused due to perturbations of superficial epithelial cells, notably the mucosal mast cells leading to the release of the vasoactive mediators including histamine, thus causing damage to gastric mucosa.[30] Mucosal blood flow has been attributed to be an important factor in the damage caused by alcohol and is modulated by prostaglandin.[31] The effectiveness of AERC in protecting against mucosal damage caused by ethanol is indication of its effect on prostaglandins. It has been also accepted that ethanol-induced ulcers are not only inhibited by anti-secretory agents such as ranitidine, but also by agents that enhance mucosal defensive factors.[32,33] Thus it is possible to propose the existence of the cytoprotective effect of compound(s) present in AERC. This is also confirmed by a significant decrease ($P<0.01$) in lipid peroxidation and an increase ($P<0.01$) in the non-protein sulfhydryl (NP-SH) and total sulfhydryl groups (T-SH) in tissue (stomach) of the animal.

AERC has shown a significant effect ($P<0.01$) in the IB-induced ulcer model in comparison to control. NSAIDs have been reported to produce ulcers by both local and systemic effects. Ibuprofen causes a direct irritant effect by increasing the H^+ ion transport. It decreases mucin, surface active phospholipids bicarbonate secretion and mucosal proliferation, and also produces damage by formation of free radicals.[34] The possible protective effect of AERC in IB could be due to increased mucus secretion and free radical scavenging activity as observed in the present study.

Our study also supports the earlier reports about anti-stress and nootropic activity of AERC which suggests the free radical scavenging effect of AERC.[35] Such activity might also be responsible for the antiulcer effect of AERC. Moreover, stress induced ulcer aare due the decrease in the mucosal defence factors[36] and there was an increase in mucin secretion in PL-induced ulcers. So cytoprotection in CRU might be due to an increase in mucosal defense.

CONCLUSION

AERC has shown a substantial and significant protection against gastric ulcers in all the models. This protective effect might have been mediated by both anti-secretory and cytoprotective mechanisms at highest dose 400 mg/kg and a cytoprotective effect at 200 mg/kg. Our study strongly suggests that active constituents of AERC have potential to be used as an antiulcer drug. Moreover, further insight into the precise mechanism of action is essential to explore the complete potency of AERC and increase its usage in contemporary medicine.

REFERENCES

1. Alkofahi A, Atta AH. Pharmacological screening of the anti-ulcerogenic effects of some Jordanian medicinal plants in rats. J Ethnopharmacol 1999;67:341-4.
2. Peskar BM, Maricic N. Role of prostaglandins in gastroprotection. Dig Dis Sci 1998;43(9 Suppl):23S-29S.
3. Konturek PC, Brzowski T, Sliwowski Z, Pajido R, Stachura J, Hahn EG, *et al*. Involvement of nitric oxide and prostaglandins in gastroprotection induced by bacterial lipopolysaccharide. Scand. J Gastroenterol 1998;33:691-700.
4. Ariyphisi I, Toshiharu A, Sugimura F, Abe M, Matsuo Y, Honda T. Recurrence during maintenance therapy with histamine H2 receptors antagonist in cases of gastric ulcers. Nikon Univ J Med 1986;28:69.
5. Hiruma-Lima CA, Gracioso JS, Toma W, Almeida AB, Paula AC, Brasil DS, *et al*. Gastroprotective effect of aparisthman, a diterpene isolated from *Aparisthmium cordatum*, on experimental gastric ulcer models in rats and mice. Phytomedicine 2001;8:94-100.
6. Gracioso JS, Hiruma-Lima CA, Souza Brito AR. Antiulcerogenic effect of a hydroalcoholic extract and its organic fractions of *Neurolaena lobata* (L.) R. Br. Phytomedicine 2000;7:283-9.
7. Hiruma-Lima CA, Gracioso JS, Bighetti EJ, Grassi- Kassisse DM, Nunes DS, Souza Brito AR. Effect of essential oil obtained from *Croton cajucara* Benth. on gastric ulcer healing and protective factors of the gastric mucosa. Phytomedicine 2002;9:523-9.
8. Antakar DS, Chintwala T, Bhatt N. Anti-inflammatory activiy of *Rubia cordifolia* Linn. in rats. Indian J Pharmacol 1983;15:185-8.
9. Joharapurkar AA, Deode NM, Zambad SP, Umathe SN. Immunomodulatory activity of alcoholic extract of *Rubia cordifolia* Linn. Indian Drugs 2003;40:179.
10. Kasture VS, Deshmukh VK, Chopde CT. Anticonvulsant and behavioral actions of triterpene isolated from *Rubia cordifolia*. Indian J Exp Biol 2000;38:675-80.
11. Adwankar MK, Chitnis MP. *In vivo* anti-cancer acivity of RC-80, a plant isolate from *Rubia cordifolia* Linn., against a spectrum tumour models. Chemotherapy 1982;28:291-3.
12. Itokawa H, Qiao YF, Takeya K, Iitaka Y. New triterpenoids from *Rubia cordifolia* var. pratensis (Rubiaceae). Chem Pharm Bull 1989;37:1670.
13. Mhaskar KS, Blatter E, Caius JF. Indian Medicinal plants – Their usage in Ayurveda and Unani Medicine. 2000;3.
14. Suleyman H, Demirezer LO, Buyukokuroglu ME, Akcay MF, Gepdiremen A, Banoglu ZN, *et al*. Antiulcerogenic effect of *Hippophae rhamnoides* L. Phytother Res 2001;15:625-7.
15. Suleyman H, Demirezer LO, Kuruuzum-Uz A. Effects of Rumex patientia root extract on indomethacin and ethanol induced gastric damage in rats. Pharmazie 2004;59:147-9.

16. Parmar NS, Desai JK. A review of the current methodology for the evaluation of gastric and duodenal anti-ulcer agents. Indian J Pharmacol 1993;25:120.
17. Shay H, Komarov SA, Fels SS, Meranze D, Gruenstein M, Siplet H. A simple method for the uniform production of gastric ulceration. Gastroenterol 194;55:43-61.
18. Goel RK, Bhattacharya SK. Gastroduodenal mucosal defense and mucosal protective agents. Indian J Exp Biol 1991;29:701-14.
19. Prino G, Paglialunga S, Nardi G, Lietti A. Inhibition of experimentally induced gastric ulcers in the rat by a new sulfated glycopeptides. Eur J Pharmacol 1971;15:119-26.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol reagent. J Biol Chem 1951;193:265-75.
21. Goel RK, Chakrabarti A, Sanyal AK. The effect of biological variables on the anti-ulcerogenic effect of vegetable plantain banana. Planta Med 1985;2:85-8.
22. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
23. Kayali RC, Akatay U, Akcay T, Altu˘g T. Effect of alpha-lipoic acid supplementation on markers of protein oxidation in post-mitotic tissue of ageing rat. Cell Biochem Funct 2006;24:79-85.
24. Srivastava SK, Nath C, Gupta MB, Vrat S, Sinha NJ, Dhawan NK, *et al*. Protection against gastric ulcer by verapamil. Pharmacol Res 1991;23:81-6.
25. Sanyal AK, Mitra PK, Goel RK. A modified method to estimate dissolved mucosubstances in gastric juice. Indian J Exp Biol 1983;21:78-80.
26. Flemstrong G, Garner A. Gastroduodenal HCO^3 transport: Characteristics and proposed role in acidity regulation and mucosal protection. Am J Physiol 1982;242:153-93.
27. Zalewsky CA, Moody FG. Mechanisms of mucus release in exposed canine gastric mucosa. Gastroenterol 1979;77:719-29.
28. Goel RK, Maiti RN, Mukhopadhyaya K. Effect of Tamrabhasma, an indigenous preparation of copper on rat gastric mucosal resistance. Indian J Exp Biol 1994;32:559-61.
29. Djahanguiri B, Taubin HL, Landsburg L. Increased sympathetic activity in the pathogenesis of restraint ulcer in rats. J Pharmacol Exp Ther 1973;184:163-8.
30. Miller TA, Henagan JM. Indomethacin decreases resistance of gastric barrier to disruption by alcohol. Dig Dis Sci 1984;29:141-9.
31. 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;19:97-102.
32. Hunt HR, Cederberg C, Dent J, Halter F, Howden C, Marks IN, *et al*. Optimizing acid suppression for treatment of acid-related diseases. Dig Dis Sci 1995;40:24s-49s.
33. Wolfe M, Sachs G. Acid suppression: Optimizing therapy for gastroduodenal ulcer healing, gastroesophageal reflux disease, and stress-related erosive syndrome. Gastroenterol 2000;118(2 Suppl 1):S9-31.
34. Scheiman JM, Dubois RN, Giardiello FM. NSAIDs, Eicosonoids, and the Gastroenteric Tract. Vol. 25. Philadelphia: Saunders; 1996. p. 279-89.
35. Patil RA, Jagdale SC, Kasture SB. Antihyperglycemic, antistress and nootropic activity of roots of *Rubia cordifoia* Linn. Indian J Exp Biol 2006;44:987-92.
36. Miller TA. Mechanisms of stress-related mucosal damage. Am J Med 1987;83(6A):8-14.

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