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Protective effect of *Indigofera aspalathoides* in chemical induced gastric mucosal lesions in rats

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SUMMARY

The plant *Indigofera aspalathoides* is used by a large number of tribes in India for the treatment of various hepatic disorders and abscesses. The methanol extract of *Indigofera aspalathoides* (MEIA) was evaluated for its protective effects on gastric mucosal lesion in Wister albino rats against indomethacin, histamine and ethanol induced gastric mucosal damage. The response to MEIA was assessed using the ulcer index, thiobarbituric acid reactive substance (TBARS), and glutahione level. MEIA pretreatment showed protection against chemical induced gastric mucosal damage, a significant reduction in the ulcer index and TBARS activity and increase glutathione level as compared with that of standard drugs.

Key words: *Indigofera aspalathoides*; Gastric mucosal damage; Indomethacin; Histamine; Ethanol; TBARS; Glutathione

INTRODUCTION

The plant *Indigofera aspalathoides* (Leguminaceae) is commonly known as 'Shivanarbembu' in Tamil. In the traditional medicinal system, the leaves, flowers and tender shoot are said to be cooling and demulcent. They are used in the form of a decoction for leprosy and cancer. (Kirtika and Basu, 2001). The whole plant is used in odematous tumors and the ashes are used in preparations for dandruff's. Recently it has been reported that the methanol extract also exhibited significant hepatoprotective properties against carbon tetrachloride induced hepatic damage in rats (Gupta *et al.*, 2004).

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MATERIALS AND METHODS

Preparation of plant extract

The plant of *Indigofera aspathaloides* was collected in the month of March from Tamilnadu, identified by Botanical Survey of India, Shibpur, Howrah. Whole plant have been shade dried, they were powdered in a mechanical grinder. The powder was then passed through sieve no # 40. The powdered material was then extracted with petroleum ether and methanol in a Soxhlet extraction apparatus. The solvent was completely removed under reduced pressure. The yield of petroleum ether extract is 7.5% and methanol extract is 4.5%. The chemical constituents of the extract were identified by qualitative analysis and confirmed by thin layer chromatography. This indicated the presence of steroids, flavonoids and tannin (Harbon, 1984). The extract was then stored in the refrigerator and a weighed amount was suspended in 0.025%

carboxy methylcellulose (CMC) for the present investigation.

Reagents for estimation of biochemical markers were obtained from Sigma Chemical Co., St. Louis. Solvents (AR grade) were obtained from E-Merck, Mumbai.

Drug induced gastric ulcer in rats (Akah *et al.*, 1990)

Male Swiss Albino rats weighing (180 - 200 g) were maintained in identical laboratory conditions and given standard food pellets (Hindustan Lever, Mumbai) and water *ad libitum*. Rats were fasted for 18 h and deprived of water for 12 h before experiment. The rats were divided into four groups (n = 6). The animal's in-group I vehicle control received 0.025% CMC solution. Group II received famotidine (30 mg/kg p.o.). Animals in Group III and IV received methanol extract of *Indigofera aspalathoides* (MEIA) at doses of 250 and 500 mg/kg 1 h before the oral dose of indomethacin (20 mg/kg p.o.). All rats in each group were sacrificed after one hour. The stomach was then opened along the greater curvature, rinsed with normal saline and examined grossly. The ulcer was graded using the following scoring system suggested by Merazzi-Uberti and Turba (Giordano *et al.*, 1990).

0- Normal mucosa
0.5- Red colouration
1- Spot ulcer
1.5- Haemarrhage streaks
2- Ulcers > 3 but < 5
2.5- Ulcers > 5

Histamine induced ulceration (Rajeshkumar *et al.*, 2001)

Rats were fasted for 18 h and deprived of water for 12 h before experiment. The rats were divided into four groups (n = 6) and received the drug interventions as describe above in the indomethacin experiment. One hour after the administration of standard and test drugs, the animals received histamine at the dose of 10 mg/kg. All rats in each group were killed and the stomach was then opened along the greater curvature, rinsed with normal saline. Then the gastric mucosa was observed and scored as mentioned above.

Ethanol induced gastric ulceration (Hollander *et al.*, 1985)

The rats were again divided into four groups (n = 6) and received the drug interventions as describe above in the indomethacin experiment. One hour after the administration of standard and test drugs, the animals received ethanol at the dose of 1 ml/ 200 g orally. One hour after the oral administration the animals were sacrificed by cervical dislocation and stomach was excised along the greater curvature and examined for ulcers. The fundic mucosal part of the stomach was homogenized (5%) in ice-cold 0.9% w/v normal saline with a Potter-Elvehjam homoginizer. The homogenate was then centrifuged at 800 g for 10 min followed by centrifugation of the supernatant at 12,000 g for 15 min and the mitocondrial fraction was used for the estimation of lipid peroxide and glutathione. Lipid peroxidation (TBARS) was estimated according to the method of Ohkawa *et al.* (1979) and Glutathione was determined by Ellman's reaction using 5'5'-dithio-bis-2-nitrobenzoic acid (DTNB) as described by Moron *et al.* (1979).

Statistical analysis

The results are expressed as mean ± S.E.M. and statistical significance by means of ANOVA followed by Dunnet's '*t*' test. $P < 0.05$ was considered significant.

RESULTS

The effect of the methanol extract of *Indigofera aspalathoides* (MEIA) on indomethacin and histamine induced ulceration was studied and the results are tabulated in Table 1 and 2. The alcohol extract significantly reduced the ulceration produced by indomethacin and histamine. The extract at the

Table 1. MEIA on indomethacin induced ulceration in rats. (The results are expressed as mean ± S.E.M.)

Treatment	Dose (mg/kg)	Ulcer Index	% Inhibition
Normal	-	0	100
Indomethacin + Vehicle	20	15.2 ± 1.2	-
Indomethacin + Famotidine	30	5.6 ± 0.3	63.15
Indomethacin + MEIA	250	10.3 ± 0.9	32.23
Indomethacin + MEIA	500	7.4 ± 0.4	51.31

Statistical analysis was done by ANOVA followed by Dunnet's '*t*' test. $P < 0.05$ was considered significant.

Table 2. MEIA on histamine induced ulceration in rats. (The results are expressed as mean ± S.E.M.)

Treatment	Dose (mg/kg)	Ulcer Index	% Inhibition
Normal	-	-	100
Histamine + Vehicle	10	22.6 ± 1.7	-
Histamine + Famotidine	30	6.8 ± 0.5	69.91
Histamine + MEIA	250	15.2 ± 0.9	32.74
Histamine + MEIA	500	10.3 ± 0.7	54.42

Statistical analysis was done by ANOVA followed by Dunnet's '*t*' test. $P < 0.05$ was considered significant.

doses of 250 and 500 mg/kg afforded 32.23% and 51.31 % ($P < 0.05$) protection where as famotidine exhibited 63.15% ($P < 0.01$) protection in indomethacin induced gastric ulceration. In histamine induced ulceration the MEIA at the dose of the 500 mg/kg afforded 54.42% protection, while the standard drug famotidine (30 mg/kg) exhibited 69.91% protection.

In the present study, MEIA was evaluated for its antiulcer activity against ethanol induced gastric ulceration in rats and the results are tabulated in Table 3. Oral administration of ethanol produced severe ulceration and significantly elevated lipid peroxide levels, while glutathione levels were significantly decreased. MEIA significantly reduced the incidence and severity of ulceration. MEIA at the doses of 500 mg/kg b.w orally afforded 52.52% protection where as the standard drug omeprazole exhibited 59.92% protection. Animals treated with MEIA at the dose of 500 mg/kg b.w orally significantly reduced the thiobarbituric acid reacting substance from 4.61 ± 0.23 to 2.38 ± 0.17 ($P < 0.01$).

Table 3. MEIA on ethanol induced ulceration in rats. (The results are expressed as mean ± S.E.M.)

Treatment	Dose (mg/kg)	Ulcer Index	% Inhibition	TBARS (n moles of MD A/mg of protein)	Glutathione (n moles/mg protein)
Normal	-	0	100	1.45 ± 0.11	10.2 + 0.9
Ethanol + Vehicle	-	25.7 ± 1.7	-	4.61 ± 0.23	4.3 + 0.2
Ethanol + omeprazole	30	10.3 ± 0.8	59.92	1.82 ± 0.14	5.2 + 0.3
Ethanol + MEIA	250	17.2 ± 1.3	33.07	3.52 ± 0.20	8.8 + 0.7
Ethanol + MEIA	500	12.2 ± 0.6	52.52	2.38 ± 0.17	6.7 + 0.3

Statistical analysis was done by ANOVA followed by Dunnet's '*t*' test. $P < 0.05$ was considered significant.

DISCUSSION

Ulcers are caused by an imbalance between aggressive and defensive factors of the gastric mucosa. Pepsin and gastric acid make up the offensive factors whose proteolytic effect is buffered by mucin secretion, mucosal glycoprotein, cell shedding, cell proliferation and prostaglandins (Goel *et al.*, 1999). Different therapeutic agents including plant extracts have been used to inhibit gastric acid secretion or to boost the mucosal defence mechanism by increasing mucus production, stabilizing the surface epithelial cells, or interfering with PGs synthesis (Goel *et al.*, 2002). Gastrointestinal injuries are known to be induced by various chemical agents (Desai *et al.*, 1996). Oxygen derived free radicals and lipid peroxidation are associated with gastrointestinal lesions (Van Kolfschoten *et al.*, 1983). Antioxidants prevent the lesion formation caused by various ulcerogens (Mizui *et al.*, 1987). Thus the present investigation was carried out to evaluate the antiulcer activity of the methanol extract of *Indigofera aspalathoides* against different animal models of ulcer.

Indomethacin is a potent prostaglandin biosynthesis inhibitor and inhibition of PG synthesis by indomethacin coincides with the earlier stages of damage to the cell membranes of mucosal, parietal and endothelial cells. It has been reported that gastric acid secretion is involved in the formation of indomethacin induced mucosal lesion (Akah *et al.*, 1999). The methanol extract of *Indigofera aspalathoides* significantly reduced the ulcer index and afforded significant protection against indomethacin induced gactric ulcers. MEIA also exhibited significant antiulcer activity against histamine induced ulceration. It is thought that histamine produce gastric ulceration by enhancing gastric acid secretion and vasospastic action (Rejeshkumar *et al.*, 2001).

Ethanol induced gastric ulcers have been widely used for the evaluation of gastro protective activity. Ethanol induces ulcers by reduction of gastric mucosal blood flow, mucus production, endogenous glutathione and prostaglandins levels.at the same timeethanol increases ischaemia, gastric vascular permeability and 'back diffusion', histamine release, efflux of sodium and potassium, influx of calcium, generation of free radicals and production of leukotrienes (Glavin *et al.*, 1992). It has been found that oxygen derived free radicals are implicated in the mechanism of acute and chronic ulceration and scavenging these free radical can play an appreciable role in the healing of these ulcers. Ethanol induces generation of free radicals, thereby elevating the lipid peroxide levels and reducing cystein which is required for GSH (glutathione) synthesis, thereby depleting glutathione levels (Loguercio *et al.*, 1993). High concentration of reduced glutathione, an antioxidant is found in the gastric mucosa of rats (Boyd *et al.*, 1979) and humans (Hoppenkamps *et al.*, 1984). GSH is important for the maintenance of mucosal integrity, and the depletion of GSH from the gastric mucosa induces macroscopic mucosal ulceration.

Oral administration of ethanol caused severe gastric damage and significantly increased the lipid peroxide level when compared to that of the control animals. Glutathione content of the gastric mucosa was decreased when compared to that of the control animals. MEIA significantly lowered the elevated lipid peroxide level and restored the altered glutathione levels when compared with the untreated animals.

The effect of MEIA on ethanol induced gastric injury may be due to its anti-oxidant activity. Phytochemical analysis by TLC & HPLC analysis of the methanol extract of *Indigofera aspalathoides* confirmed the presence of ellagic acid and gallic acid as the major components. Ellagic acid is a widely occurring poly phenol which possess strong anti oxidant activity (Festa *et al.*, 2001) which has a marked inhibitory effect on acid secretion and the occurrence of stress induced gastric lesions. These effects may be attributed to the inhibition of H(+), K(+)-ATPase activity (Murakami *et al.*, 1991). The results of biochemical estimation showed a significant anti-peroxidative effect. Thus from the

present investigation it can be concluded that the methanol extract of *Indigofera aspalathoides* afforded significant antiulcer activity by enhancing antioxidant potential of the gastric mucosa thereby reducing mucosal damage.

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