

with 1N sodium hydroxide. Resultant solution was treated with acetic acid, diluted with distilled water to obtain 1 ml of diluted solution equivalent to 1 mg of dry material. Two milliliters aliquots were made up to 5 ml with 20% acetic acid, then added acetate buffer (pH 4.7) and 1 ml of methyl orange solution (0.05%). After shaking for 10-30 s, solasodine was extracted from colored solution with chloroform. Chloroform layer was dried with anhydrous sodium sulphate and absorbances read on a spectrophotometer at 420 nm. The concentration of solasodine was determined on dry weight bases with the use of standard curve for solasodine.

Seed germination was about 92% (fig. 1). The initiation of callus and callus formation were better on slanted surface of the medium and cultures exposed to continuous light (Table 1) when compared with those developed on horizontal surface. The hypocotyl callus showed the formation of small shoots. Slanted surface of medium had better effect on the formation of shoots than the horizontal surface. BAP (4 ppm) was found to be the best growth regulator tested for the production and development of more somatic shoots (fig. 2), but IPA (2 ppm) caused the growth of the roots (fig. 3). After 2-3 days of subculturing of well developed shoots from hypocotyl callus on MS+IAA (2 ppm) shoots producing new leaves arise and the adventitious roots arisen from the callus as well as from the shoot base (fig. 4). White's medium did not show good results for the growth of somatic shoots.

The chloroform solution obtained after extraction of the powdered calli confirmed the presence of solasodine. On quantitative evaluation of callus tissues, the content of solasodine was found to be 0.52 mg/g and 0.66 mg/g in undifferentiated and differentiated callus, respectively. It shows that differentiation has caused increase in solasodine production. Differentiated tissue from hypocotyl callus of *S. eleagnifolium* also produced higher yield of solasodine than non-differentiated tissue⁹. Solasodine concentration was enhanced by the induction of organogenesis in leaf callus of *S. laciniatum*⁷.

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REFERENCES

1. Bhatnagar, J.K. and Puri, R.K., *Lloydia*, 1974, 37, 318.
2. Empe, A. and Eilert, L.I., *Plant Cell Rep.*, 1986, 5, 31.
3. Murashige, T. and Skoogs, F., *Physiol. Plantarum*, 1962, 15, 473.
4. White, P.R., In: *The Cultivation of Animal and Plant Cells*, 2nd Edn., Ronald Press, New York, 1963, 59.
5. Birner, J., *J. Pharm. Sci.*, 1969, 58, 258.
6. Nigra, H.M., Alvarez, M.A. and Giulietti, A.M., *Plant Cell Rep.*, 1989, 8, 230.
7. Chander, S. and Dadds, J., *Plant Cell Rep.*, 1983, 2, 69.

Gastric Antiulcer Activity of the Leaves of *Caesalpinia Pulcherrima*

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Petroleum ether extract of *Caesalpinia pulcherrima* was examined in HCl/ethanol and aspirin and pylorus ligation models in the rat. Pretreatment of the extract prevented the formation of gastric lesions in HCl/ethanol model. In aspirin and pylorus ligation model, the extract was able to significantly reduce the ulcer score and increase in mucus content, but had no effect on gastric juice volume or acid content. Thus the results indicate that the extracts' antiulcerogenic effect is attributable to augmentation of gastric defense mechanisms.

Caesalpinia pulcherrima (L.) Swartz belonging to

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Caesalpinaceae family is an ornamental shrub popularly known as peacock flower or *Mayuram* in Tamil Nadu, India. The leaves of *Caesalpinia pulcherrima* are used in

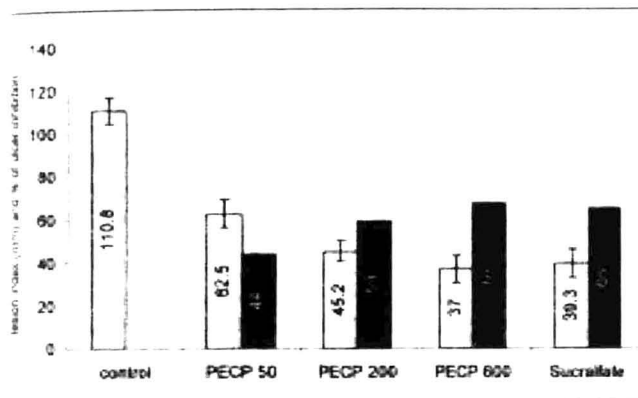


Fig. 1: Effect of pet ether extract of *Caesalpinia pulcherrima* (PECP) on EtOH/HCl-induced ulcers in rat.

Values for lesion index in mm (□) are mean±SEM of 6 animals/group. Percentage of ulcer inhibition is represented by shaded bars (■). *p<0.05 vs. control; ANOVA followed by Dunnett's multiple comparison test.

Ayurveda for the relief of fever, ulcers and tumors¹. Previous phytochemical investigations revealed the presence of phenols and related compounds in the leaves of *C. pulcherrima*². Since no pharmacological evaluation has been done for its antiulcer property we aimed in finding if the leaves possess any gastric antiulcer activity.

Caesalpinia pulcherrima leaves were collected in Chennai City in the month of December 2002 and taxonomically identified at the Department of Botany, Captain Srinvasmoorthy Drug Research Institute, Chennai. Shade dried and coarse powdered leaves of *C. pulcherrima* were

extracted in a Soxhlet with petroleum ether (40-60°) to afford a dark greenish semisolid mass, the yield of which is 11.8 % w/w on dry weight basis. Preliminary qualitative phytochemical screening of the petroleum ether extract of *Caesalpinia pulcherrima* (PECP) indicated presence of steroids, glycosides, phenols, tannins, flavonoids, alkaloids and reducing sugars. PECP was reconstituted each time in sesame oil and administered to animals orally.

Wistar rats of either sex (150-180 g) were used in this study. The animals were housed in standard environmental conditions of temperature, humidity and light and were provided with standard pelleted diet and water *ad libitum*. The experimental protocols were approved by the Institutional Animals Ethics Committee. Overnight-starved rats deprived of food (but given water *ad libitum*) were randomly distributed into 5 groups (each group consists of 6 rats). Rats received 50, 200 and 600 mg/kg p.o. of PECP. Sucralfate (100 mg/kg, p.o.) served as the reference drug and sesame oil (0.2 ml/100 g) served as solvent control. After 50 min all the animals received 1 ml p.o. of HCl/ethanol mixture³ (0.3 M HCl and 60% ethanol). After 1 h the rats were sacrificed and stomach was excised, inflated with 10 ml of normal saline and was fixed with 5% formalin for 30 min, after which it was cut along the greater curvature and lesion index (mm) was determined by measuring each lesion along its greater length; in case of peteahiae, five such lesions were taken as equivalent of a 1 mm ulcer and summed⁴.

For aspirin pylorus ligation induced ulcer model, 30 rats of either sex were divided in 5 equal groups. All the animals received 200 mg/kg, p.o. of aspirin after 1 h admin-

TABLE 1: EFFECT OF *C. PULCHERRIMA* IN ASPIRIN AND PYLORUS LIGATION MODEL IN RATS.

Treatment	Dose (mg/kg; p.o.)	Gastric volume (ml/100 g)	Acidity (mEq/l)		Ulcer score	Mucus content (µg/kg/wet glandular tissue)
			Free	Total		
Control	-	7.4±0.2	18.6±2.5	65.7±3.6	4.2±0.3	0.3±0.04
PECP	50	6.8±0.5	20.2±4.2	75.3± 6.1	3.3±0.3	0.5±0.04*
	200	7.4±0.3	19.3±3.9	69.9± 3.8	1.6±0.4*	0.7±0.08*
	600	7.8±0.4	17.8±4.1	71.6±4.4	1.0±0.0*	1.1±0.12*
Ranitidine	32	2.9±0.2*	9.0±0.8*	28.2±2.2*	1.5±0.2*	0.4±0.04

Values are mean ± SEM of 6 animals per group. * p<0.05 vs. control using unpaired student's 't' test.

istration of various doses of PECP for 5 d⁵. Sesame oil (0.2 ml/100 g) and ranitidine (30 mg/kg, p.o.) served as solvent and reference control, respectively. On the 6, 36 h starved rats were subjected to pylorus ligation⁶. After 4 h, the animals were sacrificed and the stomach contents were removed and centrifuged at 3500 rpm for 10 min and the volume of gastric juice for each rat was measured. Free and total acidity in the supernatant were determined⁷. The stomach was opened along the greater curvature and the ulcer score was determined⁸. The glandular part of the stomach was separated from the rest of the stomach and the mucus content was estimated⁹. Mucus content was expressed as alcian blue $\mu\text{g}/\text{kg}/\text{wet}$ glandular tissue.

Data was subjected to one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test and/or Student's 't' test wherever applicable. Differences below the probability levels of 0.05 were considered statistically significant.

In the HCl/ethanol-induced gastric lesion in rats, pet ether extract of *C. pulcherrima* in a dose dependant manner decreased the lesion index (fig. 1). PECP at 50, 200 and 600 mg/kg, p.o. produced 44%, 59% and 67% protection against acidified ethanol-induced ulcer lesions in rats. In aspirin and pylorus ligation model, PECP at all dose levels did not significantly reduce the gastric volume, total acidity and free acidity (Table 1), while there was significant ($p < 0.05$) increase in mucus content with PECP 200 mg/kg (0.7 ± 0.08) and PECP 600 mg/kg (1.1 ± 0.12) when compared to control (0.3 ± 0.04). PECP in a dose-dependant manner also significantly decreased the ulcer score when compared to control. The pretreatment of rats with PECP by oral administration prevented the formation of gastric lesions in HCl/ethanol model, revealing a probable cytoprotective effect of the extract, as antisecretory agents (ranitidine) are ineffective in reducing the ulcer lesion in this model^{3,10}. In aspirin and pylorus ligation model, PECP was able to significantly reduce the ulcer formation and increase in mucus content but had no effect on the gastric volume or gastric acid content. Thus the results indicate that PECP's antiulcerogenic activity might probably be attributed to potentiation of defensive mechanisms such as

gastric cytoprotection. Cytoprotection produced by certain antiulcer agents has been reported to be mediated through endogenous prostaglandins (PG) that are known to play an important role in maintaining mucosal integrity and to protect the gastric mucosa against various damaging agents¹. However since aspirin is a potent prostaglandin synthetase inhibitor, the role of PG-mediated antiulcerogenic effect of the PECP in aspirin and pylorus ligation model becomes questionable. This might indicate that a non-prostaglandin-mediated action could be involved in increase in production and strengthening the gastric mucosa. At present the cytoprotective mechanism of PECP is not clear, but the present results suggest that it might partly be due to maintaining the gastric mucosal levels. Nevertheless, the study supports the use of *Caesalpinia pulcherrima* in traditional medicine as an antiulcer agent.

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REFERENCES

1. Kirtikar, K.R. and Basu, B.D., Eds., In; Indian Medicinal Plants, 2nd Edn., Vol. 2, Periodical Experts Book Agency, New Delhi, 1991, 848.
2. Choudary, P. and Choudary, S.S., *Plant Physiol. Biochem.*, 1987, 14, 220.
3. Mizui, T. and Doteuchi, M., *Jpn. J. Pharmacol.*, 1983, 33, 939.
4. Cho, C.H. and Ogle, C.W., *Experientia*, 1978, 34, 1294.
5. Goel, R.K., Chakrabarti, A. and Sanyal, A.K., *Planta Medica*, 1985, 2, 85.
6. Shay, H., Komarov, S.A., Fels, S.S., Meraze, D., Gruenstein, I. and Siple, H., *Gastroenterology*, 1945, 5, 43.
7. Varely, H., Ed., In; Practical Clinical Biochemistry, 4th Edn., CBS Publishers and Distributors, New Delhi, 1988, 329.
8. Bonnycastle, In; Laurence, D.R. and Bacharach, A.C., Eds., Evaluation of Drug activities: Pharmacometrics, 1st Edn., Vol. II, Academic Press, New York, 1964, 510.
9. Corne, S.J., Morrissey, S.M. and Woods, R.J., *J. Physiol.*, 1974, 242, 116.
10. Yamada, H., Sun, X.B., Matsumoto, T., Ra, K.S., Hirano, M. and Kiyohara, H., *Planta Medica*, 1991, 57, 555.
11. Roobert, A., Nezamis, J.E., Lancaster, C. and Hanchar, A.J., *Gastroenterology*, 1977, 77, 433.