

ANTIULCER AND CYTOPROTECTIVE ACTION OF *WEDELIA CALENDULACEA* LESS

D.A. HEGDE, R.L. KHOSA and R.K. GOEL

Department of Pharmaceutics and Department of Pharmacology, Banaras Hindu University, Varanasi – 221 005, India.

Received: 29 June, 1994

Accepted: 4 July, 1994

ABSTRACT: The gastric antiulcer and ulcer healing effect of the ethanolic and aqueous extracts of the dried leaves of *Wedelia calendulacea*. Less was found to be significant. The effect of the aqueous extract was observed to be more pronounced in comparison with that of the ethanolic extract.

INTRODUCTION

The plant 'Bhringaraj' has been included in the category 'Dasapuspan' in Ayurveda, the members of which are credited the attributes of curing superficial wounds and ulcers as well as fevers caused by the derangement of the three doshas – vata, pitta and Kapha¹. Four plants viz *Wedelia calendulacea*, *Eclipta alba*, *Heliotropium strigosum* and *Viscum album* are known by the name Bringaraj²⁻⁴. The present paper gives an account of the systematic study of the gastric antoulcer and ulcer healing effect of the aqueous and ethanolic extracts of the leaves of *Wedelia calendulacea*. Less in rats.

MATERIALS AND METHODS

Materials

Properly identified leaves of *Wedelia calendulacea*. Less obtained from Tampco company (Madras) were air dried, powdered and defatted with petroleum ether. The defatted were extracted thoroughly and successively with alcohol (95%) and water. Both the extracts were dried and the residues suspended in water using Tween 80 (0.01%)

as suspending agent to give final concentrations of 210 mg/ml and 70 mg/ml respectively.

Animals & Methods

Healthy adult inbred albino rats of Charles-Foster strain of either sex weighing between 200 – 250 g obtained from Central animal house of the Institute of Medical Sciences, Banaras Hindu University were used for the study. The animals were kept in the departmental animal house for a week for acclimatization before submitting them to the experiment and were fed on Hindu Lever Pellet diet. They were fasted 18 – 24 hours before the day of experiment /sacrifice but water was given *ad-lib* (except in case of animals studied under the acetic acid model of ulcer healing). In each model, the animals were divided into three groups of six rats each. The animals in the first group received 1 ml of distilled water containing Tween 80 (0.01%) whereas the animals in group two and three received the suspensions of ethanolic and aqueous extracts respectively (1 ml / 100 g of rat). In case of the aspirin – induced and restraint

stress – induced ulcer experiments, the extracts were administered orally once daily for eight days. However in the acetic acid model, the animals were pretreated with the extracts for three days and then treatment was continued further for a period of 8 days after induction of ulcers by 50% acetic acid as indicated below.

Antiulcer study

Aspirin – induced ulcers were produced by administering an aqueous solution of aspirin in the dose of 200 mg/kg through an orogastric tube, once on the day of experiment. The animals were then sacrificed four hours later and the ulcers scored. The stomach was taken out, cut along the greater curvature and washed gently with running tap water to see for the ulcers in the glandular portion of the stomach. The number of ulcers per stomach was noted and the severity of ulcers was scored macroscopically with the aid of a hand lens (10X) and each given a severity rating as 0, no ulcer; + pin point ulcer and changes limited to superficial layers of mucosa; ++ Necrotic changes; +++ ulcer >1 mm but < 2 mm and more than 2/3rd of mucosal thickness destroyed with marked necrosis and congestion; ++++ ulcer > 2 mm of perforation of ulcer and complete destruction of mucosa with necrosis and haemorrhage. The ulcer index was calculated by adding the number of ulcers per stomach plus the severity of ulcers converted as 1+ (one plus) per stomach. The statistical significance was determined by Wilcoxon Rank Sum test method (non parametric).

Restrain Stress – induced ulcers were produced^{5,6} in 18 hr fasted rats by stretching and strapping them to a wooden plank with adhesive tape after securing each limb to the plank individually. The animals were then

sacrificed after 6 hours with an overdose of ether and ulcers scored using the method described above.

Ulcer healing study:

Acetic acid (50%) – induced ulcers^{7,8} were used to study the ulcer healing effect of the plant extracts. The test drugs were administered to rats once daily (1ml / 100 g of rat) by an orogastric tube for three days prior to production of ulcers by 50% acetic acid. They were then continued for another eight days to assess their effect on healing of ulcers. Since the ulcers produced by the acetic acid method are round or oval, their lengths and widths were measured and an ulcer index based on the product of length and breadth was used (mm²/rat). The statistical significance was determined by student's 't' test method.

In another series of experiments, the tensile strength which is the pressure in mm of Hg. required to rupture the gastric wall was also measured⁹. This has been used by us as an index to study the extent of healing of gastric ulcers. For this, the procedure used is as reported. The stomach was taken out, its pyloric end ligated and the cardiac end tied to the main arm of a T tube whose side arm was connected to a mercury manometer. The stomach was then completely dipped into a beaker of water. Then the pressure was gradually applied to the stomach by pumping in air through a rubber bulb attached to the T tube. The monometric reading was recorded at the instant when the stomach wall got ruptured and allowed air bubbles to pass through.

RESULTS AND DISCUSSION

The effects of ethanolic and aqueous extracts of the leaves of *Wedelia calendulacea*. Less has been studied for its

gastric antiulcer and ulcer healing properties against different ulcer models in rats. The antiulcer effects of the two extracts was studied with an arbitrarily selected oral dose of 2.1 g/kg and 0.7 g/kg respectively once daily for 8 days prior to experimentation. The respective doses significantly reduced the mean incidence, severity and ulcers in rats (Table 1), indicating their gastric mucosal protective effects. This could be attributed to their effect on either gastric mucosal offensive acid-pepsin or on defensive gastric mucosal factors like mucin secretion, cell proliferation, cell shedding or mucosal glycoproteins. The exact mode of actions of the plant is still under investigation.

The ulcer healing study has been undertaken to assess the effect of the two extracts using the acetic acid induced gastric ulcer model

in rats. The test drugs were given once daily orally for 3 days before and 8 days after induction of ulcers by 50% acetic acid (Table 2). Results indicate that both the extracts were effective in the healing of gastric ulcers and a similar finding was observed when tensile strength of stomach was measured in control and extract treated groups. Treatment with both the extracts showed significant increase in tensile strength of stomach musculature in comparison to those observed for control group animals (Table 2).

Thus, the results of this study do indicate the antiulcerogenic and ulcer healing properties of both the ethanolic and aqueous extracts of the plant. The effect of the aqueous extract seems to be more pronounced than that of the ethanolic extract.

TABLE 1. EFFECT OF ETHANOLIC (E.E) AND AQUEOUS EXTRACT (A.E.) OF *Wedelia calendulacea* LESS ON ASPIRIN (200 mg/kg, P.O... 4h BEFORE) INDUCED AND RESTRAINT STRESS INDUCED GASTRIC ULCERS IN RATS.

S. No.	Model	Oral treatment g/kg; O.D. x 8 days	No. of Animal	Ulcer / Stomach	Severity / Stomach	Ulcer Index
				A	B	A – B
I	Aspirin induced ulcers	Control (Distilled water + 0.01% Tween 80)	6	19.3 ± 4.3	27.7 ± 4.9	47.0 ± 9.1
		E. E (2.1)	6	9.5 ± 1.7 ^a	13.2 ± 3.1 ^b	22.7 ± 4.8 ^b
		A.E. (0.7)	6	8.8 ± 1.7 ^b	10.5 ± 2.2 ^d	17.7 ± 3.6 ^c
II	Restraint stress induced	Control (Distilled water + 0.01% Tween 80)	6	13.4 ± 2.32	30.14 ± 6.16	43.5 ± 8.0
		E.E	6	4.67 ± 2.33 ^a	8.83 ± 5.50 ^b	13.5 ± 7.8 ^b
		A.E	6	4.00 ± 0.93 ^b	6.31 ± 1.65 ^d	10.3 ± 2.4 ^c

Results are mean ± SEM. p. values a<0.1; b<0.05; c<0.02; d<0.01 compared to respective control analysed by Wilcoxon Rank sum test method (non parametric).

TABLE 2. EFFECT OF AQUEOUS (A.E) AND ETHANOLIC (E.E.) EXTRACTS OF *Wedelia calendulacea* (g/kg; O.D. 3 DAYS BEFORE PLUS EIGHT DAYS AFTER INDUCTION OF ULCER) ON HEALING OF GASTRIC ULCERS INDUCED BY 50% ACETIC AND IN RATS.

Oral treatment	Number of animals	Ulcer index	Tensile strength in mm of Hg
Control (Distilled water + 0.01% Tween 80)	6	43.2 ± 6.1	46.3 ± 4.8
E.E (2.1 O.D)	6	13.5 ± 5.6 ^d	91.7 ± 9.8 ^d
A.E (0.7 O.D)	6	14.7 ± 6.7 ^b	88.0 ± 12.5 ^b

Results are mean ± SEM. p. values a<0.1; b<0.05; c<0.02; d<0.01 compared to respective control analysed by student's 't' test method.

REFERENCES

1. Aiyer N.K. and Kolammal M; *Pharmacognosy of Ayurvedic Drugs* (1962), 1(5); Kerala, Department of Pharmacognosy, University of Kerala, Trivendrum, 117.
2. Anonymous; *The Wealth of India* Vol. III, CSIR, New Delhi, 1952, 127.
3. Chandra S; *Quart. Jour. Crude Drug. Res.*; 1969; 9(4), 1461.
4. Anonymous; *The useful plants of India*; Publication and Information Directorate, CSIR, New Delhi, 1986, 681.
5. Fregly M.J.; *Amer J. Physiol*, 1953, 173, 393.
6. Sanyal A.K., Pandey B.L. and Goel R.K.; *J. Ethanopharmacol.*, 1982, 5, 79.
7. Okabe S. and Pfeiffer C.J.: *Peptic Ulcer*, Ed. Pfeiffer C.J.; Munksgaard Copenhagen, 1971, 45.
8. Goel R.K. and Maiti R.N.; 'Proceedings of 1st International Symposium on Natural drugs and the digestive tract' Naples Italy, 26 – 28th July 1992.
9. Ezer and Soorny L. ; *J. Pharm. Pharmacol*; 1975, 27, 866.