ANTI-ULCER ACTIVITY OF HENNA (LAWSONIA INNERMIS L.) LEAVES IN RATS

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Abstract:

The anti ulcer effects of aqueous, chloroform and ethanol extracts prepared from the henna leaves was evaluated in rats employing the pylorus ligation, stress induced and aspirin induced models. The parameters taken to assess anti ulcer activity were volume of gastric juice, free acidity, total acidity and ulcer index. The results indicated that chloroform extract significantly ($p<0.001$) decreased the volume of gastric acid secretions, free acidity and total acidity and ulcer index.

Keywords: Henna, Anti ulcer effect, Pylorus ligation, Aspirin, Stress

Introduction

Lawsonia innermis (Henna) are commonly called as Mahendi which belongs to the family lythraceae grows as a glabrous much branched shrub or small tree. It is cultivated and warm template regions as a hedge plant, Henna leaves have long been used in India and middle East countries For colouring palms of hands, sole of feet Leaves contain important cosmetic dye. The principal colouring matter is a lawsone, which is used as a tropical sunscreen and as a prophylactic against skin diseases. They have astringent property. They have been used in the form of paste or ducoction against boils, bruises and skin inflammation Ali et al., (1) have reported analgesic, anti-inflammatory and anti pyre-tic effects of henna in rats Bhuvaneshwari et al., 121 have reported antimicrobial property of henna Widespread efforts have been launched to identify novel ulcer drug tium natural resources. A study of efficacy of different extracts of henna against gastric slots with three models in rats is an effort made in the same direction.

MATERIALS AND METHODS

Henna leaves were procured from Gulbagra University, Gulbarga campus in summer. Herbarium was submitted to Department of Botany, Gulbarga University. Gulbarga, India and was identified as Lawsonia innermis (HGUG-554). The leaves were allowed to shade dry in open air for 3-4 weeks. Dried leaves were powered mechanically.

Preparation of extract

400 gm of powder was suspended in 500ml of distilled water at room temperature. The mixture was sieved through a muslin cloth, followed by filtration using filter paper. The filtrate was mixed with chloroform in a separating funnel and shaken until separation was observed in two layers. The extract of two layers were run out into separate beaker and placed in an oven to dry at 50°C. Residues of extracts were made into suspensions using sterile distilled water and chloroform in concentration of 100 mg/ml. ethanolic extract was also prepared similarly [3].

Animals

The Albino-Wister rats of either sex weighing between 150-200 g were used. The animals were acclimatized the for 10 days before and during study in the Central Animal House of Luqman College of Pharmacy, Gulbarga. under standard conditions at room an temperature of 24±2 C. relative humidity of 45-55% and 12:12 light/dark cycle. The animals were food with standard rodent pellet (Sai Durga Feeds & Food, Bangalore, India (Pranav Agro Industries Ltd., Sangli, India). Water was supplied ad libitum under strict hygienic conditions. The Institutional Animal Ethics Committee, Luqman College of Pharmacy, Gulbarga approved the protocol of the study. All the chemicals T used were of analytical grade.

Acute Toxicity

Acute toxicity studies of henna extracts were performed on rats and the lethal dose was estimated using the method described by Miller and Tainter [4].

Experimental Procedure

Animals were divided into five groups (n=6), Group- 1 received 2% gum acacia that served as control, gitup-II received ranitidine orally (20mg/kg), group- III. IV and V received aqueous, chloroform and ethanolic extracts (1g/kg) respectively. Animals were divided into five groups (n=61. Group- I received 20 gm acacia that served as control, T group-II received ranitidine orally (20mg/kg), groups III. IV and V received aqueous, chloroform and ethanolic extracts (1g/kg) respectively

Study of anti ulcer activity using pylorus ligation method

The method of Shay et al.. [5] was adopted. Animals were fasted for 24 h and the dose was administrated 30 min prior to pylorus ligation. Animals were sacrificed 4 h later and the stomach was removed. The gastric content was collected and centrifuged. The volume, free acidity, total acidity of gastric fluid was determined. The stomach was then incised along the greater curvature and observed for ulcers. The number of ulcers was counted using a magnifying glass. Mean ulcer score for each animal was expressed as ulcer index. The ulcers were graded using the following scoring system-0= Normal mucosa; 0.5- Red colouration; 1.0-Spot ulcer; 1.5-Hemorrhagic streaks; 2.0-Ulcere"3 mm but d"5 mm: 3.0-Ulcer e" 5 mm

Study of anti ulcer activity using stress induced ulcers

The method of Nagura (6) modified by Bauchi (7) was adopted.The Animals were fasted fis 24 b before d the experiment. The annuals were treated with respective dose They were anaesthetized with ether Bai upper and lower extremities were fixed together and the animals were wrapped in a wire gaze. They were horizontally suspended in the dark at 20°C for h24h, and finally sacrificed. The stomach was removed and severity of ulcers was registered and the ulcer index was calculated as mentioned earlier.

Study of anti ulcer activity using aspirin induced ulcers

The method of Hedge et al., [8] was adopted. The animals were treated with respective dose of 8 days as mentioned in previous model. After 8 days of treatment animals were fasted for 24 h. ulcer was induced by administration of aqueous suspension of aspirin (200 mg/kg) 4 h later. The animals were sacrificed and stomach was opened to calculate the ulcer index as given earlier.

Photo-chemical analysis

The henna leaves extract (chloroform) was analysed using TLC plates (0.1 mm thick silica gelj eluted with ethyl acetate-glacial acetic acid-water (100:11:1126). The spots were identified under short wave UV light.

Statistical analysis

The results of the above experiment are indicated in seven of Mean & SEM, Statistical difference between mean were calculated using one-way analysis of variance followed by Dunnet's 't' test, $p<0,05$ were considered statistically significant.

RESULTS AND DISCUSSION

Traditionally medicinal plants have been used in folk medicine through out the world to treat various diseases, especially gastric ulcers. We evaluated preventive effects of aqueous, chloroform and ethanol extracts obtained from henna leaves in animals using the different standard experimental models of induced gastric ulcers. Pylorus ligation model resulted the accumulation of gastric secretary volume and an increase in the total acid output of the gastric juice. Circular and linear lesions were frequently seen in the stomach of all the control animals. Administration of henna extracts resulted in a significant reduction

Table 1: Effect of henna (Lawsonia innermis) leaves on gastric ulcers induced by pylorus ligation

Group No.	Treatment	Vol.of Gastric Juice	Free Acidity	Total Acidity	Uleer Index	%Protection Ulcer
I	Control	9.25	20.65	56.6	2.5	…….
II	Ranitidine	3.625	5.825	14.8	0.75	70.0
III	Aqueous extract	5.625	10.120	24.77	1.62	35.2
IV	Chloroform extract	4.375	7.875	19.025	1.12	31.92
V	Ethanol extract	6.750	19.27	44.12	2.0	00.0

Table 2 : Effect of henna leaves on gastric ulcer induced by stress

Group	Treatment	Ulcer index mean	% Protection from ulcer
I	Control	12.5	…….
II	Ranitidine	1.5	87.98
III	Aqueous extract	7.5	39.90
IV	Chloroform extract	5.5	54.92
V	Ethanol extract	8.5	31.92

Table 3: Effect of henna leaves on gastric ulcer induced by aspirin

Group no	Treatment	Ulcer index mean	% Protection from ulcer
I	Control	12.5	……
II	Ranitidine	2.5	79.17
III	Aqueous extract	4.0	66.60
IV	Chloroform extract	2.5	79.17
V	Ethanol extract	7.5	37.50

In ulcer index when compared with control (Table 1). Although in most of the cases the aetiology of the ulcers is unknown, it is generally accepted that it results from an imbalance between aggressive factor and the maintenance of the mucosa l integrity through the endogenous defence mechanism[9]. Henna prevented the mucosa l lesions induced by pylorus ligation. This suggests that the components present in the extract must be suppressing gastric damage. Henna was also found to decrease the acid volume, total acidity and free acidity. These effects of henna treatment on the parameters that influence the intiation and induction of ulceration may be considered as high desirable property of anti- ulcerogenic agent In this study our investigations continued with stress induced model.

Ulcers induced by stresses in rats, which was protected by our extract, With stress-induced model too, it was the chloroform extract of the henna leaves that offered maximum protection of 54.92% against the ulcer observed (Table 2). It has been reported that stress induced gastric lesions develop as a result of

multifunctional impairment of mucosa l defence system [10], vagal nerve which increases gastric secretion [11] and gastric mobility [12]. Apart from peripheral events, central mechanism including over acidity have also been considered for the pathogenesis of stress ulcers [13.14]. Based on results of this study it could be suggested that inhibition of acid hyper secretion might be involved in the protection an afforded by henna extract in this model.

Further it has been postulated that histamine might be involved in the formation of pylorus ligated ulcers and plays a mediating a role in gastric secretions stimulated by gastrin vagal excitation |15,16](Glick et al, 1966, Rangachari, 1975). Endogenous histamine formation and the release from mast cells in the gastric mucosa have also been implicated in the pathogenesis of gastric ulcers produced by stress [16](Guth and [3 Hall, 1960), Thus, the effects of henna extract on gastric lesion induced by the above two models could [4 be due to the histamine inhibition.

he efficacy of henna extract against gastric ulcers led us to perform yet another model ie. aspirin induced. This model too resulted in a significant percentage protection (17) against gastric ulcers. The percentage protection observed was very much the same as that of standard drug ranitidine (Table 3). A few reports have been implicated focal mucosal ischemia as a major event in the development of aspirin induced acute erosive gastritis [17,18].

Photochemical screening of henna extract (chloroform) revealed presence of flavonoids. Rajkapoor et al., [19] have reported anti ulcer effects of Nigella Sativa to be due to due to flavonoids. Various flavonoids have been reported for their antioxidants property [20]. It has been demonstrated that many drugs or formulations possess potent antioxidant actions and are effective in healing experimentally induced gastric ulcers. Batra and Balraman [21] have reported that the anti ulcer effect of peptic are was due to its antioxidants mechanism of action. We have not carried out studies pertaining to the anti-oxidant nature of our extract however the presence of flavonoids can be considered as one of the bio active responsible for the effects presented in this investigation[22].

CONCLUSION

This study reveals significant antiulcer effects of aqueous, chloroform and ethanol extracts from henna leaves in experimental models of gastric lesionsinduced by pylorus ligation, aspirin and stress. Further er studies using more specihe methods are required to explore the flavanoid responsible for the activity and the mechanism of this activity which might prove important and improved therapies for the treatment and prevention of ulcers.

REFERENCES

1. Ali. B.H.. Bashir A. K., Tantra, M.O. Pharmac 51:356- 359(1995)

2. Bhuvaneshwari, S. 5.. Poongthai, K. A.. Appla Raju. B. Ind J of Pharmacol. 34, 260-263 (2002).
3. Wealth of India, the dictionary of Indian raw material and industria products vol 9 (CSIR) New Delhi Publication(1969).

4. Muhammad. H.S., Muhammad. S. Afrj Biotechnol. 934-937 (2005).

5. Miller. L.C.. Tainter, M.L. Proc Soc. Rep. Biol Med. 5726-264(1994).

6. Shay. J., Komarow. S.A., Fels, S.S. Meanze, D., Gruestein, M., Siplet. H.A. Gasteroenterol. 5. 43-510 1945)

7. Nagura, M. Effects of psychotropic drugs on catecholamines in brain and adrenal medulla of rats under stress producing peptic ulcers. JPN. Pharmaco (1972)

8. Bacchi, E.M. Estudo farmacologico da acao antiulcer dos extratos Stros camporum Pohle Caesalpina ferrea martius. Ph. D. Thesis Instituto de Ciencias Biomedicas da Sao Paulo University (1988).

9. Hegde. D.A., Khosa, R.L.. Goul. R.K. (1994) Ane Sci Lite, 14:77-81 (1994).

10. Piper. D. W., Stiel. D.D. Med. pro 2:7-10 (1986).

11. Guth, P. H. Dig Dis and Sci. 17:807-813(1972).

12. Kitgawa, H., Fujiwara, M. O. Gasteroenterol 77.298- 302 (1979)

13. [12], Gamck, T., Busak. S. Bass, PAM J Physiol. 250:G191- G199(1986).

14. [13]. Henke, P.G., Ray, A. Exp and clin. Gasteroenterol. 50:562-569(1992).

15. [14]. Bhatnagar, M., Sissodia, S., Bhatnagar, R. Annl. New York Acad. Sci. (2005)

16. [15]. Glick. D. Bon Redlick. D.. Bevis, S., Jones, L. Gastroenterol. 51:18-23(1996).

17. [16]. Rangachari, P.K. Nature, 253:53-55 1975(1975).

18. 117]. Ashley. S. W., Cheung, L.Y. Am J of Physiol 247:G339- G345 (1984).

19. [18], Robins. P. G. Br J Exp Path 61:497-504(1980)

20. [19]. Rajkapoor, B., Anandan, R., Jayakar, B. (2002). Curr Sci, 82:177-178 (2002)

21. 20. Mitchel,J.J. H., Gardner, P.T., Me Phadi, P.C., Morris, A.R., Duthie,GGArch. Biochem. Biophys, 360: 142- 148(1998).

22. 21. Batra, P.A., Balraman, R. Phytomed 12:264-270 (2005).