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Evaluation of Total Phenolic Contents and Antiulcerogenic Activity of Root Bark of Azadirachta Indica

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Abstract

The effect of methanol extract of root bark of Azadirachta indica was investigated in mice to evaluate the antiulcerogenic activity. Total phenolics were also determined. The root barks of have been extracted by Azadirachta indica successive solvent extraction method. Extracts were subjected to phytochemical analysis and total phenolics were also determined by the modified Folin-Ciocalteu method. The parameters taken to assess anti-ulcer activity were volume of gastric secretion, pH, free acidity, total acidity, and ulcer index and

Index terms— Azadirachta indica, Root bark, Total phenolics, antiulcerogenic activity, Omeprazole, Methanol extract.

1 Introduction

zadirachta indica **??**AI) has been advocated for the treatment of disorders like cough, nausea, vomiting, fever, jaundice, gonorrhea, intestinal warm infestation and leprosy in indigenous system of medicine 1 and reported to have antiulcerogenic property. [2][3] The biological, medicinal and industrial uses of various parts of AI and the compounds isolated from it have been reviewed. [4][5][6] AI barks contained condensed tannins to the extent of 15% along with other nonisoprenoid constituents like flavonoids and phenolics. 7 Peptic ulcer, one of the most common gastrointestinal disease, is caused by multiple factors including stress, smoking, nutritional deficiencies, noxious agents such as alcohol, NSAID and Helicobacter pylori infection, among others. [8][9] Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of gastric ulcers. 10 This is an important reason to investigate the antiulcer effect of AI bark extracts that have been used traditionally against gastric diseases.

As to pharmacological effects, different extracts of leaves, seeds and stem barks of AI showed antimicrobial [11][12] , antioxidant 13 and antiulcer activities. [14][15] In our previous study, antioxidant effect of hydro alcoholic root bark extract was tested. 16 Given the association between biological constituents present and antiulcerogenic effects of the AI root bark, the present study was carried out by two approaches. First, we performed phytochemical screening of root bark successive solvent extracts. Second, we selected root bark methanol extract to assess its antiulcerogenic activity in ethanol induced gastric ulcer in mice.

2 II.

3 Materials and Methods

4 a) Chemicals and reagents

All reagents and chemicals used were of analytical grade. Folin-ciocalteu reagent (Merck Pvt. Ltd. India), Sodium carbonate (Merck Pvt. Ltd. India), standard omeprazole was the kind gift from Aurobindo Pharma Ltd., Hyderabad.

5 b) Plant material

The root bark of AI was collected from agriculture land of Deshmukhi village of Andhrapradesh, India and the authentication of plant material was done by a botanist at Osmania University, Hyderabad and the voucher no was 0125.

6 c) Preparation of root bark extracts

Root barks were shade dried and powdered mechanically after cutting into small pieces. The powdered plant material was extracted in a soxhlet extractor by successive soxhlet extraction method based on polarity order of solvents. Solvents employed were pet ether, chloroform, ethyl acetate and methanol. The extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator 17 .

Resultant successive extracts of root barks were subjected to qualitative chemical analysis for the presence of biologically active constituents. **??**8 Thin layer chromatography was performed for all the extracts by taking Quercetin as biomarker. Mobile phase employed was ethyl acetate: formic acid: glacial acetic acid: water (100: 11: 11: 26). 19 e) Determination of total phenolics content The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolics and polyphenolic antioxidants. It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent. 20,21 Total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method. An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40 o C for color development. Absorbance was then measured at 765 nm using the Shimadzu UV-1800 spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1 mg/ml. Total phenolics content were expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: y = 0.1216x, R 2 = 0.9365, where x was the absorbance and y was the tannic acid equivalent (mg/g). 22 f) Animals Swiss albino mice (24-30 g) of either sex maintained under standard husbandry conditions (temp 23±2 o C, relative humidity 55±10% and 12 hours light dark cycle) were used for the screening. Animals were fed with standard laboratory food and ad libitum during the study period. The experimental protocol has been approved by Institutional Animal Ethics Committee (IAEC NO.1330/AC/10/CPCSEA). g) Ethanol induced gastric ulcer 23,24 The methanol extract of root bark of AI was selected as it is having significant amount of biologically active constituents (from the results of phytochemical analysis) to evaluate anti ulcer activity by ethanol induced gastric ulcer in albino mice. After 12 hour of fasting Swiss albino mice weighing 24-30 g of either sex were divided into 5 groups, each group consists of 6 animals.

Group 1 served as a control received 1.0 ml/kg p.o 80% Tween 80.

Group 2 served as standard control received 30 mg/kg, p.o Omeprazole. After 1h all the animals were treated with 0.2 ml of ethanol p.o to induce gastric ulcer. Animals were sacrificed by cervical dislocation one hour after administration of ethanol. The stomach was excised and lesion index was determined by measuring each lesion in mm along its greater length.

7 h) Determination of gastric parameters

Collection of gastric juice: After post operative period, animals were sacrificed by cervical dislocation and the stomach was dissected out as a whole by passing a ligature at the esophageal end. Gastric content was evacuated into graduated tube by cutting along the greater curvature of the stomach, and was centrifuged at 3000 rpm for 10min.

Volume of gastric juice: The volume of the centrifuged sample was expressed as ml/ 100 g body weight.

pH of gastric juice: pH of gastric juice was measured with the help of pH meter.

Free and total acidity: Gastric juice (1ml) was pipette into a 100ml conical flask and diluted with 9ml distilled water. Two or three drops of Topfer's reagent was then added and titrated with 0.01 N sodium hydroxide until all traces of red colour disappeared and the colour of the solution was yellowish-orange. The volume of alkali added was noted. This volume corresponds to free acidity. Two or three drops of phenolphthalein were then added and the titration was continued until a definite red ring appeared; the volume of alkali added was noted. The volume corresponds to total acidity. The sum of the two titrations was total acidity. Acidity was expressed in terms of mEq/L. Acidity was expressed as:Acidity = 0.1

Estimation of gastric ulcerative index changes: The stomach was opened along the greater curvature and it was washed with running tap water. Then the ulcerative area was counted by placing it on a flat wooden plate.

The following arbitrary scoring system was used to grade the incidence and severity of lesion.0 = Normal, 1 = Red coloration, 2 = Spot ulcers, 3 = Hemorrhagic streaks, 4 = Ulcers $>$ 3 but $<$ 5 and 5 = Ulcers $>$ 5. Ulcer index and % protection were calculated by following formulas.

8 Volume of

9 Results and Discussion

Qualitative chemical analysis results (table-2) were exhibiting the presence of alkaloids, glycosides, flavonoids, tannins, saponins and terpenoids. TLC results (table-1) were qualitatively confirming the presence of flavonoids

in successive extracts of root bark by using quercetin as biomarker. Total phenolic contents were quantified by standard procedures and results were given in table 1. Results depicts that phenolic content was significantly found in ethylacetate extract followed by methanol extract. The anti-ulcer activity of root bark of AI was evaluated by employing ethanol induced gastric ulcer in mice. Ethanol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane. 26 Pretreatment of mice with root bark extracts produced a dose dependent protection in the ethanol induced ulceration model as compared to control group. However the protection was statistically significant reduced the severity of ulcer and caused a significant reduction of ulcer index in this model. Omeprazole produced significant gastric ulcer protection as compared to control group (Table 3). Ethanol damages the plasma membrane and leads to intracellular accumulation of sodium and water by increasing the membrane permeability. These changes ultimately cause cell death and gastric mucosal exfoliation. 27 Ethanol is also known to release the endogenous ulcerogenic mediators. These could precipitate mucosal injury either by causing vascular changes like mucosal edema and increased mucosal permeability or by nonvascular effects like mucus depletion and enzyme release in the stomach. 28 The decrease in volume of gastric juice may also attributed to its anti secretory ear 2012 Y potential of the drug. The anti secretory potential may also relate towards gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration. 29 AI root bark methanol extract significantly decreased the gastric juice volume as compared to control. Methanol extract significantly increased p H as compared to control and nearer to standard. The excessive secretion of hydrochloric acid in the stomach was considered to be an important factor in the formation of peptic ulcer. Hydrochloric acid is known to produce ulceration and digestion of the stomach tissues as well as to reduce the neutralizing capability of the stomach mucus secretions. [30][31][32] As a measurement of free hydrogen ion, pH indirectly represents the hydrochloric acid concentration in the stomach. Increase in pH is usually affected by either the reduction of the acid secreted in the stomach or the increase in the volume of alkaline and neutral fluids (mucus). The variation in the pH level among the groups shows tendency of protective effects of them towards gastric ulceration. The decrease in acidity was at its maximum level for the reference standard group followed by extract treatment group. The least decrease in acidity was shown by methanol extract treated group at its 500mg/kg dose. Macrocsopic examination of ethanol induced gastric ulcer in mice was shown in figure 1. suggest that flavonoid quercetin promotes a decrease in ulcerative lesions due to its antioxidant effect. In addition, a review of antiulcer drugs of plant origin shows that triterpenes, because of their ability to strengthen defensive factors such as stimulation of mucus synthesis or maintenance of the prostaglandin contents of gastric mucosa at high levels, are compounds with potential antiulcerogenic activity (Lewis and Hanson, 1991). Dose dependent ulcer index results were given in figure 2. Comparison of ulcer protection of root bark methanol extract with that of standard control was shown in figure **??**. Based on this data, it is suggested that the gastro protection observed in this study could be related to the presence of phenolics and flavonoids in the methanol extract of root bark of AI extract.(a) (b) (c) IV.

10 Conclusion

In conclusion, the results show that the methanol extract of root bark of Azadirachta indica present antiulcer activity, as evidenced by ethanol induced gastric ulcer model in albino mice. Results suggest that the effectiveness of the extract as anti ulcerogenic agent may be due to presence of flavonoids and phenolics compounds. The results of this study showed that the root bark of Azadirachta indica contains appreciable amount of phenolic contents along with other biologically active constituents. [1] [2]

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Figure 1:

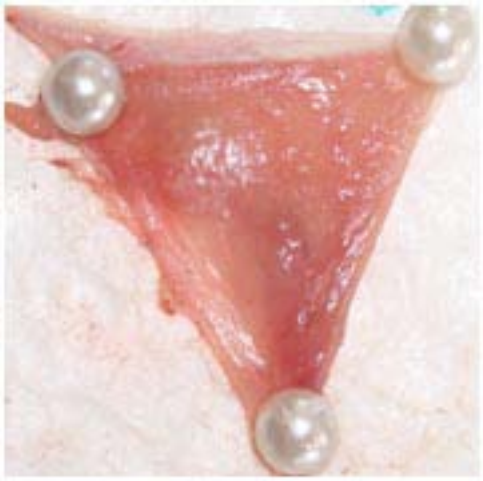

Figure 2: 25

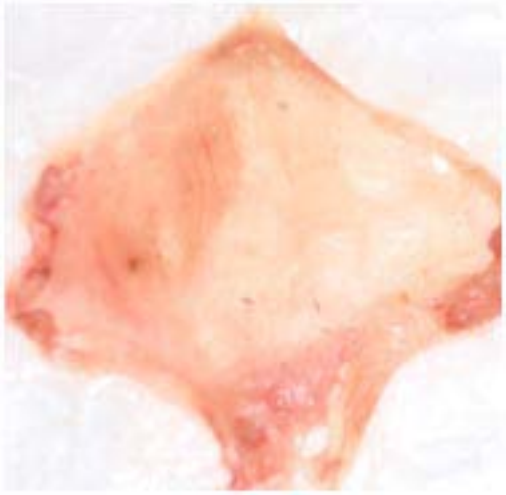

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Figure 3: Figure 1 :

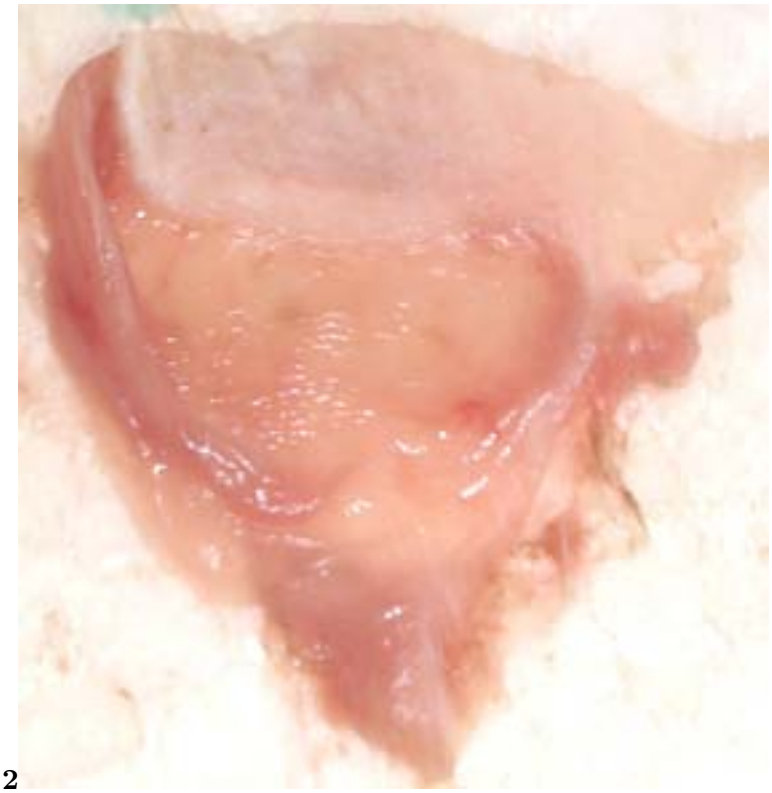

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Figure 4: Figure 2 :

d) Phytochemical evaluation Ulcer index= Arithmetic mean of intensity in group+ Number of ulcer positive

$$\% \text{ Protection} = \frac{\text{Control mean index-Test mean index}}{\text{Control mean index}} \times 100$$

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NaOH×Normality×100mEq/L/100
Ulcer Index
25

Figure 5:

1

Extract	Petether	Chloroform	Ethylacetate	Methanol	% 80 Ethanol
% Yield w/w	2.50	3.80	1.72	4.70	1.29
R f Value	0.87	0.89	0.92	0.89	0.89
Total phenolics content, µg/ml	98.19±1.66	19.73±0.41	821.54±2.70	740.10±0.13	380.75±2.78

Total phenolic contents were expressed in Mean±SEM.

Figure 6: Table 1 :

2

Figure 7: Table 2 :

3

Treatment	Dose (mg/kg)	Vol. of gastric juice(ml)	pH	Free Acidity (mEq/L)	Total Acidity (mEq/L)	Ulcer index	%Protection
Control	-	1.65±0.81	2.9±0.20	31±0.89	74.5±1.87	5.25±0.48	0
Std.control	30	1.48±0.07	3.9±0.13	9.3±0.81	23.3±2.75	1.02±0.2 ***	75
Treated	100	1.53±0.05	2.77±0.08	28.8±0.98	61.3±1.86	3.16±0.214	21
	250	1.53±0.08	3.22±0.11	16±3.57	42.3±5.68	2.33±1.70	41.75
	500	1.23±0.2	3.70±0.10	11.21±1.23	29.5±6.10	1.58±0.47 ***	60.50

Values are expressed in mean±SEM Statistical comparison was performed by using ANOVA coupled with st test. *** P<0.001 were consider statistically significant when compared to control group.

Figure 8: Table 3 :

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