ejpmr, 2017,4(2), 438-442

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

ANTI-ULCER EFFECT OF ETHANOLIC EXTRACT OF *ACACIA AURICULIFORMIS*

[1]Ramandeep Kaur* and [2]Amanpreet Singh

[1]Adesh Institute of Pharmacy and Biomedical Sciences, Adesh University, Bathinda.
[2]Department of Pharmacy, Max Super Speciality Hospital, Bathinda.

***Corresponding Author: Ramandeep Kaur**

Adesh Institute of Pharmacy and Biomedical Sciences, Adesh University, Bathinda.

Article Received on 16/12/2016 **Article Revised on 05/01/2017** **Article Accepted on 26/01/2017**

ABSTRACT

The investigation was to evaluate gastroprotective effects of ethanolic extract of *A. auriculiformis* bark on pylorus induced and swimming induced gastric mucosal injuries in rats. Sixty rats were divided into 2 models containing 30 rats each with 5 groups per model, Normal control, experimental control, standard and extract treated groups. Standard control was administered with rabeprazole (20mg/kg) and experimental with two doses of extract of EAA at dose of 100mg/kg and 200mg/kg respectively. Ulcers were induced by pylorus ligation and forced swimming for 3 hours. Rats were sacrificed 3 hours after pylorus ligation and forced swimming. Experimental group exhibited significant mucosal injuries, lesions in both models which were reduced in extract groups of 100mg/kg and 200mg/kg. The increase in the levels of catalase and glutathione and decrease in lipid peroxidation in both models proved the antioxidant activity of extract.Thus it can be concluded that ethanolic extract of *A. auriculiformis* have antiulcer activity, which can be attributed to its antioxidant mechanism of action.

KEYWORDS: *A. auriculiformis*, gastroprotective, rabeprazole.

INTRODUCTION

A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases. Several hundred genera are used medicinally, mainly as herbal preparations in the indigenous systems of medicine in different countries and are sources of very potent and powerful drugs which have stood the test of time and modern chemistry has not been able to replace most of them.[1] The world is now moving towards the herbal medicine or phytomedicines that repair and strengthening bodily systems (especially the immune system, which can then properly fight foreign invaders) and help to destroy offending pathogens without toxic side effects.[2] The use of conventional medications is often unsatisfactory for many patients because of adverse effects and loss of effectiveness on long term uses. As a result, current trends indicate that people are shifting to non-conventional therapy. Moreover, the problem of resistance to current conventional therapy has become far more serious. Therefore, it has become necessary to search for an alternative and equally effective means of tackling the problem. As part of the efforts in searching for new alternatives, upgrading and integration of traditional medicine is considered to be one among the possible solutions.[3] Today, it has been developed as a separate industry as many people favour herbal medicine over synthetic medicine. Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects. Several regulatory models for herbal medicines are currently available including prescription drugs, over-the-counter substances, traditional medicines and dietary supplements. Harmonization and improvement in the processes of regulation is needed, which combines scientific studies and traditional knowledge. Finally, the trend in the domestication, production, biotechnological studies and genetic improvement of medicinal plants, instead of the use of plants harvested in the wild, will offer great advantages, since it will be possible to obtain uniform and high quality raw materials which are fundamental to the efficacy and safety of herbal drugs.[2] Gastric ulcer is an illness that affects a considerable number of people worldwide associated with potentially life threatening complications, including bleeding, perforation penetration and obstruction.[4] Although the etiology of gastric ulcers is still debated, it is accepted that gastric ulcers are triggered by an imbalance between factors that damage and those that protect the stomach. Mucosal damage, an initial step in gastric ulcer development, has been known to be due to hypersecretion of HCl through H+, K+-ATPase action, harbouring of H. pylori on the damaged mucin layer, the blockade of the cyclooxygenase enzyme system by NSAIDs, and oxidative stress by reactive oxygen species. Reactive oxygen species (ROS) and free radicals play an important role in the pathogenesis of several human diseases including GUD. Various studies have also shown that the endogenous anti-oxidant defense enzymes

play a principal role in eliminating ROS and free radicals generated from the action of factors that damage the stomach. An approach to manage GUD, therefore, is through the scavenging of ROS and the stimulation of the endogenous anti-oxidant enzymes in the stomach in addition to the other approaches like the inhibition of gastric H+, K+-ATPase and the elimination of *H. pylori* using antibiotics.[5] Since decades many indigenous drugs have been known to possess anti-ulcer activity. The antiulcer properties of *Emblica officinalis* and *Glycyrrhiza glabra* have been mentioned. The anti-oxidant properties of *Tinospora cordifolia*, *E. officinalis* and *G. glabra* were earlier investigated and were found to possess free radical scavenging property. These ingredients were also found to produce significant induction in the levels of various endogenous antioxidant enzymes[5]. *Acacia auriculiformis* A. Cunn is a vigorously-growing deciduous or evergreen tree and belongs to the family Mimosaceae. It is rich in methylglucuronic acid, glucuronic acid. In addition, extracts from the *Acacia* species are rich in phenols and polyphenols and have strong antimutagenic and antioxidant activities.[6]

METHODS

1.1 Phytochemical screening of extracts

Ethanolic extract of bark of *Acacia auriculiformis* was subjected to various chemical tests to detect the chemical constituents present in it.[7]

Tests for Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a. Molisch's test: To 2ml of the extract, 1ml of α-naphthol solution, concentrated sulphuric acid was added through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of sugars.

b. Fehling's test: To 1ml of the extract, equal quantities of Fehling's solution A and B was added, upon heating formation of a brick red precipitate indicates the presence of reducing sugars.

c. Benedict's test: To 5ml of Benedict's reagent,1ml of extract solution was added and boiled for 2 minutes and cooled. Formation of red precipitate shows the presence of reducing sugars.

Tests for Flavonoids

a. Shinoda test: The alcoholic extract was treated with magnesium turnings and concentrated HCl. Intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

b. Lead acetate test: The extract was treated with few drops of lead acetate solution; formation of yellow colour precipitates indicates the presence of flavonoids.

c. Zinc-HCl reduction test: To the alcoholic solution of extracts, a pinch of zinc dust and concentrated HCl was added. Appearance of magenta colour after few minutes indicates the presence of flavonoids.

Tests for Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

a. Dragendorff's test: To 1 ml of extract, 1 ml of Dragendorff's reagent (potassium bismuth iodide solution) was added. Orange-red precipitates indicate the presence of alkaloids.

b. Mayer's test: To 1 ml of extract, 1 ml of Mayer's reagent (potassium mercuric iodide solution) was added. Whitish yellow or cream coloured precipitates indicate the presence of alkaloids.

c. Hager's test: To 1 ml of extract, 3ml of Hager's reagent (saturated aqueous solution of picric acid) was added. Yellow coloured precipitates indicate the presence of alkaloids.

d. Wagner's test: To the 1 ml of extract, 2 ml of Wagner's reagent (iodine in potassium iodide) was added. Formation of reddish brown precipitates indicates the presence of alkaloids.

Tests for Tannins

a. Gelatin test: To the test solution, 1% gelatin solution containing 10% sodium chloride was added. Formation of white precipitates indicates the presence of tannins.

b. Ferric chloride test: To 1ml of the extract, ferric chloride solution was added; formation of a dark blue or greenish black colour product shows the presence of tannins.

Test for Saponins

a. Froth test: Extracts were diluted with distilled water to 20 ml and were shaken in measuring cylinder for 15 minutes. Formation of foam of height of 1cm indicates the presence of saponins.

1.2. In-vitro study

a. Determination of the total flavonoid content

One milliliter of ethanolic extract of *Acacia auriculiformis* containing 1mg/mL of dry matter was placed in a 10 mL volumetric flask, then 5 mL of distilled water was added followed by 0.3 mL of 5% NaNO2. After 5 min, 0.6 mL of 10% AlCl3 was added. After another 5 min 2 mL of 1 M NaOH was added and volume was made up with distilled water. The solution was mixed and absorbance was measured at 510 nm. TF amounts were expressed as catechin equivalents per dry matter. All samples were analyzed thrice and results averaged.[8]

b. Determination of total phenolic content

The total phenolic content (TPC) of the extract of *Acacia auriculiformis* was determined by the method of Folin–Ciocalteu reaction using gallic acid as standard. To 100 µl of extract 500 µl of (50%) Folin–Ciocalteu reagent was added, followed by the addition of 1 ml of 20% Na2CO3 solution. After 20 min incubation at room temperature the absorbance was measured at 730 nm. The total phenolic content was expressed as Gallic acid equivalents (GAE) in mg/g samples.[9]

c. Determination of DPPH radical scavenging activity

The extract was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Scavenging of DPPH represents the free radical reducing activity of extract based on a one-electron reduction. Briefly, the reaction mixture contained 300 µl of extract concentrations (1– 100 µg /ml) and 2 ml of DPPH (0.1 mM in methanolic solution). The reaction mixture was then placed in the cuvette holder of the spectrophotometer against the blank, which did not contain the extract. The Lascorbic acid was used as the positive control. The percent DPPH decolourization of the sample was calculated by the equation.[10]

DPPH (%) =A Control-A test/A Control X 100

The decolouration was plotted against the sample extract concentration in order to calculate the IC50 values (inhibitory concentration 50 µg /ml), which is the amount of sample necessary to decrease the absorbance of DPPH by 50%.

d. NO scavenging assay

Sodium nitroprusside (5µM) in standard phosphate buffer solution was incubated with different concentration of the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 5 hr. After 5 hr, 0.5 ml of incubation solution was removed and diluted with 0.5 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of chromophore formed was read at 546 nm. The control experiment was also carried out in similar manner, using distilled water in the place of extracts. The experiment was performed (in triplicate) and % scavenging activity was calculated using the formula.

100 − [100/blank absorbance × sample absorbance].

1.3 Experimental protocol

Table 1Pylorus ligation method

Table 2 Swimming induced model

For both models rats were divided into five groups, containing six animals in each group. Group I served as normal control which received vehicle. Animals of group II served as experimental controlpylorus ligated. Animals of group III received standard drug Rabeprazole (20mg/kg). Animals of Groups IV and V received ethanolic extract of *Acacia auriculiformis* bark at low and at high dose (100 mg/kg and 200 mg/kg).

1.3.1 Pylorus ligation method

Pylorus ligation was carried out in anaesthetized rats. The pylorus of each rat was tied/ligated under light ether anesthesia and the abdominal incisions were closed. In treatment group the animals were administered orally 1hr before pylorus ligation and 3 hr after pyloric ligation the rats were sacrificed. The stomach was removed and opened along with the greater curvature.[13]

1.3.2 Swimming induced model

Stress ulcers were introduced by forcing swimming in the glass cylinder (height 35 cm, diameter 25 cm) containing water to the height of 35 cm maintained at 250C for 3 hours. Animals were fasted for 24 hours prior to the experiment.[11] The volume of gastric content and its pH was recorded. Biochemical parameters like GSH, Catalase, lipid peroxidation and histopathology was done.

1.4 *In vivo* study

1.4.1 Determination of ulcer index

Scoring of ulcer

0 = Normal coloured stomach 1.5 = Haemorrhagic streaks

0.5 = Red colouration 1 = Spot ulcer

2 = Ulcers ≥ 3 but ≤ 5mm 3 = Ulcers >5mm

Calculation of ulcer Index

UI = UN + US + UP x 10-1

UI = Ulcer Index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animals with ulcer[12]

1.4.2 Determination of pH and free acidity

Gastric juice was collected from rat's stomach. The gastric juice thus collected was centrifuged and the volume of gastric juice as well as pH of gastric juice was measured. Free acidity was measured Tropfer's reagent is used. 0.01N NaOH used to titrate gastric juice until canary yellow colour observed. Volume of 0.01N NaOH consumed was noted (Golbabapour et al., 2013)

1.4.3 Determination of total acidity

An aliquot of 1ml of gastric juice was taken in to a 50 ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink color wasestablished. The volume of 0.01N NaOH consumed was noted, total acidity was calculated and expressed as meq/l.

1.4.4 Determination of percentage protection

The percentage protection[14] produced by plant extract against ulcer determined by using a formula

Control - Treatment × 100

Control

(Control- UI in control group; Treatment- UI in treated group)

1.4.5 Determination of lipid peroxidation

In vitro lipid peroxidation levels of homogenate were measured as TBARS. Ten percent of homogenate was prepared in 20mM phosphate buffer saline (PBS), pH 7.4 (36). Briefly, 0.25mL of homogenate was incubated with 5–25 μgmL of extract in 20mM PBS, pH 7.4. After 5min of pretreatment, 0.5mL each of ferric chloride (400mM) and ascorbic acid (400mM) was added and incubated at 37◦C for 1 h. The reaction was terminated by addition of 2.0mL of TBA reagent (15% TCA, 0.37% TBA in 0.25N

HCl) and tubes were boiled for 15 min at 95◦C, cooled, centrifuged and read at 532 nm. TBARS was measured by using a standard TMP (1,1,3,3 tetramethoxy propane) calibration curve (0.1– 0.5 μg) and expressed as percent inhibition of lipid peroxidation by extracts.

1.4.6 Estimation of reduced glutathione

To 0.1 ml of different tissue homogenate 2.4 ml of 0.02M EDTA solution was added and kept on ice bath for 10 min. Then 2 ml of distilled water and 0.5 ml of 50 % TCA were added. This mixture was kept on ice for 10-15 min and then centrifuged at 3000 rpm for 15 min. 1 ml of supernatant was taken and 2ml of Tris-Hcl buffer was added. Then 0.05 ml of DTNB solution (Ellman's reagent) was added and vortexed thoroughly. OD was read (within 2-3min after the addition of DTNB) at 412 nm against a reagent blank. Absorbance values were compared with a standard curve generated from known GSH.[16]

1.4.7 Estimation of Catalase

A volume of 0.1 mL of supernatant was added to a cuvette containing 1.9 mL of 50 mM phosphate buffer pH 7.0). A reaction was started by the addition of 1.0 mL of freshly prepared 30 mM H2O2. The rate of decomposition of H2O2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of CAT was expressed as µmol H2O2 metabolized/mg protein/min.[15]

Catalase activity = change in absorbance/min

E x volume of sample x mg protein

4.5 Histopathology Study

Specimens of the gastric walls were fixed in 10% bufered formalin for 18h at 4◦C and were processed using the paraffin tissue-processing machine. Stomach section was stained with hematoxylin and eosin for histological evaluation.

ACKNOWLEDGEMENTS

At the start of every major task initially difficulties seem insurmountable. But as we begin treading on the chosen path with honesty and perseverance we find that God is there to help us. Towards the completion of my article it is a pleasurable aspect that I have now the break to communicate my thankfulness to all those who have been influential in the completion of this task.

CONCLUSION

Consequently all the data displayed that the ethanolic extract of *Acacia auriculiformis* is a potential source to treat ulcer. The above activity might be due to availability of phytoconstituents specially polyphenolic component present in the plant extract. EAA at both the doses showed prominent activity as a good source for antisecretory and antiulcer activity.

REFRENCES

1. Ahmad I, Mehmood Z, Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology*, 62: 183-193.
2. Pandey M, Debnath M, Gupta S, Chikara S.K (2011). Phytomedicine: An ancient approach turning into future potential source of therapeutics. *Journal of Pharmacognosy and Phytotherapy*, 3(3): 27-37.
3. Bothiraja C, Joshi P.P, Dama G.Y, Pawar A.P (2011). Rapid method for isolation of plumbagin, an alternative medicine from roots of *Plumbago zeylanica*. *European Journal of Integrative Medicine*, 3: 39-42.
4. Nartey E.T, Ofosuhene M, Agbale C.M (2012). Anti-ulcerogenic activity of the root bark extract of the African laburnum "*Cassia sieberiana*" and its effect on the anti-oxidant defence system in rats. *BMC Complementary and Alternative Medicine*, 12: 247.
5. Bafna P.A, Balaraman R (2005). Anti-ulcer and anti-oxidant activity of Pepticare, a herbomineral formulation. *www.elsevier.de/phymed*, 12: 264-270.
6. Krishnaiah D, Sarbatly R, Nithyanandam R (2011). A review of the antioxidant potential of medicinal plant species. *www.elsevier.com/locate/fbp*, 89: 217–233.
7. Singh R, Singh S, Kumar S, Arora S (2006). Studies on antioxidant potential of methanol extract /fractions of *Acacia auriculiformis* A. Cunn. *Elsvier*, 103(2007): 505-511.
8. Saiprasanna, B; Babu, S.M; Ramani, Y.R (2012). Phytochemical investigation and study on antioxidant properties of Pongamia pinnata hydroalcohlic leaf extract. *Plant Sciences Feed*, 2: 74-78.
9. Singh L.P.K, Mishra A, Saha D, S Snehasikta (2011). Doxycycline blocks gastric ulcer by regulating matrix metalloproteinases-2 activity and oxidative stress. *World J of gastroenterol*, 17(28): 3310-3321.
10. Singh, D., Yadav, P., Nayak, S., Hatwar, B., 2012. Anti-acne, preliminary phytochemical and physico-chemical investigation of *Saraca asoca* bark. *Journal of Natural Products and Plan Resources*, 2: 119-126.
11. Nanjundaiah S.M, Annaiah H.N.M, Dharmesh S.M (2011). Gastroprotective effect of ginger rhizome (*Zingiber officinalis*) Extract: Role of Gallic acid and Cinnamic Acid in H+,K+- ATPase/H. pylori Inhibtion and Anti-Oxidative Mechanism. *Evidence-Based Complementary and Alternative Medicine*, 2011: 1-13.
12. Manoharan, P; John, S; Golla, U; Thangathirupathi, A (2010). Antiulcer effect of Coccinia grandis (Linn.) on pylorus ligated (albino) rats. *IJPRD*, 6(2): 1-9.
13. Golbabapour S, Hajrezaie M, Hassandarvish P, Majid N. A, Hadi A H.A, Nordin N, Abdulla M.A (2013). Acute Toxicity and Gastroprotective Role of

M. pruriens in Ethanol-Induced Gastric Mucosal Injuries in Rats. *BioMed Research International*, 2013: 1-13

14. Rao, P.R; Viswanath, R.K (2007). Cardioprotective activity of silymarin in ischemia-reperfusioninduced myocardial infarction in albino rats. Exp Clin Cardiol, 12(4): 179-187.
15. Dash, D.K; Yeligar, V.C; Nayak, S.S; Ghosh, T; Rajalingam , D; Sengupta, P; Maiti, B.C; Maity, T.K (2007). Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus rutescens* (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. *Tropical Journal of Pharmaceutical Research*, 6(3): 755-765.