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EVALUATION OF ANTI-ULCER ACTIVITY OF ACTIVE FRACTION OF *HELICTERES ISORA* LINN. FRUIT EXTRACT

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ABSTRACT

Peptic ulceration is considered to be one of the modern age epidemics, has been affecting approximately 10% of world population. Peptic ulcer occurs due to an imbalance between acid and pepsin along with the weakness of the mucosal barrier. Due to these, it is commonly associated with damage of the stomach's mucosal layer, which is simply generated via excess generation of exogenous and endogenous active oxygen and free radicals. Some of the main causes of gastric ulcers include chronic use of alcoholic beverages and anti-inflammatory drugs, as well as stress and *Helicobacter pyroli* infection In this study phytochemical screening of the fruits of *Helicteres isora* Linn. were performed to find out the existence of various phytoconstituents. Then *in vitro* studies were carried out to check various antioxidant property using hydrogen peroxide scavenging assay, DPPH radical scavenging activity, estimation of total phenolic content and total flavonoid content. Animal models were developed by aspirin+pyloric ligation method and ethanol ligation method on Wistar rats. The chosen drug showed a potent antiulcer activity, the effect may be attributed to the presence of flavonoids in the extract. Flavonoids are responsible for the free radical scavenging activity and are believed to be one of the important components in antiulcer activity. Potent free radical scavenging activity was found for active sub-fractions of ethyl acetate extract of *Helicteres isora* Linn. This study showed a potent antiulcer activity of the most active fraction of *Helicteres isora* Linn. that suggests, ethnopharmacological approach in selecting the plant for study may be useful.

KEYWORDS: *Helicteres isora* Linn. fruit extract, Pylorus ligation, HCl/ethanol induced ulcer, Flavonoids, Anti-ulcer activity, Ranitidine, Omeprazole.

INTRODUCTION

An ulcer is a discontinuity or break in a bodily membrane that impedes the organ of which that membrane is a part from continuing its normal functions. A peptic ulcer is a distinct breach in the mucosal lining of the stomach (gastric ulcer) or the first part of the small intestine (duodenal ulcer), a result of caustic effects of acid and pepsin in the lumen. *Helicobactor pylorus is* one of the most common causes of peptic ulcer. Ulcers can be worsened by drugs such as aspirin, ibuprofen, and other NSAIDs.

Helicteres isora Linn. (East-Indian screw tree, Nut-leaved screw tree) is a species of small tree or large shrub found in Asia including Indian Subcontinent, South China, Malay Peninsula, Java and Saudi Arabia. Also, found in Australia. It is a Large shrub with grey bark and alternately arranged hairy, ovate shaped leaves with serrate margins. Flower are brick red or orange-red in color. Fruits are compound pod, twisted like screw with pointed end, signifying the name "Indian Screw Tree". Raw fruits are greenish in color, brown or grey when dried. Seeds are black-brown, highly polished, roughly rhomboid, rectangle or triangular in shape.

MATERIALS AND METHODS

Plant authentication and extraction: Fruits of *Helicteres isora* Linn. were collected from Birla Institute of Technology, Mesra, Ranchi, Jharkhand in the month of August and was identified and authenticated from Central National Herbarium, P.O.: Botanical Garden, Howrah. [No- CNH/20/2014/TECH.II/60]. The plant specimen (Herbarium) was also submitted in Dept. Of Pharmaceutical Sciences, BIT, Mesra, Ranchi. The powdered material was carried out using 4 solvents namely- Isopropyl alcohol, Acetone, n-Hexane and Methanol by cold maceration process in an air tight, clean flat bottomed container for 7 days at room temperature with occasional stirring and shaking and then it was clarified by filtration and was concentrated in a rotary evaporator and then the fractions were kept in a desiccator for future use. Further sub-fractions F1, F2, F3, F4 were obtained through column chromatography.

***In-vitro* studies:** were carried out of the active fraction of *Helicteres isora* Linn. fruit extract to check various antioxidant property using hydrogen peroxide scavenging assay, DPPH radical scavenging activity, estimation of total phenolic content and total flavonoid content.

Hydrogen Peroxide Scavenging Activity: The ability of sample extracts to scavenge hydrogen peroxide was estimated by following the method of Ruch *et al* with slight modifications. A solution of hydrogen peroxide (40mmol/L) was prepared in phosphate buffer (50 mmol/L, pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. For preparing test samples, different concentration of sample extracts (50, 100 and 250µg/ml) in distilled water was prepared and 0.5 ml of each concentration was added to hydrogen peroxide (0.6 ml) in respective test tubes. The volume was achieved up to 5 ml using phosphate buffer. Absorbance at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide.

$$\% \textbf{ Scavenged } [\mathbf{H2O2}] = \frac{Ac - As}{Ac} * \mathbf{100}$$

Where, AC= absorbance of control

AS= absorbance of sample

DPPH (1,1-diphenyl-2-picrylhydrazyl) Free Radical Scavenging Activity: The free radical scavenging activity of the extract was assessed on the basis of the radical-scavenging effect of the stable 1,1 Diphenyl-2-picryl hydrazyl (DPPH) free radical. A series of extract concentration was prepared (100,200,300,400,500 mcg/ml).Then 1ml of extract at different concentrations was mixed with 4ml of 0.004% (0.1mM) DPPH in methanol. The disappearance of DPPH was read spectrophotometrically at 517nm after 30min of incubation at room temperature in the dark. A purple to yellow colour change is observed. The same solvent was used as control instead of extract .The same procedure was repeated with methanolic solutions of synthetic antioxidant ascorbic acid as positive control. Methanol was used as blank. The measurements were performed in triplicate and the results were averaged.

Calculation: Free radical scavenging capacity was expressed as percentage inhibition of DPPH radical and was calculated by the following equation:

$$\mathbf{I\ (\%) = 100 * \left(1 - \frac{Abs\ of\ sampl}{Abs\ of\ control}\right)}$$

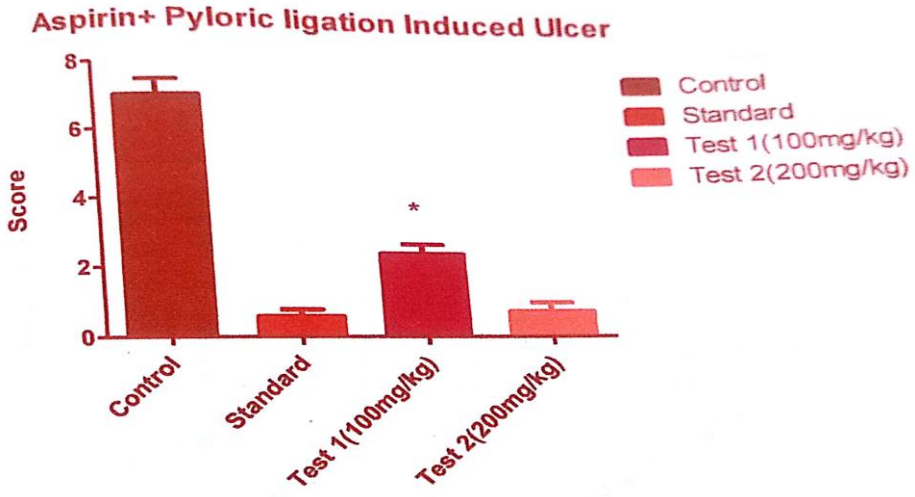
Determination of Total Flavonoid Content: Estimation of total flavonoid content of both the sample extract was measured with the help of an aluminium chloride colorimetric assay described by Woisky and Salatino. In a test tube containing 0.5 ml of sample extract, 1.5 ml of methanol (75 %), 0.1 ml potassium acetate (1 M), 0.1 ml of aluminium chloride (10 % w/v) and 2.8 ml of distilled water was added. Mixture obtained was kept to stand for 30 minutes at room temperature and absorbance was measured at 435 nm using UV visible spectrophotometer. The quercetin was taken as standard and stock solution of 10µg/ml was prepared by dissolving quercetin in methanol. A different dilution of 6.25, 12.5, 25, 50, 80, 100µg/ml was prepared from stock solution and a calibration curve of quercetin was made. The results of total flavonoid contents were expressed in term of milligrams of quercetin equivalent (QE) per gm extract (mg QE/gm of dry extract)

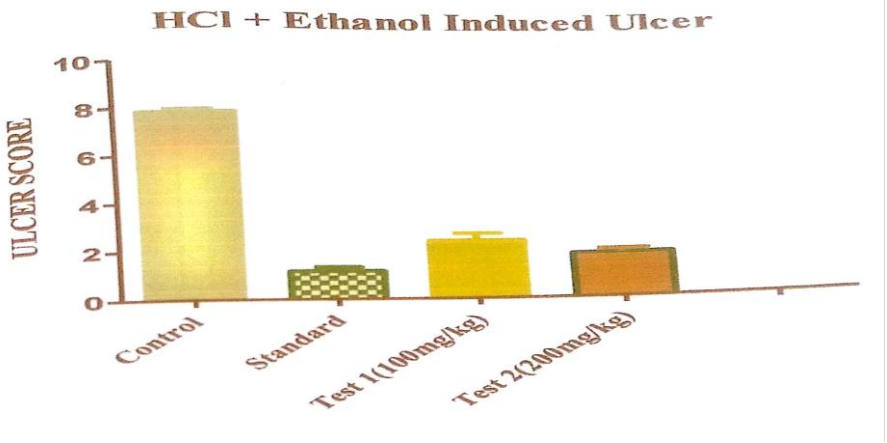
Estimation of total phenolic content: The concentration of phenolic in plant extract was determined using spectrophotometer method. Ethyl Acetate extract and methanolic extract of *Withaniasomnifera* and *Terminalia arjuna* respectively was prepared by mixing 0.5ml of extract with 2.5ml of 10% Folin Ciocalteau reagent dissolved in and2.5ml of 7.5% Sodium Carbonate. Blank was prepared without extract .The samples were incubated for 45minutes.The absorbance was determined using spectrophotometer. Standard solution of Gallic acid (2, 4, 6, 8, 10, 12mcg/ml) were pipetted into concentrated 2- 12µg/ml in a series of test tubes .The absorbance of Gallic acid is taken at 765nm against ethanol.

***In-vivo* studies:** Two different sets of models were used, one is the aspirin + pyloric ligation induced model in which after 12 h of fasting, rats were randomly divided into four groups of six animals each. Group I – 1ml of vehicle (dist.H2O/NS), Group II – Ranitidine (50mg/kg), Group III & Group IV - 100 & 200 mg/kg of F4 fraction of HL fruit extract, respectively for 5 days. The other set of model is HCl / Ethanol induced ulcer model in which after 12 h of fasting, rats were randomly divided into four groups of six animals each. Group I – 1ml of vehicle (normal saline), Group II – Omeprazole (30mg/kg), Group III & Group IV - 100 & 200 mg/kg of F4 fraction of HL fruit extract, respectively for 5 days. All the animals were sacrificed and the stomachs were removed, opened at the greater curvature and the content was measured before drained into a centrifuge tube and subjected to centrifugation at 3000rpm for 10 min.

RESULTS

The study revealed that in case of aspirin + pylorus ligation induced ulcers the F4 sub-fraction of *Helicteres isora* fruit treated groups showed a significantly ($p < 0.05$) increase in gastric juice pH, reduction in the gastric volume and total acidity when compared to control and in case of HCl / Ethanol induced ulcer model, F4 sub-fraction significantly ($p < 0.01$) reduced the ulcer index and afforded significant protection against HCl / Ethanol induced ulcer in a dose dependent manner when compared to control group.





CONCLUSION

The chosen drug showed a potent antiulcer activity, the effect may be attributed to the presence of flavonoids in the extract. Flavonoids are responsible for the free radical scavenging activity and are believed to be one of the important components in antiulcer activity. Potent free radical scavenging activity was found for active sub-fractions of ethyl acetate extract of *Helicteres isora* Linn. This study showed a potent antiulcer activity of the

most active fraction of *Helicteres isora* Linn. that suggests, ethnopharmacological approach in selecting the plant for study may be useful.

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