GASTRO PROTECTIVE POTENTIAL OF PREMNA SERRATIFOLIA LINN. LEAVES AGAINST ASPIRIN INDUCED ULCER IN ALBINO RATS.

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Summary

Premna serratifolia Linn. is an important medicinal herb (synonym- Premna integrifolia) known as “Agnimantha” in Ayurveda and traditionally used for cardio tonic, antibiotic and antihyperglycemic properties. Plant has shown anticoagulant activity and the decoction of leaves exhibited anti-inflammatory and anti arthritic activity. However, its antiulcer activity has not been investigated still now. Hence it was considered to evaluate the antiulcer activity of ethanol extract of leaves of Premna serratifolia Linn. Results revealed that the ethanolic extract possess significant antiulcer and antisecretory activity when compared to Ranitidine, the activity may be due to the presence of phytoconstituents like alkaloids, irridoid glycosides and flavonoids in it.

Key words; Premna serratifolia Linn. Leaves, Ethanol extract, Anti ulcer activity

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Introduction

Premna serratifolia Linn., is a plant species (Verbenaceae), popularly known as Munnai in Tamil and Agnimantha in Ayurveda, is a large shrub 9m in height, with a comparatively having short trunk and numerous branches. It is widespread throughout Micronesia and much of the tropical Pacific and Asia. Infusion of the leaves is administered with pepper in cold and fever. Leaves are used to cure “weakness of limbs” and the leaves and leaf sap were used to alleviate headache. Root and wood forms an ingredient of “Dasmulam” an Ayurvedic formulation which is used in a variety of affections. From the leaves, a Verbascoside - iridoid glycoside conjugate was isolated along with Premnafolioside, a new Phenylethanoid, and other Phenolic compounds were isolated from stem. Anti-inflammatory and anti-arthritis activity against acute, sub-acute and chronic inflammation induced in both immunological and non-immunological experimental models, anticoagulant activity and anti-hyperglycemic activity have also been reported, but its antiulcer activity has not been studied till now. In the present study, the antiulcer activity of leaf of ethanolic extract of Premna serratifolia Linn., against Aspirin induced ulceration in albino wistar strain rats have been evaluated.

Materials and methods

Plant Material and Authentication: Fresh leaves of Premna serratifolia Linn. were collected in the month of November from Sirkazi, TamilNadu. The plant was authenticated by Dr. P. Jayaraman, Plant Anatomical Research Centre, Chennai and the voucher specimen (Herbarium No. PARC/2007/80) have been kept in the Department of Pharmacy, Annamalai university, Chidambram.

Preparation of Extracts: Freshly collected leaf materials were cut into small pieces, shade dried until a constant weight was obtained and coarsely powdered. The air-dried powdered material was extracted successively with ethanol in a Soxhlet extractor. The extract was concentrated, evaporated to dryness, until semi-solid masses were obtained and then extractive value calculated as 2.72 %.

Drugs and chemicals

Premna serratifolia Linn. leaves extract were obtained from vignan pharmacy college, Guntur, India. Aspirin and Ranitidine was obtained from Hetero drugs Ltd, Hyderabad. Toffer's reagent Nice chemicals pvt.Ltd., cochin. All other chemicals were of analytical grade.

Dose fixation

Premna serratifolia Linn. leaves extract was administered at different doses (200,400mg/kg/p.o./day). The doses and duration of the treatment period that exhibited the maximum antiulcer activity (based on ulcer index, volume of gastric juice, free acidity, total acidity, $pH$) was fixed as the optimum dose schedule for the drug.

Grouping

The following groups of animals were used. GroupI-Normal control, GroupII-Possitive control, GroupIII-Ranitidine(), GroupIV- Premna serratifolia Linn leaves extract(200 mg/kg/ P.o/day), GroupV- Premna serratifolia Linn leaves extract(400 mg/kg/ P.o/day)

Phytochemical screening

The ethanolic extract of Premna serratifolia Linn leaves is intended to investigate various phytochemicals in the leaves by standard chemical tests.

Acute oral toxicity study

The acute oral toxicity study was done according to the OECD guidelines 423 (Acute toxic class method). A starting dose of 2000 mg/kg body weight p.o of ethanolic extract of leaves of Premna serratifolia Linn. was administered to 3 male rats, observed for three days. There was no considerable change in body weight before and after treatment of the crude extract and no signs of toxicity were observed. When the experiments were repeated with the same dose level, for further 3 days, and observed for 4 days, no changes were observed from first set of experiment. LD50 cut off mg/kg body weight was observed. Based on this study, it was assumed that the dose of 200mg/kg body weight or 400 mg/kg body weight can be safely administrated.

Evaluation of anti-ulcer activity

The modified method of was used for the production of experimental gastric ulceration, that is, in rats, by administering aspirin (200 mg/kg) suspended in 1% sodium carboxymethyl cellulose. The aqueous suspension of aspirin was administered with the help of a round tip cannula at 12.00 h. Ethanolic extracts of Premna serratifolia Linn. (200 and 400 mg/kg), were administered orally 3 h prior to and after the aspirin treatment. This regimen was continued for 3 consecutive
days, following 36 h fasting. Four hours after the aspirin administration the animals were sacrificed by decapitation. The stomach was opened and the percentage inhibition of ulcer was determined\(^1\). Mean ulcer score for each animal was expressed as ulcer index. The maximum length of each lesion was determined and the sum of the lengths of all lesions in each stomach was expressed as the ulcer index\(^2\).

**Determination of free acidity and total acidity**

1ml of gastric juice is pipette out in 100ml conical flask, 2-3 drops of toffer’s reagent is then added and titrated with 0.01 N sodium hydroxide until all traces of pink colour disappears and the colour of the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Titration is continued until pink colour of solution reappears. Again the total volume of alkali added is noted, this volume corresponds to total acidity. Acidity (MEq/1/100g) can be calculated by using the formula.

\[
\text{Acidity} = \frac{\text{volume of NaOH} \times \text{Normally of NaOH} \times 100}{01} \text{ MEq/1/100gm}
\]

**Histopathology study**

Stomach was dissected from the rabbit carcass along with the heart and was fixed in 10% neutral buffered formalin for 48 hours. The organs were then washed in running tap water for overnight. The organs were then trimmed and were processed in Yorco automatic tissue processor. The processed tissue sections were then embedded in paraffin wax using Leica embedding station. Three micron thick sections were prepared using Leica microtome. The sections were stained using routine Haematoxylin and eosin technique. The stained sections were observed for any changes under light microscope.

**Statistical analysis**

The data were expressed as mean+SEM. Results were analyzed statistically by one-way ANOVA followed by DUNNETT’s test using SSPS software version. The difference was considered significant if \(P<0.05\) and highly significant if \(P<0.01\).
Results

Phytochemical screening

The screening results were as follows: alkaloids + ve; carbohydrates; -- ve; proteins and amino acids +ve; steroids - ve; phenols +ve; flavonoids +ve; glycosides + ve; saponins -ve; tannins +ve. Were +ve and -ve indicated the presence and absence of compounds.

Acute oral toxicity study

There was no considerable change in body weight before and after treatment of the experiment and no signs of toxicity were observed. When the experiments were repeated with the same dose level, for further 3 days , and observed for 4 days, no changes were observed from first set of experiment. LD50 cut off mg/kg body weight was observed . Based on this study , it was assumed that the dose of 200mg/kg body weight or 400 mg/kg body weight can be safely administrated.

Evaluation of anti-ulcer activity

In the aspirin induced ulcer model, it was observed that the treatment with ethanolic leaves extract of Premna serratifolia Linn. 200 and 400 mg/kg and ranitidine (200 mg/kg) significantly reduced the lesion index, the total, free acidity and the percentage of ulceration, in comparison with negative control group (p < 0.01). The percentages of inhibition of ulcers were The result of the effect of Ethanolic extract of leaves of Premna serratifolia Linn. on gastric secretion, ulcer index, free acidity, total acidity and pH are shown in Table No1, Oral administration of test extract to albino rats caused significant decrease in ulcer index and the percentage of gastric protection was 40.8%(Standard), 17.3%(positive control), 48.6%(low dose), 58.3%(high dose). When compared to control there was also significant decrease in volume of gastric juice and increase in pH. The free and total acidity were also decreased to a significant extent, The statistical analyses were carried out by using one-way ANOVA followed by DUNNETT’s test using SSPS software version. 68.72%, for the test groups with 200 mg/kg and 62.13% for 400 mg/kg Premna serratifolia Linn ethanolic extract.

Table 1. Effect of Ethanolic extract Of Premna Serratifolia Linn. leaf on Aspirin Induced ulceration in albino rat. (Treatment Vs. Control)

<table>
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<tr>
<th>Group No</th>
<th>Body wt. gms</th>
<th>Normal coloured stomach</th>
<th>Red. Colouration</th>
<th>Spot ulcer</th>
<th>Streaks hemottnagic</th>
<th>U≥3 ≤5</th>
<th>U&gt;5</th>
<th>Total Score</th>
<th>Mean ulcer I ± Sem</th>
<th>Total Protection</th>
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Histopathological study

The Histopathological studies revealed that high dose of *Premna serratifolia* Linn leaves prevented ulcer formation where as low dose could not prevent ulcer formation. The above facts are shown in figure 1-5.
Figure 1. Section of glandular stomach of control group showing normal histology. HE x 5.

Figure 2. Ulcerated gastric mucosa of the diseased control group with frank microscopic ulcer. HE x 5.
Figure 3. Gastric mucosa of standard drug treated group showing no ulceration in the mucosa of glandular stomach. HE x 5.

Figure 4. Low dose group revealing moderate erosion in the glandular stomach. HE x 5.
Figure 5. Section of glandular stomach of high dose group showing only congestion of mucosa with no ulceration. HE x 5.

Discussion

The leaves of *Premna serratifolia* Linn. was found to be rich in phytochemical constituents which may have variety of pharmacological actions. In the literature survey the revealed reported the presence of Alkaloids, Tannins, and Proteins in the entire plant. The result of the present study indicates that Ethanolic extract of leaves of *Premna serratifolia* Linn. exhibited anti-ulcer activity against Aspirin Induced ulcers in rats. Table no.1 and Fig. No 1 to 5 show the results obtained from the Ethanolic extract of Leaf of *Premna serratifolia* Linn. on albino wistar rats at doses 200mg, 400mg/kg body weight and had shown a significant graded and dose dependent decrease in ulcer index, gastric acid secretion, free acidity and total acidity and there was a significant increase in pH gastric juice of aspirin Induced ulcer rats. The Histopathological studies revealed that high dose of *Premna serratifolia* Linn. leaves prevented ulcer formation where as low dose could not prevent ulcer formation. The above facts are shown in figure 20-24. Cytoprotection by drugs has been considered due to the generation of prostaglandin by anti ulcer drugs when used in these non-secretory doses. Alkaloids have been reported to possess a significant anti-ulcer activity, which are active chemical ingredients of leaf of *Premna serratifolia* Linn.

Conclusion

In the Ethanolic extract of leaves of *Premna serratifolia* Linn. at doses of 200mg/kg and 400mg/kg P.O were found to be having significant, graded and dose dependent anti-ulcer and anti secretory activity when compared to control group and using Ranitidine 200mg/kg P.O as standard. Thus from the present study it can be concluded that the Ethanolic extract of leaves of *Premna serratifolia* Linn. leaf exhibits anti-ulcerogenic and antisecretory activity in Aspirin induced ulceration in rats.
Acknowledgement

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