IN VIVO STUDIES ON AQUEOUS EXTRACT OF GUM RESIN OBTAINED FROM COMMIPHORA WIGHTII FOR THE TREATMENT OF PEPTIC ULCER

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ABSTRACT

The objective of this study was to investigate the Antiulcer activity of gum resin of Commiphora wightii (bhandaari) in rats. The antiulcer activity of aqueous extract was evaluated in indomethacin & ethanol induced gastric ulcer model in wistar rats. The aqueous gum resin extract was prepared by cold maceration extraction process using water as solvent. Phytochemical analysis & acute oral toxicity were carried out using standard methods. The preliminary phytochemical screening of C.wightii revealed the presence of sterols, glycoside & flavonoids. The Aqueous extract at a concentration of 250 mg/kg & 500 mg/kg exhibit a protective effect on ulcer induced models & was compared with standard drugs Ranitidine & Misoprostol. Percentage ulcer inhibition of aqueous Extract at 500 mg/kg for Indomethacin & ethanol induced ulcer model were 23.27% & 40.55% respectively. Among the doses studied aqueous extract 500 mg/kg body weight was found to be optimum for significant ulcer reduction. The flavanoids & Sterols present in the aqueous extract might be responsible for the antiulcer action probably by maintaining the antioxidant level in the gastrointestinal tract there by reducing mucosal damage. The extract exhibit ulcer protection activity in dose dependent manner.

KEYWORDS: Antiulcer activity, Ranitidine, Misoprostol, Ulcer Index, C.wightii.

INTRODUCTION

Peptic ulcer is the most common GIT disorder in the present day life of the industrialized and civilized world. The prevention or cure of peptic ulcers is one of the most important
challenges confronting medicine nowadays, as it is certainly a major illness affecting 8 to 10% of the global population and of these 5% suffer from gastric ulcer.[1] Thus, there is demand to find additional information regarding the antiulcer potential of plant species. *Commiphora wightii* is vata and kapha suppressant and is widely used in the disease caused by vata. It is a good pain reliever and also acts as an anti-inflammatory. It promotes wound healing and is very effective as vermicidal.[2,3]

In India, Guggul is mainly produced by 4 species: *C. mukul, C. wightii, C. berryi, and C. agallocha*.4,5,6 It is used in hypercholesterolemia, obesity, rheumatism, liver diseases, digestive problems, acne, tumour fever etc. Because the phytochemical investigation of *Commiphora wightii* also revealed the presence of flavonoids[7] & Sterols an attempt has been made to investigate the antiulcer activity of aqueous extract of *Commiphora wightii* gum resin in various experimental animal models for gastric ulcer.

**MATERIALS AND METHODS**

**PLANT COLLECTION & IDENTIFICATION**

The gum resin of concerned plant; *Commiphora wightii*, Burseraceae was collected from local nursery situated in Bhopal city, M.P. Resin was shade dried to prevent any type of moisture presence and degradation of active constituents. Authentication was done by Dr. Zia-ul Hasan, Professor & head of department botany, Saifia college of science, Bhopal (MP), India. (Voucher specimen No. 332|Bot|Saifia|2012). The gum resin were dried as quickly as possible in shade & stored in airtight glass jars until use.

**PREPARATION OF EXTRACT**

The collected gum resin of *Commiphora wightii* were shade dried & powdered coarsely & then passed through 40 mesh sieve. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of *Commiphora wightii* was cold macerated with the solvent distilled water for 7 days. The solvent was removed at low temperature under reduced pressure or the extract was evaporated to dryness using water bath (60-70\(^\circ\)C) & rotary evaporator. The extract obtained was stored in labeled, airtight, amber colored bottle in the refrigerator until use for phytochemical analysis & pharmacological studies. The extract obtained was weighed to a constant weight & the percentage yield w/w basis was calculated.
Determination of percentage yield

\[
\% \text{ Yield} = \frac{\text{weight of extract}}{\text{Weight of powdered drug}} \times 100
\]

PHYTOCHEMICAL SCREENING

The screening was carried out on the aqueous extracts of gum resin of *Commiphora wightii* to determine the active principles or secondary plant constituents. Tests were carried out for carbohydrates, reducing sugar, tannins, polyphenols, flavanoids, alkaloids, gum, saponins, amino acid, resins & steroids.

ANIMALS

Healthy albino wistar rats of either sexes weighing 150-250 g were used for the evaluation of acute oral toxicity test and anti ulcer activity. The animals were used after an acclimatization period of 10 days to the laboratory environment. Animal was obtained from animal house of VNS institute of pharmacy, Bhopal. Ethical clearance for the handling of animals and procedure used in study was obtained from institutional animal ethical committee prior to the beginning of the study (VNSIP/IAEC/2012/6871). All animals were stored in standard cages and maintained at 25\(^\circ\)C, under 12 hrs dark/ light cycle. The animals were fed with standard rat feed and water was given after specific interval prior to experiment animal were kept for 12 hrs fasting.

ACUTE TOXICITY STUDY\(^[8]\)

Young healthy female wistar rats weighing 200-250 g selected by random sampling technique were used in the study. The animal were fasted prior to dosing overnight, provided only water after which extract were administered to the groups orally at the single dose 5 mg/kg body weight by gavage using a stomach tube or a suitable intubation canula and the group were observed for 14 days. If mortality was observed in 2-3 animals out of 6, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, 2000 mg/kg body weight. The animals were observed for toxic symptom such as behavioral changes, locomotion, convulsion, sleep, comma and mortality for 24 hours (in every 30 min interval for first 4 hours after dosing).
ANTIULCER ACTIVITY
Two models (Indomethacin & ethanol) with effective induction of ulcer experimentally in rats were employed to evaluate the antiulcer activity of aqueous extract of Commiphora wightii to treat peptic ulcer.

Indomethacin Induced Ulcers[9]
Five groups of 6 Wistar rats weighing 150-200 g are used. The standard (Misoprostol- 0.012 mg/kg P.O) and test extract (aqueous-250 mg/kg, 500 mg/kg) were administered orally in 0.5% CMC solution 10 min prior to oral Indomethacin administration (40 mg/kg). Six hours later, the rats were sacrificed and their stomachs removed. Formal-saline (2% v/v) is then injected into totally ligated stomachs for overnight. The next day, the stomachs were opened along the greater curvature, then washed in warm water and examined to determine the ulcer scores.

Ulcer index was determined using the formula:

\[
\text{Ulcer index} = U_N + U_S + U_P \times 10^{-1}
\]

Where, \(U_N\) = Average of number of ulcers per animal, \(U_S\) = Average of severity scores, \(U_P\) = Percentage of animals with ulcers.

Based on the intensity, the ulcers were given scores as follows: 0 = no ulcer, 1 = superficial mucosal erosion, 2 = deep ulcer or transmural necrosis, 3 = perforated or penetrated ulcer

\[
\text{Percentage inhibition} = \frac{UIC - UIT}{UIC} \times 100
\]

Where UIC=Ulcer index of control group, UIT= Ulcer index of test group.

Ethanol Induced Ulcers[10]
Five groups of 6 wistar rats weighing between 150-200 g were fasted before the administration of ethanol. The standard drug (Ranitidine- 50mg/kg P.O) or extract (aqueous-250 mg/kg & 500 mg/kg, P.O) was administered 1 hour before ethanol administration. Ethanol (90%) was administered to all the animals at a dose of 0.5ml/100g & after 1 hour, all the animals were sacrificed & ulcer index was determined.
STATISTICAL ANALYSIS
Ulcer indices were shown as the mean ± SEM & level of ulcer protection presented as percentage inhibition. Statistical comparisons were performed by one way ANOVA followed by Dunnet’s “t” test. The results were considered statistically significant if p-values were less than 0.05.

RESULTS
The percentage yield of aqueous extract was 31%. Phytochemical screening showed that the extract contain sterols, tannins & flavonoids. Acute toxicity results showed that the LD_{50} was greater than 2000mg/kg.

Indomethacin Induced Ulcers
In Table 1, ulcer inhibition was evident in treatment groups of the aqueous extract of Commiphora wightii compared to the negative control. However statistically significant ulcer inhibition (22.32% and 23.27%, *p< 0.05) could be seen at doses of 250mg/kg & 500 mg/kg of the Aqueous extract.

Ethanol Induced Ulcers
The Aqueous extract of Commiphora wightii at all the doses provided protection from ulcer & the protection was dose dependent. The Aqueous extract at doses of 250 mg/kg & 500 mg/kg provides statistically significant protection (20.88% & 40.55%, *p<0.05) when compared with negative control. (Table 2).

Table 1: Effect of aqueous extract on Indomethacin induced ulcer in rats (n=6).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment groups</th>
<th>Doses</th>
<th>Ulcer Index</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups I</td>
<td>Vehicle control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>Negative control(CMC+ Indomethacin)</td>
<td>40 mg/kg</td>
<td>11.6 ±0.22</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>Positive control(Misoprostol)</td>
<td>0.012 mg/kg</td>
<td>6.9± 0.11*</td>
<td>40.51</td>
</tr>
<tr>
<td>Group IV</td>
<td>Aqueous extract</td>
<td>250 mg/kg</td>
<td>9.0±0.17*</td>
<td>22.32</td>
</tr>
<tr>
<td>Group V</td>
<td>Aqueous extract</td>
<td>500 mg/kg</td>
<td>8.9±0.13*</td>
<td>23.27</td>
</tr>
</tbody>
</table>

Data were expressed as mean±SEM. Significant at *p<0.05 (ANOVA followed by Dunnett’s test) when compared to negative control, n=6.
Table 2: Effect of Aqueous extract on Ethanol induced ulcer in rats (n=6).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment groups</th>
<th>Doses</th>
<th>Ulcer Index</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups I</td>
<td>Vehicle control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>Negative control (CMC + Ethanol)</td>
<td>5 ml/kg</td>
<td>9.0±0.17</td>
<td>-</td>
</tr>
<tr>
<td>Group III</td>
<td>Positive control (Ranitidine)</td>
<td>50 mg/kg</td>
<td>5.24±0.10*</td>
<td>41.77</td>
</tr>
<tr>
<td>Group IV</td>
<td>Aqueous extract</td>
<td>250 mg/kg</td>
<td>7.12±0.15*</td>
<td>20.88</td>
</tr>
<tr>
<td>Group V</td>
<td>Aqueous extract</td>
<td>500 mg/kg</td>
<td>5.35±0.18*</td>
<td>40.55</td>
</tr>
</tbody>
</table>

Data were expressed as mean±SEM. Significant at *p<0.05 (ANOVA followed by Dunnett’s test) when compared to negative control, n=6.

DISCUSSION

Ulcer is a recurrent disease affecting large populations in all geographical regions and reactive oxygen species have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric damage. Peptic ulcers results from an imbalance between defensive (cytoprotective) and offensive factors (gastric acid), association with \textit{H. Pylori} infection and increased use of NSAIDS like aspirin, Indomethacin that causes damage by inhibiting biosynthesis of cytoprotective prostaglandins. The Antiulcer activity of the aqueous extract of \textit{C. wightii} against Indomethacin & ethanol induced ulcers was established. Our finding showed that the aqueous extracts at doses of 250 mg/kg & 500 mg/kg body weight had antiulcer effect on acute experimental gastric ulcer in rats. The present finding concluded that aqueous extract had potential Antiulcer activity, which was not superior to the respective effect observed with standard drug (Ranitidine & misoprostol).

ACKNOWLEDGEMENT

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