ANTI-INFLAMMATORY AND ANTI-OXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF EUPHORBIA THYMIFOLIA LINN WHOLE PLANT

NAGARAJU GARIPELLI1, CHINNALALAIAH RUNJA2, NAGARAJU POTNURI3 and RAVI KUMAR PIGILI4

1Dept. of Medicinal Chemistry, Mother Teresa College of Pharmacy, Ghatkesar, R. R. Dist., A.P, India, 2Dept. of Medicinal Chemistry, 3Dept. of Pharmaceutics, Jinnapally B.R. Pharmacy College, Yenkapally (V), Moinabhad, R.R. Dist., AP, India, 4Dept. of Bio analytical, Alizant Drug Research Solutions Private Limited, Dulpally (V), R.R. Dist., AP, India. Email: gnraj_elixir@yahoo.com

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ABSTRACT

Inflammation is often associated with the free radicals which are very reactive and harmful. This indicates the inflammation and oxidation in biological systems requires simultaneous attention. Many plants from Euphorbia species which were available in semi-arid regions like India had shown a variety of pharmacological activities for different disorders. The present work involved in the evaluation of the antioxidant and anti-inflammatory activity of the plant Euphorbia thymifolia linn. The ethanolic extract of the whole plant studied for the proposed activities. The extract in the dose of 100 mg/kg body weight caused a comparable reduction in edema with that of standard drug, Indomethacin (10mg/kg) when evaluated for anti-inflammatory activity by carrageenan-induced rat paw edema method. The extract also inhibited Nitric Oxide free radical which was estimated by Griess's method which involves the use of gries reagent (1% Sulphanilamide, 2% Phosphoric acid and 0.1% Naphthyl ethylenediamine dihydrochloride). The extract was found to produce significant Anti-inflammatory & Antioxidant activities and phytochemical screenings could also be conducted.

Keywords: Euphorbia Thymifolia Linn (ETL), Carrageenan, Anti-inflammatory, Antioxidant, Nitric Oxide (NO) and EEET (Ethanolic Extract of Euphorbia Thymifolia).

INTRODUCTION

Euphorbia thymifolia linn is a small annual herb whose stems are prostrate, dichotomously branched, slender, cylindrical and less hairy. Leaves are opposite, very small, numerous, 3-6 by 2.5-4mm obliquely oblong or elliptic-oblong, rounded at the apex, glabrous above, slightly pubescent beneath. It consists of 0.8mm long stalk which is very sharp; capsules of the plant are 1.5mm long obtusely keeled, pubescent. Styles are short. Seeds are 1.25 mm long quadrangular bluntly pointed with 5 or 6 transverse furrows. The seeds are conical, measure 0.5mm in diameter, acutely 4-angled, shallowly transversely wrinkled, reddish brown without caruncle.

Euphorbia thymifolia linn is a member of the Euphorbiaceae family. The Euphorbiaceae family is one of the largest families of higher plants, about 300 genera and 7,500 species have been reported. There are about 2,160 species in genus Euphorbia and it is a large family of flowering plants (300 genera and over 5000 species). Some of the plants belonging to the genus Euphorbia have been used in folk medicine for hundreds of years. They have been used for the treatment of cancers, tumors, migraines, skin diseases, gonorrhea, intestinal parasites and warts.

E. antisiphilitica is a popular herbal remedy in India, where it is used for the treatment of liver ailments. E. prostrata is also applied in Indian folk medicines as an anti-inflammatory and blood purifier.

Preparation of Ethanolic Extract of Euphorbia Thymifolia Linn

The whole plant of ETL was collected and coarsely powdered. The powder was successively extracted with ethanol using soxhlet extractor. The Ethanolic extract of Euphorbia thymifolia was dried under reduced pressure using a rotary flash evaporator and was kept under the refrigeration. The percentage yield was 6%. The ethanolic extract thus obtained was used for the preliminary phytochemical screening and pharmacological studies.

In-Vitro Antioxidant Studies

Nitric Oxide scavenging activity

Nitric oxide scavenging activity was measured by using UV-Visible spectrophotometer. Sodium nitroprusside (5mM) in phosphate buffer was mixed with different concentrations of EET (25-800 µg/ml), dissolved in normal saline and incubated at 25°C for 30 min. Control without test compound but with equivalent amount of sodium nitroprusside was taken. After 30 min 1.5 ml of the

mixture was layered onto 5 ml of Griess reagent (1% Sulphanilamide, 2% Phosphoric Acid and 0.1% Naphthyl ethylenediamine dihydrochloride). The absorbance was read at 540 nm against control. Percentage inhibition was calculated against control.

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incubation solution was removed and diluted with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% Phosphoric acid, and 0.1% Naphthyl ethylenediamine dihydrochloride). The adsorbents of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with Naphthyl ethylenediamine dihydrochloride was measured at 546 nm. Vitamin E was used as a reference standard.

In-Vitro Anti-inflammatory activity

Wistar albino rats (150-200g) of either sex were housed under uniform environmental conditions. They were divided into three groups (Control, Test, and Standard) of six animals each and the following regimen of treatment was instituted. The animals were maintained in well ventilated room temperature with natural day/night cycle in polypropylene cages. They were fed balanced rodent pellet diet and tap water ad-libitum throughout the experiment. The animals were housed for one week prior to the experiments to acclimatize to laboratory conditions. Control was given with only ethanol. Test group was given by EEET (100mg/kg) and the standard group has been given by Indomethacin (10mg/kg). The reduction of volume of paw edema for different groups was measured by plethysmograph at equal intervals.

Table 1: Phytochemical screening of Ethanolic Extract of whole plant of ETL

<table>
<thead>
<tr>
<th>Chemical Tests</th>
<th>Inference</th>
<th>Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Mayer’s test</td>
<td>Positive</td>
<td>Alkaloids Present</td>
</tr>
<tr>
<td>b. Dragendorff’s test</td>
<td>Positive</td>
<td>Carbohydrates Present</td>
</tr>
<tr>
<td>c. Hagner’s test</td>
<td>Negative</td>
<td>Proteins Absent</td>
</tr>
<tr>
<td>a. Molkich’s test</td>
<td>Positive</td>
<td>Steroids Present</td>
</tr>
<tr>
<td>b. Fehling’s test</td>
<td>Positive</td>
<td>Flavonoids Present</td>
</tr>
<tr>
<td>c. Benedict’s test</td>
<td>Positive</td>
<td>Phenols Present</td>
</tr>
<tr>
<td>d. Barfoed’s test</td>
<td>Positive</td>
<td>Gums &amp; Muclilage Absent</td>
</tr>
<tr>
<td>e. Liermann-Burchard test</td>
<td>Positive</td>
<td>Glycosides present</td>
</tr>
<tr>
<td>f. 5% KOH test</td>
<td>Negative</td>
<td>Saponins Absent</td>
</tr>
<tr>
<td>g. Biuret test</td>
<td>Negative</td>
<td>Terpenes Present</td>
</tr>
<tr>
<td>h. Millon’s test</td>
<td>Negative</td>
<td>fixed oils Absent</td>
</tr>
</tbody>
</table>

Nitric Oxide scavenging activity

EEET showed promising free radical scavenging effect against nitric oxide induced release of free radicals in a concentration dependent manner. The IC_{50} values of EEET was found to be 638.36µg/ml (r = 0.921) and 645µg/ml (r = 0.921), respectively. The IC_{50} value of vitamin E was 142.2µg/ml (r = 0.909).

Carrageenan induced paw edema

Acute inflammation was tested on edema induced by Carrageenan in rats. The animals were fasted and divided in to groups. All the animals received their respective doses of test drugs 1 hr prior to the administration of the phlogistic agent. After 1 hr, 0.1ml of 1% solution of freshly prepared Carragenen in normal saline was injected in the sub plantar surface of the white hind paw of the rats. The paw volume was measured before and each hour afterwards for a period of 6 hrs using mercury displacement Plethysmograph. The difference between left and right paw volumes indicated the degree of inflammation and % inhibition of paw volume by the standard and test drugs was calculated.

RESULTS

Phytochemical Screening of Ethanolic Extract of whole plant of ETL

The results of preliminary phytochemical screening of the ethanolic extract of whole plant ETL was shown the presence of alkaloids, carbohydrates, phenols, sterols, terpenes and flavonoids by doing various confirmatory tests for each type of chemical constituents and shown in Table I.

Table 2: Effect of EEET on Carrageenan induced paw edema

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg b.wt, p.o)</th>
<th>EDEMA Volume (ml)**</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>5 hr</th>
<th>6 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>10</td>
<td>0.2±</td>
<td>0.24±</td>
<td>0.34±</td>
<td>0.36±</td>
<td>0.4±</td>
<td>0.4±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.19±</td>
<td>0.22±</td>
<td>0.21±</td>
<td>0.19±</td>
<td>0.17±</td>
<td>0.2±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(6.14%)</td>
<td>(10.56%)</td>
<td>(37.24%)</td>
<td>(47.1%)</td>
<td>(56.6%)</td>
<td>(64.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group-II</td>
<td>10</td>
<td>0.19±</td>
<td>0.20±</td>
<td>0.18±</td>
<td>0.16±</td>
<td>0.13±</td>
<td>0.12±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.04±</td>
<td>0.008</td>
<td>0.001</td>
<td>0.01</td>
<td>0.001</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(15.98%)</td>
<td>(55.5%)</td>
<td>(67.5%)</td>
<td>(68.5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** All values expressed Mean±SEM of 6 animals per group; ap<0.001; bp<0.01; cp<0.05; comparison-- group II, III Vs group-I
ACKNOWLEDGEMENT

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DISCUSSION

Indigenous drug system can be a source for variety of new drugs which can provide relief to pain and inflammation but their claimed reputation has to be verified on a scientific basis. The present investigation reveals that plant ETL was a significant Anti-inflammatory activity. Recent studies suggest that inflammation and tissue damage are due to the liberation of free radicals. The free radicals have been implicated in the pathophysiology of the various clinical disorders including inflammation, acute hypertension, cancer, etc, normally endogenous intracellular antioxidants protects the tissue from injury by free radicals.

The study on Nitric Oxide scavenging demonstrates that ETL is a potent scavenger of nitric oxide. NO generated from sodium nitroprusside reacts with oxygen to form nitrite ions which can be estimated by the use of gries reagent. Scavengers of NO compete with oxygen leading to reduced production of NO.

Carrageenan induced edema of the rat paw is used widely as a working model of inflammation in the search for new anti-inflammatory drugs. This method is appearing to be the basis for the discovery of new anti-inflammatory drug indomethacin. All Non-steroidal anti-inflammatory drugs are effective in inhibiting edema formation especially in the late phase in which the prostaglandins are involved. In this study indomethacin showed maximum inhibitory effect on edema formation at 6th hour, this corresponds to the period of prostaglandins phase. EEET also inhibited edema formation with maximum activity at the same period as Indomethacin when compared to control. The ethanolic extract of Euphorbia thymifolia showed the presence of alkaloids, carbohydrates, phenols, sterols, terpenes and flavonoids. The ethanolic extract possess various active constituents, a need arises for further pharmacological studies there by revealing the mechanism of action of anti-inflammatory role of ethanolic extract of Euphorbia thymifolia.

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Cooperation from the colleagues and various departments is appreciated.

REFERENCES


Fig. 1: Effect of EEET on Carrageenan induced paw edema