Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

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

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Received: 1 Feb 2012, Revised and Accepted: 21 March 2012

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 griess reagent (1% Sulphanilamide, 2% Phosphoric acid and 0.1% Naphtyl ethylenediamine dihydrochloride). The extract was found to produce significant Anti-inflammatory & Anti-oxidant 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, divaricately 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[1, 2]. There are about 2,160 species in genus Euphorbia and it is a large family of flowering plants (300 genera and over 5000 species)[3]. 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, migraine, skin diseases, gonorrhea, intestinal parasites and warts [4,5].

E. antisyphi1itica is a popular herbal remedy in India, where it is used for the treatment of liver ailments[6]. *E. prostrate* is also applied in Indian folk medicines as an anti-inflammatory and blood purifier[7]. The extract of *E. fischeriana* is used in the manufacture of an ointment for psoriasis (a chronic skin disease in which red scaly pustules and patches appear)[8]. Latex of *E. lateriflora* is used as a treatment for ringworm and in dilute aqueous solution, as a purgative[9]. It is also considered as a remedy for enlargement of the liver and spleen[10]. Roots of *E. wallichii* have been traditionally used in Tibetan folk medicines for the treatment of edema and skin disease such as furuncle, exanthema and cutaneous anthrax[11]. Members of Euphorbia are rich in phenolics, aromatic esters[12], steroids[13], diterpenoids[14,15], tetracyclic triterpenoids[13], pentacyclic triterpenoids[12,14], essential oils[16] and several bioactive constituents [17,18]. In several studies different extracts and isolated compounds from Euphorbia showed cytotoxic activity against cell lines such as KB, K562, CNE2, ANA-1, B16, CHO, Hep-2, and Hela[19-20]. Some of the Euphorbia species have cytotoxic, antiviral, antibacterial, and antifungal activities[21-22].

E. neriifolia linn commonly known as "Sehund or thohar" in Hindi and has an antioxidant activity[23] in ethanolic leaf extract. Alkaloid extracts of different parts of *E. hirta* showed significant antibacterial activity against Enterobacter aerogens, Bacillus subtilis, Klebsiella pneumoniae, Raoultella planticola and Agrobacterium tumefaciens[24].

Euphorbia thymifolia Linn in Ayurveda is called as – Chhoti dudhi, Laghu dugdhikaa in Bengal - Dudiya, shweetkeruee, swetkerua, in Ceylon – Cgittirapalavi English – Chicken weed, dwarf spurge, red caustic creeper,in Gujarat – Nahanidudheli In Hindi –Chhoti–dudhi, in Marathi - Ghakdidudhi, Chothadudhi, in Sidha– Ammanptharisi, in Sanskrit - Laghududhika, Raktavindachada, in Spanish – Golondrina Unani - Dudhi khurda Charaka prescribed Dugdhika as an ingredient of vegetable soup for diarrhoea, painful bleeding piles[25].

Hence, the purpose of present work is to evaluate the Anti-inflammatory and Anti-oxidant activities and phytochemical screenings of the ethanolic extract of whole plant of ETL.

MATERIALS & METHODS

Plant collection and phytochemical screening

The whole plant of ETL used for the investigation was collected from the plains and lower hills of Yacharam (Haliya), Nalgonda (District), Andhra Pradesh (State), India, during the rainy season in the month of August and the plant was authenticated by plant research officer, Botanical Garden, Hyderabad, A.P, India.

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 EEET (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 30min 1.5 ml of the

incubation solution was removed and diluted with 1.5 ml of griess reagent (1% Sulphanilamide, 2% Phosphoric acid, and 0.1% Naphtyl 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 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 experimental period. 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 given by Indomethacin (10mg/kg). The reduction of volume of paw edema for different groups was measured by plethysmograph at equal intervals.

Carrageenan induced paw edema in rats

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 1hr prior to the administration of the phlogistic agent. After 1 hr, 0.1ml of 1% solution of freshly prepared Carrageenen 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 1: Phytochemical screening of Ethanolic Extract of whole plant of ETL

Chemical Tests		Inference	Constituents
a.	Mayer's test	Positive	Alkaloids Present
b.	Dragendorff's test	Positive	
c.	Hagner's test	Positive	
a.	Molisch's test	Positive	Carbohydrates Present
b.	Fehling's test	Positive	
c.	Benedict's test	Positive	
d.	Barfoed's test	Positive	
a.	Libermann-Burchard test	Positive	Steroids Present
b.	5% KOH test	Positive	
a.	Biuret test	Negative	Proteins Absent
b.	Millon's test	Negative	
a.	10% Lead acetate	Positive	Tannins Present
b.	10% NaCl,	Positive	
c.	Aq. Bromine solution tests	Positive	
a.	Ferric Chloride	Positive	Phenols Present
b.	10% Sodium Chloride tests	Positive	
a.	Conc. Sulpuric acid	Positive	Flavonoids Present
b.	Magnesium turnings test	Positive	
c.	Shinoda test	Positive	
d.	Lead acetate test	Positive	
e.	Sodium hydroxide test	Positive	
a.	Swelling test	Negative	Gums & Mucilage Absent
1.	Baljet test	Positive	Glycosides present
2.	Borntrager's test	Positive	
3.	Modifies borntrager's test	Positive	
a.	Froth test	Positive	Saponins Absent
a.	Tin + Thionyl Chloride test	Positive	Terpenes Present
a.	Spot test	Negative	fixed oils Absent

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 = 0.932) 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

Effect of EEET on Carrageenan induced paw edema was shown in Table-II. The extract showed significant anti inflammatory activity ($p<0.001$) when compared to control but less activity when compared to indomethacin ($p<0.001$) and showed in Figure-I.

Table 2: Effect of EEET on Carrageenan induced paw edema

Groups	Dose (mg/kg b.wt, p.o)	EDEMA Volume (ml)**					
		1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Group-I	10	0.20± 0.004	0.24± 0.008	0.34± 0.001	0.36± 0.01	0.4± 0.001	0.41± 0.01
Group-II	200	0.19± 0.004[c] (6.14%)	0.22± 0.008[b] (10.56%)	0.21± 0.001[a] (37.24%)	0.19± 0.008[a] (47.1%)	0.17± 0.006[a] (56.6%)	0.20± 0.013[a] (64.64%)
Group-III	10	0.19± 0.004[b] (7.76%)	0.20± 0.006[a] (15.98%)	0.18± 0.008[a] (47.05%)	0.16± 0.005[a] (55.55%)	0.13± 0.008[a] (67.5%)	0.12± 0.008[a] (68.5%)

** All values expressed Mean±SEM of 6 animals per group; [a]$p<0.001$; [b]$p<0.01$; [c]$p<0.05$; comparison-- group II, III Vs group-I

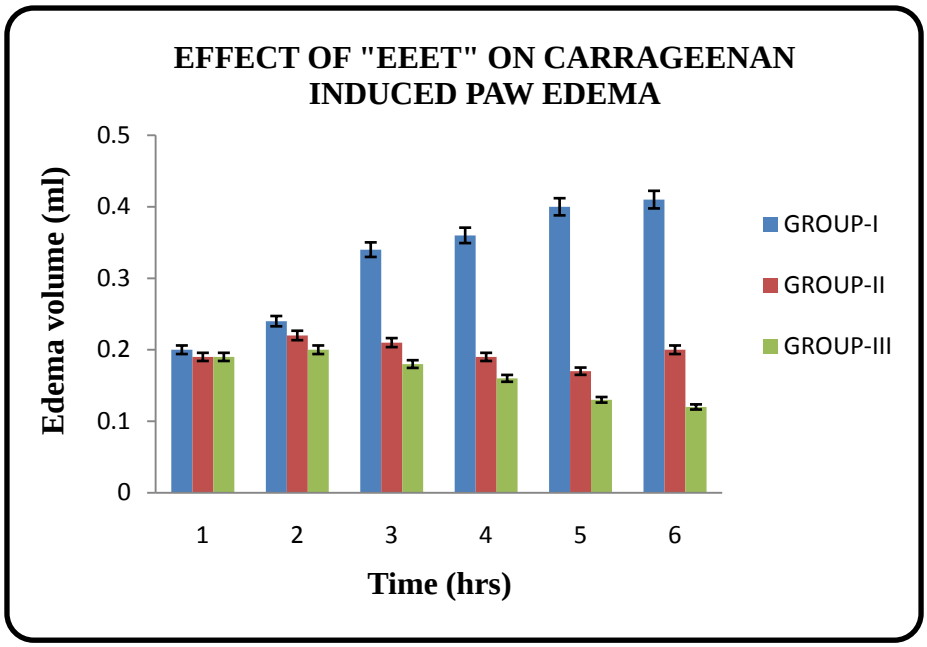

Fig. 1: Effect of EEET on Carrageenan induced paw edema

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 griess 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 of *Euphorbia thymifolia* at a dose of 200mg/kg body weight administered orally to rats produced significant anti inflammatory activity in all the experimental models. As 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*.

ACKNOWLEDGEMENT

The study was supported by the fund from the Department of Pharmacognosy, Mother Teresa College of Pharmacy, India and the authors wish to thank the management of Mother Teresa College of Pharmacy, Ghatkesar, Hyderabad, India for supporting this work. Cooperation from the colleagues and various departments is appreciated.

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