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Research Article

EVALUATION OF IN VITRO ANTI INFLAMMATORYACTIVITY OF ELAEOCARPUS GANITRUS OF BARK EXTRACT BY HRBC MEMBRANE STABILIZATION

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ABSTRACT

To evaluate the in-vitro anti inflammatory activity of Elaeocarpus ganitrus roxb of bark extracts by HRBC membrane stabilization. The plant Elaeocarpus ganitrus roxb (Elaeocarpaceae) commonly known as Rudraksha the king of herbal medicines. The plants are distributed throughout India, Southeast Asia, Japan, Malaysia, Southern China, Australia etc. The leaves, fruits, seeds and stems of the plant have been used widely in traditional folk medicine in many parts of India. In the information regarding the use of bark of *Elaeocarpus ganitrus Roxb* for several disorders systemic in vitro studies were carried out in our laboratory to establish the anti inflammatory potential by using HRBC membrane stabilization method. HRBC membrane is equals to lysosomal membrane which plays the role in the process of inflammation. The in-vitro HRBC membrane stabilization method showed significant anti inflammatory property of various Elaeocarpus ganitrus tested extracts. It was found that the chloroform extract shows significant anti inflammatory activity at the concentration of 500µg/ml which is comparable to the standard Diclofenac sodium.

Keywords: *Elaeocarpus ganitrus Roxb,* Anti inflammatory, HRBC membrane stabilization.

INTRODUCTION

Inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality"[1], or the reaction to injury of the living microcirculation and related tissues[2]. Inflammatory response to tissue injury involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair[3] which are aimed at host defense and usually activated in most disease conditions. HRBC (human red blood cell) membrane is similar to the lysosomal membrane and the prevention of hypo tonicity induced membrane lysis can be taken as an in vitro measure of anti inflammatory activity of the plant extracts. Several experimental protocols of inflammation are used for evaluating the potency of drugs. The management of inflammation related diseases is a real issue in the rural community; the population in these areas uses many alternative drugs such as substances produced from medicinal plants. Herbs play a major role in modern medicines. Elaeocarpus ganitrus roxb is known as rudhrakha plant belongs to the family Elaeocarpaceae[4].It is distributed throughout in India, China and Malaysia. It is a large evergreen broad leaved medium sized tree [5]. This grows in the area from the gangetic plain to the foothills of great Himalaya[6]. *E. ganitrus* is an important medicinal plant with several curative properties so that it is used in traditional systems of medicine. It is been used to cure many health problems in different parts of the World. Leaves, seeds and fruits are used for various medicinal properties and traditionally used to cure anxiety, depression, stress, migraine, palpitation, epilepsy, migraine, nerve pain, asthma, hypertension, liver diseases, lack of concentration and arthritis. The present investigation aimed to evaluate anti inflammatory activity of bark extracts of rudhrakha by in-vitro anti inflammatory screening

MATERIALS AND METHODS

Plant material

The bark of Elaeocarpus ganitrus was collected in 22-1-2016 at our college garden Tadepalligudem, west Godavari district, A.P India. The plant material was identified and authenticated by Department Of Botany, K.G.R.L College, Bhimavaram.

Preparation of extracts

The collected bark was dried under shade and powdered. 50gm of powder was loaded in to soxhlet extractor and subjected to hot percolation with chloroform, methanol for 48 Hrs and macerated with water for 12 Hrs. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator. The % of yield values obtained were2.06, 0.16 and 5.43 for aqueous and methanol, chloroform respectively.

PHYTOCHEMICAL ANALYSIS

The phytochemical analysis of methanolic, chloroform, aqueous extracts of bark of Elaeocarpus ganitrus was analysed for the compounds such as tannins, saponins, flavonoids, phenols, terpenoids,glycosides and alkaloids.

In the present study the phytochemicals occurring in the various solvent extracts of bark of Elaeocarpus ganitrus (methanol, chloroform and aqueous extracts) were analyzed qualitatively (Fig:1)by standard procedures for phytochemical screening (Table-1**)**. The results revealed that the presence of various secondary metabolites of therapeutical importance. The major phytochemicals found were tannins, flavonoids, terpenoids, sterols, triterpinoids and alkaloids.

IN -VITRO ANTI-INFLAMMATORY ACTIVITY

Blood sample

Blood samples were collected from healthy human volunteers (n=3) by maintaining aseptic condition without a history of anti-histamine drugs, steroids and oral contraceptive.1ml of blood was collected from each volunteer according to protocol accepted by institutional committee of Sri Vasavi institute of pharmaceutical sciences.

Preparation of extracts

Plant extracts of various concentrations (100, 200, 300, 400, 500 µg/ml) was prepared (Fig. 2) by serial dilutions.

Preparation of Human Red Blood Cells (HRBC) Suspension

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged (Fig. 3) at 3000 rpm for 10 min and packed cells were washed three times with isosaline(0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline[7].

Hypotonicity Induced Hemolysis

The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hyposaline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with 0.5 ml of plant extracts and standard drug diclofenac sodium of various concentrations (100, 200, 300, 400, 500 µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37^{o}C for 30 min and centrifuged respectively. The hemoglobin content in the suspension (Fig. 4) was estimated using spectrophotometer at 560 nm.

The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization or protection was calculated by using the formula,

The percentage of hemolysis of HRBC membrane can be calculated as follows:
% Hemolysis = (Optical density of Test sample / Optical density of Control) X 100
The percentage of HRBC membrane stabilisation can be calculated as follows:
% Protection = 100 – [(Optical density of Test sample / Optical density of Control) X100]

RESULTS

The lysosomal enzymes released during inflammation produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The non steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane[8]. Since HRBC membrane are similar to lysosomal membrane components the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti inflammatory activity of drugs. The results were reported in table 2 &3. It was observed from the Fig.5 that the

chloroform extract shows significant anti inflammatory activity at the concentration of 500 µg/ml which is comparable to the standard Diclofenac sodium. The anti inflammatory activity of the extracts were concentration dependent, with the increasing concentration the activity is also increased. The chloroform extract of Elaeocarpus ganitrus has significant anti inflammatory activity in comparison to the aqueous, methanolic extract of the same plant.

CONCLUSION

Through our study it was found that aqueous,methanolic and chloroform extracts of Elaeocarpus ganitrus possesses anti inflammatory activity but chloroform extract shows better activity as compared to standard and other extracts of same plant . By the above obtained results, it can be suggested that the application of the Elaeocarpus ganitrus component may be accessible for greater section of the society for the treatment of various inflammatory diseases. In addition, positive result in anti inflammatory activity test led us to the interference that the plant extract may contain various phyto chemical compounds. Hence, further in-vivo studies are suggested to be undertaken to pin point the exact compounds and to better, understand its actions.

Table 1: Preliminary Phytochemical analysis of Methanolic, Chloroform, Aqueous extracts of bark of Elaeocarpus ganitrus

S. No.	Test	Methanolic extract	Chloroform extracts	Aqueous extracts
1.	Tannins	+	-	+
2.	Saponins	-	-	-
3.	Flavanoids	-	+	-
4.	Terpenoids	+	-	+
5.	Glylosides	-	-	-
6.	Alkaloids	+	+	+
7.	Sterols	+	+	+

+ Present, - Absent

Table 2: Optical density of various concentrations of bark extracts of Extracts of E.ganitrus for Invitro Anti inflammatory activity at various concentrations

S. No	Concentration of extract(µg)	Aqueous extract of E.G	Methanolic extract of E.G	Chloroform extract of E.G	Standard Diclofenac
1	100	0.343±0.02	0.546±0.03	0.194±0.01	0.193±0.02
2	200	0.272±0.03	0.492±0.02	0.091±0.01	0.182±0.01
3	300	0.263±0.02	0.441±0.01	0.089±0.03	0.159±0.03
4	400	0.224±0.01	0.419±0.01	0.074±0.01	0.125±0.04
5	500	0.122±0.01	0.222±0.02	0.071±0.01	0.074±0.02

(O.D-Optical density, Values are expressed as SEM of 3 readings)

Table 3: % protection of various extracts of E. ganitrus at various concentrations

S. No	Concentration (µg/ml)	Activity (%protection of heamolysis)			
		Aqueous extract of E.G	Methanolic extract of E.G	chloroform extract of E.G	Standard Diclofenac
1	Control	-	-	-	-
2	100	38.59	2.673	65.4	65.6
3	200	51.51	12.2	83.77	67.5
4	300	53.11	21.39	84.13	71.6
5	400	60.07	25.31	86.80	77.70
6	500	78.25	60.42	87.34	86.81

E.G: Elaeocarpus ganitrus

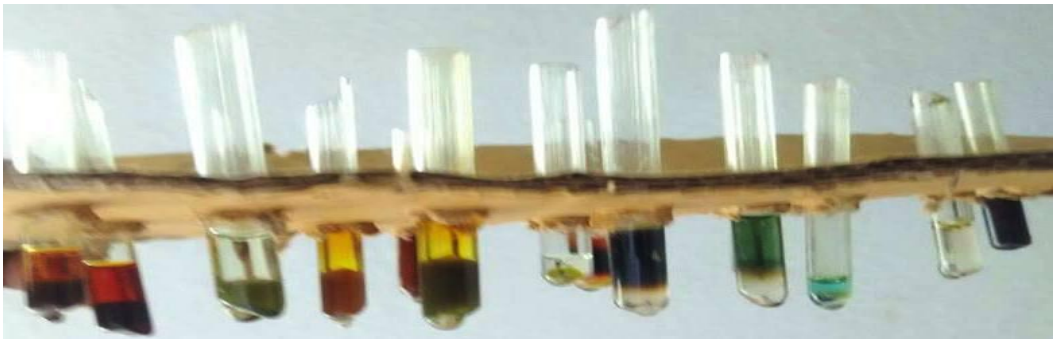

Fig. 1: Phytochemical screening

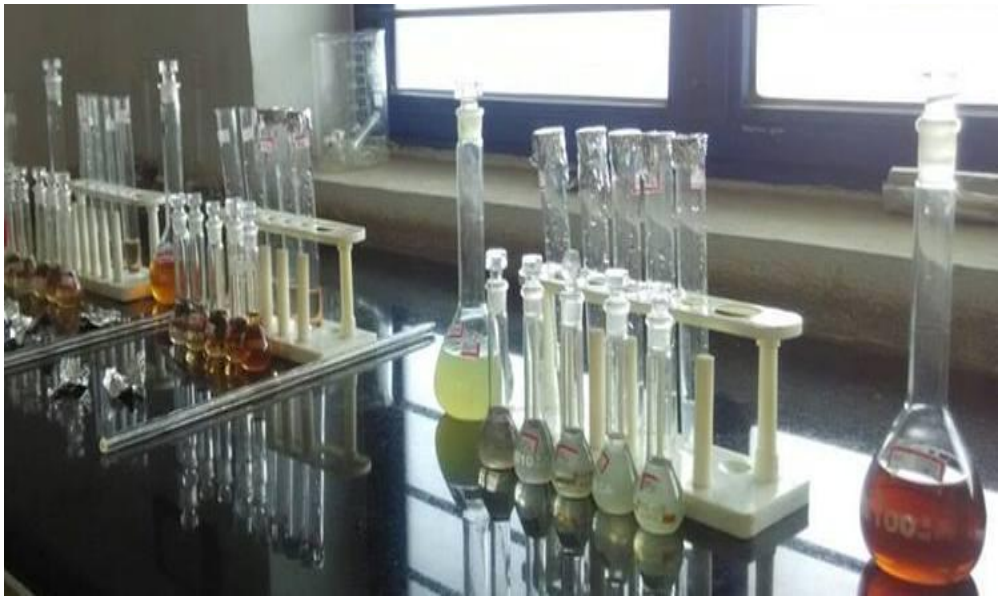

Fig. 2: Plant extracts of various concentrations (100, 200, 300, 400, 500 µg/ml) was prepared by serial dilutions

Fig. 3: Hypotoncity induced Samples

Fig. 4: Centrifuged samples

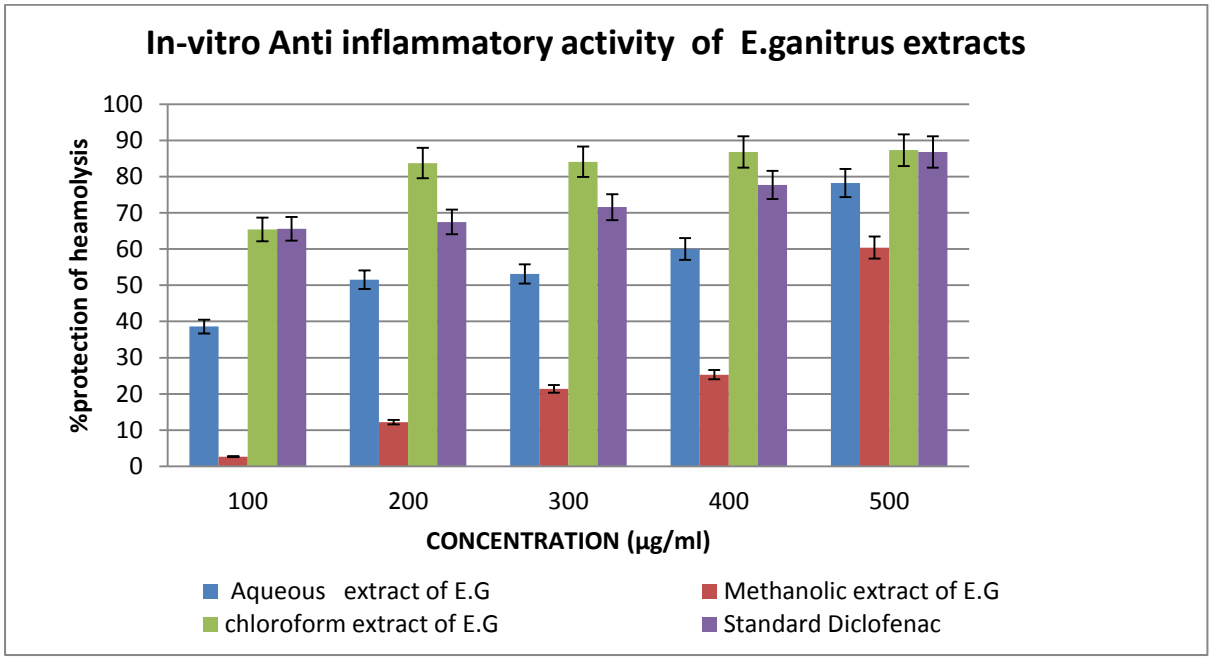

Fig. 5: Invitro anti inflammatory activity of various extracts of E. granitrus

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