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Anti-inflammatory activity of the leaf extracts of *Gendarussa vulgaris* Nees

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ABSTRACT

Objective: To evaluate the anti-inflammatory property of the leaf extracts of *Gendarussa vulgaris* (*G. vulgaris*) Nees. **Methods:** *G. vulgaris* Nees of the family Apocynaceae is a medium sized tree grown in semishade or no shade and is common in the Ernad and Nilambur taluks of Kerala. Various parts of this plant have been used in the treatment of ulcers, sores, inflammation, dyspepsia, healing of wounds, etc. The present study aimed at the evaluation of anti-inflammatory property of the aqueous and alcoholic extracts of the leaves by both *in vitro* and *in vivo* methods. *In vitro* method was estimated by human red blood cell membrane stabilisation (HRBC) method and *in vivo* method was estimated on the carrageenan induced paw oedema. **Results:** Both the methods showed significant anti-inflammatory property of the different extracts tested. **Conclusions:** The alcoholic extract at a concentration of 300 mg/mL showed potent activity on comparing with the standard drug diclofenac sodium.

1. Introduction

Inflammation is a reaction of living tissues towards injury, and it comprises systemic and local responses^[1]. In spite of our dependence on modern medicine and the tremendous advances in synthetic drugs, a large number of the world populations (80% of people) cannot afford the products of the western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material. The fact is well recognized by the WHO which has recently compiled an inventory of medicinal plants listing over 20 000 species. The family Apocynaceae consists of several important medicinal plants with wide range of pharmacological, biological activities and interesting phyto chemical constituents. The main action of anti-inflammatory agents is the inhibition of Cyclooxygenase enzymes which are responsible for the conversion of Arachidonic acid to prostaglandins. Since human red blood cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of

hypotonicity induced HRBC membrane lysis was taken as a measure in estimating the anti-inflammatory property of various extracts of *Gendarussa vulgaris* (*G. vulgaris*) Nees. Thus, Human red blood cell membrane stabilization (HRBC method)^[2] has been used as a method in estimating the anti-inflammatory property. In certain parts of Malabar the leaf of this plants was traditionally used in the treatment of inflammation. The present study aimed to authenticate that traditional information by both *in vitro* and *in vivo* anti-inflammatory screening.

2. Materials and methods

2.1. Preparation of extracts

Fresh leaves of *G. vulgaris* Nees were collected from Kizhattur area of Malappuram district Kerala and were authenticated by botanist. The leaves were dried in shade and powdered to a coarse form. It was then successively extracted with petroleum ether, chloroform, ethyl acetate, ethanol and water using continuous hot soxhlet extract as each of their increasing order of polarity. The extracts were concentrated under reduced pressure and preserved at low temperature.

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2.2. Chemicals and instruments

All chemicals used in the estimation were of analytical grade. Carrageenan was purchased from sigma chemicals. Reference standard diclofenac sodium was obtained as gift sample from MRL labs Chennai. Shimadzu 1701 UV Visible spectrophotometer was used for the *in vitro* study.

2.3. Animals

Adult Wister albino rats (80 g –120 g) of either sex were used for the *in vivo* evaluation. They were housed under standard laboratory conditions and were fed with standard animal feed and water ad libitum. The experimental protocol was approved by institutional animal ethical committee.

2.4. Acute toxicity test

Acute toxicity study was performed as per OECD guidelines 423[2]. (Acute toxicity class method).

2.5. In vitro Anti-inflammatory activity

HRBC method was used for the estimation of anti-inflammatory activity *in vitro*[3]. Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution. This blood solution was centrifuged at 3 000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline. This HRBC suspension was used for the estimation of anti-inflammatory property. Different concentrations of extract, reference sample and control were separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC suspension. All the assay mixtures were incubated at 37 °C for 30 minutes and centrifuged at 3 000 rpm. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

Table 2

Anti-inflammatory activity by *G. vulgaris* induced oedema.

Drug	Dose (mg/ kg)	Carrageenan induced oedema (Volume in mL)			
		60 min	120 min	180 min	240 min
Control		0.40± 0.21	0.42±0.093	0.47±0.16	0.47±0.21
Diclofenac	5	0.19± 0.33 [*]	0.17±0.078 [*]	0.15±0.28 [*]	0.12±0.66 [*]
Ethanollic Extract	200	0.30±0.33 [*]	0.26±0.28 [*]	0.22±0.064 [*]	0.20±0.023 [*]
Ethanollic Extract	300	0.17±0.45 ^{*a}	0.15±0.52 ^{*a}	0.14±0.21 ^{*a}	0.12±0.71 ^{*a}
Ethanollic Extract	400	0.24±0.71 ^{*a}	0.21±0.091 ^{*a}	0.21±0.16 ^{*a}	0.17±0.33 ^{*a}
Aqueous Extract	200	0.31±0.45 [*]	0.28±0.66 [*]	0.25±0.023 [*]	0.24±0.16 [*]
Aqueous Extract	300	0.21±0.33 ^{*a}	0.18±0.45 ^{*a}	0.17±0.33 ^{*a}	0.16±0.055 ^{*a}
Aqueous Extract	400	0.23±0.71 ^{*a}	0.22±0.37 ^{*a}	0.20±0.18 ^{*a}	0.20±0.24 [*]

Values are expressed in mean ± SEM (n=6); *– P<0.05 with control; a– P<0.05 with standard.

Percentage protection= 100– (OD sample/ OD control) × 100

2.6. In vivo Anti-inflammatory activity

Paw oedema was induced on each rat by injecting 0.1 mL of carrageenan on physiological saline to the left hind paw[4]. The extracts at different concentrations were administered orally 30 minutes prior to carrageenan administration. Paw volumes were measured at 60, 120, 180 and 240 minutes by mercury displacement method using plethysmograph. The percentage inhibition of paw volume in extract treated groups was compared with control. Diclofenac sodium (5 mg/kg) was used as the standard.

2.7. Statistical analysis

Statistical analysis was done using one way analysis of variance followed by Dunnetts test. P values greater than 0.05 were considered as significant.

3. Results

3.1. Acute toxicity studies

The extracts of *G. vulgaris* nees did not show any sign of toxicity up to 2000 mg/kg body weight and hence it was considered to be safe.

Table 1

In vitro anti-inflammatory activity of leaf extracts of *G. vulgaris* Nees.

Sl. No	Extract type (mg/mL)	Percentage protection
1	Ethanollic– 200	27.43
2	Ethanollic–300	33.88
3	Ethanollic–400	28.15
4	Aqueous–200	24.66
5	Aqueous–300	29.32
6	Aqueous–400	26.71
7	Diclofenac sodium (5mg/mL)	34.66

3.2. *In vitro* anti-inflammatory activity

G. vulgaris Nees ethanolic and aqueous extracts at different concentrations (200, 300, 400 mg/mL) showed significant stabilization towards HRBC membranes. The percentage protection of ethanolic extract at concentration 300 mg/mL was higher than that of concentrations. However the percentage protection was found to be decreased at higher concentration. The results were tabulated in Table 1.

3.3. *In vivo* anti-inflammatory activity

The extracts of *G. vulgaris* Nees at different concentrations showed significant reduction in the paw volume of rats. The ethanolic extract at concentration of 300mg/mL showed potent activity compared with the reference standard Diclofenac sodium. The results were tabulated in Table 2.

4. Discussion

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. *G. vulgaris* Nees of the family Apocynaceae is a common plant of North Kerala. Phytochemical evaluation of the various extracts of *G. vulgaris* Nees reveals the presence of flavonoids, glycosides, saponins, steroids, tannins and polyphenols. Here anti-inflammatory activity was performed based on the folk lore information using two methods. HRBC method was selected for the *in vitro* evaluation of anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane^[5] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release. The result indicted that the leaf extract of *G. vulgaris* Nees at various concentrations has significant anti-inflammatory property. Carrageenan induced inflammation is a useful model for the estimation of anti-inflammatory effect. The development of oedema in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin, prostaglandin and the like^[6-8]. Leaf extract of *G. vulgaris* Nees showed significant anti inflammatory activity. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the glycosides or steroids^[9] present in the extract. The present result indicates the efficacy of *G. vulgaris* Nees as an effective therapeutic

agent in the treatment of acute inflammations. The result of present study authenticates the folk lore information on the anti-inflammatory property of the leaf extract of *G. vulgaris* Nees. Further and detailed studies are in process for the isolation of active constituent responsible for this property and to identification of the possible mechanism of its anti inflammatory property.

Conflict of interest statement

We declare that we have no conflict of interest.

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