Original Article

Anti-Inflammatory Activity Of Alga, *Gelidiella Acerosa*

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Abstract
The anti-inflammatory activity was shown in carrageenan (acute) and cotton pellet induced granuloma model (sub-acute). Thus this activity can be contributed to the phytochemicals present in the extracts like alkaloids, glycosides, phenolic compounds, flavonoids and tannins. The methanolic extract of *Gelidiella acerosa* can be concluded to possess highest amounts of phenolics, flavonoids and DPPH free radical scavenging activities from the present studies. It also possess the highest anti-inflammatory potential followed by the ethyl acetate and hexane respectively. Therefore the antioxidant and anti-inflammatory activity of the extracts of *Gelidiella acerosa* can be attributed to its phytochemical compounds present in the extracts.

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 Cyclooxegenase 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 To evaluate the anti-inflammatory property of the Alga, ***Gelidiella acerosa***
. 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 anti-inflammatory screening.

MATERIALS AND METHODS

Preparation of extracts

The plants of *Gelidiella acerosa* were dried in shade and powdered. Dried algal powder (550 g) was extracted with n- Hexane, three times each by cold maceration at room temperature. In cold

maceration whole powdered plant drug is kept in contact with the solvent in a stoppered container for 48-72 hours with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile drugs. For extraction, the powder was taken in round bottom flask and macerated for 72-48 hours at room temperature with all material defatted. The algae was extracted with three solvents with increasing polarity in order of n-hexane, ethyl acetate and methanol respectively at the room temperature three times each. The extracts thus obtained were concentrated by distilling off the solvent under reduced pressure by using Rota vacuum Evaporator. The defatted marc thus obtained was air dried and then the percentage yields of all three extracts i.e. n-hexane, ethyl acetate and methanol of *Gelidiella acerosa* was calculated.

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.

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.

Acute toxicity test

Acute toxicity study was performed as per OECD guidelines 423[2]. (Acute toxicity class method).
In vitro Anti-inflammatory activity

Carrageenan-induced paw edema:

Wistar Rats of either sex (150-200 g) were divided into five groups containing six animals in each. The rats were fasted for 12 h prior to induction of edema. Rats were deprived of water only during the experiment to ensure uniform hydration and minimize variability in edematous response. Inflammation of hind paw was induced by injecting 0.1 ml of 1% w/v carrageenan in normal saline into the sub plantar region of right hind paw by Winter, *et al*. (1962)

The control group received Saline: 0.9% Nacl solution by Moraes, *et al*. (2007) and the standard group received Diclofenac sodium (10 mg/kg) p.o. Saneja, *et al.* (2009). The other three groups orally received extracts at doses 400 mg/kg, respectively. All the drug treatments were given 1 hr before the carrageenan injection; edema was expressed as the increase in paw volume due to carrageenan injection. The paw volume was measured with a digital plethysmometer (Ugo Basile, 7140) before and 1, 2, 3, 4 and 5 h after carrageenan injection.

Percentage rise in paw volume was calculated by using following formula. % $rise\ in\ paw\ volume$=Vt – Vc Vc x 100

Where, Vt = Paw volume post carrageenan injection t Phytochemical investigation & Pharmacological screening of marine alga, *Gelidiella acerosa.* Vc = Paw volume before carrageenan injection.

Cotton Pellet Induced Granuloma

The method of Winter and Porter with slight modification was used to study chronic inflammation Five groups of six animals in each group were taken, anaesthetized with ether. The skin of the flank region was shaved and disinfected with 70% ethanol. An incision was made and by a blunt forcep subcutaneous tunnels were formed and a sterilized cotton pellet (35±1mg) was placed in the flank region. The saline solution, standard drug (Diclofenac sodium), and the algal extracts i.e. n-hexane extract, ethyl acetate extract and the methanolic extracts of *Gelidiella acerosa* were administered for 7 consecutive days starting from day of cotton implantation. At 8th day rats were anaesthetized again and the cotton pellet (along with granular tissue formed around) were removed surgically and

freed from extraneous tissue. The pellets were weighed immediately for wet weight. Then, pellets were dried in an incubator at 60º C until a constant weight was obtained.

Statistical analysis

Statistical analysis was done using one way analysis of variance followed by Dunnets test. ***Gelidiella acerosa*** greater than 0.05 were considered as significant.

RESULTS

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.

Carrageenan-induced paw edema

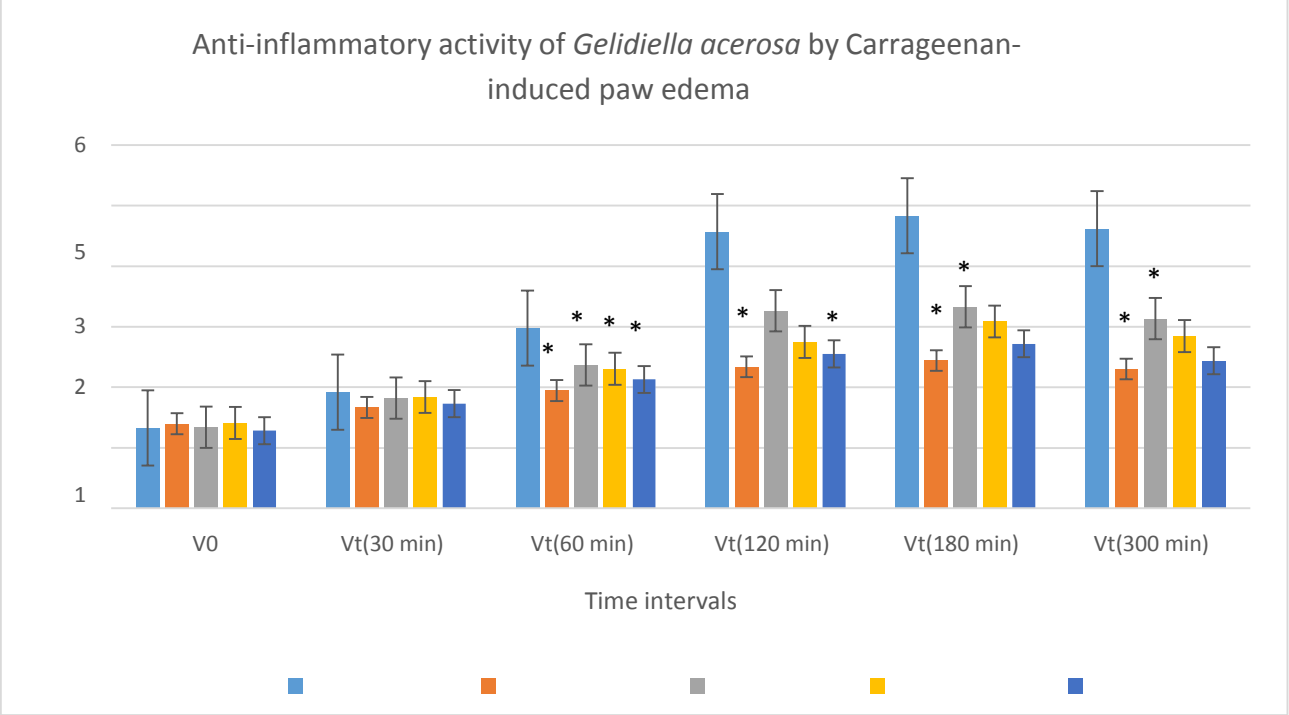
Carrageenan induced paw edema is a conventional model of acute inflammation which has a substantial extrapolative value for anti inflammatory agents acting by inhibiting the mediators of acute inflammation by Badole S., *et al*. (2011). Carrageenan is a family of linear sulphated polysaccharides extracted from the red seaweed marine alga *Chondrus crispu*. Lambda carrageenan is used in animal models of inflammation to test anti-inflammatory activity because dilute carrageenan solutions (1-2%) injection causes swelling and pain The edema produced by sub plantar injection of carrageenan in rat hind paw is biphasic over 4 or more hours. The early phase is attributed due to release of serotonin and histamine while later phase is sustained by prostaglandins.

Anti-inflammatory activity and continuity between two phases is provided. The second phase is sensitive to most clinically effective anti-inflammatory drugs. All the three extracts of *Gelidiella acerosa* were found to significantly inhibit carrageenan induced rat paw edema in the late phase regulated by prostaglandins and leukotrienes. The methanolic extract of *Gelidiella acerosa* exhibited the anti-inflammatory effect better than the effect of the ethyl acetate and hexane extract of *Gelidiella acerosa*.

Table 5.12: Anti-inflammatory activity of *Gelidiella acerosa* by Carrageenan paw edema.

Groups	**Dose**	**Paw volume after drug/ extract administration (ml) (mean ± SD)**					
		0 min	30 min	60 min	120min	180min	300 min
Control (Normal Saline)	0.2 ml	1.33±0.35	1.92±0.40	2.97±0.33	4.56±0.28	4.83±0.30	4.61±0.27
Standard (Diclofenacsodium)	10mg /kg	1.39±0.40	1.66±0.40	1.94±0.34 *	2.33±0.40 *	2.44±0.41 *	2.29±0.38 *
Hexane extract of *Gelidiella acerosa*	400m g/kg	1.34±0.29	1.82±0.27	2.36±0.25 *	3.26±0.51 *	3.32±0.36 *	3.13±0.31 *
Ethyl acetate extract of*Gelidiella acerosa*	400m g/kg	1.40±0.43	1.83±0.36	2.30±0.42 *	2.74±0.43 *	3.08±0.18 *	2.84±0.14 *
Methanolic extract of *Gelidiella acerosa*	400m g/kg	1.28±0.38	1.72±0.33	2.12±0.29 *	2.55±0.36 *	2.71±0.32 *	2.43±0.34 *

Values are expressed as mean ± SD for six animals and analyzed by One way ANOVA followed by Dunnett's test, *p< 0.001 when compared to vehicle control.



Anti-inflammatory activity of *Gelidiella acerosa* by Carrageenan-inducedpaw edema

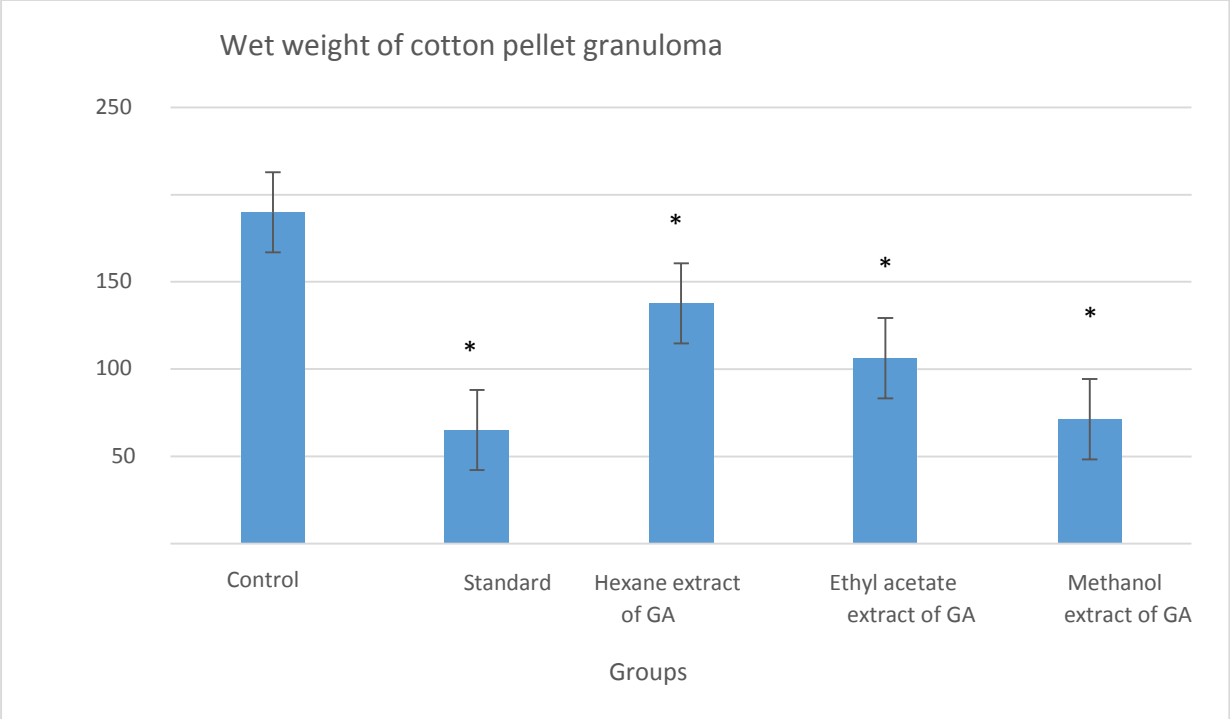
Cotton pellet induced granuloma

Cotton Pellet induced granuloma in rats is a prolonged model of inflammation which has been widely used to evaluate activity of anti- inflammatory drugs on proliferative phase of inflammation. Propagation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels which are the basic sources of highly vascularized reddish mass is termed as granulation tissue is seen during repair process of inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and the dry weight correlates well with the amount of granulomatous tissue formed. It was noted from the present study that all the three extracts of *Gelidiella acerosa* possess significant activity against cotton pellet induced granuloma in rats indicating ability to reduce number of fibroblasts and synthesis of collagen and mucopolysaccharide, natural proliferative events of granulation tissue formation.

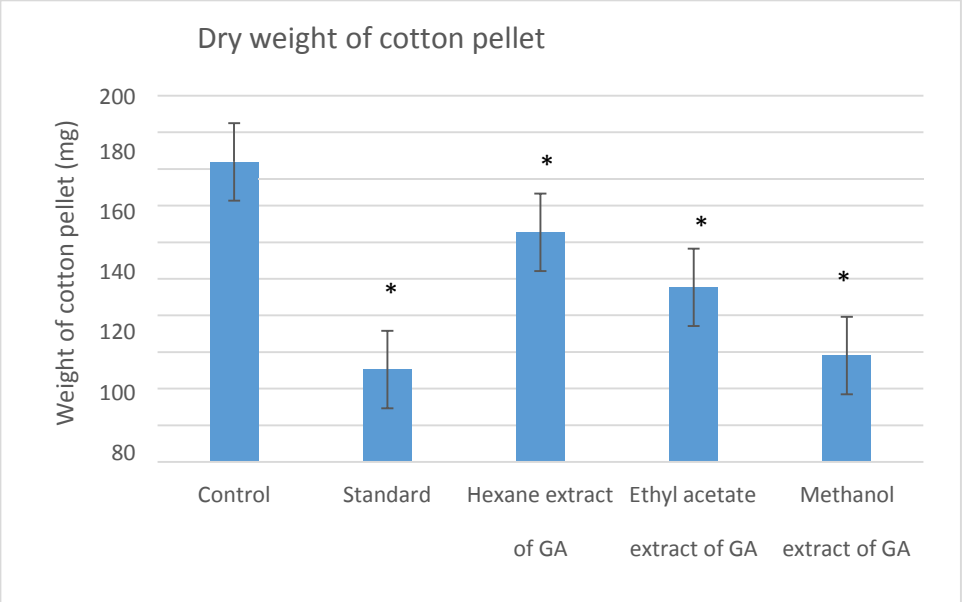
Anti-inflammatory activity of *Gelidiella acerosa* by cotton pellet granuloma.

Groups	Dose	Wet weight of cotton pellet (mg) (Mean ± SD)	% Inhibition	Dry weight of cotton pellet (mg) (Mean ± SD)	% Inhibition
Control (NormalSaline)	0.2 ml	189.85±1.56	------	163.85±2.53	------
Standard (Diclofenacsodium)	10mg/kg	65.11±3.46*	65.7	48.41±3.22*	70.45
Hexane extract of *Gelidiella acerosa*	400mg/kg	137.67±2.19*	27.48	110.26±3.06*	32.70
Ethyl acetateextract of *Gelidiella acerosa*	400mg/kg	106.23±2.77*	44.04	81.34±2.10*	50.35
Methanolicextract of *Gelidiella acerosa*	400mg/kg	71.33±3.03*	62.42	58.00±1.27*	64.60

Values are expressed as Mean ± SD (n=5), *p<0.001 denotes significance with respect to the control group using one way ANOVA followed by Dunnet's test.



Anti-inflammatory activity of *Gelidiella acerosa* by cotton pellet inducedgranuloma.



Anti-inflammatory activity of *Gelidiella acerosa* by cotton pellet inducedgranuloma.

5.2 DISCUSSION

The extracts of *Gelidiella acerosa* were screened for the presence of the phytoconstituents both qualitatively and quantitatively. Phytochemical screening showed the presence of saponins, phenols, flavonoids, alkaloids and tannins. The flavonoid content in the methanolic extract was calculated to be 48.5 mg/g Quercetin equivalent, ethyl acetate contained 43.5 mg/g Quercetin equivalent while the hexane extract contained 37.5 mg/g Quercetin equivalent.

The phenolic content was estimated to be 34.34 mg/g gallic acid equivalent in methanolic extract of *Gelidiella acerosa*, ethyl acetate extract of *Gelidiella acerosa* contained 22.6 mg/g gallic acid equivalent while the hexane extract of *Gelidiella acerosa* contained 16.4 mg/g gallic acid equivalent phenolic content.

All the three extracts of *Gelidiella acerosa* were also evaluated for the in-vitro DPPH free radical scavenging activity and the hexane, ethyl acetate and methanolic extract of the *Gelidiella acerosa* were found to exhibit 32.77%, 50.71% and 68.42% free radical scavenging activity as compared to the highest percentage free radical scavenging activity of ascorbic acid (standard) 76.55%.
On acute oral toxicity the extract was found to be safe up to 4000 mg/kg and thus the 1/10th of the dose 400mg/kg was selected for the studies.
The present examination was carried out to scientifically evaluate the hexane, ethyl acetate and methanolic extracts of *Gelidiella acerosa* for the carrageenan induced paw edema and cotton pellet induced granuloma models of anti-inflammatory activity. Diclofenac sodium (10 mg/kg) was used as the standard drug for both the models.
All the three extracts were found to significant at $p<0.001$ and inhibit the paw edema volume in the carrageenan induced paw edema model in the later hours of the carrageenan administration i.e. after one hour time period. The methanolic extract was found to inhibit the paw edema volume with a maximum range followed by the ethyl acetate and hexane extracts against the diclofenac sodium as the standard which showed the highest inhibition of the paw edema. Phytochemical investigation & Pharmacological screening of marine alga, *Gelidiella.*
The cotton pellet induced granuloma was assessed with both wet and dry weight of the cotton pellet along with the granulomatous tissue. The proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels which are the basic sources of highly vascularized reddish mass is termed as granulation tissue is seen during repair course of inflammation. The fluid absorbed by the pellet greatly impacts the wet weight of the granuloma and the dry weight associates well with the amount of granulomatous tissue formed. The percentage inhibition of the wet and dry weight of the cotton pellet was found to be maximum for the diclofenac sodium 70.45%, followed by the methanolic extract of *Gelidiella acerosa* 64.60%, ethyl acetate extract of *Gelidiella acerosa* 50.35% and the least percentage inhibition was found in hexane extract of *Gelidiella acerosa* 32.70%.

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