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Research Article

Exploration of possible mechanisms for anti-inflammatory activity of *Ipomoea aquatica* Forsk. (Convolvulaceae)

Mital N. Manvar[1*] and T. R. Desai[2]

[1]*PhD Scholar, School of Pharmacy, RK University, Rajkot, Gujarat, India and Assistant Professor, Department of Pharmacognosy, Atmiya Institute of Pharmacy, Rajkot, Gujarat, India.*
[2]*Dean and Director Faculty of Pharmacy, RK University, Rajkot, Gujarat, India*

*Correspondence Info:
Mital N. Manvar
PhD Scholar,
School of Pharmacy,
RK University, Rajkot, Gujarat, India.
E-mail - mital_manvar@rediffmail.com

Abstract
Currently used steroidal and non steroidal anti-inflammatory drugs have severe side effects. These side effects are very difficult to manage than the disease itself. Hence, there is to search new safe resources to cure such diseases that the use of plant based drugs. This study deals with anti-inflammatory evaluation of the hydroalcoholic extract of *Ipomoea aquatica* leaves as well as their possible mechanism of action. A carrageenan-induced rat paw oedema model was used for anti-inflammatory study. The mechanism/s by which *Ipomoea aquatica* is mediated the ant-inflammatory activity was determined by its effects in antihistamine activity, prostaglandin synthesis inhibition activity, membrane stabilizing activity and protein denaturation inhibition activity. Dose dependent anti-inflammatory activity was found with HAEIA in rat paw oedema model using carrageenan. HAEIA effective to suppressed the wheal area formed by histamine. HAEIA revealed dose dependent prostaglandin synthesis inhibition activity. HAEIA was effectively inhibited the heat induced hemolysis of HRBCs as well as heat induced albumin denaturation. Therefore, it was concluded that the HAEIA has anti-inflammatory activity possibly mediated through inhibition of release of mediator histamine and prostaglandin and has also HRBCs membrane stabilization and protein denaturation inhibition properties.
Keywords: *Ipomoea aquatica*, Convolvulaceae, anti-inflammatory, mechanisms

1. Introduction
Inflammation is the tissue reaction to foreign substances or infection [1]. It is a part of the host defense mechanisms [1]. It involved inflammatory reactions due to release of histamine, bradykinin and prostaglandins like substances [1]. Many biological models have been described for study of paw oedema. An acute phase of inflammation was studied by many researchers using carrageenan-induced paw oedema model [1-2]. The mediators detected in early phase of carrageenan-induced inflammation are histamine, 5-hydroxytryptamine and bradykinin, whereas in the late phase of inflammation prostaglandins are detected [1-2].

During the past decades, there is much progress in medical research even though the treatment of many serious diseases remains problematic [3]. A chronic inflammatory disorder is a major health problem in the world [4]. Many medications are available to prevent or minimize progression of the inflammatory disease like Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids [5]. NSAIDs are associated with several side effects; their prolonged use often leads to bone marrow depression, gastric intolerance, water and salt retention [4-

5]. The most serious side effects are liver and kidney failure, ulceration and prolonged bleeding after surgery or injury[5]. Therefore, in current situation, it is important to developed new component with more powerful anti-inflammatory activities without side effects. Many of the plant drugs used in modern medicine had been mention in traditional systems of medicine.

Ipomoea aquatica Forsk. (Family- *Convolvulaceae*) is green leafy perennial herb. It is commonly known as Nalanibhaji and found throughout India, Ceylon, Tropical Asia, Africa and Australia [6]. *I. aquatica* use as carminative, lessens inflammation; useful in fever, Jaundice, biliousness, bronchitis, liver complaints in Unani system of medicine [7]. The present study have been deal with inflammatory evaluation of hydroalcoholic extract of *Ipomoea aquatica* leaves (HAEIA) using the carrageenan-induced paw oedema assay. In addition different methods were used to assess the mechanism/s by which *Ipomoea aquatica* has mediated the anti-inflammatory activity.

2. Materials and methods

2.1 Plant material

The leaves of *I. aquatica* were collected from Rajkot district of Gujarat, India. The plant material was identified by Faculty in botany, Biology Department, Gyanyagna College of Science & Management, Rajkot and voucher specimens (Voucher No. AIP/12/02) has been retained in Department of Pharmacognosy, Atmiya Institute of Pharmacy, Rajkot, Gujarat, India.

2.2 Animals

Albino Wistar rats of either sex weighing 230–260 g were provided by the Animal House of Atmiya Institute of Pharmacy, Rajkot. Twelve hours before the experiments they were maintained only with water. This experiment approved by Institutional Animal Ethics Committee (IAEC) (Protocol number: PG 1004).

2.3 Reagents and chemicals

Carrageenan (Sigma Chemical Company, USA), Bovine serum albumin (Chiti Chem Corporation, Baroda, Gujarat, India), Histamine dihydrochloride, all the reagents were of analytical grades. Indomethacin capsules (25 mg), chlorpheniramine tablets (4mg), aspirin tablets (50mg) and diclofenac sodium tablets (50 mg) standard non-steroidal anti-inflammatory drugs were purchased from a pharmaceutical shop at Rajkot, Gujarat, India**.**

2.4 Preparation of *I. aquatica* extracts

Air-dried leaves of *I. aquatica* were ground into powder using an electric grinder. Thousand gram of dried leaves powder were exhaustively extracted by maceration with aqueous ethanol (70%). Thereafter, filtered and extract was concentrated on water bath to a dry residue (yield 20.1% w/w).

2.5 Anti-inflammatory activity

2.5.1 Carrageenan induced rat paw oedema model

The rats were fasted 24 h prior to the study. They were divided into six groups (each group with 6 animals). Group 1 treated as control and group 2 treated with standard drug indomethacin. Groups 3, 4, 5 and 6 received 50, 100, 500 and 1000 mg/kg suspension of hydroalcoholic extract of leaves of *I. aquatica* (HAEIA) respectively. The right hind paw of the rats were injected with 0.1 ml of a 1% (w/v) solution of carrageenan in saline into the subplantar aponeurosis to induce edema. The phlogestic agent injected after 60 min of oral administration of the vehicle, extracts and the standard drug. By using plethysmometer, the volumes of paw edema were measured at every hour upto 5h after the induction of inflammation. The following equation was used to determine % inhibition of oedema. % Inhibition of oedema = (1-Vt/Vc) ×100. Where, Vt is paw volume of the rats of test groups. Vc is the paw volume of the rats of control groups [8].

2.5.2 Antihistamine activity assay

Eighteen rats were anaesthetised and shaved on posterior left lateral side. After 24 hours, rats were divided into three groups (each with 6 animals). Group 1 treated orally with 1000 mg/kg of suspension of HAEIA, group 2 treated with chlorpheniramine (antihistamine receptor antagonist) 0.7 mg/kg and group 3 received vehicle. After 1 h, the rats were injected subcutaneously with 0.05 ml of histamine dihydrochloride(200 mcg/ml) at the area of the shaved skin under mild ether anaesthesia. After 2.5 min, measured the wheal radius and the areas were calculated[9].

2.5.3 Prostaglandin (PG) synthesis inhibition model

The uteri spontaneous contractions were measured using isometric sensor for 10 min. The normal activity of the uteri was recorded for a further 10 min after the contractions become regular. Sequentially added HAEIA to make concentrations 2.5, 5, and 7.5mcg/ml in organ bath (each dose repeated 4 times). Also the reference drug

aspirin 5µg/ml (repeated 4 times) was given same manner and calculated the percent reduction in frequency and amplitude of contractions as compare to normal contractions[9].

2.5.4 Membrane stabilizing activity assay

The reaction mixture (2ml) prepared using 1ml of 10% human red blood cells (HRBCs) suspension and 1ml of test samples of HAEIA (50,100,250,500,750 and 1000 mcg/ml). The control sample consist only saline instead of test sample while the standard sample consists indomethacin (200mcg/ml). The all reaction mixtures were incubated for 30min at 56 ºC and coole.. The reaction mixtures were centrifuged for 5 min at 2500 rpm. Then measure the absorbance at 560 nm of collected supernatants. The experiment was performed in triplicates for all the test samples. The following equation was used to determine % inhibition haemolysis. %membrane stabilization = $[\{A_C - A_T\}/ A_C] \times 100$. Where, A_C is the absorbance of control, A_T is the absorbance of test sample [9].

2.5.5 Inhibition of albumin denaturation

The test mixture prepared usign 2ml test extracts of different concentrations (50, 250, 500 and 1000 mcg/ml) and 3ml of 1% aqueous solution of bovine albumin fraction (each repeated 3 times). Small amount of 1N HCl was added to the test mixture to adjust pH 6.4. After that the test mixtures were incubated for 20 min at 37 ºC. Then test mixtures were heated to 51ºC for 20 min. Test mixtures were cooled and their turbidity measured at 660nm using UV-Visible Spectrophotometer. Diclofenac sodium (200mcg/ml) used as standard while control did not contain drug. The following equation was used to determine %inhibition of protein denaturation. % inhibition= $[\{A_C - A_T\}/ A_C] \times 100$. Where, A_C is the absorbance of control, A_T is the absorbance of test sample [10].

2.6 Data analysis

Data analyses are presented as means±SEM of measurements. One way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test used for the statistical analysis. All data were analyzed using the GraphPad Prism 6 Demo computer software. Statistical differences were considered to be significant at $P < 0.01$.

3. Results

The results of anti-inflammation activity of the HAEIA using carrageenan induced oedema assay are given in Table 1. The present study indicated that the HAEIA have a marked and dose dependent anti-inflammation**.** The highest % inhibition of oedema by the 1000 mg/kg body weight of HAEIA was 87.24% at 5 h. while 100 mg/kg body weight of HAEIA was showed 67.01% inhibition of oedema which was comparable with that of the indomethacin (68.03%) at 5 h.

Table 1: Effects of the *I. aquatica* leaf extract on the carrageenan-induced rat hind paw edema

Treatment& Dose	**Paw edema volume (%**inhibition**)**				
(mg/kg b.w.)	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control	1.54±0.040	1.91±0.038	2.30±0.055	2.32±0.064	1.96±0.050
Indomethacin	0.83±0.011***	0.78±0.040***	0.78±0.017***	0.76±0.066***	0.63±0.059***
(10 mg/kg b.w.)	(46.10)	(59.16)	(66.09)	(67.24)	(68.03)
HAEIA	1.21±0.046**	1.39±0.044***	1.55±0.061***	1.33±0.083***	0.89±0.024***
(50 mg/kg b.w.)	(21.21)	(27.40)	(32.85)	(42.75)	(54.42)
HAEIA	0.98±0.049***	0.93±0.033***	0.81±0.058***	0.79±0.051***	0.65±0.030***
(100 mg/kg b.w.)	(36.58)	(51.31)	(64.69)	(66.00)	(67.01)
HAEIA	0.91±0.046***	0.83±0.037***	0.75±0.079***	0.66±0.055***	0.35±0.030***
(500 mg/kg b.w.)	(40.69)	(56.54)	(67.29)	(71.45)	(82.31)
HAEIA	0.89±0.077***	0.68±0.050***	0.68±0.062***	0.54±0.035***	0.25±0.030***
(1000 mg/kg b.w.)	(42.42)	(64.22)	(70.33)	(76.90)	(87.24)

[Figures in parenthesis indicate oedema inhibition percentage. Values are expressed as mean±SEM, (N=6). *Significantly different from control group (P<0.01)**]**

As shown in Table 2, HAEIA effectively suppressed the wheal area formed by histamine.

Table 2: Effects of the *I. aquatica* leaf extract in antihistamine activity assay

Treatment & Dose (mg/kg b.w.)	**Area of wheal (mm^2)**	**Reduction of area (%)**
Control	73.52±3.14	-
Chlorpheniramine (0.7 mg/kg b.w.)	42.39±2.48***	42.34
HAEIA (1000 mg/kg b.w.)	46.83±5.29***	36.29

[Values are expressed as mean±SEM, (N=6). *Significantly different from control group ($P < 0.01$)**]**

The results of prostaglandin synthesis inhibition activity of HAEIA and aspirin were demonstrated in Table 3. HAEIA showed dose dependent reduction of spontaneous contractions of isolated dioestrus uterus of rat.

Table 3: Effects of the *I. aquatica* leaf extract in prostaglandin (PG) synthesis inhibition model

Treatment & Concentration	Amplitude of contractions (mm)	Reduction of amplitude (%)	Frequency of contractions	Reduction of frequency (%)
Normal	18±1.73	-	27.33±1.76	-
Aspirin (5 mcg/ml)	5±0.58***	72.22	4.00±0.68***	85.36
HAEIA (2.5 mcg/ml)	12±0.52**	33.34	19.00±0.58***	30.49
HAEIA (5 mcg/ml)	9±0.57***	50.00	8.67±0.68***	68.29
HAEIA (7.5 mcg/ml)	6±0.34***	64.81	6.00±0.57***	78.05

[Values are expressed as mean±SE.M, (N=4). *Significantly different from normal contraction ($P < 0.01$)]

HAEIA was effectively inhibits heat induced hemolysis of HRBCs (Table 4). HAEIA showed dose dependent membrane stabilizing activity.

Table 4: Effects of the *I. aquatica* leaf extract in membrane stabilizing activity assay

Test sample (Concentration in µg/ml)	Mean absorbance	% membrane Stabilization
Control	0.834	-
Indomethacine (200 mcg/ml)	0.015***	98.24±0.10
HAEIA (50 mcg/ml)	0.500**	39.98±0.14
HAEIA (100 mcg/ml)	0.385***	53.82±0.18
HAEIA (250 mcg/ml)	0.233***	72.09±0.10
HAEIA (500 mcg/ml)	0.142***	83.01±0.22
HAEIA (750 mcg/ml)	0.084***	89.88±0.21
HAEIA (1000 mcg/ml)	0.047***	94.40±0.11

[Values are expressed as mean±SEM, (N=3). *Significantly different from control ($P < 0.01$)**]**

HAEIA showed significant effectiveness in inhibition of heat induced albumin denaturation (Table 5). The maximum %inhibition of protein denaturation was 96% at 1000 mcg/ml of HAEIA.

Table 5: Effects of the *I. aquatica* leaf extract on albumin denaturation

Test sample (Concentration in µg/ml)	Mean absorbance	%Inhibition
Control	0.093	-
Diclofenac sodium (200 mcg/ml)	0.012***	87.14±0.62
HAEIA (50 mcg/ml)	0.063**	32.85±0.38
HAEIA (250 mcg/ml)	0.024***	73.92±0.94
HAEIA (500 mcg/ml)	0.008***	91.07±0.36
HAEIA (1000 mcg/ml)	0.003***	96.78±0.61

[Values are expressed as mean±SEM, (N=3). *Significantly different from control ($P < 0.01$)**]**

4. Discussion

The anti-inflammatory activity was studied using *in vitro* and *in vivo* models at different doses of the hydroalcoholic extract of *I. aquatica* leaf**.** The present study revealed the significant reduction ($P < 0.01$) in the paw volume at lower dose 100 mg/kg HAEIA as compared control. Carrageenan-induced rat paw oedema assay comprises two phases [11]. The first phase (1–2 h) is linked with serotonine and histamine while late phase related with neutrophil infiltration, free radicals production, eicosanoid release and release of mediators derived from neutrophil[12]. The kinin like substances is release in between early and late phase to produce oedema[13]. To determine the mode of drug action it is important to identify the mediators involved in different phases. The paw edema is formed due to increase vascular permeability or increase blood flow which may due to synergism between various inflammatory mediators [14-15]. The development of carrageenan induced oedema is generally correlated with the early exudative stage of inflammation [14-15]. Inhibition of paw oedema in rat at early phase was demonstrated by HAEIA.

A vascular permeability is increase by the histamine and it is responsible for the formation of a wheal around the histamine injected skin[9]. The substances work as histamine antagonists are reduced wheal area

foromation[9]. Significant reduction of wheal area formation in HAEIA treated rats was found as compare to control (Table 2). Prostaglandin synthesis is responsible for the spontaneous activity of isolated rat uterus [16]. The HAEIA showed inhibition of spontaneous contractions of isolated uterus dose dependently (Table 3) thus the results indicated the ability of HAEIA to inhibit prostaglandin synthesis. Hence anti-inflammatory activity of HAEIA was possibly due to antihistamine activity and inhibition of prostaglandin synthesis.

The erythrocyte membrane is analogous to the lysosomal membrane [17]. These neutrophil lysosomal constituents include protease and bactericidal enzymes, which cause further tissue damage and inflammation upon extracellular release [17]. The stabilization of erythrocyte membrane indicated that HAEIA may stabilize membrane of lysosomal and inhibit the release of lysosomal content at the site of inflammation.

Protein denaturation is one of well documented causes of inflammation in conditions like rheumatoid arthritis [18]. Many plant extracts have ability to inhibit protein denaturation dose dependently [19]. Thus, HAEIA has one of the mechanism of action was protection against protein denaturation.

5. Conclusion

The results of present study showed that HAEIA may be potent inhibitor of inflammation. The study suggested that the inhibition of prostaglandin and histamine synthesis, protein denaturation inhibition and the stabilization of lysosomal membrane could be probable mechanisms for anti-inflammatory action of *I. aquatica*.

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