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Research Article

ANTIINFLANNATORY AND MEMBRANE-STABILIZING ACTIVITY OF ETHANOLIC EXTRACT AND ITS ETHYLACETATE AND N-BUTANOL FRACTIONS OF *IPOMOEA STAPHYLINA*

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ABSTRACT

The ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions were evaluated for formalin induced paw oedema and membrane-stabilizing property. The result obtained indicates that the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fraction (200 mg/kg, p.o.) significantly decreased the formalin induced paw oedema. The extract and its fractions also exhibit membrane-stabilizing property, as significantly (P<0.001) reduced the levels of haemolysis of erythrocytes exposed to hypotonic solution.

Keywords: *Ipomoea staphylina*, formalin, paw oedema, membrane-stabilizing property, haemolysis.

INTRODUCTION

The treatment of inflammatory diseases is mainly dependent on nonsteroidal anti-inflammatory drugs (NSAID) [1]. Nonsteroidal anti-inflammatory drugs (NSAID) act by blocking cyclooxygenase enzyme (COX) thus inhibit the conversion of arachidonic acid to prostaglandin [2]. But long-term administration of NSAID induces gastro-intestinal ulcers and bleeding due to their non-selective inhibition of both isoforms of the COX enzyme (COX-1 and COX-2 isoform) [3-5]. Selective COX-2 inhibitors are associated with adverse cardiovascular effects [6]. On the other hand, steroidal drugs as anti-inflammatory agents are not safe due to their multiple side effects [7,8]. Therefore, developing new agents with more powerful anti-inflammatory activity with lesser side effects is, at present, of great interest.The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane [9]. Therefore, as membrane stabilizes that interfere in the release and or action of inflammatory mediators like histamine, serotonin, prostaglandins, leukotrienes etc [10].

Ipomoea staphylina is an extensive climber belonging to the family convolvulaceae. A literature review reveals antiulcer property [11] of *Ipomoea staphylina.* Other species of genus Ipomoea like *Ipomoea pes-caprae*, *Ipomoea imperati*, *Ipomoea involucrate* and *Ipomoea asarifolia* has been reported for anti-inflammatory activity [12-15]. So, the present study was carried out to evaluate the anti-inflammatory activity of ethanolic extract and its ethyl acetate and n-butanol fractions of leaves of *Ipomoea staphylina.*

MATERIALS AND METHODS

Plant Material

Leaves of *Ipomoea staphylina* were collected from forest area of Karnataka near to Bangalore. The *Ipomoea staphylina* plant taxonomically identified and authenticated by Dr. K. Karthigeyan at Central National Herbarium, Botanic Garden, Howrah, where the voucher specimen is conserved under the reference number SMF-01. The leaves of *Ipomoea staphylina* were cleaned and dried under shade at room temperature for several days and powdered. The powder was defatted with petroleum ether (60-80 GR) for 72 h and then the dried powder was extracted with ethyl alcohol to get a yield of 10.2 % w/w. The ethanolic extract was dispersed in distilled water and partitioned with ethyl acetate in a separating funnel till the colourless ethyl acetate fraction is obtained.

Then the aqueous part is then partitioned with n-butanol to get the butanol fraction. Ethyl acetate and butanol fraction so obtained was concentrated by keeping in boiling water bath to get the solid residue. The dried extracts were stored in airtight container and placed in refrigerator.

Phytochemical screening

Preliminary phytochemical screening of ethanolic extract of *Sechium edule* fruits and its ethyl acetate and n-butanol fractions were performed for the presence of alkaloids, phenolics, flavonoids, saponins, proteins, carbohydrates and glycosides [16].

Drugs and chemicals

Diclofenac sodium and aspirin were obtained from Micro Labs, Bangalore, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

Experimental animals

In-breed wistar rats (150-200 g) of either sex weighing 20-25g maintained under controlled conditions of temperature (23± 2°C) and humidity (50 ± 5%) and a 12-hour light–dark cycle, were used for the experiment. They were housed in sanitised polypropylene cages containing sterile paddy husk as bedding. They had free access to standard rat pellet diet and water *ad libitum*. The animals were given a week's time to get acclimatized with the laboratory conditions. All the experimental procedures were performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

Mice were kept overnight fasting prior to drug administration. Animals were received a single oral dose (2000 mg/kg, b.w.) of ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions. After the administration of *Ipomoea staphylina* leaves extract and its different fractions food was withheld for further 3–4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, piloerection, urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes. Mortality, if any, was determined over a period of 2 weeks [17].

Formalin induced inflammation

In this method rats (n=6) were pre-treated orally with the ethanolic extract of leaves of *Ipomoea staphylina* its ethyl acetate and n-butanol fractions (200 mg/kg, p.o and 100 mg/kg, p.o), Diclofenac (10 mg/kg, p.o) and saline (10 ml/kg, p.o) respectively. Thirty minutes after administration of different substances, 0.1ml of 1% of

formaline subcutaneously into the planter region was injected to all animals in the left hind paw. The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer (model 7150, Ugo Basile, Italy). The paw volume was determined at 0 h (before formalin injection) and 0.5, 1, 2, 3 and 4 h later [18, 19]. % Inhibition of oedema = $100[1-(V_t/V_c)]$ Where V_t and V_c are volume of formalin injected paws of drug treated group and control group respectively.

Membrane stabilizing activity

Preparation of erythrocyte suspension

Whole blood was obtained with heparinized syringes from rats through cardiac puncture. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4).The blood was centrifuged each time for 10 minutes at 3000 g.

Hypotonic solution-induced rat erythrocyte haemolysis

Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte haemolysis. The test sample consisted of stock erythrocyte (RBC) suspension (0.50 ml) mixed with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the ethanolic extract of leaves of *Ipomoea staphylina* its ethyl acetate and n-butanol fractions (0.25-1.0 mg/ml) or aspirin (0.1 mg/ml) . The control sample consisted of 0.5 ml of RBC mixed with hypotonic - buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated [10].

% Inhibition of heamolysis = 100 x {OD1-OD2/OD1}

Where: OD1 = Optical density of hypotonic-buffered saline solution alone OD2 **=** Optical density of test sample in hypotonic solution

Statistical analysis

All data were represented as mean±S.EM. Results were statistically evaluated using one way analysis of variance (ANOVA) followed by Tukey-Kramer (post tests) using INSTAT software. $P < 0.05$ was considered as statically significant.

RESULTS

Preliminary phytochemical analysis

The preliminary phytochemical analysis of the extracts has shown the presence of alkaloids, tannins, flavonoids, saponins, proteins and carbohydrate. The ethyl acetate fraction and n-butanol fraction has shown the presence of flavonoids and saponins respectively.

Table 1 : Effect of *Ipomoea staphylina* (IS) leaves extract and its fractions on formalin induced paw oedema

Treatment (mg/kg)	Mean increase in paw volume (ml)						% Inhibition at 4 h
	0 h	0.5 h	1 h	2 h	3 h	4 h	
Control	0.49± 0.015	0.70± 0.026	1.04± 0.012	1.15± 0.019	1.27± 0.014	1.33± 0.019	-
Diclofenac sodium (10)	0.50± 0.022	0.57± 0.016***	0.82± 0.012***	0.93± 0.013***	1.04± 0.012***	0.91± 0.016***	31.57
IS Extract (200)	0.53± 0.031	0.62± 0.010*	0.89± 0.011***	0.98± 0.014***	1.12± 0.017***	0.92± 0.018***	30.82
IS Extract (100)	0.51± 0.022	0.66± 0.012	0.96± 0.016	1.06± 0.010***	1.17± 0.014***	0.99± 0.015***	25.56
Ethyl acetate fraction of IS (200)	0.48± 0.022	0.63± 0.012*	0.90± 0.017***	0.99± 0.022***	1.16± 0.014***	0.93± 0.021***	30.07
Ethyl acetate fraction of IS (100)	0.49± 0.027	0.67± 0.015	1.00± 0.011	1.07± 0.013**	1.19± 0.018*	1.02± 0.012***	23.30
n-Butanol fraction of IS (200)	0.48± 0.017	0.64± 0.011	0.91± 0.017***	0.99± 0.015***	1.16± 0.015***	0.95± 0.016***	28.57
n-Butanol fraction of IS (100)	0.51± 0.024	0.66± 0.014	0.99± 0.025	1.09± 0.013*	1.22± 0.013	1.09± 0.013***	22.01

Values are expressed as mean ± SEM, n=6; *** P<0.001, ** P<0.01, * P<0.05 considered statistically significant as compared to control group.

Acute toxicity studies

In LD50 studies, it was found that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.

Effect on formaldehyde-induced paw oedema

The ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions were evaluated for formalin induced paw oedema. The result obtained indicates that the ethanolic extract of leaves of *Ipomoea staphylina* (200 mg/kg, p.o.) significantly decreased the formalin induced paw oedema at 0.5 h ($P<0.05$), 1 h, 2 h and 4 h ($P<0.001$). The ethyl acetate fraction (200 mg/kg, p.o.) also significantly decreased the paw oedema at 0.5 h ($P<0.05$), 1 h, 2 h and 4 h ($P<0.001$). The n-butanol fraction of *Ipomoea staphylina* (200 mg/kg, p.o.) also significantly decreased the formalin induced paw oedema at 1 h, 2 h, 3 h and 4 h ($P<0.001$). Maximum inhibition of paw oedema was observed with the ethanolic extract of leaves of *Ipomoea staphylina* and its ethyl acetate and n-butanol fractions (200 mg/kg, p.o.) at 4 h when compared to the control group (Table 1). Diclofenac sodium (10 mg/kg, p.o.) and the ethanolic extract of leaves of *Ipomoea staphylina* (200 mg/kg, p.o.) inhibited paw oedema by 31.87% and 30.82% respectively.

Effect on erythrocyte membrane stability

The extract and its fractions at concentration range of 0.50-1.0 mg/ml significantly ($P<0.001$) protect the rat erythrocyte membrane against lysis induced by hypotonic solution. The standard drug aspirin (0.10 mg/ml) also offered a significant ($P<0.001$) protection of the rat erythrocytes membrane against the damaging effect of hypotonic solution. The ethanolic extract of *Ipomoea staphylina* and its ethyl acetate and n-butanol fraction shows 56%, 49% and 40% inhibition of haemolysis at a concentration of 1.0 mg/ml, whereas the standard drug aspirin (0.10 mg/ml) produced 72% inhibition of RBC haemolysis (Table 2).

Table 2: Effect of *Ipomoea staphylina* (IS) leaves extract and its fractions on rat erythrocyte haemolysis

Sample	Concentration	Optical density (OD)	%Inhibition of heamolysis
Hypotonic medium	50 mM	0.83±0.018	-
IS Extract	0.25 mg/ml	0.64±0.010***	22
	0.50 mg/ml	0.55±0.010***	33
	0.75 mg/ml	0.42±0.010***	49
	1 mg/ml	0.36±0.011***	56
Ethyl Acetate Fraction	0.25 mg/ml	0.68±0.011***	18
	0.50 mg/ml	0.58±0.018***	30
	0.75 mg/ml	0.49±0.013***	40
	1 mg/ml	0.42±0.020***	49
n-Butanol fraction	0.25 mg/ml	0.72±0.018***	13
	0.50 mg/ml	0.65±0.017***	21
	0.75 mg/ml	0.57±0.024***	31
	1 mg/ml	0.49±0.019***	40
Aspirin	0.10 mg/ml	0.23±0.017***	72

Each value represents the mean ± SEM of 6 experiments. ***P<0.001 considered statistically significant as compared to Hypotonic medium.

DISCUSSIONS

The results of the study suggest that the *Ipomoea staphylina* leaf extract and its ethyl acetate and n-butanol fractions possesses anti-inflammatory activity, as it significantly inhibited paw oedema induced by formalin in rats. The extract and its fractions also shown membrane stabilizing effect, as it offer significant protection of the erythrocyte against lysis induced by hypotonic solution.

Formalin, a potent oedematous agent, produced inflammation through the release of several inflammatory mediators including prostaglandins [20]. Injection of 1% formalin subcutaneously into hind paw of rats produced localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response. Chemical mediators such as substance P and bradykinins followed by a tissue mediated response where histamine, 5-HT, prostaglandins and bradykinin are known to be involved [21]. The initial phase of the oedema is due to the release of histamine and serotonin and the oedema is maintained during the plateau phase by kinin like substance [22] and the second accelerating phase of swelling due to the release of prostaglandin like substances. In this study, we found that the administration of the the ethanolic extract of leaves of *Ipomoea*

staphylina its ethyl acetate and n-butanol fractions (200 mg/kg, p.o.) reduced the oedema in the later phase of inflammation.

The strength of cells depends on the integrity of their membranes. Exposure of erythrocytes to hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Such injury to erythrocyte membrane will further make the cell more susceptible to free radical -induced lipid peroxidation [23, 24].

Compounds with membrane-stabilizing properties are well known for their ability to interfere with the release of phospholipases that trigger the formation of inflammatory mediators such as histamine, serotonin, prostaglandins, leukotrienes etc [25]. The extract and its fractions at concentration range of 0.50-1.0 mg/ml significantly (P<0.001) protect the rat erythrocyte membrane against lysis induced by hypotonic solution which suggests that its anti-inflammatory activity observed in this study, may be related to the inhibition of the early phase of inflammatory events, the release of chemical mediators.

CONCLUSION

In conclusion, the results of the study suggest that *Ipomoea staphylina* leaf extract its ethyl acetate and n-butanol fractions possess anti-inflammatory and membrane stabilizing activity.

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