

Anti-Inflammatory Activity of the Fruit Extract of *Benincasa hispida*

Deepak Kadam and Smita Lele*

Food Engineering and Technology Department, Institute of Chemical Technology, N. P. Marg, Matunga, Mumbai 400019, India

Abstract: *Benincasa hispida* (Ash gourd, family: Cucurbitaceae) is well known for its medicinal property. In the Ayurvedic system, it is used as a rejuvenating agent and in the treatment of nervous disorders. Methanolic and petroleum fruit extracts were subjected to evaluation of phytochemicals and acute oral toxicity study. Further, Anti-inflammatory activity was performed using human red blood cell (HRBC) membrane stabilization assay and carrageenan induced paw edema in rat. Oral administration of methanolic extract of concentration (350 mg/kg) significantly reduced the paw edema ($P < 0.05$) in a dose dependent manner as compared to carrageenan induced rats.

Keywords: *Benincasa hispida*, Phytochemicals, Anti-inflammatory, HRBC membrane stabilization, Carrageenan.

1. INTRODUCTION

Nature has provided many compounds of significant medicinal application that play an important & often dominant role in the maintenance of human health. Natural plant products such as herbs, fruits and vegetables have become more popular in recent years due to public awareness and developing significance in the pharmacological evaluation. Now days, Indian traditional Ayurvedic systems of medicine has increasing interest among consumers and the scientific community prefer to control their health with the help of natural plant medicines [1]. Plant medicines has wide variety of free radical scavenging molecules, such as phenolic compounds, amines, vitamins, terpenoids, carotenoids [2] and some endogenous metabolites, which are rich in antioxidant activity and anti-inflammatory activity [3].

Inflammation is a complex part of the body's immune response to a native substance which produces excessive amount of free radical causing lipid peroxidation resulting in membrane destruction followed by production of mediator and chemotactic factor [4]. It is also correlated with the different disease such as rheumatism, encephalitis, fibrosis, cancer [5], heart problem etc. NSAIDs anti-inflammatory drugs are most widely used for the treatment of inflammation of various kinds, but can cause many adverse effects. Therefore there is a need to emphasize on research for discovery of anti-inflammatory agent from natural sources. Herbal extracts and treatments are found to be safer alternatives so extensive research can be done for potential anti inflammatory activity.

Benincasa hispida also known as Ash gourd belongs to the family of Cucurbitaceae. They are of great interest due to their wide range of biological activity. It is considered as a good source of valuable nutrients including organic acids, natural sugars, amino acids, vitamins and mineral elements. The fruit possesses anti-ulcer [6], anti-angiogenic [7], antihistamines [8], anti-inflammatory [9] antioxidant and angiotensin-converting enzyme (ACE) inhibitors [10], anorectic [11], and hypoglycemia [12] properties. The large fraction that are responsible for the above mentioned activities in ash gourd fruits are flavonoids [13], saccharides, triterpenoids, proteins, carotene, glycosides, vitamins, minerals, β -sitosterin, uronic acids, lupeol and sterols [14].

In this present work, an attempt has been made to investigate the phytochemicals and anti-inflammatory activity of methanolic and petroleum ether extracts of *Benincasa hispida* fruit by Human red blood cell (HRBC) membrane stabilization and carrageenan induced paw edema in rat methods.

2. MATERIALS AND METHODS

2.1. Preparation of Extract

Fresh fruit, *Benincasa hispida* was collected from the Matunga market, Mumbai, INDIA. The fruit was chopped and seeds were separated. Further, fruit were air dried, powdered and passed through the 60 mesh size sieve. It was then sequentially extracted using hot Soxhlet method with solvents like petroleum ether, methanol, ethyl acetate and water, each used in their increasing order of polarity. The extracts were dried under reduced pressure at temperature 42 °C. The crude extract was vacuumed dried and stored at low temperature.

*Address correspondence to this author at the Food Engineering and Technology Department, Institute of Chemical Technology, N. P. Marg, Matunga, Mumbai 400019, India; Tel: +91 22 3361 1111; Fax: +91 22 2414 56 14; E-mail: dr.smita.lele@gmail.com

2.2. Chemical and Instrument

All chemicals used in the experiment were of analytical grade (HiMedia, Mumbai Chapter). Carrageenan was procured from Sigma Chemicals. Reference standard acetylsalicylic acid (ASA; Ecosprin) was obtained from USV Ltd (Mumbai, India). Detection for the *in vitro* assay was performed by UV Visible spectrophotometer.

2.3. Phytochemical Evaluation

Standard phytochemicals screening tests were performed to identify the different constituents present in methanol and petroleum ether extracts of fruit [15].

2.4. Animal

Healthy Albino Wistar rat (150-250gm) and Albino Swiss mice (18-22gm) of female sex were procured from the Haffkine Biopharmaceuticals (Mumbai, India). They were housed at the Animal Testing Centre (CPCSEA/315). Procedure for animal maintenance and experimentation was carried out as per guidelines of CPCSEA, Government of India. Animals were given free access to standard laboratory food and water. Animals were maintained in polypropylene cages. Standard conditions of temperature (22 + 5°C) and humidity (55 + 15%) were maintained with a 12 h light-dark cycles. All animals were acclimatized for 1 week before experimental procedures were carried out. The Experimental study procedure was approved by the Institutional Animal Ethics Committee.

2.5. Acute Toxicity Study

Acute toxicity study was performed as per OECD guidelines 420.

2.6. *In-Vitro* Anti-Inflammatory Activity

The HRBC membrane stabilization assay was used for the estimation of *in vitro* anti-inflammatory activity according to TK Mohamed Saleem *et al.* (2011) with some modification. Whole human blood was freshly collected and mixed with equal volume of sterilize Alsever solution. The sample was centrifuged at 3000 rpm for 10 min. Further supernatant was separated and packed cells (sediments) were washed with isosaline for three consecutive times. Quantification of red blood cell was determined and redeemed with isosaline (10% v/v). The principle behind this is; the hypotonicity triggers membrane lysis which in-turn stabilises human RBC membrane. Different concentration of fruit extract, reference sample and control samples were mixed with

(1ml) phosphate buffer, (2 ml) hyposaline and (0.5 ml) HRBC suspension. Samples were further incubated at 37 °C for 30 min and centrifuged. The haemoglobin quantity in the sample was estimated by the spectrophotometric analytical method at 560 nm. The percent hemolysis was evaluated by considering the hemolysis generated in the control as 100%.

$\% \text{ Protection} = 100 - [(\text{OD of Test sample} / \text{OD of Control}) \times 100]$

2.7. *In Vivo* Anti-Inflammatory Activity

Paw oedema was induced on each rat by transfusing 0.1 ml of 1% w/v carrageenan on physiological saline. The methanolic and petroleum ether extract at different concentration were administered orally for 60 minutes after carrageenan administration. Paw volume was measured immediately (0 h) and thereafter measured at 1 h intervals for 4 h by method based on the Archimedes' principle of buoyancy and hydrostatic pressure [16]. The percentage inhibition of paw volume was compared between control and both the extract. Acetylsalicylic acid (300mg /kg) was used as the standard.

2.8. Statistical Analysis

Statistical analysis and interpretation was done using one way analysis of variance followed by Dunnett's test. $P < 0.05$ were considered as significant.

3. RESULTS

3.1. Phytochemical Evaluation

The phytochemicals characteristics of petroleum ether and methanolic extract of the edible part of the fruit were tabulated in Table 1. Analysis of the edible part of fruit extracts revealed the presence of phytochemical such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids.

3.2. Acute Toxicity Studies

The concentration of methanolic and petroleum fruit extract of *Benincasa hispida* up to 2000 mg/kg body weight did not show any sign of toxicity and hence it was considered to be safe.

3.3. *In Vitro* Anti-Inflammatory Activity

To measure the anti inflammatory activity, the protection of membrane stabilization of RBC was

Table 1: Phytochemical Constituents of Petroleum Ether and Methanolic Extract of *Benincasa hispida* Fruit

Phytochemical	<i>Benincasa hispida</i> fruit	
	Petroleum ether extract	Methanol extract
Alkaloids	+ve	- ve
Flavonoid	+ ve	+ ve
Glycoside	- ve	+ ve
Saponine	- ve	- ve
Phytosteroid	+ ve	+ ve
Polyphenol	+ ve	+ ve
Triterpenoid	+ ve	+ ve

Note: (+ve) Indicates presence of phytoconstituent, (-ve) Indicate absence of phytoconstituent.

considered. The components present in the extract possibly have maintained the tonicity and may have balanced the membrane which prevented the inhibition of HRBC membrane lysis due to reduction of hypotonicity. The percent of membrane stabilization for methanolic and petroleum ether extracts were studied at different concentration (150, 250, 350 mg/ml). The percent protection of methanolic extract at concentration 350mg/ml for membrane stabilization

was found to be higher. The results were tabulated in Table 2.

3.4. *In Vivo* Anti-Inflammatory Activity

The methanolic and petroleum extract of *Benincasa hispida* fruit at different concentration showed significant reduction in the paw volume of rat. The methanolic extract at a concentration of 350mg/ml showed significant activity in comparison to the

Table 2: *In-Vitro* Anti-Inflammatory Activity of Fruit Extract of *Benincasa hispida*

Sr. No	Extract type(mg/ml)	Percentage of protection
1	Methanolic-150	12.16
2	Methanolic-250	15.74
3	Methanolic-350	17.31
4	Petroleum ether- 150	7.41
5	Petroleum ether- 250	10.04
6	Petroleum ether- 350	14.23
7	Ecosprin tablet(acetylsalicylic acid) -25	24.57

Table 3: *In-Vivo* Anti-Inflammatory Activity of Fruit Extract by *Benincasa hispida*, Carrageenan-Induced Rat Paw Edema

Group	Treatment Design	Dose(mg/kg)	Carrageenan induced oedma (volume in ml)				
			0 min	60 min	120 min	180min	240min
i	Induced control (1% carrageenan in saline)		1.05±0.08	1.26±0.09	1.45±0.08	1.53±0.13	1.75±0.14
ii	Ecosprin tablet (acetylsalicylic acid)	300	0.98±0.07	1.11±0.17	1.25±0.11*	1.10±0.10*	0.96±0.15*
iii	Methanolic extract	250	1.06±0.12 ^a	1.21±0.08	1.35±0.14	1.28±0.12*	1.23±0.05 ^{a*}
iv	Methanolic extract	350	1.03±0.46 ^a	1.13±0.11	1.23±0.11*	1.21±0.10*	1.18±0.08*
v	Petroleum ether extract	250	0.97±0.13	1.11±0.10	1.35±0.11	1.43±0.08 ^a	1.41±0.12 ^{a*}
vi	Petroleum ether extract	350	1.00±0.10	1.18±0.10	1.38±0.14	1.38±0.08 ^a	1.28±0.08 ^{a*}

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

reference standard Ecosprin. The results are presented in Table 3.

4. DISCUSSION

Inflammation is a response of living tissue towards injury, infection or irritation. During the inflammation process the activated neutrophils such as bactericidal enzymes and protease are released by lysosome which may cause various disorders. The activated neutrophils released enzymes can cause the tissue injury which may damage the macromolecules and lipid peroxidation of membranes that are assumed to be responsible for globally prevalence disease, such as atherosclerosis, rheumatoid arthritis and asthma. Phytochemical evaluation of petroleum extract and methanolic extract of *Benincasa hispida* revealed the presence of alkaloid, flavonoid, glycoside, phytosteroid, polyphenol and tryterpenoid.

In the present study, in-vitro anti-inflammatory assay was studied based on HRBC membrane stabilization method. As the erythrocyte membrane possesses a similar structure with the lysosomal membrane, [17] therefore the stability suggests that the extract may have maintained lysosomal membranes. The application of extract indicated the stability of lysosomal membrane that is important in restricting inflammatory response by inhibiting the release of lysosomal enzymes. The result indicates that fruit extract of *Benincasa hispida* has significant in-vitro anti-inflammatory property.

The carrageenan-induced inflammation is practical and significant model for the evaluation of in-vivo anti-inflammatory effect [17]. The expansion of carrageenan induced edema in the paw of rat is represented by a biphasic curve [18]. The first phase involves the release of histamine, 5-HT and kinins [19] along with platelet activating factor and arachidonic acid metabolites [20]. Edema is a result of the liberation of Prostaglandins (PGs) as they are the most significant factor in the advancement of the second phase of the reaction that is estimated at around 3 h times [21]. Fruit extract of *Benincasa hispida* showed a significant anti-inflammatory effect and it may be due to the inhibition of any inflammatory mediators by the glycoside or steroid [18]. Flavonoids, triterpenoids and polyphenols are active components widely found in the fruit extracts which may be responsible for anti-inflammatory activity [2, 13]. The present work reveals that the methanolic fruit extract of *Benincasa hispida* is a potential therapeutic agent for the treatment of inflammation.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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