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Research Article

ANTI-INFLAMMATORY ACTIVITY OF *CICER ARIETINUM* SEED EXTRACTS

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ABSTRACT

Inflammation is a part of complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells or irritants. *Cicer arietinum* which is generally consumed as a seed food is a good source of protein and traditionally used in pacifying the burning sensation in stomach, hepatomegali, stomatitis, inflammations, skin diseases and bronchitis. In present study, the anti-inflammatory potency of methanolic and ethanolic extracts of *Cicer arietinum* seeds at different doses were investigated against carrageenan and histamine induced paw edema in rats. The paw edema was measured using a digital plethysmometer. After performing the acute toxicity studies, no mortality was observed even at highest dose of 2 g/kg, p.o. So, we have selected two different doses 250 mg/kg and 500 mg/kg body weight of rats for the present study and they are administered orally. The results were analyzed by one way analysis of variance (ANOVA). Almost all the treatments of extracts showed a significicant $p<0.001$ anti-inflammatory activity when compared to control groups and with standard drug (Indomethacin 10 mg/kg. p.o). Both the methanolic and ethanolic extracts showed the dose dependant activity. Among these extracts, the methanolic 500 mg/kg and ethanolic 500 mg/kg extracts of *Cicer arietinum* showed maximum anti-inflammatory activity from 2nd to 5th hours. There by the findings concluded that *Cicer arietinum* seeds exhibit an anti-inflammatory activity and further studies were suggested to isolate the active principles responsible for the activity.

Key words: *Cicer arietinum*, Carrageenan, Histamine, Anti-inflammatory, Paw edema

INTRODUCTION

Inflammation is a basic way in which a body reacts to infection, irritation or other injury, the key feature being redness, warmth, swelling and pain. However inflammation is a stereotyped response and therefore it is considered as a mechanism of innate immunity as compared to adaptive immunity, which is specific for each pathogen[1]. At present, the drugs which are useful in treatment of this condition include narcotics e.g. opiods and non-narcotics e.g. salicylates. Almost all of these drugs were known to possess some of the common and deleterious side effects[2]. So, the purified drugs that are obtained from the natural sources can serve as better anti-inflammatory agents with low toxicity and high therapeutic value[3]. Natural products play a significant role in human health in relation to the prevention and treatment of inflammatory conditions[4]. One of such natural product which is used as an anti-inflammatory agent is *Cicer arietinum*.

Cicer arietinum which is most commonly called as chick pea or Bengal gram or Indian gram is an edible legume of the family Fabaceae. They are high in protein and one of the earliest cultivated vegetables; 7,500-year-old remains have been found in the Middle East[5]. *Cicer arietinum* is an annual, branching from the base, several stems erect, angular or winged, densely glandular-pubescent, as are also the leaves[6]. The seeds are typically colored; mostly brown somewhat angular shaped with a prominent characteristic "beak" that house the embryonic axis. Sometimes they are consumed whole as such. But most of the times, the seeds are decorticated and used[7]. The ethnomedical uses of seeds includes the treatment of conditions like hyperdipsia, burning sensation, leprosy, splenomegalopathy, pharyngitis, bronchitis, inflammation and skin diseases[8]. In current study, the desi variety seeds are taken which have a brownish seed coat over the seed.

Carrageenan-induced rat paw edema is a widely used test to determine the anti-inflammatory activity and it has been fully characterized in the past[9, 10, 11]. More recently, it has been shown that cyclooxygenase-2 (COX-2) reaches maximal expression 1 hour from carrageenan local injection[12]. The carrageenan induced paw edema is believed to be a biphasic response. The first phase is attributed to the release of histamine, serotonin and kinin and the second phase is related to the release of prostaglandin and bradykinins[13, 14]. All these mediators produce inflammation when they are injected subcutaneously in the rat paw[15].

Histamine is an important inflammatory mediator, potent vasodilator substance and also increases the vascular permeability[16]. So, it can be used as an inflammatory agent. Literature review also indicated that the anti-inflammatory property of *Cicer arietinum* has not been clinically evaluated so far. So the present study aimed to evaluate the anti-inflammatory potency of methanolic and ethanolic extracts of *Cicer arietinum* seeds at two different doses of 250 mg/kg and 500 mg/kg body weights against carrageenan and histamine induced inflammation.

MATERIALS AND METHODS

Plant material

Collection and authentication of plant materials

The seeds of *Cicer arietinum* (desi variety) belonging to the family Fabaceae were collected in May, 2011 at the local areas of Anantapur district, Andhra Pradesh, India. The plant material was identified and authenticated by Dr. J. Raveendra Reddy, M.Pharm., PhD, Department of Pharmacognosy, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur and voucher specimen (12/11) was preserved in Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, India.

Processing of sample

The seeds of *Cicer arietinum* (desi variety) were collected, pulverized into fine powder along with the husk and used for extraction.

Preparation of extracts

Preparation of methanolic extract of *Cicer arietinum* seeds

The powdered dried seeds were subjected to extraction process by maceration with methanol at room temperature for 48 hours with occasional stirring. The extract was filtered and concentrated to dryness at room temperature.

Preparation of ethanolic extract of *Cicer arietinum* seeds

The powdered seeds were loaded into the soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off and the extract was concentrated to dryness at room temperature.

Phytochemical analysis

The methanolic and ethanolic extracts of *Cicer arietinum* seeds were subjected to preliminary phytochemical screening.

Experimental animals

Male albino rats (Wistar strain) of 240–300 g were used to carry out the anti-inflammatory activity. The animals had free access to standard commercial diet and water *ad libitum* and were housed in cages under standard laboratory conditions i.e., 12:12 hour light/dark cycle at 25 ± 2^{0}C.

Acute toxicity studies

Each of the seed extracts of *Cicer arietinum,* up to a higher dose of 2 g/kg were administered orally to normal rats. During the first four hours after the drug administration, the animals were observed for gross behavioral changes. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, body weight and mortality were observed up to 14 days. No mortality observed with oral administration of all the extracts even at the highest dose of 2 g/kg, p.o. Institutional Animal Ethical Committee (IAEC) had approved the experimental protocol and care of animals was taken as per the guidelines of CPCSEA, Department of animal welfare, Government of India.

Ethical approval

The Institutional Animal Ethics Committee (878/ac/05/CPCSEA/013/2011) has approved the experimental protocol at Post Graduate Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh, India.

Drugs and chemicals

Indomethacin (Abbott Pvt. Ltd., Goa), Carrageenan (Hi Media Laboratories Pvt. Ltd., Mumbai), Histamine (Hi Media Laboratories Pvt. Ltd., Mumbai), Normal saline, Ethanol (Merck Specialities Pvt. Ltd., Mumbai) and Methanol (Merck Specialities Pvt. Ltd., Mumbai).

Anti-inflammatory activity

These extracts were tested for anti-inflammatory activity by carrageenan[17] and histamine[18] (inflammagens) induced paw edema method in rats.

Different groups of animals taken for experiment are as follows:

Control group

Group I (C-I): Control: Carrageenan (s.p) + Normal saline (p.o)

Group II (C-II): Control: Histamine (s.p) + Normal saline (p.o)

Standard group

Group III (SD-I): Standard: Carrageenan (s.p) + Indomethacin 10 mg/kg (p.o)

Group IV (SD-II): Standard: Histamine (s.p) + Indomethacin 10 mg/kg (p.o)

Test group

Group V (ME): Test: Carrageenan (s.p) + Methanolic extract 250 mg/kg (p.o)

Group VI (ME): Test: Carrageenan (s.p) + Methanolic extract 500 mg/kg (p.o)

Group VII (EE): Test: Carrageenan (s.p) + Ethanolic extract 250 mg/kg (p.o)

Group VIII (EE): Test: Carrageenan (s.p) + Ethanolic extract 500 mg/kg (p.o)

Group IX (ME): Test: Histamine (s.p) + Methanolic extract 250 mg/kg (p.o)

Group X (ME): Test: Histamine (s.p) + Methanolic extract 500 mg/kg (p.o)

Group XI (EE): Test: Histamine (s.p) + Ethanolic extract 250 mg/kg (p.o)

Group XII (EE): Test: Histamine (s.p) + Ethanolic extract 500 mg/kg (p.o)

Carrageenan-induced paw edema in rats

The animals were pre-treated with vehicle/indomethacin/extracts 30 minutes before the injection of 0.1 ml of 1% carrageenan into the s.p (sub-plantar) region in right hind paw to the rats. Paw volumes were measured by the dislocation of water column in a plethysmometer at 1, 2, 3, 4 and 5 hours after the administration of test materials. Reduction in the paw volume compared to the control animals was considered as the anti-inflammatory response.

Histamine induced paw edema in rats

The animals were pre-treated with vehicle/indomethacin/extracts 30 minutes before the injection of 0.1 ml of 1% histamine into the sub-plantar region in right hind paw to the rats[19]. Paw volumes were measured by the dislocation of water column in a plethysmometer at 1, 2, 3, 4 and 5 hours after the administration of test materials. Reduction in the paw volume compared to the control animals were considered as anti-inflammatory response.

Finally, the anti-inflammatory activity was calculated by using the percentage inhibition of edema which can be given by the formula,

% Inhibition of edema =

$$\frac{\text{Paw edema in control group – Paw edema in test group}}{\text{Paw edema in control group}} \times 100$$

In-vitro study

Heat induced haemolysis

20µL of uncoagulated fresh rat blood was added to vials containing 1 mL of 0.1 M PBS (Phosphate Buffered Saline, pH 7.4). Methanolic and ethanolic extracts were added to the vials (in triplicate), so as to achieve the final concentrations of 200, 250, 300, 350 and 400 µg/ml of each extract. PBS (1ml) and rat blood was added to control vials. Then the drug solutions were subjected to centrifugation at 3000 rpm in micro centrifuge for 10 minutes to make sure that all the materials had completely dissolved. No residues were ever observed after centrifugation indicating complete solubility of the drug preparations used. After mixing the contents in vials and pre-incubating at 37^{0}C for 15 minutes. Then the mixtures were heated for 25 minutes at 54^{0}C. After spinning down the precipitate, the absorbance of the supernatant was measured at 540 nm in a spectrophotometer. The percentage inhibition of haemolysis of test should be compared with respect to the control.

% Inhibition of haemolysis =

$$\frac{\text{Absorbance in control group – Absorbance in test group}}{\text{Absorbance in control group}} \times 100$$

Statistical analysis

The results were expressed as mean ± S.E.M. The differences were compared using one way analysis of variance (ANOVA) and subsequently followed by Bonferroni's test.

RESULTS

Physical properties of the extracts

The color, texture and the percentage yield of the methanolic and ethanolic extracts of *Cicer arietinum* were tabulated in table 1.

Table 1: Physical Properties Of *Cicer Arietinum* Seed Extracts.

Plant part	Type of extract	% yield	Texture	Colour
Cicer arietinum seeds	Methanolic extract	13.51	Gummy	Brownish
	Ethanolic extract	4.75	Oily gum	Yellowish brown

Phytochemical analysis

After subjecting to screening, both the methanolic and ethanolic extracts revealed the presence of carbohydrates, flavonoids and saponins, where as phenols and tannins are present only in methanolic extract. However, alkaloids and phytosterols were not detected in the seeds of the plant. The details of phytochemical constituents are given in table 2.

Table 2: Phytochemical Analysis of *Cicer Arietinum* Seed Extracts.

Phytochemicals	Methanolic extract	Ethanolic extract
Alkaloids	–	–
Carbohydrates	+	+
Flavanoids	+	+
Phenols	+	–
Phytosterols	–	–
Saponins	+	+
Tannins	+	–

+ indicates presence; – indicates absence of the phytochemical constituents which were screened using various identification tests.

Acute toxicity

The acute toxicity study revealed the non toxic nature of all the extracts even at a higher dose of 2 g/kg body weight of rats.

Anti-inflammatory activity

Effect on carrageenan-induced paw edema

The effect of methanolic and ethanolic extracts of *Cicer arietinum* on carrageenan-induced edema in albino rats is shown in the table 3. The results obtained indicate that both of the above extracts had significant anti-inflammatory activity in albino rats when compared with that of the control groups ($p<0.001$). The potency was found to be inversely proportional to the time taken for reduction in paw volume. The methanolic and ethanolic extracts showed the dose dependant activity. The methanolic extract of *Cicer arietinum* reduced edema 63.15%, near to the standard group induced by carrageenan on oral administration 500 mg/kg when compared with untreated control groups. Methanolic 250 and ethanolic 250, 500 mg/kg extracts shows 25.26, 17.89 and 56.84% inhibition respectively. Whereas the standard group shows 67.36% inhibition in paw edema.

Table 3: Effect of *Cicer Arietinum* Seed Extracts on Carrageenan-Induced Paw Edema in Rats.

Treatment groups (n=6)	Dose (mg/kg)	Paw edema (ml) Mean ± S.E.M (% inhibition)				
		1st hour	2nd hours	3rd hours	4th hours	5th hours
C-I	Saline 5 ml	0.60±0.008	0.95±0.012	1.02±0.008	0.90±0.005	0.82±0.008
SD-I	10	0.25±0.003** (58.33)	0.31±0.002** (67.36)	0.37±0.010** (63.72)	0.37±0.010** (58.88)	0.30±0.002** (63.41)
ME	250	0.40±0.002** (31.66)	0.71±0.011** (25.26)	0.80±0.008** (21.56)	0.80±0.006* (11.11)	0.66±0.005** (19.51)
ME	500	0.29±0.011** (51.66)	0.35±0.020** (63.15)	0.42±0.011** (58.82)	0.42±0.008** (53.33)	0.37±0.005** (54.87)
EE	250	0.51±0.005** (15.00)	0.78±0.005** (17.89)	0.88±0.005** (13.72)	0.87±0.006 (03.33)	0.69±0.005** (15.85)
EE	500	0.34±0.011** (43.33)	0.41±0.012** (56.84)	0.53±0.014** (48.03)	0.53±0.020** (41.11)	0.39±0.003** (52.43)

n=6, **p<0.001, *p<0.01 (significant) when compared to C-I; statistical analysis was done by one way analysis of variance (ANOVA) followed by Bonferroni's test.

Table 4: Effect of *Cicer Arietinum* Seed Extracts on Histamine Induced Paw Edema in Rats.

Treatment groups (n=6)	Dose (mg/kg)	Paw edema (ml) Mean ± S.E.M (% inhibition)				
		1st hour	2nd hours	3rd hours	4th hours	5th hours
C-II	Saline 5 ml	0.68±0.005	1.02±0.010	1.18±0.011	0.96±0.008	0.85±0.008
SD-II	10	0.30±0.003* (55.88)	0.39±0.003* (61.76)	0.49±0.003* (58.47)	0.49±0.005* (48.95)	0.36±0.003* (57.64)
ME	250	0.45±0.008* (33.82)	0.80±0.005* (21.56)	0.89±0.005* (24.57)	0.85±0.008* (11.45)	0.68±0.005* (20.00)
ME	500	0.28±0.005* (58.82)	0.37±0.008* (63.72)	0.47±0.003* (60.16)	0.45±0.005* (53.12)	0.34±0.005* (60.00)
EE	250	0.55±0.006* (19.11)	0.84±0.011* (17.64)	0.91±0.008* (22.88)	0.88±0.005* (08.33)	0.75±0.008* (11.76)
EE	500	0.30±0.003* (55.88)	0.39±0.005* (61.76)	0.49±0.012* (58.47)	0.47±0.008* (51.04)	0.39±0.005* (54.11)

n=6, *p<0.001 (significant) when compared to C-II; statistical analysis was done by one way analysis of variance (ANOVA) followed by Bonferroni's test.

Effect on histamine-induced paw edema

The effect of methanolic and ethanolic extracts of *Cicer arietinum* on histamine-induced edema in albino rats is shown in the table 4. The results obtained indicate that both of the above extracts had significant anti-inflammatory activity in albino rats when compared with that of the control groups ($p<0.001$). The potency was found to be inversely proportional to the time taken for reduction in paw volume. The methanolic and ethanolic extracts showed the dose dependant activity. The methanolic extract of *Cicer arietinum* reduced edema 63.72%, more than the standard group induced by histamine on oral administration 500 mg/kg when compared with untreated control groups and thus it is considered as a significant treatment. Methanolic 250 and ethanolic 250, 500 mg/kg extracts shows 24.57, 22.88 and 61.76% inhibition respectively. Whereas the ethanolic extract 500 mg/kg produced an inhibitory effect equal to that of the standard group which shows 61.76% inhibition in paw edema.

Effect on heat induced haemolysis

The effect of methanolic and ethanolic extracts of *Cicer arietinum* on heat induced haemolysis in albino rats is shown in the table 5. The results obtained indicate that both of the above extracts shows significant anti-inflammatory activity in albino rats by protecting the red blood cells from haemolysis. The methanolic and ethanolic extracts showed the dose dependant hemolytic protection. The methanolic extract of *Cicer arietinum* at 400 µg/ml inhibited hemolytic damage 99.72% which is nearly equal to standard group treated with indomethacin. Whereas the ethanolic extract also at its higher concentration 400 µg/ml produced 95.65% inhibition on heamolysis.

Table 5: Effect of *Cicer Arietinum* Seed Extracts on Heat Induced Haemolysis of Erythrocyte Membrane.

S. no	Concentration (µg/ml)	% Inhibition of Heat Induced Haemolysis		
		SD (indomethacin)	Methanolic extract	Ethanolic extract
1	200	42.26	49.64	40.36
2	250	68.74	71.23	65.67
3	300	89.62	90.26	86.46
4	350	98.32	98.86	92.34
5	400	99.69	99.72	95.65

SD indicates the standard drug.

DISCUSSION

Carrageenan-induced inflammation is most commonly used as an experimental model for evaluating the anti-inflammatory potency of compounds or natural products because it produces reproducible results[20]. Development of edema in the paw of the rats is due to the release of histamine, serotonin and prostaglandin like substances[21].

The knowledge of these mediators involved in different phases is an important step for interpreting the mode of drug action. The methanolic and ethanolic extracts of *Cicer arietinum* showed more significant reduction in paw edema at 2nd hours or more after when injected with carrageenan, suggesting that *Cicer arietinum* produces an anti-edematous effect during the second phase similar to that of the indomethacin.

Histamine which acts as a vasodilatory agent easily produces the inflammatory response. But by our study it was observed that histamine is not as effective as carrageenan in producing the inflammation to the paw of rats. Although it is less efficacious in producing inflammation, it is beneficial as an inflammagen in particular cases. The methanolic and ethanolic extracts of *Cicer arietinum* showed significant reduction in paw edema at 2nd hours or more with histamine injection, suggesting that *Cicer arietinum* shows reduction in edema which was caused due to the vasodilatory action of the histamine and here it can be better compared with that of the indomethacin.

Both the methanolic and ethanolic extracts of *Cicer arietinum* showed dose dependant anti-inflammatory activity. Among these, in both the cases of carrageenan and histamine induced inflammations, the methanolic extract 500 mg/kg showed more significant reduction in paw edema when compared to the other treatments.

So, the present activity of *Cicer arietinum* may be due to the presence of flavonoids, phenols and saponins in both the methanolic and ethanolic extracts. An extra presence of the tannins in methanolic extract may contribute somewhat major anti-edematous activity when compared to that of the ethanolic extract. This can be better supported by the previous studies which have showed that the flavanoids[22, 23, 24] and tannins[25] in plants attribute for the anti-inflammatory activity.

Apart from these invivo studies, an invitro study, i.e., the heat induced haemolysis is also performed on the methanolic and ethanolic extracts of *Cicer arietinum*. In this, lysosomes play a major role in the inflammatory reaction[26]. The vitality of cells depends upon the integrity of their membrane, exposure of RBCs to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin[27, 28]. 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[29]. As both the extracts of *Cicer arietinum* seeds significantly decreased the heat induced lysis of red blood cells, it may be inferred that stabilization of lysosomal membrane is one of the mechanisms by which the *Cicer arietinum* seed extracts mediates their anti-inflammatory activity.

Thus it can be concluded that the methanolic and the ethanolic extracts of the seeds of *Cicer arietinum* antagonizes the initial and late phases of inflammation that was caused due to carrageenan and another inflammatory mediator, histamine. But in these, the highly polar methanolic extract was more efficacious than the ethanolic extract. The results support the traditional use of this plant in inflammatory conditions and suggest the presence of biologically active compounds which may be worth for further investigation and elucidation. However further investigations are needed to explore the exact active constituents and mechanisms responsible for the anti-inflammatory activity which in turn may result in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

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