WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 8, 503-510 Research Article ISSN 2278 – 4357

ANTI-INFLAMMATORY ACTIVITY OF *SYZYGIUM CERASOIDEUM* LEAF EXTRACT BY CARRAGEENAN INDUCED PAW OEDEMA IN ALBINO RATS

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Article Received on
11 June 2018,

Revised on 01 July 2018,
Accepted on 22 July 2018

DOI: 10.20959/wjpps20188-12035

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ABSTRACT

Syzygium cerasoideum (Myrtaceae) is an Indian medical plant used traditionally for its anti rheumatic, hypoglycemia and rubifacient activities. It is also used in dysentery and the root infusion is applied over painful joints. It is used as pain reliever by Ayurveda, Siddha and Unani practitioners, but lacks scientific validation. The Anti-inflammatory property of the aqueous (AQSC) and methanolic extract (MESC) of *Syzygium cerasoideum* was evaluated using carrageenan induced paw oedema model in wistar albino rats. The AQSC and MESC leaf extracts at 200 and 400 mg/kg showed significantly ($P<0.001$) at 3rd and 4th h after administration. MESC at 400 mg/kg showed significant anti-inflammatory activity at 4th h, where it caused 55.95% inhibition. The Anti-inflammatory effect of both the extracts at 400 mg/kg *b.w.p.o* are comparable to that of standard drug Diclofenac (5 mg/kg *b.w.p.o*). The results of present study demonstrate that aqueous and the methanolic extracts of leaves of *Syzygium cerasoideum* possess significant anti-inflammatory potential and explicate justification of the use of this plant in the treatment of inflammatory disease conditions.

KEYWORDS: Syzygium Cerasoideum, Diclofenac, Carrageenan.

INTRODUCTION

Inflammation is the response to injury of cells and body tissues through different factors such as infection, chemicals, and thermal and mechanical injures.[1] Most of inflammatory drugs

now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythema, edema, and pain. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required.[2] Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the treatment of inflammation related diseases like arthritis, asthma, and cardiovascular disease.[3] Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for wide range of pain and inflammatory conditions.[4] However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes.[5-7] Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates.[8,9] Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects.

The plant *Syzygium cerasoideum* has a wide medicinal uses as anti rheumatic, hypoglycemic, rubifacient, back-bitter astringent given in dysentery and bronchitis. A concentrate of the root infusion is applied and rubbed over painful joints. Aerial parts exhibits hypoglycemic activity. *Syzygium cerasoideum* consist of phenolic compounds cyanidin, 3-gluciside, delphinidin 3- glucoside, ellagic acid, kaempferol, myricetin, quercetin, and rutin.[10] Based on the above findings, *Syzygium cerasoideum* leaf extract was evaluated for it's anti-inflammatory effects on experimental induced inflammation.

2. MATERIAL AND METHODS

2.1. *Plant material:* *Syzygium cerasoideum* belongs to Myrtaceae found in Uttar pradesh, Bihar, Assam, Orissa upto 600m and Western ghats upto 900m. The plant was collected and authenticated by Dr. Madhava setty, Sri venkateshwar university, Thirupathi were voucher specimen number 1320 was deposited for future reference.

2.2. *Preparation of extract:* The shade-dried leaves were ground homogenously using a mixer-grinder and approximately 100g of the powder subjected to Soxhlet extraction, for 16 hours using 5L of distilled water and 5L of 99.9% Methanol (Scientific OEM, Mumbai, India) as solvents. The dark green, semi-solid extracts obtained were made free from the solvents by placing them in an incubator at 60_C for 12 hours. The yield of the AQSC was 21% and that of the MESC was 12.298%. Previous toxicity studies of the leaves of *Syzygium*

cerasoideum did not show any toxicity and behavioral changes bin rats up to 2000 mg/kg p.o.(oral administration) dose, put ethanolic paper, hence doses of 200 and 400 mg/kg p.o. were selected for the present study.

2.3. *Animals:* Wistar rats (100-150g) were obtained from the Animal House, Dayananda sagar university, Bengaluru. They were housed at a temperature of 24 ± 2 C, 12-hour light/dark cycles, 35-60% humidity, in polypropylene cages, and fed a standard rodent diet with water ad libitum. Animals were deprived of food but not water 4 hours before the experiment.

2.4. *Drugs*: Diclofenac (Reckitt Benckiser, Gurgaon, India), and Carrageenan (sigma Chemicals,St.Louis, MO, USA) were procured from the respective companies and were used in the study.

2.5. *Ethical considerations*: Experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics committee of the DSU 606/20/c/CPCSEA conform to the 'Guidelines for care and use of animals in scientific research' (Indian National Science Academy 1998, Revised 2000).

2.6. *Carrageenan –induced rat paw edema model:* The procedure described by Di rosa. M *et al*[11],was used. Albino rats (Wistar strain) of either sex selected by random sampling technique weighing between 180-200gm body weight seleted for the study. They were divided into six groups of six animals each. Treatment schedule was as follows

Group I: Control (1% SCMC 10 ml /kg *p.o*).
Group II: Standard(Diclofenac 5mg/kg *p.o).*
Group III: Aqueos extract (AQSC) at the dose of 200mg/kg *b.w.p.*o.
Group IV: Aqueos extract (AQSC) at the dose of 400mg/kg *b.w.p.o.*
Group V: Methanolic extract (MESC) at the dose of 200mg/kg *b.w.p.o.*
Group VI: Methanolic extract (MESC) at the dose of 400mg/kg *b.w.p.o.*

The 200,400 mg/kg *b.w. p.o* dose of the AQSC and MESC of *Syzygium cerasoideum* Respectively. Carrageenan (0.1 mL of 1%) was injected into the sub plantar tissue of the right hind-paw of each rat. The paw volume was measured initially at 0, 1, 2, 3 and 4 hr after carrageenan injection using a plethysmometer (Biodevices, New Delhi, India). The difference between the initial and subsequent values gives the actual odema volume which was

compared with control. The inhibition of inflammation was calculated using the formula[12] % inhibition =100(1-Vt /Vc), Where Vc represents oedema volume in control and Vt oedema volume in group treated with test extracts.

2.7. *Estimation of median effective dose:* The median effective dose (ED50) values were estimated using Graph Pad Prism software version 5.03 (Graph Pad Software Inc., San Diego, CA, USA). The PI values obtained from the carrageenan induced paw edema model were initially normalized to percentage activity assuming that the maximal response (100%) is seen at the dose of 400 mg/kg and the minimal response (0%) is seen at the dose of 0 mg/kg of the AQSC and MESC. The log dose response curves were then generated using a normalized nonlinear regression curve model, and by interpolation of the log dose (best-fit value) 50% activity was obtained. The antilog of the obtained log dose produced the ED50 value.

2.8. *Statistical analysis:* Results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA). A p value < 0.05 was considered statistically significant.

3. RESULTS

3.1. *Carrageenan-induced paw edema model:* The AQSC, MESC of the leaf of *Syzygium cerasoideum* 200, 400 mg/kg *b.w*. *p.o* showed a dose-dependent, significant inhibition of carrageenan-induced rat paw edema from 0.5 hours to 3 hours following drug administration, compared to the control group.

The maximum PI of paw edema by the *Syzygium cerasoideum* was observed with AQSC and MESC leaf extracts at 200 and 400 mg/kg decreased oedema significantly (P<0.001) at 3rd and 4th h after administration. The MESC at 400mg/kg showed significant anti-inflammatory activity at 4thh, where it causes 55.95% inhibition are comparable to that of standard drug Diclofenac (5 mg/kg *b.w.p.o*). The ED50 values of the AQSC and the MESC were 28.91 mg/kg and 37.23 mg/kg, respectively.

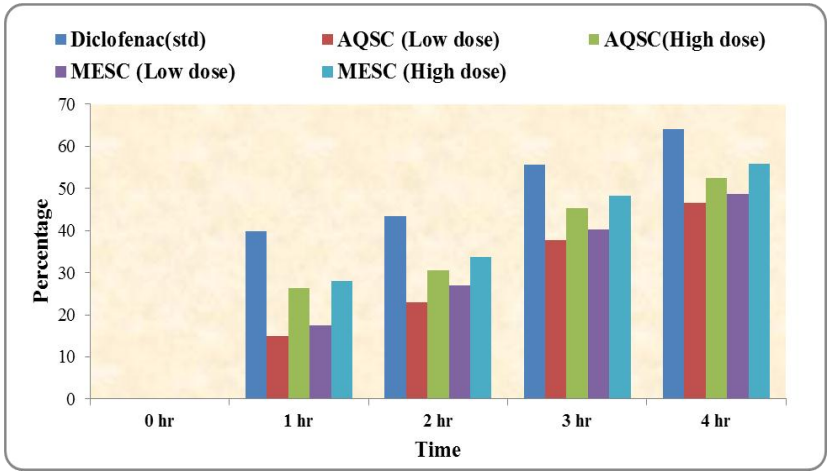
Evaluation of AQSC and MESC on carrageenan induced paw oedema (Percentage inhibition).

Groups	0 hour		1 hour		2 hour		3 hour		4 hour	
	Paw oedema (ml) Mean± SEM	% ROV	Paw oedema (ml) Mean± SEM	% ROV	Paw oedema (ml) Mean± SEM	% ROV	Paw oedema (ml) Mean± SEM	% ROV	Paw oedema (ml) Mean± SEM	% ROV
Control	0.1566±0.022	-	0.4182± 0.014	-	0.4882±0.022	-	0.5681±0.045	-	0.6531±0.058	-
Diclofenac 5mg/kg	0.1566±0.022	-	0.2500±0.002***	39.87	0.2666±0.020***	43.31	0.2366±0.011***	55.66	0.2182±0.007***	64.08
AQSC 100mg/kg	0.2050±0.008	-	0.3449±0.039$	14.89	0.3700±0.022***	22.87	0.3439±0.010***	37.70	0.3282±0.025***	46.65
AQSC 400mg/kg	0.1782±0.017	-	0.3049±0.025*	26.36	0.3349±0.028***	30.59	0.3016±0.030***	45.23	0.2882±0.012***	52.53
MESC 100mg/kg	0.2082±0.024	-	0.3366±0.018$	17.53	0.3466±0.012***	27.01	0.3182±0.016***	40.18	0.3115±0.005***	48.64
MESC 400mg/kg	0.1982±0.041	-	0.2931±0.018**	28.06	0.3165±0.012***	33.64	0.2815±0.017***	48.26	0.2665±0.010***	55.95

n=6, Significance at *P<0.05, **P<0.01, ***P<0.001 & $ not significant v/s control.

AQSC- Aqueous extract of *Syzygium cerasoideum* leafs

MESC- Methanolic extract of *Syzygium cerasoideum* leafs



Std-Standard drug, AQSC- Aqueous extract of Low dose-100, High dose-400, MESC- Methanolic extract of Low dose-100, High dose-400.

4. DISCUSSION

In this study, we evaluated the anti-inflammatory activity of the AQSC and MESC of the leaves of *Syzygium cerasoideum* by carrageenan-induced paw edema model. The carrageenan-induced paw edema model is used to screen the anti-inflammatory activity of a drug in the acute phase of inflammation. Edema induced by carrageenan is believed to be biphasic.[13] The first phase (1 hour) involves the release of serotonin and histamine and the second phase (> 1 hour) is mediated by cyclo oxygenase products. Continuity between the two phases is provided by kinin[14] The AQSC and MESC of the leaves of *Syzygium cerasoideum* significantly inhibited the edema formation in both the first and second phases. The anti-edematous activity of *Syzygium cerasoideum* in the first phase could be due to the possible suppression of histamine signaling by the mast cell stabilizing effect[15,16,17] and direct inhibition of histamine H1 receptor and histidine decarboxylase gene transcriptions[18] Another possible explanation could be the corticotrophic action of as evidenced by a raise in some plant species in plasma cortisol levels[19] which antagonizes nuclear factor-kappa-B (NFkB)[20] In the present study, the anti-edematous activity of the AQSC and MESC persisted in the second phase with the maximal effect observed at 3 and 4 hours.

It is difficult to attribute the observed effects of the leaf of *Syzygium cerasoideum* to any one particular chemical moiety. Flavonoids and saponins are known to exhibit their anti-inflammatory effect by several mechanisms[21,22] along with a wide spectrum of other pharmacological effects such as analgesic, antioxidant, antimicrobial, antiviral, anticancer, antidiabetic, and antiplatelet activities.[23] The anti-inflammatory activity of the leafs of *Syzygium cerasoideum* could be attributed to its flavonoids and saponins.

5. CONCLUSION

It can be concluded that the AQSC and the MESC of the leaves of *Syzygium cerasoideum* possess anti-inflammatory activity thus validating the ethno pharmacological claims. This knowledge could be tapped to formulate new agents to treat inflammatory and allergic ailments.

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