ANTI-INFLAMMATORY PROPERTIES OF ZANTHOXYLUM OVALIFOLIUM WEIGHT METHANOLIC EXTRACT ON ALBINO WISTAR RATS

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ABSTRACT

Objective: The object of the present study, the methanolic leaf extract of Zanthoxylum ovalifolium was examined for anti-inflammatory activity and tested using carrageenan-induced paw edema in albino Wistar rats.

Materials and Methods: The rats were grouped into five, each group consisting of six animals, Group I (negative control) received 1 ml of saline, Group II (standard) received 10 mg/kg, o. of indomethacin, and remaining three groups received three different doses (100, 200, and 400 mg/kg, p.o.) of the methanolic leaf extract after 1 h 0.1 ml of 1% carrageenan injection. The result of anti-inflammatory activity was set up to be dose-dependent in the carrageenan-induced paw edema model.

Results: The methanolic leaf extract has shown significant (p<0.001) inhibition of paw edema, 66%, 66.03%, and 69.29% on the 3rd hat the doses of 100, 200, and 400 mg/kg, p.o., respectively. The results were expressed as the Mean standard error of mean and statistical significance was analyzed by ANOVA followed by Dunnett’s test. The methanolic leaf of Z. ovalifolium extract showed significant anti-inflammatory activity compared with the standard drug indomethacin.

Conclusion: Methanolic leaf extract of Z. ovalifolium showed excellent results in the anti-inflammatory experiment so, the plant can be explored as a potential medicine in suppressing inflammation.

Keywords: Zanthoxylum ovalifolium, Methanolic leaf extract, Anti-inflammatory activity, Paw Edema, Carrageenan.

INTRODUCTION

Medicinal plants play a very important role in human life. The use of herbal plants is prevalent and has used for medicinal purposes long before the prehistoric period. In India, it has been reported that 2500 plants and 100 species were supplied as a source of traditional medicine [1]. Medicinal plants were widely practiced in traditional system of medicine on many accounts. People are using numerous medicinal plants and its derived products to cure and relief from various physical and mental disorders. Herbal plants were used in traditional system of medicine such as Ayurveda, Siddha, Unani, Chinese, and Tibetan medicines. The ancient literature Rigveda, Yajurveda, Atharva veda, Charaka Samhita, and Sushruta Samhita describes the use of medicinal plants for the treatment of various health issues [2].

Zanthoxylum genus belongs to the family Rutaceae and is commonly known as the citrus family, which has been composed of 160 genera and about 2070 species. Most plant species of this family are trees, shrubs, or herbs. Aromatic oil glands and sometimes thorns are present on the surface of the leaves. The aroma of the plant is due to the presence of hygienic oil cavities in the leaves and other young organs of the plant body.

The Zanthoxylum species are deciduous and evergreen shrubs and trees and they are inhabitants to warm temperate and subtropical region of the world [3]. Zanthoxylum is the largest genus consisting of about 250 species [4]. Many species of this genus have been largely studied and used in the traditional systems of medicine to cure many diseases such as cholera, colic asthma, cancer, snakebite, cold, microbial infections, diabetes, cough, fever, headache, and toothache [5]. On study, the species of Zanthoxylum have demonstrated antimicrobial, anti-inflammatory, analgesic, antimarial, and cytotoxic properties [6-10]. The plant Zanthoxylum ovalifolium is a large genus which is commonly called as Thorny Yellowwood. The plants are an aromatic tree, bark is smooth, greenish-brown and covered with scattered short and straight prickles [11]. Both simple and compound leaves present on each twig, leaf about 6-16.5 cm. Oil dots quite large, numerous and conspicuous. The small spiny structure presents on the lower surface of the leaves. Fruits are subglobose having about 6-7 mm in diameter. Plants are distributed in the Western Ghats, Himalayan region, Central, South, South-East, and East Asia. Most of the Zanthoxylum species are widely distributed over Asia but are not used as a spice throughout the region. The fruits of this plant are used to prepare pickles. The essential oil extracted from the plant has 2,4,6-Trimethoxy-Styrene compound which has biological properties [12].

Inflammation is a part of the biological system’s response to injury and infection; generally occur by physical, chemical, and biological agents either externally or internally. The usual sign of inflammation is pain, swelling, heat, and redness ultimately aiming to perform the dual role of reducing the damage and promoting tissue repair [13]. The inflammation is formed by cyclooxygenase (COX). There are two different types of COX, COX1, and COX2, both enzymes which synthesis (NSAIDs) are widely used as a medication to treat the inflammation. This NSAIDs drug reduces the inflammation by inhibiting both COX1 and COX2. As a result over use of these NSAIDs medication which leads to the adverse side effects and injure human biological system mainly affects the human organs such as liver, ulcer, gastrointestinal tract and kidney. [1, 6, 17]. Hence, there is a need to discover the alternative inflammatory medication which is most safe, less toxic, and potent from the unfavorable side effects. However, so far no study has been reported of anti-inflammatory. Hence, the present study was, therefore, undertaken to evaluate the anti-inflammatory activity of the methanolic leaf extract of Z. ovalifolium.

MATERIALS AND METHODS

In this present investigation, the selected plant Z. ovalifolium weight was collected from different forest region of Srigeri and Agumbe region in
Karnataka during July 2017. The plant was identified and authenticated
with the help of flora (Gamble JS). The herbarium specimen has been
deposited in the Department of Postgraduate Studies and Research in
Applied Botany Jnanasahyadri, Kuvempu University, Shankaraghatta.
The collected plant parts were dried for 1 week and ground to a coarse
powder with the help of a suitable grinder. The powder was stored in an
airtight container at room temperature before extraction.

Animal
Wistar rats of either sex of approximately the same age, weighing about
125–150 g were used for the study were housed in polypropylene cages
and fed with standard pellet diet and water ad libitum. The animals
were under an alternate cycle of 12 h of darkness and light each. Before
each test, the animals were fasted for at least 12 h. The experimental
protocols according to the Committee for the Purpose of Control and
Supervision of Experiments on Animals guidelines and Institutional
Animal Ethics Committee (IAEC) clearance were taken before the
commencement of the study. Ethical guidelines governing the use
of animals for conducting experiments was strictly followed and was
approved by NITTE Ethical Committee (NGSM/14/2017).

Acute toxicity studies
According Organisation for Economic Co-operation and Development
guidelines 425 the animals were divided into control and test groups
containing six animals each. The control group received the vehicle
(1% acacia) while the test groups received graded doses (5, 50, 100,
500, and 5000 mg/kg) of different extracts orally and were observed
for mortality until 48 h and found safe up to the last dose the lethal dose
for 50% [18] was calculated by taking 5000 mg/kg as a maximum dose
1/10 was selected as a therapeutic dose.

Anti-inflammatory activity by carrageenan-induced rat paw
edema model
The animals were divided into five groups each group consisting of six
rats. Group I (negative control) received 1 ml of normal saline, Group II
(Standard) received 10 mg/kg p. o. indomethacin, and Groups III–V
received methanolic leaf extract of Z. ovalifolium (100, 200, and
400 mg/kg, p. o.) of the sample, respectively. After 1 h, the rats were
challenged with subcutaneous injection of 0.1 ml of 1% w/v solution of
carrageenan (Sigma chemical co, StLouis MO, USA) into the subplantar
side of the left hind paw [19]. The paw was marked in the cup of the
apparatus with water. The rat paw volume was measured using
digital plethysmograph by the mercury displacement method. The
paw volume was measured immediately after injection (0 h) and then
the same procedure was repeated every hour until 3 h after injection
of carrageenan to each group. The difference between the initial and
subsequent reading gave the actual edema volume.

Percent inhibition of inflammation was calculated using the formula,

\[
\% \text{edema inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

Where, \(V_c\) = edema volume in control and \(V_t\) = edema volume in the test
extracts treated group [20].

 Statistical analysis
Data obtained were studied statistically by calculating standard error of
the mean (SEM) with respect to the control group for each participating
group. Result of anti-inflammatory activity of the methanolic leaf
effluents of Z. ovalifolium by carrageenan-induced paw edema method
(Table 1).

RESULTS
Acute toxicity studies
The methanolic leaf extract of Z. ovalifolium did not show any sign of
toxicity up to 5000 mg/kg body weight, and hence, it was considered
to be safe.

Anti-inflammatory activity
The present in vivo anti-inflammatory experiment was carried out by
carrageenan-induced paw edema method. The effect of the methanolic
extract on carrageenan-induced in rats is shown in Table 1 and Fig. 1.
Here, Group 1 served as a control, Group 2 received 10 mg/kg p. o. of
standard indomethacin, and Group 3 of experimental animals receive
a dose of (100, 200, and 400 mg) of Z. ovalifolium extract. The paw
volume was measured for control, standard and three different dose
levels of test crude methanolic at 1 h interval for 4 h which is tabulated in
Table 1.

From Table 1 and Fig. 2, the methanolic leaf extract of Z. ovalifolium
showed significant anti-inflammatory activity on carrageenan-induced
paw edema and the results were comparable to that of control. It
was observed that decreasing inflammation of paw volume after
administration of the extract. The methanolic leaf extract at a dosage
of 100 and 200 mg/kg p. o. observed inhibition of paw edema 17.26%,
56.53%, 50%, and 66% and 29.49%, 54.06%, 52.61%, and 66.03% at
0, 1, 2, and 4 h correspondingly. At the highest dose level of leaf
effluent 400 mg/kg p. o. result obtained was 10.07%, 60.07%, 61.76%,
and 69.29% at 0, 1, 2, and 3 h, respectively. At 4 h of dosage 400 mg/kg p.
o. was seen 69.29%, p<0.01, while the standard drug indomethacin
at a dose of 10 mg/kg was produced inhibition of paw edema 13.66%,
46.99%, 50.98%, and 66.03% at 1, 2, 3, and 4 h, respectively.

DISCUSSION
Inflammation is a vital part of the immune system’s response to
stimuli, for example, pathogens, damaged cells, or irritants. The
swelling, heat, redness, immobility, mouth sore, joint pain, abdominal
pain, and fever are the symptoms of the inflammation. Inflammation
is differentiated into two types, acute and chronic inflammation.
Nowadays, inflammation is treated by NSAIDs. However, as a result
of long-term use of this NSAID damage human organic system and
associated with adverse effects, high blood pressure, skin problems,
reducing liver and kidney functions, diarrhea, gastrointestinal damage,
and heart failure. There is been report that inflammatory response
directly related to chronic diseases such as cardiovascular diseases,
rheumatoid arthritis, and many types of cancer [21]. There is a
direct link between anti-inflammatory activities with the anti-tumor
activity [22]. Now, there is a need for alternative therapeutic selection
to establish anti-inflammatory drug as they are safe, less toxic, effective,
inflammation suppression. In the initial hour, there is no significant
The treated rats were observed for 3 h to evaluate the percentage of
different compounds present in the crude extract directly responsible for
anti-inflammatory, antioxidant, analgesic, and anticancer [25]. Hence, the
show a number of pharmacological activities such as antimicrobial,
ring. Depending on the substituted present in it, the compounds
produced dose-dependent and significant (p<0.001) inhibition of
carrageenan-induced paw edema at the 3rd h (69.29% at 200 mg/kg) over
a period of 3 h. The different aliphatic and aromatic compounds present in
in treating inflammation [5,6], this plant Z. ovalifolium is unknown as an
values obtained from the anti-inflammatory activity of Z. ovalifolium is subjected to statistical analysis by one-way ANOVA using Dunnett’s
c ontrol of inflammation. Further work is needed to place the active
The result of the present study on the methanolic leaf extraction of
important for further postclinical studies.
CONCLUSIONS
Many medicinal plants were used in the traditional Indian system of
medicine. These medicinal plants are rich sources of bioactive compounds, used for treating many different kinds of diseases. Herbal
medicine of anti-inflammatory drugs can be a good option to avoid adverse side effect which is caused by a synthetic one.
The result of the present study on the methanolic leaf extraction of
Z. ovalifolium possesses significant anti-inflammatory activity by
carrageenan-induced paw edema method. Therefore, the present study scientifically confirmed the use of Z. ovalifolium using as a traditional
medicine of inflammation. Further work is needed to place the active
principle from the methanolic leaf extract of Z. ovalifolium and its
phystopharmaceutical studies.
CONFLICTS OF INTEREST
There are no conflicts of interest.
AUTHOR’S CONTRIBUTIONS
Pavani carried out the experiment and wrote the manuscript meanwhile, Dr. Raja Naika helped supervise the project and conceived the original.
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