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Research Article

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 p. 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 h at 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.

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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 Tibetian 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 lysigenous 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, antimalarial, 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 the prostaglandins [14,15]. Nonsteroidal anti-inflammatory drugs (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. [16,17]. 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 Sringeri 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 $1/10^{th}$ 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, St. Louis 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,

$$\% \text{ edema inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc=edema volume in control and Vt=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 extracts 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 extract 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^{th} h of dosage 400 mg/kg p. o. was seen 69.29%, $p<0.001$, 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,

Table 1: Effects on methanolic extract of *Z. ovalifolium* on carrageenan-induced paw edema in Wistar rats

Treatment (mg/kg)	Mean increase in paw volume (ml) (%)				% decrease in paw volume in 3 h
	0 h	1 h	2 h	3 h	
Control	0.139±0.0077	0.283±0.0161	0.306±0.0189	0.368±0.0136	-
STD	0.12±0.0059 (13.66)	0.15±0.0072 (46.99)	0.15±0.0075 (50.98)	0.125±0.0067** (66.03)	50
ZO-100	0.115±0.0469 (17.26)	0.123±0.0504 (56.04)	0.153±0.0626 (50)	0.125±0.051* (66)	52
ZO-200	0.098±0.0401 (29.49)	0.13±0.0531 (54.06)	0.145±0.0592 (52.61)	0.11±0.0449* (66.03)	56
ZO-400	0.125±0.051 (10.07)	0.113±0.0463 (60.07)	0.117±0.0476** (61.76)	0.113±0.0463*** (69.29)	57

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett's test and differences between means were regarded significant at P<0.015*, P<0.01**, and P<0.001***, *Z. ovalifolium: Zanthoxylum ovalifolium*, SEM: Standard error of mean

Figure 1: *Zanthoxylum ovalifolium* plant

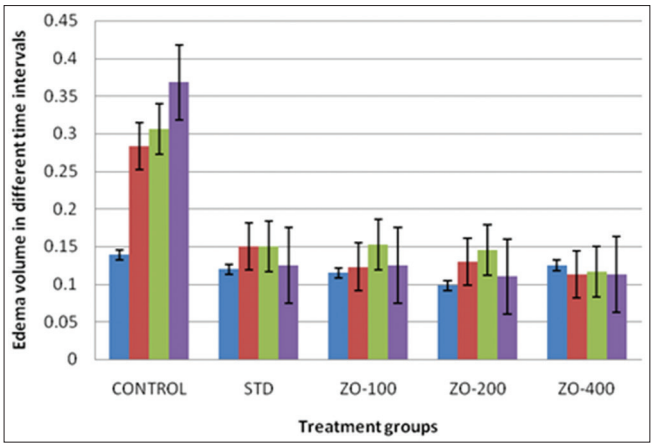

Fig. 2: Anti-inflammatory activity of *Zanthoxylum ovalifolium* methanolic extracts in carrageenan-induced paw edema method

and abundantly available, moreover, being reasonably affordable. Therefore, plants medicine is very much important in developing countries as primary health care. The present study was conducted to evaluate anti-inflammatory activity of *Z. ovalifolium* methanolic leaf extract using carrageenan-induced rat hind paw edema.

Carrageenan-induced paw edema inflammation is one of the most satisfactory animal test procedures to screen anti-inflammatory drugs. The time course of edema development in carrageenan-induced edema is reported as biphasic event [23]. The beginning phase inflammation occurs within 1 h of carrageenan injection and is characteristic to the release of cytoplasm enzymes, histamine, and serotonin from the mast cells. The late phase (>1 h) carrageenan-induced paw edema in animals is very sensitive to cyclooxygenase and is continued by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandins produced by tissue macrophages [24]. The *Z. ovalifolium* 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 the crude extract have different substitutes in different positions on the ring. Depending on the substituted present in it, the compounds show a number of pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, analgesic, and anticancer [25]. Hence, the different compounds present in the crude extract directly responsible for the positive anti-inflammatory activity in the crude extract.

The treated rats were observed for 3 h to evaluate the percentage of inflammation suppression. In the initial hour, there is no significant inflammation suppression observed in the extracts treated rats, but after successive hours there is noticeable inflammation suppression.

The methanolic extract of *Z. ovalifolium* at 100 and 200 mg/kg p. o. showed moderate inflammation suppression which is not comparable with the standard indomethacin, but in 400 mg/kg p. o. There is a drastic reduction of hind paw edema in the carrageenan treated animals which is more significant than that of standard indomethacin results (Table 1).

From the results, it is clear that the anti-inflammatory activity of *Z. ovalifolium* methanolic extract is concentration dependent, i.e., anti-inflammatory activity increase with the increase in the concentration. Acute toxicity results of this methanolic extract also tell that dose can be used up to 5000 mg/kg body weight so it is medicinally important for further postclinical studies.

The traditional herbal healer from India used other species of *Zanthoxylum* genus such as *Zanthoxylum armatum, Zanthoxylum rhoifolium, Zanthoxylum nitidum, Zanthoxylum chalybeum, Zanthoxylum schinifolium, Zanthoxylum Riedelianum,* and *Zanthoxylum usambarense* in treating inflammation [5,6], this plant *Z. ovalifolium* is unknown as an anti-inflammatory agent and other biological properties.

The values obtained from the anti-inflammatory activity of *Z. ovalifolium* is subjected to statistical analysis by one-way ANOVA using Dunnett's multiple comparison test obtained significant values for the methanolic extract at 400 mg/kg as well as for standard. The values were triplicated and expressed in terms of mean±SEM and $p<0.015$*, $p<0.01$**, and $p<0.001$ were considered as significance value.

The values were subjected to graphical analysis by plotting treatment groups along X-axis and reduction of paw edema in the different time intervals along Y-axis got an inclined exponential curve showed that increase in the concentration also increases the inflammation suppression.

CONCLUSION

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 phytopharmaceutical 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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