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Research Article

Evaluation of Anti-inflammatory Activity of *Trichosanthes dioica* Roxb. Seeds

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ABSTRACT

Inflammation a complex disease associated with various symptoms. These symptoms can be managed with non-steroidal and steroidal anti-inflammatory drugs, which lead to severe side effects on long-term use. Natural products are used as an alternative source of the discovery of new entities to be used as compounds anti-inflammatory compounds to overcome this problem. *Trichosanthes diocia (T. diocia)* belongs to Cucurbitaceae family. *T. dioica,* a dioecious perennial herbaceous vegetable, is a rich source of vitamin C and minerals. In addition to fruits, leaves and tender shoots have been used in the indigenous system of medicine. The objective of the present study was to evaluate the anti-inflammatory activity of methanolic extract of *T. dioica* seeds for scientific validation of the folklore claim of the plant

The extract was evaluated at three different concentrations of 100, 200, and 400 mg/kg, p.o. Significant dose-dependent anti-inflammatory activity was observed, comparable with that of the standard drug indomethacin at a dose of 10 mg/kg.

Introduction

Inflammation, a great challenge for humanity, consists of several symptoms evoked by ailment affecting the human body.[1] It activates the host defense mechanism, a necessary process that assists organisms to react to harmful and injurious stimuli[2] like irritants, pathogens, chemical and physical assaults, including immunological responses.[3] Human health care has a great dilemma in understanding the processes associated with inflammation.[4]

Exhibition of inflammation produces doleful conditions like redness, fever, pain, and swelling with some changes in the skin covering at the site,[5] which needs timely pharmacological treatment.[6] The induction process is initiated by the release of various mediators like leukotrienes, prostaglandin, cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), histamine, nitric oxide, serotonin, tumor necrosis factor-alpha (TNF-α), gamma (IFN-γ),[2], substance P and platelet-activating factor.[7]

Excessive production of these mediators results in chronic hepatitis, rheumatoid arthritis, pulmonary fibrosis, and atherosclerosis being a major target for treating inflammatory disorders.[8] Further physiopathology of inflammation and the role of free radicals in the pathogenesis of the disease is greatly emphasized for studies over decades. Free radicals produce reactive oxygen species (ROS) by acting on molecular oxygen, which increases inflammation, creating an imbalance between the body's antioxidant system and oxidizing molecules, causing oxidative stress. This oxidative stress causes inflammatory cascades resulting in the damage of cellular components.[9] Blocking of these causative agents

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of inflammation is considered a potential candidate to be used as anti-inflammatory drugs.

Clinically steroidal and non-steroidal steroidal agents (NSAIDs) have been employed therapeutically to treat these inflammatory diseases.[10] NSAIDs express their effects by blocking the metabolism of arachidonic acid by lipoxygenase and cyclooxygenase enzyme pathways.[11] Associated with the drugs' controversial multiple side effects, an hour is needed to develop alternative safer new compounds discovered from plants with better therapeutic index with fewer side effects. According to the WHO report, 70–80% of the world population is based on nonconventional medicine derived from plant sources as their primary health care system.[12,13] As in emergent nations price of medicines and the cost of consulting a physician are beyond the limit of most people,[14] drugs derived from plants appear as a boon for treating various ailments.

It is worth mentioning here that Cucurbitace family plants have gained importance due to their high nutritive value. *T. dioica* (pointed gourd), cucurbita species is an important medicinal plant with synonyms patol, potala, sespadula, and parwal.[15] It is an important ingredient of preparations stew, curry and soup. Sometimes it is eaten as fried and as dolma with meat stuffing, fish and roe.[16] The fruits are diuretic and have anti-ulcer effects.[17] Sap of leaves was utilized to cure the expansion of liver and spleen and be used as tonic and febrifuge. Its leaves and fruits cure jaundice and treat alcoholism. Leaves are used as antimicrobial, anti-pyretic, and cardio tonic. They are employed in the treatment of edema, epilepsy, alopecia and skin disease.[18]

T. dioica possesses alkaloids, flavonoids, cucurbitacins, proteins, amino acids, triterpenoids, and phytosterols responsible for their pharmacological activities like antioxidant, anti-inflammatory, and ameliorative anti-pyretic, antidiarrheal, antimicrobial, cholesterol-lowering, hypoglycemic, and wound healing, anti-worm, and laxative action. The fruits and leaves are used to treat edema and inflammation ethno pharmacologically (Shaarangadhara Samhita). Seeds are generally thrown and used as byproducts. Based on literature and traditional uses of the plant, the seeds are reported to have anti-hyperglycemic properties. As no scientific data was available for the anti-inflammatory activity in seeds, the present study was hypothesized and designed to evaluate the potent anti-inflammatory potential of seeds in experimental models

Materials and Methods

Plant Material

The fully ripe fruits of *T. dioica* have been taken from the nearby grain market of Chandigarh in the month of September.2020. After cleaning, washing, and drying, healthy fruit seeds were collected. The verification was carried out by the Department of Botany and Environment, Guru Nanak Dev University, Amritsar, Punjab. A voucher specimen used in the present study (DUL.Sc1811) was deposited for record at the department's Herbarium.

Drug and Chemicals

The drug carrageenan was purchased from S. D. Fine-Chemicals, and other chemicals were purchased from commercial sources of analytical grade. All drug solutions which were used in the study were freshly prepared before use.

Extract Preparation

The coarse crude seeds were cleaned, shade dried, and grounded to powder. The powder was immersed in methanol using the cold maceration method for 72 hours. After extraction, filtration has been done by employing a Whatman No.1 filter paper, and solvent has been evaporated to dryness under reduced pressure using a rotary evaporator.

Animal Care and Handling

Albino Wistar rats healthy rats (female or male) weighting range 150–200 g were obtained from Central Research Institute, Kasauli, India. The animals used in the study has been housed in standard metal cages under controlled environmental temperature (23 ± 2)°C, humidity (50 ± 5)%, and were fed with a standard laboratory diet obtained from (Kisan Feeds Ltd., Mumbai, India) and water *ad libitum*. Light dark cycle for 12 hours has been perpetuated during the experimental protocol. The semi-sound proof laboratory has been utilized to carry out the experiments. The observer was blind to the treatment group assignment. The approval of the experiment protocol was taken from the institutional animal ethical committee (IAEC) and care of the animals done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. IAEC/CCP/19/PR-12).

Anti-inflammatory Activity

Footpad edema, or paw swelling, is one of the most suitable techniques for estimating inflammatory responses to irritants and antigenic trials. Here, test extract at different doses is estimating for acute anti-inflammatory activity. Prevention of carrageenan-induced paw swelling indicates the occurrence of the activity. This study relates to indicating the therapeutic effect of the anti-inflammatory activity of this medicinal plant.[19] The *in vivo* acute anti-inflammatory screening was performed to specific doses of methanol extract by employing the functional model Carrageenan-induced rat paw edema, and the percentage inhibition of edema is expressed at the right hind paw in contrast to the control. Carrageenan-induced edema is a nonspecific inflammation however is extremely sensitive

to NSAIDs. A reference standard, indomethacin was used, which is a potent NSAID. An increase in prostaglandin (PG-E2) level is attributed to the extremity of the inflammatory response to pain in this model. Winter et al., (1962) procedure was used to investigate the anti-inflammatory effect of indomethacin (standard) and methanol extracts on Carrageenan-induced rat paw edema in albino rats.[20]

Carrageenan-induced Rat Paw Edema

The selected model contained six groups, and every group has six animals. Group I functioned as the vehicle control group (1% CMC, p.o.), group II functioned as a disease control group (0.1 mL of carrageenan (1%) injected at the plantar surface of the right hind paw), and group III behaves as a reference standard (indomethacin, 10 mg/kg, p.o.) for estimating comparative pharmacological importance. In addition to this, groups IV-VI present as MeTD (methanol extract of *T. diocia* seeds) pre-administered at different dose levels (100, 200 and 400 mg/kg, p.o.). In group III-VI pretreatment to indomethacin, distinct doses of MeTD were conducted before 1-hour of the carrageenan administration. The paw volume was measured plethysmo-graphically immediately after injection at 1, 2, and 3 hours intervals.[21] The edema was expressed as an increase in paw volume. After that percentage of inhibition of edema was determined for every group concerning the control group as follows:

Percentage of inhibition of paw edema = (1- Vt/Vc) ×100

Where Vc and Vt represent the average paw volume of control and drug-treated animals, respectively.[22]

Statistical Analysis

Statistical evaluation was done by presenting the values as mean ± SEM. One-way ANOVA followed by Student's t-test has been employed to calculate the statistical significance of the difference between the control groups and the test compounds. A value of $p < 0.01$ was believed to be significant.

Results

For the acute phase of inflammation, carrageenan-induced paw-volume increase and the effects of the indomethacin and methanolic extract of *T. diocia* seeds were analyzed. As the most potent effect was observed in the third hour compared to others in a dose-dependent manner, statistical data for that hour was considered to evaluate the acute anti-inflammatory effect. Analysis of data cleared that indomethacin was the most effective drug with (percentage inhibition of edema to 76% of acute anti-inflammatory effect) and the most efficient MeTD extract has been observed to be potent at a dose of 400 mg/kg body wt., with (percentage inhibition of edema to 59% of acute anti-inflammatory effect). The same extract exhibited (percentage inhibition of edema to 21% of acute anti-inflammatory effect) at the dose of 100 mg/kg body wt. (Table 1, Fig. 1) It is clear from the table that although MeTD extract had an anti-inflammatory effect, it was weaker when compared with the standard drug indomethacin.

Discussion and Conclusion

Inflammatory conditions are accepted as one of the most identified reasons for co-morbidity among the population.[23] Inflammation occurs in three phases, the first phase specifies enhancement in vascular permeability, the second phase shows infiltration of leucocytes, and the third indicates granuloma formation.[5] Current treatment of inflammation is by NSAIDs, leading to enhanced risk of blood clots consequence in strokes and heart attacks. As a result, natural products these days are considered a better source for the treatment of inflammation.[19]

With the repeated use of synthetic chemicals, more side effects were noticed, reflecting the growing interest in using herbal drugs.[23] This fact is now well accepted that these drugs are considered a boon for treating various diseases. However, clinically, therapeutically,

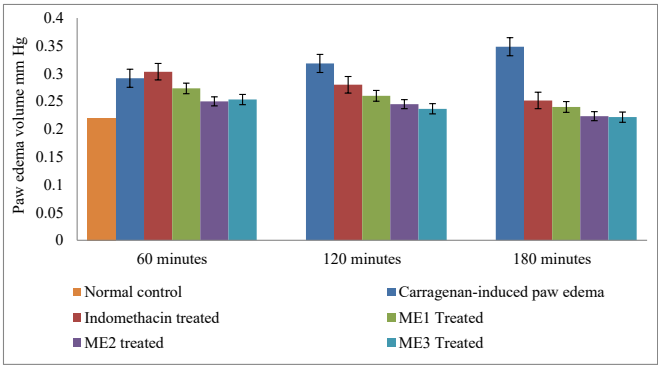

Fig. 1: Anti-inflammatory influences of different methanolic extracts (MeTD) of *T. dioica* seeds on carrageenan-induced paw edema.

Table 1: Effect of methanolic extract of *T. diocia* seeds (MeTD) in Carrageenan- induced paw edema in rats.

Groups	*1 hrs (60 mins)*	*2 hrs (120 mins)*	*3 hrs (180 mins)*
Control	0.22 ± 0.0	0.22 ± 0.0	0.22 ±0.0
Carrageenan-induced paw-edema	0.29 ± 0.015[a]	0.318 ± 0.028[a]	0.348 ± 0.029[a]
Indomethacin treated rats	0.33 ± 0.031[b]	0.28 ± 0.019[b]	0.251 ± 0.025[b]
MeTD1 (100mg/kg)	0.27 ± 0.016	0.26 ± 0.011	0.24 ± 0.014
MeTD2 (200mg/kg)	0.25 ± 0.036	0.245 ± 0.022	0.23 ± 0.022
MeTD3 (400mg/kg)	0.25 ± 0.016	0.23 ± 0.012	0.22 + 0.019

Values are mean ± SEM. Sample size = 6; p < 0.01 vs Control.

Table 2: Inhibition of inflammation vs doses of indomethacin and extract (%).

Sr. No.	*Treatment*	*Dose (mg/kg)*	*Average % inhibition of inflammation (%)*
1	Indomethacin	10	76
3	MeTD1	100	21
3	MeTD2	200	36
4	MeTD3	400	59

and diagnostically, this herbal system does not correlate well with the modern system of medicine. As disease classification is emphasized on symptoms, it becomes difficult to evaluate pharmacological activity about the traditionally prescribed use of indigenous drugs. Nevertheless, presumptions based on the traditional use of natural drugs with specific beneficial activity have been regarded as a successful approach in medicinal plant research.[24]

Combining natural products with modern medicine leads to the production of the most suitable drugs in treating various diseases like diabetes mellitus, pepulcerslcer, bronchial asthma, hypertension, and inflammation.[20] The emphasis of the study was to ascertain the scientific foundation for the folk utilization of *T. diocia* seeds. The present data illustrates, for the first time, that systemic use of the MeTD seed extract at different doses that indicated anti-inflammatory effects in Carrageenan-induced rat paw edema model of inflammation. To justify the anti-inflammatory activity of MeTD we determined the influences of MeTD treatment on the carrageenan-induced paw edema in rats served as the foremost eminent experimental model in search for noble evaluation of anti-inflammatory influences of natural products.[10] Carrageenan has very strong chemical releases inflammatory and pro-inflammatory mediators, causing an acute and local inflammatory response and is used to prove the efficacy of orally active anti-inflammatory agents.[24]

However, it is also sensitive to cyclooxygenase inhibitors making it useful in evaluating NSAIDs.[18] Results here clearly infers that anti-inflammatory activity of the methanolic extract of the seeds of *T. diocia* showing inhibition range from 21 to 59%, which is, however, lower than 76% of that of indomethacin, a standard drug for anti-inflammatory effect (Table 2). Hence it is clear that methanolic extract of *T. diocia* seeds possessed distinct anti-inflammatory activity. Recent studies with other plant extracts have shown that phytochemical constituents like flavanoids, triterpenoids, and phytosterols are known to promote anti-inflammatory activity. Preliminary phytochemical screening of methanolic extract of seeds of *T. diocia* showed the presence of flavanoids, triterpenoids, and phytosterols, and these constituents may be responsible for the remarkable dose-dependent anti-inflammatory activity of the plant. Hence, from the results data, it is concluded that methanolic extract of T. diocia seeds has properties of being potent moiety for promoting anti-inflammatory activity to support the presumed utilization of this plant species in folklore claims.

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