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ANTI-INFLAMMATORY ACTIVITY OF *TRICHOLEPIS GLABERRIMA* ON CARRAGEENAN-INDUCED RAT PAW OEDEMA MODEL

Veena Rani I.*, Kiranmai G. and Hafza Meeraj

Department of Pharmacology, SSJ College of Pharmacy, Vattinagulapally, Hyderabad-90.

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***Corresponding Author**
Veena Rani I.
Department of Pharmacology, SSJ College of Pharmacy, Vattinagulapally, Hyderabad-90.

ABSTRACT

Methanolic and aqueous extract of *Tricholepis glaberrima* were investigated for anti-inflammatory activity by carrageenan induced right hind rat paw odema method in albino rats at the dose of 100mg/kg, 200mg/kg and 400mg/kg, p.o. (per orally). In vivo anti-inflammatory activity by carrageenan-induced rat paw oedema model was selected to induce the inflammation. The methanolic and aqueous extract of aerial parts of *Tricholepis glaberrima* showed significant anti-inflammatory activity at the dose of 200mg and 400mg/kg body weight when compared to control and standard. According to the results of the present investigation, it can be concluded that the different doses of methanolic and aqueous extracts of *Tricholepis glaberrima* have been shown to be effective against carrageenan induced paw oedema in rats. There is no doubt that the extracts of *Tricholepis glaberrima* possess significant anti-inflammatory activity which has confirmed in our study.

KEYWORDS: Anti-inflammatory activity, *Tricholepis glaberrima*, carrageenan, diclofenac sodium.

INTRODUCTION

Inflammation is defined as a local and protective response of living mammalian tissues to injury or wound due to any harmful stimuli or agent.[1] It is body defense mechanism in order to eliminate or limit the spread of injurious agent followed by removal of dead cells and tissues and initiates heal and repair.[2] It is a basic way in which body reacts against injury, irritation and other stimuli. Tissue damage caused by wound or invading pathogenic

organisms induces a complex sequence of events during inflammation.[3] It is the body's immune response.[4]

It is protective mechanism of body against invasive organisms which eventually lead to redness, pain, swelling, and temperature that evokes inflammatory cells namely., macrophages, monocytes, neutrophils, and mast cell to invade at the site of wound or injury establishing an inflammatory microenvironment, that leads to the death and degradation of the organism, agent or affected cells and eventually restoration of cellular or organ repair process.[5]

Inflammation is either acute or chronic, in nature. Various chemical mediators are released during inflammation such as bradykinin, prostaglandin, histamine etc. Cyclooxygenase (COX) is a key enzyme in the synthesis of prostaglandin, thromboxanes, and prostacyclins.[1] It is regulated by growth factors and different cytokines such as IL6, ILβ, TNF alpha[6], is essential for the conversion of arachidonic acid in to prostaglandin.[7] Among the various mediators, prostaglandin is of major responsible for the generation of pain and itching during inflammation.[8] NSAIDs like Asprin, Parcetamol, and ibuprofen are more commonly prescribed medications for the treatment of acute and chronic conditions, relief pain and itching during inflammation. Long term uses of these medication affects the human biological system; there is need for safe and nontoxic anti-inflammatory drugs.

Plant medicines are of great importance in the primary health care. Plant has the ability to synthesize phytochemical compounds as secondary metabolites. Different parts of the medicinal plants are used as raw drugs and they possess the various medicinal properties.[4] Plant have a great potential for producing new drugs used in traditional medicine to treat various acute and chronic Inflammatory disorders.[9] Due to fewer side effects of herbal medicine, now a day's herbal medications uses are increased.[10]

Tricholepis glaberrima belongs to family Asteraceae and is commonly known as "Brahmadandi". The plant contains several constituents like saponin glycosides, flavonoids, triterpenoids, and sterols betulin, spinasterol, stigmasterol, ariterpenoid-cycloart-23-en-3 beta, 25 diol and stigma 7 enol.[11] Chloroform, Methanol and aqueous extract of the plant had proved aphrodisiac activity[12], antioxidant activity[13], ameliorative effects in hepatic damage[14], neuropharmacological activity[15] and antiobesity activity.[16]

MATERIAL AND METHODS

Sample collection and authentication

The fresh aerial parts of plant *Tricholepis glaberrima* were collected from the Tirupathi and were authenticated by Botanist Dr K Madhava shetty at Sri Venkateshwara University.

Preparation of extracts

Preparation of Methanolic extract

The fresh aerial parts of plant ***Tricholepis glaberrima*** were collected, dried under shade and then coarsely powdered with a mechanical grinder. The powdered material was filtered with sieve of mesh no.20. 250gms of the filtered powder was used for preparing extracts. Methanolic extract of plant *Tricholepis glaberrma* was prepared by soxhletion using soxhlet apparatus with methanol as a solvent at a temperature range of 60^{o}c for 24hrs. Further, the extract was concentrated by evaporating on water bath at 40^{o}c. It was stored in air tight container in refrigerator. The concentrated extract was weighed and suspended in normal saline to get the desire concentration for the experiment.

Preparation of Aqueous extract

The Aqueous extract of fresh aerial parts of plant ***Tricholepis glaberrima*** was prepared by maceration process by treating 100mg of fresh powder with 500ml of distilled water along with 10ml of chloroform as a preservative. The maceration process was carried out for 7 days with occasional stirring. The extract was filtered and evaporated to a thick paste.

Drugs, chemicals and instrument

All chemicals and drugs are used in this study were of the high analytical grade.

Carrageenan, Standard drug (Diclofenac sodium), Vernier caliper.

Animals

30 Healthy adult, albino rats of either sex weighing between 200 to 250 gms were used in pharmacological studies. The animals were maintained in a well ventilated room under standard conditions of temperature 22±1ºC and relative humidity 55±5% with 12:12 hr light/ dark cycles in polypropylene cages. The animals were freely accessed to a standard commercial pellet diet and water *ad libitum.* The rats were acclimatized to laboratory environment for 15 days before initiation of experiment.

The experimental protocol was approved by the institutional animal Ethics Committee (IAEC), SSJ College of Pharmacy and the reference number is 1448/PO/Re/S/11/CPCSEA /04/2016.

In vivo anti-inflammatory activity by carrageenan-induced rat paw oedema model

The method of winter *et al.*,[17] was used to evaluate anti-inflammatory activity with some minor modifications. The animals were divided into six groups, each group containing 5 animals.

Group-I animals received 0.5ml normal saline and served as control. Group –II of animals were treated with fleshly prepared 0.1 ml of 1% solution of carrageenan and served as toxic group. Group- III animals were administered diclofenac sodium (10mg/kg) and considered as standard. Group IV, V, and VI animals received the methanolic and aqueous extract of aerial parts of plant *Tricholepis glaberrima* respectively at the dose of 100mg/kg; 200mg/kg; 400mg/kg p.o. once and considered as test groups.

A mark was made on the right hind paws of rats just below the tibio tarsal junction. Oedema was induced by sub plantar injection of 0.1ml of 1% freshly prepared solution of carrageenan in normal saline into the right-hind paws of each rat of all the groups except the group I. Animals of group III, IV and V were treated with the single dose of extract, 30 minutes prior to carrageenan injection. Paw thickness were measured just before the carrageenan injection, that is, at "0 hour" and then at 1, 2, 3, 4, and 24th hour after carrageenan injection. Increase in paw thickness was measured by vernier caliper as the difference in paw thickness at "0 hour" and paw thickness at respective hours.

EXPERIMENTAL STUDY PROTOCOL OF METHANOLIC AND AQUEOUS EXTRACT OF PLANT *TRICHOLEPIS GLABERRIMA*

Group	Treatment	Dose (Once daily) oral
Group I	Control (normal saline)	0.5 ml
Group II	Carrageenan	0.1 ml of 1% solution
Group III	Diclofenac sodium	10 mg/kg
Group IV	METG AETG	100mg/kg 100mg/kg
Group V	METG AETG	200 mg/kg 200mg/kg
Group VI	METG AETG	400 mg/kg 400mg/kg

STATISTICAL ANALYSI

The statistical analysis of the evaluation of anti-inflammatory activity of aerial parts of *Tricholepis glaberrima* in methanolic and aqueous extract against the carrageenan-induced paw oedema in albino rats were analyzed using one-way Anova followed by Dunnett's test and expressed as mean ± SEM. Values expressed Mean± SEM $^{***}p< 0.001$, $^{**}p< 0.01$, $^{*}p< 0.05$ when compared to Standard group.

RESULTS

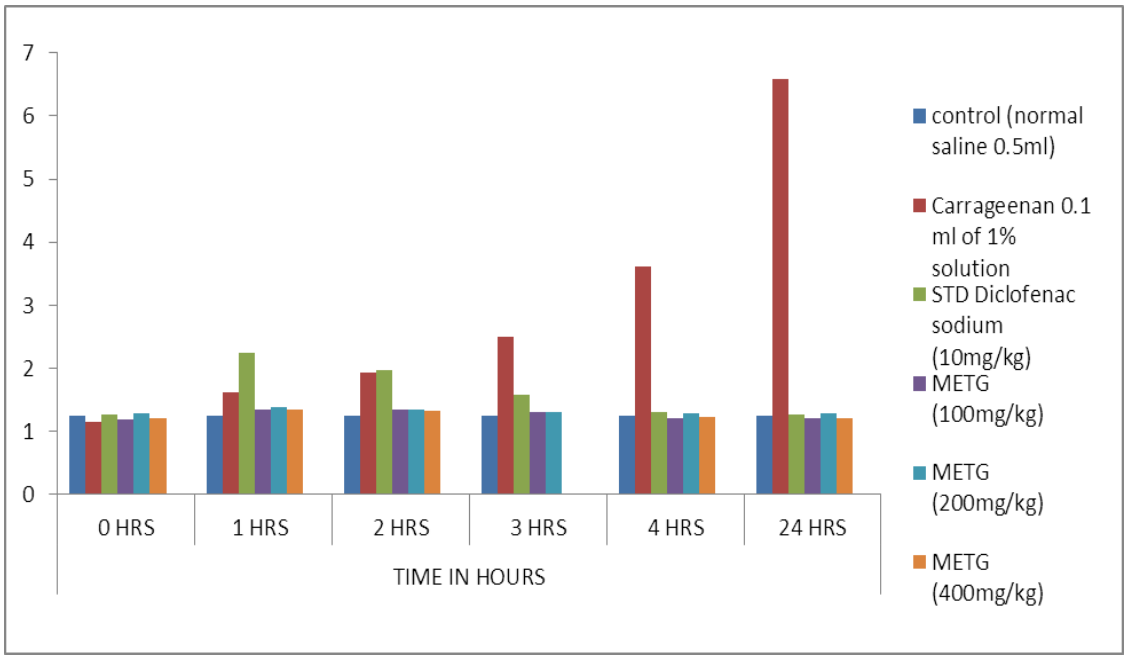
To assess the anti-inflammatory activity of the extracts, the groups were treated with different doses of methanolic and aqueous extracts of *Tricholepis glaberrima*. The difference in paw oedema thickness was calculated in each control, test, standard and toxic groups at different time intervals. Results of the evaluation of anti-inflammatory activity revealed that there was a significant increase in the paw thickness with animals treated with toxicant carrageenan (0.1ml of 1 %) at different time intervals. At the end of the 24th hr, the thickness of paw was higher than the initial paw thickness. Significant reduction in the thickness of paw was observed when the animals were treated with different doses of methanolic and aqueous extract of *Tricholepis glaberrima.* They had successfully reversed the carrageenan induced toxicity, which was evident by the significant reduction or changes in the thickness of paw oedema at different hours, which were brought down significantly and are comparable to that of control animals.

Thus, it was demonstrated that the methanolic and aqueous extracts of *Tricholepis glaberrima* have the anti-inflammatory action. The thickness of paw oedema in rats treated with different doses (100mg/kg, 200mg/kg, and 400mg/kg) of methanolic and aqueous extracts has drastically decreased and is less than the control group. Here the 1st hr and 2nd hour showed elevated values of paw thickness as compared to 6th and 24th hour. After that, the values were lowered in 3rd, 4th, and 24th hr respectively, and comparable to that of standard diclofenac sodium and control groups. From the statistical analysis we concluded that both methanolic and aqueous extracts of *Tricholepis glaberrima* at different doses 100, 200 and 400mg/ kg body weight showed significant anti inflammatory activity in rats paw oedema induced by carrageenan. Both extracts had showed same anti-inflammatory activity in carrageenan induced animals at different doses and time intervals and compared to standard groups treated with diclofenac sodium (10mg/kg) and control groups.

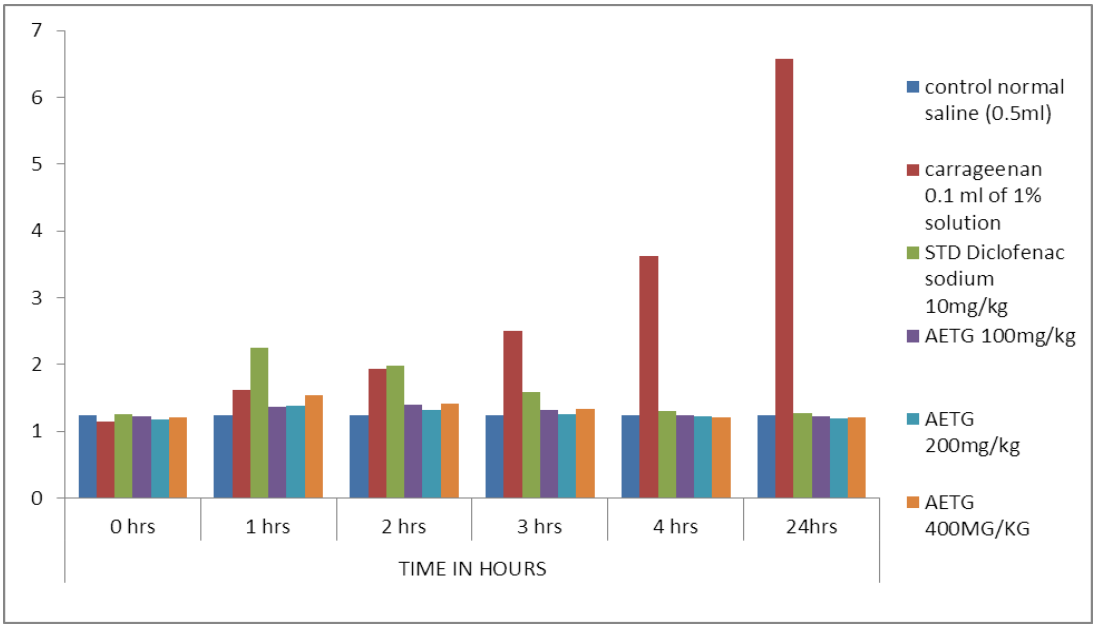
Anti-inflammatory activity of methanolic and aqueous extract of *Tricholepis glaberrima* by carrageenan-induced rat paw oedema method.

Groups	Dose	Change in Paw thickness					
		0hr	1hr	2hr	3hr	4hr	24hr
Control (normal saline)	0.5 ml	1.24 ± 0.14*	1.24 ± 0.14*	1.24 ± 0.14*	1.24 ± 0.14*	1.24 ± 0.14*	1.24 ± 0.14*
Carrageenan	0.1 ml of 1% solution	1.15± 0.18*	1.62 ± 0.16	1.94 ± 0.65	2.50 ± 0.98*	3.62 ± 1.02**	6.58 ± 1.52**
Diclofenac sodium	10 mg/kg	1.26 ± 0.25	2.25 ± 0.36	1.98 ± 0.56	1.58 ± 0.18	1.30 ± 0.51	1.27 ± 0.15
Methanolic Extract of *Tricholepisglaberrima*	100mg/kg	1.19 ± 0.12*	1.35 ± 0.11*	1.34 ± 0.16**	1.30 ± 0.11**	1.21 ± 0.80**	1.20 ± 0.11
Methanolic Extract of *Tricholepisglaberrima*	200 mg/kg	1.28 ± 0.23*	1.39 ± 0.12**	1.35 ± 0.17**	1.31 ± 0.40*	1.29 ± 0.15**	1.29 ± 0.15**
Methanolic Extract of *Tricholepisglaberrima*	400 mg/kg	1.21 ± 0.14*	1.34 ± 0.15*	1.32 ±0.15**	1.28 ±0.15**	1.22 ± 0.15**	1.21 ± 0.12*
Aqueous Extract of *Tricholepisglaberrima*	100mg/kg	1.23±0.02**	1.36 ± 0.21**	1.40 ± 0.24*	1.31 ± 0.01**	1.24 ± 0.20*	1.23 ± 0.07*
Aqueous Extract of *Tricholepisglaberrima*	200 mg/kg	1.18 ± 0.14*	1.38 ± 0.15*	1.32 ± 0.12**	1.26 ± 0.20**	1.22 ± 0.18**	1.19 ± 0.36**
Aqueous Extract of *Tricholepisglaberrima*	400 mg/kg	1.20±0.12**	1.54 ± 0.15**	1.42 ±0.18**	1.33 ±0.15**	1.21 ± 0.19***	1.20 ± 0.12***

Values expressed Mean± SEM *** p< 0.001, ** p< 0.01, * p< 0.05 when compared to Standard group. One-way ANOVA followed by Dunnett's.



Graph 1: Effects of Methanolic extract of *Tricholepis glaberrima* on carrageenan-induced paw oedema in rats.



Graph 2: Effects of Aqueous extract of *Tricholepis glaberrima* on carrageenan-induced paw oedema in rats.

DISCUSSION

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants etc characterized by redness, warmth, swelling and pain at the site of inflammation.[18] It is body defense mechanism in order to eliminate or limit the spread of injurious agent followed by removal of dead cells and tissues and initiates heal and repair. Inflammation is either acute or chronic depending upon the defense capacity of host.[2]

Acute inflammation may be primary the response of the body to any harmful stimuli. It is characterized by the exudation of fluids and plasma proteins and the migration of leukocytes, most importantly neutrophils into the injured area. This acute inflammatory response is useful to the defense mechanism aimed at killing of bacteria, virus and parasites while still facilitating wound repairs.[19]

In the present study, we have investigated the effects of methanolic and aqueous extract of aerial parts of *Tricholepis glaberrima* on carrageenan- induced paw edema in rats. Carrageenan-induced paw edema which is an in vivo model is frequently employed to assess the anti-edematous effects.[20, 21] Oedema formation in the carrageenan-induced paw edema model is a biphasic response.[22] In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin and bradykinin on vascular permeability. Histamine is a primary vasoactive amine[23], plays an important role in early and immediate acute

inflammatory responses.[24] The main actions of histamine during inflammation are vasodilation, increased vascular permeability, which causes itching and pain.[25]

The inflammatory edema reached its maximum level at the 1st hr and after that it started declining. The late phase of the inflammatory response has been shown to be due to potentiating effect of bradykinin on mediator release and prostaglandins, producing edema (swelling) after mobilization of the leukocytes.[26] It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents and they are releated to COX inhibition, specially COX-2.

Qualitative phytochemical analysis of the extracts of plant *Tricholepis glaberrima* were confirmed the presence of various phytochemical constituents like alkaloids, carbohydrates, tannins, glycosides, saponins, flavanoids etc., whereas triterpenoids, steroids and proteins were found to be absent. Secondary metabolites are of great importance in the field of drug research.

The methanolic and aqueous extract of aerial parts of *Tricholepis glaberrima* was capable of reducing both early and delayed phases of carrageenan- induced inflammation. The effectiveness of plant extracts to suppress inflammatory responses may be due to the presence of secondary metabolites and their inhibitory action on COX enzymes.

In carrageenan-induced rat paw oedema model methanolic and aqueous extract of aerial part of *Tricholepis glaberrima* were showed significant inhibition on the thickness of paw. Both extracts showed varying degree of anti-inflammatory activities with statistical significance at all tested dose levels i.e. 100mg, 200mg and 300mg/kg of body weight. At the same time, diclofenac sodium the standard drug also showed the same significant inhibitory activity at the dose of 10mg/kg body weight.

This study revealed that the extract of *Tricholepis glaberrima* showed possible significant inhibitory action on paw edema, due to presence of phytochemical constituents like glycosides, alkaloids, saponins flavanoids and tannins etc by inhibiting the action of cox-2[27], thereby inhibits the synthesis of prostaglandins an inflammatory mediator.[28]

CONCLUSION

In this present work the attempt was made to study the anti-inflammatory activity of methanolic and aqueous extracts of plant *Tricholepis glaberrima*. According to the results of

the present investigation, it can be concluded that the different doses of methanolic and aqueous extracts of *Tricholepis glaberrima* have been shown to be effective against carrageenan induced rat paw oedema. There is no doubt that the extracts of *Tricholepis glaberrima* possess significant anti-inflammatory activity which has confirmed in our study. The present study supports the claim in the use of the extract of *Tricholepis glaberrima* in traditional medicines for the treatment of inflammatory diseases.

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