

A Study to Compare the Anti Inflammatory Activity of *Tinospora Cordifolia* with Sterile Normal Saline and Diclofenac Sodium in Male Wistar Albino Rats

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Received: 25-09-2023 / Revised: 28-10-2023 / Accepted: 30-11-2023

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Conflict of interest: Nil

Abstract

Introduction: Herbal medication is commonly used in ancient as well as modern in many parts of the world in the treatment of acute inflammation (AI). A study was taken to compare the anti-inflammatory (AnI) effect of *Tinospora cordifolia* (TC) with sterile normal saline and diclofenac sodium in male wistar albino rats.

Methods: It was a prospective research conducted in the department of Pharmacology, Dr Pinnamaneni Institute of Medical Sciences & Research Foundation between October 2014 and 2016. Male wistar albino rats weighing about 250-300gm were included. Animals were divided in to test, standard and control groups, sterile normal saline, diclofenac sodium and TC were administered, respectively; 6 animals each, respectively. TC were studied at 19.95mg/kg, 25.69mg/kg and 33.25mg/kg, categorized as 2A, 2B and 2C, respectively and 6 animals in each category. The medicament extracts were prepared as per the standard guidelines and administered intraperitoneal route. Carrageenan induced rat paw oedema model was used. Paw oedema volume up to the ankle joint was measured in the test animals at 0 and 3hours following carrageenan challenge by using mercury Plethysmograph filled with mercury. ANOVA, was used for the data analysis; P <0.05 was considered statistically significant.

Results: The herbal medicament showed significant reduction in rat paw volume. When the test group was compared with the control and standard, there was a significant reduction in the paw volume. Comparison between the standard and test also showed statistically significant reduction in the paw volume.

Conclusion: In the animal models also TC act as AnI agent. Due to the precautions there was minimal animal deaths. But small sample size, aqueous solution alone are the limitations of this research.

Keywords: Medicament, Animal, Mean, Inflammation.

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Introduction

Inflammation, response of vascularized tissue may initiate the healing process of the damaged tissues. Rubor, tumor, calor, dolor and functio laesa are the different signs of inflammation. Clinically, acute and chronic are the 2 different forms and the acute is the prominent. [1] Urticaria is an acute inflammation (AI), prevalent throughout the globe. [2] Asthama is a chronic inflammation (CI), the incidence in India is 15-20 million. [3] Rheumatoid arthritis, tuberculosis, glomerulonephritis, inflammatory bowel disease (IBD) are some other clinical forms of CI. Due to the environmental as well as genetic influences, there is a significant rise in some CIs such as IBD. [4]

AI response is host protective against the invasion of foreign bodies. Hence the treatment protocols are not changed inspite of the availability different therapeutic options. [5] Herbal medication is commonly used in ancient as well as modern in many parts of the world. Different experimental models of *Tinospora cordifolia* (TC) were reported in the literature but limited animal studies from this setting. With this back ground, a study was taken to compare the anti-inflammatory effect of *Tinospora cordifolia* (TC) with sterile normal saline and diclofenac sodium in male wistar albino rats.

Methods:

It was a prospective research conducted in the department of Pharmacology, Dr Pinnamaneni Institute of Medical Sciences & Research Foundation, chinnaoutpally. Approval of the Institutional Animal Ethical Committee (IAEC) was obtained prior to the study. The study was conducted between October 2014 and 2016. Male wistar albino rats weighing about 250 – 300gm were included in this research. The animals were divided in to test, standard and control groups. In the control group, sterile normal saline was injected and diclofenac sodium injection in the standard animal; 6 animals each, respectively in the control and standard. Necessary precautions were taken during the experiment to minimize the mortality.

In the test group of animals, TC was studied at different concentration. Simultaneously TC was also studied in three concentrations, at 19.95mg/kg, 25.69mg/kg and 33.25mg/kg on the paw volume of the animal; these were categorized in to 2A, 2B and 2C, respectively and 6 animals in each category. The medicament extracts were prepared as per the standard guidelines.

Carrageenan induced rat paw oedema model was used to study the AI and sub-acute phase of inflammation in this research. [6] Based on the weight of the animal, the test drug was administered intraperitoneally or orally. Mode of administration was selecting based on the body weight of the animal and just half an hour before the carrageenan challenge. A mark was made at the tibio tarsal joint of the animal. Paw oedema volume up to the ankle joint was measured in the test animals at 0 and

3hours following carrageenan challenge by using mercury Plethysmograph filled with mercury. [7] The animals were maintained in well ventilated animal house with light and dark cycle, 12 hours each. Plethysmograph was used to measure different parameters of the animal. [8]

Statistical Analysis

The data were analyzed using SPSS software version 20. The analyzed data was presented in mean, standard deviation (SD). Descriptive statistics such as ANOVA, Dunnett's test, Tukey post hoc test were used for the data analysis; P <0.05 was considered to be statistically significant.

Results

In the control animal, the mean \pm SD of the paw volumes were 4.03 \pm 0.05 and 4.8 \pm 0.09, respectively at 0 hrs and 3 hrs. The difference in the paw volumes were statistically significant (t value = 36.37; P <0.001). In the standard with diclofenac sodium, the mean \pm SD of the paw volumes were 4.07 \pm 0.05 and 4.25 \pm 0.05, respectively at 0 hrs and 3 hrs. The difference in the paw volumes were statistically significant (t value = 11; P <0.001). The herbal medicament showed significant reduction in rat paw volume (Table 1). When the test group was compared with the control and standard, there was a significant reduction in the paw volume. Comparison between the standard and test also showed statistically significant reduction in the paw volume.

Table 1: Mean change in the paw volume among the study animals.

Group	Mean \pm SD			Inhibition (%)	Statistical analysis
	0 hr	3 hr	Change		
Control	4.03 \pm 0.05	4.8 \pm 0.09	0.77 \pm 0.05	0%	t = 36.37; P <0.001
Standard	4.07 \pm 0.05	4.25 \pm 0.05	0.18 \pm 0.04	77%	t = 11; P <0.001
Test2A	4.08 \pm 0.04	4.7 \pm 0.09	0.62 \pm 0.12	19%	t = 12.92; P <0.001
Test2B	4.07 \pm 0.08	4.45 \pm 0.1	0.38 \pm 0.15	51%	t = 6.38; P < 0.001
Test2C	4.13 \pm 0.12	4.48 \pm 0.15	0.35 \pm 0.05	55%	t = 15.65; P <0.001

Discussion

Inflammation is an innate attempt, helpful for self-protection. Numerous anti-inflammatory (AnI) agents are available in allopathic as well as herbal medicine. The herbal formulations are relatively free from adverse side effects and have less drug dependence causing tendency. So in the present study AnI effect of TC was studied. [9]

Carrageenan induced rat paw oedema is one of the standard methods used to evaluate AnI effects. [6] The method is simple, easy and short lasting as well as reproducible. TC (Willd.) Miers is an inevitable

ingredient in Ayurveds in the treatment of inflammatory disorders. [10]

In the present study diclofenac sodium was used as standard dose at a concentration of 5.14mg/kg, administered intra peritoneal (IP) route. In the mean rat paw volume, there was 77% reduction. Gupta mradu et al. [11], SR Deorukhakar et al. [12], also used carrageenan induced rat paw oedema method and the investigators used indomethacin as standard dose at 5, 10 mg/kg, oral route, respectively. The percentage of inhibition was reported to be 78.65 and 61.29, respectively.

In the present research, aqueous solution of TC extract was used at 3 different concentrations; the percentage of inhibitions was 19, 51 and 55, respectively. It was reported to be 33.06% and 67.97%, in the literature. [11, 13] The initial disparity in the reported studies with the current research is not clear. But the difference in the concentration of TC and next is the adoptability of animal. Though AnI action of TC was reported in the literature, how the recipient reacts and the changes in the body against any chemical can be influence by so many factors. In addition, Kwatha was used by Biswajyoti Patgiri et al. [13] In another report by Utpalendu Jana et al. [14] study 50 mg/kg of TC extracts had 67.28% inhibition by using carrageenan induced rat paw oedema method which was higher compared to the current research.

Due to the precautions there was minimal animal death, this is a strength of the current research. But small sample size, aqueous solution alone are the limitations of this research. Even in the animal models also TC act as AnI agent.

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