Research Article *ISSN: 2277-8713*
Vipin Kumar, IJPRBS, 2012; Volume 1(5): 193-204 *IJPRBS*

ISSN: 2277-8713
IJPRBS
A Path for horizing your innovative work
INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

EVALUATION OF ANTI-INFLAMMATORYACTIVITY OF TINOSPORACORDIFOLIA: ROLE IN ARTHRITIS

Dr. VIPIN KUMAR[1], Dr. PANKAJ KUMAR MODI[2], Dr. SANJEEV SHARMA[3]

1. **MD, Assistant Professor, Department of Pharmacology, Teerthanker Mahaveer Medical College, Moradabad.**
2. **MD, Assistant Professor, Department of Pharmacology, National Institute of Medical Science, Jaipur.**
3. **Professor and Head, Department of Pharmacology, Teerthanker Mahaveer Medical College, Moradabad.**

Accepted Date:
07/09/2012
Publish Date:
27/10/2012

Keywords
Anti-inflammatory activity,
Arthritis
Immunological

Corresponding Author
Dr. VIPIN KUMAR
MD, Assistant Professor, Department of Pharmacology, Moradabad.

Abstract

Objectives: To evaluate the anti-inflammatory activity of Tinosporacordifolia in experimentally induced inflammation in albino rats. **Methods**: The study was conducted on albino rats (150-200 gm) of either sex. Inflammation was induced by non-immunological (acute, sub acute, chronic) and Immunological methods. For each set of experiment, animals were divided in five groups of six animals each. 1st group was given normal saline, 2nd group standard anti inflammatory drug and 3rd, 4&5th group Tinosporacordifolia (T. cordifolia) in doses of 100, 200 &400 mg/kg respectively. In both experiments, inflammation was measured by standard methods.

Results: In both Non-immunological (acute, sub acute, chronic) &immunologically induced inflammation *T. cordifolia* treated groups show significant anti-inflammatory activity in comparison to control group **Conclusion:** *T. cordifolia* exhibited significant and promising anti-inflammatory activity in all the experimental models covering the different phases of inflammatory response.

INTRODUCTION

Arthritis, a joint disorder that involves inflammation of one or more joints, is characterized by joint stiffness, swelling, redness, and warmth. Although pathogenesis of arthritis is not fully understood but inflammation is an important event. So anti-inflammatory drugs form the mainstay in the treatment of arthritis.

Anti-inflammatory agents limit, control or modify the normal inflammatory reaction. An ideal anti-inflammatory drug is one which limits destructive and harmful complications of arthritis as well as has few side effects. Corticosteroids demonstrate the anti-rheumatic action[1] but show various serious side effects.[2] So many non-steroidal anti-inflammatory drugs (NSAIDs) have come to light but most of them exhibit gastrointestinal tract (GIT) toxicity.[3] Recently, selective COX-2 inhibitors have been introduced as newer NSAIDs. This group is safe with regard to GIT side effects but is facing increased scrutiny due to its cardiovascular toxicity.[4]

There are some Ayurvedic drugs which have potential for anti-inflammatory action and at the same time they are also claimed to be free from side effects which limit the usage of the above mentioned conventional allopathic drugs. Moreover, synthetic drugs are very expensive to develop, it is, therefore, essential that efforts should be made to introduce new drugs which are safer, cheaper and more effective.

T. cordifolia, an herbal plant has been claimed by ayurvedic physicians to be effective in the treatment of various types of arthritis.[5] However; there is lack of scientific studies to ratify these claims. Therefore, the present study is undertaken to explore the anti-inflammatory activity of

T. cordifolia in experimentally induced inflammation in albino rats.

MATERIALS & METHODS

The study was conducted on healthy albino wistarrats (100 – 150 gm) of either sex, maintained at an ambient temperature of 25 – 35ºC with food and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee and was executed according to the guidelines of the committee for the purpose of control and supervision of the experiments on animals (CPCSEA), India.

Dried stem of the plant were pulverized in an electric blender to form a powder. 100 g of the prepared powder weighing was macerated and soaked in 500 ml of distilled water for 24 h. It was then filtered through a 1mm mesh sieve and the filtrate was concentrated to a dark green residue by heating at 40 °C, till complete evaporation of water was achieved. . Finally, the aqueous extract was kept in desiccators for 2 weeks to remove the excessive moisture and was used for further studies.

For inducing inflammation, both non-immunological and immunological methods were employed. In each experiment animals were divided in 5 groups. 1st group was given normal saline, 2nd standard anti-inflammatory drug and 3rd, 4 and 5th group *T. cordifolia* in doses of 100, 200 &100 mg/kg respectively. Standard agent used in acute inflammation was indomethacin (5 mg/kg) and for sub acute, chronic and immunological method, dexamethasone (0.5mg/kg). In acute inflammation & in immunological method, drugs were given for 1 day and in sub acute& chronic experiment drugs were given for 5 &10 days respectively. In all experiments drugs were given orally.

(A)Non-immunological inflammation methods

(a) Acute inflammation

Carrageenan induced hind paw edema method

Initially animals of each group were treated with test drugs. After 1hr all these animals were challenged with carrageenan 0.1 ml of 1% solution in distilled water, injected into the sub planter tissue of the right hind paw to induce paw edema. The paw volume was measured before giving test drugs and 3 hrs

after the carrageenan administration, by plethysmometric method.[6, 7]

The average paw swelling in the group of the drug treated rats was compared with that of untreated rats (control group) and the percent of inhibition of the edema was determined.

(b) Sub acute inflammation method

Cotton pellets granuloma method

Four cotton pellets, each weighing 10mg were sterilized at 121°C under 15 lbs pressure for 30 minutes. Rats were anaesthetized under ketamine (100mg/kg, i. m.) and a 15 mm ventral incision in both axillae & groins were made, four cotton pellets were implanted per rat in axilla & groin and were sutured. The respective groups of cotton implanted rats were treated orally with test drugs for 5 days, from the day of implantation. Cotton pellets were dissected out on 6th day from the day of implantation. Each pellet and associated tissue was weighed wet and then dried for 24hrs and reweighed.[8]

(c) Chronic inflammation method:

Formaldehyde induced arthritis method

Animals of each group were challenged with inflammation inducing agent formaldehyde 0.1ml of 2% solution, injected into the sub planter tissue of the right hind paw on day 1 and day 3 to induce paw oedema and the respective groups were treated orally followed by daily measuring the paw volume for 10 days.[9]

(B) Immunological inflammation method

Tuberculin sensitivity method

Animals of each group were treated with 0.25 ml of Complete Freund's Adjuvant (1 mg killed mycobacterium per ml) subcutaneously into the planter surface of the left hind paw on day 1. On day 14 respective groups of animals were treated with test drugs orally once and followed by 0.1 ml of 10 TU (Purified Protein Derivative-P.P.D.) was injected intradermally into the flanks which were previously depilated. After 24 and 48 hr diameter of tuberculin reaction was measured.[10]

Statistics

Results were expressed as mean ± SD. Statistical differences between the groups were tested by one way analysis of variance

(ANOVA) followed by Newman-Keuls Multiple Comparisons.

RESULTS AND DISCUSSION

Carrageenan induced hind paw edema method

Indomethacin (5mg/kg) and *T. cordifolia* in the doses of 100, 200, 400 mg/kg significantly decreased the paw edema volume induced by carrageenan as compared with saline treated group. *T. cordifolia* was found to suppress the carrageenan induced edema significantly (P<0.05) at 100mg/kg dose. However at the dose of 200, 400 mg/kg the inhibition was highly significant (P<0.001) like indomethacin. (Table 1)

Cotton pellet induced granuloma method

T. cordifolia was found to reduce exudates as well as the granulation tissue formation in cotton pellets test highly significantly (P<0.001) in all doses of *T. cordifolia* like dexamethasone. (Table 2)

Formaldehyde induced arthritis method

There was significant inhibition of inflammation by *T. cordifolia* throughout the duration of experiment (P<0.001). In *T. cordifolia* treated group maximum Inhibition was observed at the 400 mg/kg dose that was comparable with standard anti-inflammatory agent dexamethasone. (Table 3)

Tuberculin sensitivity test

T. cordifolia in the dose of 100mg/kg showed no significant suppression of tuberculin reaction at 24hr and at 48hr while at the dose of 200mg/kg *T. cordifolia* showed significant suppression (p<0.05) at 24 hours, while at 48 hr suppression is highly significant(p<0.001). Dexamethasone as well as *T. cordifolia* (400 mg/kg) markedly suppressed the tuberculin reaction both at 24 hr and 48 hr interval (P <0.001). (Table 4)

Discussion

Although NSAIDs and corticosteroids are used for treatment of arthritis, but due to their various serious side effects, ideal and safe drugs for a patient suffering from different stages of arthritis are yet to be found.

A number of compounds viz. alkaloids, glycosides, diterpenoid lactones, sesquiterpenoids, steroids, phenolics, aliphatic compounds, polysaccharides and

flavonoids has been confirmed in aqueous extract of T. cordifolia by phytochemical analysis.[11, 12]

The inflammatory response is a polyphasic tissue reaction, which ranges from short lived increase in vascular permeability to a prolonged cellular infiltration and proliferation. So it is important to evaluate the performance of anti-inflammatory drug via a battery of tests valid for various phases of inflammation.

Few studies[13, 14] have been done about for the anti-inflammatory activity of T. cordifolia but in this study we combined both immunological and nonimmunological models to strengthen the possible role of T. cordifolia in arthritis.

Carrageenan induced edema has a biphasic effect. The first phase is due to release of autacoids, histamine and serotonin (0-2hr), plateau phase is maintained by a kinin like substance which increase the vascular permeability up to two and a half hours. The maximum inflammation is seen approximately three hours post the carrageenan injection (which is attributed to PG release), after which it begins to decline.[15] Edema induced by the sub plantar injection of carrageenan in the rat hind paw is reported to have been inhibited by a number of steroidal and non-steroidal anti-inflammatory drugs.[16]

*T. cordifolia sig*nificantly decreased the paw edema induced by carrageenan as compared with saline treated group which may be due to inhibition of cyclo-oxygenase enzyme.[17]

For further verification of the anti-inflammatory activity of *T. cordifolia* and its effects on the transudative and proliferative components of chronic inflammation, the cotton pellet granuloma model was used. The wet weight of the cotton pellets represents transudative component and the dry weight of the pellets represent granulomatous component of inflammation. *T. cordifolia* was found to reduce transudates as well as the granulation tissue formation in cotton pellets test significantly ($P<0.001$) with all 3 doses used, as compared to saline treated group. (Table 2) As Kinin is the main mediator of this granulomatous reaction, [18] the effect of *T. cordifolia* on this model of sub acute inflammation confirmed that it

might have inhibited the chemical mediators of inflammation.

In formaldehyde-induced paw edema model, the anti-inflammatory activity was evident from day 3 to 11 of treatment, indicating that *T. cordifolia* is effective against the chronic inflammation. *T. cordifolia* was found to reduce formaldehyde-induced paw edema significantly (P<0.001) with all 3 doses used as compared to saline treated group (Table-3). Oxidative stress is one of the responsible factors for establishment of chronic arthritis.[19] Antioxidant property of *T. cordifolia* has been proved by some studies which explain the protective role of this plant in chronic inflammation.[20]

Immunological mechanism (delayed hypersensitivity) is responsible for some inflammatory diseases.[21] it can be easily elicited in sensitized rats as evidenced by the tuberculin reaction. At the dose of 100 mg/kg *T. cordifolia* showed insignificant activity but was found to be significantly effective (P<0.001) in the doses of 200mg/kg and 400mg/kg as compared to saline treated group. (Table4) Steroidal constituent of *T. cordifolia* may be responsible for this because steroid suppress T cell activity, responsible for delayed hypersensitivity.[22] an arabinogalactan has been isolated from the dried stems and examined by methylation analysis, partial hydrolysis and carboxyl reduction. Purified polysaccharide showed polyclonal mitogenic activity against B-cells; their proliferation did not require macrophages.[23]

All anti-inflammatory drugs are not equally effective in suppressing all components of the inflammatory response, but in the present investigation. *cordifolia* exhibited significant and promising anti-inflammatory activity in all the experimental models which cover the different phases of inflammatory response. So if the pure active principle of *Tinosporacordifolia* could be isolated and evaluated, the constituents of *Tinosporacordifolia* could be used more rationally for treatment of arthritis.

Table-1

Effect of *T. cordifolia* and indomethacin on carrageenan induced hind paw volume in albino rats (n=6)

Drug	Dose (mg/kg, oral)	Paw Volume (ml) ± SD		% inhibition in Paw Volume
		Initial	3hrs	
Saline	5 ml	0.61±0.03	0.96±0.02	
Indomethacin	5	0.61±0.02	0.79±0.03**	48.57
T. cordifolia	100	0.59±0.03	0.86±0.02*	23.01
T. cordifolia	200	0.57±0.04	0.82±0.03**	28.57
T. cordifolia	400	0.59±0.03	0.81±0.04**	37.14
**P<0.001 and *P<0.05 (as compared to saline treated group)				

Table-2

Effect of *T. cordifolia* and dexamethasone on cotton pellets implantation in albino rats (n=6)

Drug	Dose (mg/kg, oral)	Weight of cotton pellets (wet) mg ± SD	% inhibition	Weight of cotton pellets(dry) mg ± SD	% inhibition
Saline	5 ml	312.55±2.82		83.51±3.12	
Dexamethasone	0.5	134.68±4.14*	56.91	40.32±3.75*	51.72
T. cordifolia	100	205.35±5.34*	34.30	60.01±3.80*	28.15
T. cordifolia	200	178.61±3.91*	42.86	46.70±3.47*	44.08
T. cordifolia	400	177.25±4.33*	43.29	46.32±3.91*	44.56

*P<0.001 (as compared to saline treated group)

Table 3

Effect of T. cordifolia and dexamethasone on formalin induced arthritis in albino rats (n=6)

Drug	Dose (mg/kg,oral)	% increase in paw volume ± SD									
		Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
Saline	5 ml	113.09± 4.23	105.08± 5.18	115.98± 5.75	94.97 ± 3.31	93.30± 4.67	90.77± 3.45	89.09± 3.43	86.56 ± 3.21	82.37± 3.35	80.69± 3.33
Dexamethasone	0.5	50.29±2.58*	39.21±3.83*	55.01±2.57*	23.46±1.76*	16.80±1.30*	14.40±1.20*	12.01±0.98*	10.40±1.10*	8.76±1.10*	8.68± 1.18*
T. cordifolia	100	75.45±3.67*	60.21±3.35*	82.86±2.57*	70.18±3.06*	66.86±3.04*	62.99±2.19*	59.68±3.42*	56.92±3.96*	54.16± 4.31*	49.75±4.8*
T. cordifolia	200	66.33±3.49*	55.24±4.57*	69.88±5.03*	47.24±2.96*	43.56±2.81*	37.81±3.38*	34.67±3.20*	32.82±1.88*	29.32± 2.03*	26.77± 1.67*
T. cordifolia	400	55.05±2.90*	50.60±4.67*	58.19±4.59*	31.29±2.64*	25.19±2.06*	22.68±1.43*	20.17±1.10*	17.64±1.61*	15.13± 1.12*	13.61± 1.16*

*P<0.001 (as compared to saline treated group)

Table 4

Effect of *T. cordifolia* and dexamethasone on tuberculin sensitivity test in albino rats (n=6)

Drug	Dose (mg/kg, oral)	24 hrs		48 hrs	
		Diameter of wheal (mm) ±SD	% inhibition	Diameter of wheal (mm) ±SD	% inhibition
Normal Saline	5 ml	11.33±0.60		11.33±0.62	
Dexamethasone	0.5	3.66±0.66**	67.70	3.34±0.56**	70.53
T. cordifolia	100	10.33±0.62	8.83	9.67±0.52	14.66
T. cordifolia	200	8.60±0.56*	24.10	8.32±0.55**	26.57
T. cordifolia	400	6.30±0.49**	44.40	5.66±0.60**	51.05

**P<0.001 and *P<0.05 (as compared to saline treated group)

REFERENCES

1. Hench PS, Kendall EC, Slocumb CH and Polley HF: The effect of a hormone of the adrenal cortex (17-hydrooxy-11-dehydro-corticosterone; compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis. Proc Staff Meet Mayo Clin 1949; 24: 181.

2. Richard AH and Pamela CC: Adrenal corticosteroids. 4th ed. Lippincott's illustrated reviews of pharmacology. Baltimore, Maryland. 2009; 316.

3. Nikander R, McMohan FG and Ridolfo AS: Non steroidal anti-inflammatory agents. Ann Rev Pharmacoltoxicol 1979; 19:469-90.

4. Shawn XS, Kwan YL, Carl TB and Jay LG: Withdrawal of COX-2 selective inhibitors rofecoxib and valdecoxib: impact on NSAID and gastro protective drug prescribing and utilization. Current Medical Research and Opinion 2007; 23: 1859-66

5. KirtiSinha, N P Mishra, J Singh and SPS Khanuja: Tinosporacordifolia (Guduchi), a reservoir plant for therapeutic applications: A Review. Indian Journal of Traditional Knowledge. 2004; 3: 257-270.

6. Winter CA, Risley EA and Nuss GW: Carrageenan induced edema in the hind paw of the rats as assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962; 111: 544-7.

7. Harris JM and Spencer PSJ: A modified plethymographic apparatus for recording volume changes in the rat paw. J Pharm Pharmac 1962; 14:464-466.

8. Kellet DN: The anti-inflammatory activity of protamine sulphate and of hexadimethrine bromide, inhibitors of plasma kinin formation. Brit J Pharmacol 1965; 24: 705-13.

9. Brownlee G: Effect of deoxycortone and ascorbic acid on formaldehyde induced arthritis in normal and adrenalectomized rats. Lancet 1950; 1: 157-159.

10. Satyavati GV, Prasad DN, Das PK and Singh HD: Anti-inflammatory activity of Semecarpusanacardium Linn. A preliminary

study. Ind J Physiol Pharmacol 1969; 13:37-45.

11. Javeed Ahmed Wani, Rajeshwara N Achur and R K Nema: Phytochemical screening and aphrodisiac property of Tinosporacordifolia. International Journal of Pharmaceutical and Clinical Research 2011; 3:21-26.

12. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B and Ghosh AC: Chemistry and medicinal properties of Tinosporacordifolia (Guduchi) Indian J Pharmacol.2003; 35:83-91

13. Jana U, Chattopadhyay RN and Shw BP: Preliminary studies on anti-inflammatory activity of *ZingiberofficinaleRosc., Vitexnegundo* Linn. andTinosporacordifolia (Willid) Miers in albino rats. Indian J Pharmacol. 1999; 31:232–3

14. Gulati OD and Pandey DC: Anti-inflammatory activity of Tinosporacordifolia. Rheumatism. 1982; 17:76–83.

15. Vinegar R, Schreiber W and Hugo RJ: Biphasic development of carrageenan edema in rats. J PharmacolExpTher 1969;166:96-103

16. Vagel HG: Analgesic, anti-inflammatory and antipyretic activity in Drug discovery and evaluation pharmacological assays, 2nd Ed. New York: Springer. 2002: 759-67.

17. Prakash Kumar B, Jenny Jacob and Jorny Augustine: Dual inhibition of lipoxygenase/cyclooxygenase by an ayurvedic herb, Tinosporacordifolia (WILLD.); possible explanation of anti-inflammatory activity. Journal of Pharmacy Research 2011; 4: 1779-1780.

18. Margolius HS: Tissue Kallikrienes and kinnins regulation and roles in hypertensive and diabetic disease. Ann Rev Pharmacol Toxicol 1989; 29: 343-8.

19. Paul PT, Nathan JZ, Dougulas RG and Gary FS: Rheumatoid arthritis and p 53: how oxidative stress might alter the course of inflammatory diseases. Immunology today 2000; 21:78-82.

20. Jasbir Singh, AkashBagla and Vikas Pahal: Hepatoprotective activity of herbal extracts in carbon tetra chloride intoxicated albino rats by measuring anti-oxidant enzymes. International Journal of Pharm Tech Research. 2010; 2:2112-2115.

21. Cotran, Kumar and Collins: Diseases of immunity. 7th ed. Robbins Pathologic Basis of Disease. Philadelphia, Pennsylvania. 2005; 252.

22. Leung DY and Bloom JW: Update on glucocorticoid action and resistance. J. Allergy Clin. Immunol. 2003; 111: 3–22.

23. Chintalwar G, Jain A, Sipahimalani A, Banerji A, Sumariwalla P, Ramakrishnan R and Sainis K: An immunologically active arabinogalactan from Tinosporacordifolia. Phytochemistry. 1999;52:1089–93