Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com (ISSN : 2277 –4572)
Research Article

IN VITRO AND *IN VIVO* ANTI-INFLAMMATORY EFFICIENCY OF *TRICHODESMA INDICUM* (L.) LEAF EXTRACTS

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DOI: 10.7897/2277-4572.04670

Received on: 25/10/15 Revised on: 06/11/15 Accepted on: 13/11/15

ABSTRACT

Objective of the study was to screen four extracts (HETI, ACTI, METI and AQTI) of *Trichodesma indicum* leaves (Family: Boraginaceae) and its different solvent soluble concentrations for possible *In vitro* enzyme assay, *In vivo* anti-inflammatory activity in experimental rats. *In vitro* anti-inflammatory activity was evaluated by 5-Lipoxygenase Enzyme Assay; *In vivo* anti-inflammatory activity was determined by carrageenan induced rat paw oedema method in experimental rats. *In vitro* anti-inflammatory enzymatic assay of four extracts showed significant inhibition against lipoxygenase. METI has less IC_{50} (133.55µg/ml) when compared to other three extracts. In *In vivo* analysis the methanol extracts of 200 & 400 mg/kg body weight showed significant inhibition of paw oedema by 55.61% and 71.43% ($P < 0.01$) respectively at 3rd hr compared to standard drug. The findings of studies demonstrated both *In vitro* and *In vivo* anti-inflammatory activity of the leaves of Trichodesma indicum.

KEYWORDS: *Trichodesma indicum* (L.), 5-Lipoxygenase, Carrageenan, paw oedema.

INTRODUCTION

Plants are also an important source of fine chemicals, which find their application in pharmaceutical industries across the globe. Plants have been the traditional source of raw materials and finished medicinals, since many centuries. A rich heritage of knowledge on preventive and curative medicines is available in ancient scholastic works. The development of the science of phyto pharmaceuticals and the hope for remedies for chronic diseases has generated new enthusiasm among researchers to develop herbal medicines.

Medicinal plants have pivotal role in the natural drug discovery. These are less toxic to humans, more effective in nature, economically less cost and freely available in nature[1]. Due to its wide range of activities people in olden days used plants for therapy even without the knowledge of the chemical constituents present in the plant.

Inflammation is a defence reaction of the organism and its tissue to injurious stimuli that lead to the local accumulation of plasmatic fluid and blood cells. Although it is a defence mechanism, the complex events and mediators involved in the inflammatory reaction can be included, maintained or aggravated by many diseases[2]. Inflammation is the starting process of any biological discomfort of the body. The anti-inflammatory activity was measured through *In vitro* and *In vivo* process. In the *In vitro* lipoxygenase enzyme assay was performed and *in vivo* carrageenan induced rat paw oedema method was followed.

Trichodesma indicum belongs to the family Boraginaceae. It is commonly known as Indian borage. This is having significance in traditional healing of diseases. *T. indicum* leaves and roots were used for snake bite, diuretic, dysentery and diarrhoea. Its root decotion is used for anti-inflammatory drug in folklore medicine. Previous studies also stated that chloroform root extract have shown anti-inflammatory activity in both acute and chronic models[3]. *T. indicum* whole plant has antitussive activity[4], anti-diarrheal activity[5,6], insecticidal activity[7], metal chelating activity[8] and corrosive inhibitor[9]. *T. indicum* aerial parts are effective against cancerous cell lines and showed highest cytotoxicity against human breast cell line MCF-7[10]. In the present study anti-inflammatory activity of *T. indicum* leaves was evaluated by both *in vitro* and *in vivo* methods.

MATERIALS AND METHODS

Plant material and Extraction

T. indicum plant material was collected from sheshachalam Hills, Tirupathi, Andhra Pradesh, India. Plant material was taxonomically identified and authenticated by the botanist. Leaves were shade dried and powdered with pulveriser.

Leaves were extracted with four solvents such as Hexane, Acetone, Methanol and Aqueous. The crude extracts were extracted with Soxhlet apparatus and condensed with rotary evaporator. The four crude extracts were condensed and lyophilized to obtain powder form for animal studies.
HETI : Hexane Extract
ACTI : Acetone Extract
METI : Methanol Extract
AQTI : Aqueous Extract

Animals

Male Sprague Dawley rats weighing between 150-200 g were obtained from Sainath Agency, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 25 + 2 °C with an alternating 12h light-dark cycle and relative humidity of 50 ±15%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and of the Regulatory body of the government. They were fed with standard laboratory diet and water *ad libitum* during the experiment. The experimental protocol was approved by the institutional animal ethical committee (IAEC) No: ANUCPS/IAEC/AH/P/13/2015 dated 13/03/2015.

Acute oral toxicity study

Oral toxicity test was performed according to OECD guide lines 423. The overnight fasting Rats were given oral dosage of 2000mg/kg body weight observed for 14 days for morbidity and mortality. The four extracts were analysed for the toxicity study and behavioural changes.

***In-vitro* Anti-inflammatory Analysis**
Evaluation of 5-Lipoxygenase Inhibitory Activity

Products of 5-LOX pathway of arachidonic acid metabolism may mediate some pathological events associated with acute inflammation and reversible airways obstruction of asthma[11,12]. Thus, activity of various extracts of *T. indicum* on 5-LOX inhibition was studied. 5-LOX enzyme inhibitory activity of *T. indicum* extracts were measured using the method of Reddanna[13] modified by Ulusu[14]. AQTI, HETI, ACTI & METI extracts were tested. The assay mixture contained 80mM linoleic acid, 10 µl potato 5 – LOX in 50mM phosphate buffer (pH 6.3). The reaction was initiated by the addition of enzyme buffer mixture to linoleic acid and the enzyme activity was monitored as the increase in absorbance at 234nm. The reaction was monitored for 120 Sec and the inhibitory potential of extracts was measured by incubating various concentrations of test for two minutes before addition of linoleic acid. All assays were performed in Triplicate. The percentage inhibition was calculated by comparing slope of test substance with that of enzyme activity.

$$\% \text{ of inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and average.

***In vivo* Anti-inflammatory analysis**

Effect of *Trichodesma indicum (L)* Extracts on Carrageenan-induced rat paw oedema[15,16]

Carrageenan induced hind paw oedema was determined according to the method of Winter and Vinegar. Albino rats of male sex weighed 150-200gms were divided into groups of six animals each the dosage of the drugs administered to the different groups were as follows group 1-control, group 3 to 4 – plant extracts, group 2-indomethacin (10 mg/kg) all the drugs were administered orally.

After one hour of the administration of the drugs, dose 0.1ml of 1% w/v carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rats and right hind paw served as the control. The paw volume of the rats was measured in the digital plethysmography, at the end of 0 min,60 min and 120 min., 180min the % increase in paw edema of the treated group was compared with that of the drugs under investigation were calculated based upon the percentage inhibition of the inflammation.

The extracts were administered orally in the following order
Group-I Received 0.1 ml of Drug vehicle (1% gum Acacia)
Group-II Received Indo methacin (10mg/Kg b.wt).
Group-III Received extract of *Trichodesma indicum (L)* 200 mg/kg
Group-IV Received extract of *Trichodesma indicum (L)* 400 mg/kg
The experiment was performed for the four extracts of HETI, ACTI, METI, AQTI with their two different concentrations of 200 and 400mg/kg body weight.

$$\text{Percentage inhibition} = \frac{\text{Control (increase in paw volume in 3rd h)} - \text{Test (increase in paw volume in 3rd h)}}{\text{Control (increase in paw volume in 3rd h)}} \times 100$$

Statistical Analysis

Values Expressed as Mean ±SEM (no of animals per group=6). The significance of various treatments and evaluation of data were calculated using One-way analysis of variance ANOVA method followed by dunnett's multiple comparison test and unpaired student's t test method in graph pad prism 5 analysis software and Microsoft Excel software; positive control group was calculated with reference to normal group; experimental groups were calculated with the positive control group & $P<0.05$ was accepted as significant.

RESULTS AND DISCUSSION

***In-vitro* Anti-inflammatory Activity**

Bioactive constituents finding has been increased in the view of anti-inflammatory compounds due to their usage in infectious diseases. Lipoxygenase and xanthine oxidase are the two enzymes responsible for the inflammatory mediated diseases like atherosclerosis, cancers, diabetes and hypertension[17]. In course of enzyme peroxidation a lipid peroxy radical was formed by scavenging of molecules.

In the present study four extracts of Trichodesma indicum were tested for *in vitro* anti-inflammatory activity. The previous studies also stated that roots are having anti-inflammatory activity. The *in vitro* inflammatory activity was performed by the enzyme lipoxygenase assay. The inhibitory action of extract towards lipoxygenase enzyme was calculated by percentage of inhibition and inhibitory concentration of half-life was measured based on those values. The four extracts have the better IC_{50} values when compared with the standard indomethacin. The IC_{50} values of methanol extract was 133.55 µg/ml, hexane 280 µg/ml, acetone 210.75 µg/ml and aqueous has 159.72 µg/ml was shown in Table 1 and Graph 1.

Comparison of four extracts showed dose dependent nature along with concentration. Percentage of inhibition was directly proportional to concentration of extract.

Acute Toxicity Study

In the evaluation of acute oral toxicity test dose up to 2,000 mg/kg body weight of all four extracts didn't cause any mortality in rats during 14 days of observation. Rats didn't show any signs of toxicity or behavioural changes or other physiological activities.

***In vivo* Anti-inflammatory activity**

The anti-inflammatory activity in the rats was performed by the carrageenan induced rat paw oedema method. Anti-inflammatory activity was measured with the plethysmometer apparatus. The average paws volume and percentage of inhibition of four extracts were given in table 4.19 to 4.23 and graph 4.20 to 4.24. Control group was treated with only carrageenan which causes localized oedema. Swelling of paw volume increased in this group due to untreated nature so that paw volume progressively reached maximum at 3rd hour after injection. The pre-treated crude extracts of *T. indicum* were found to be more significant in reduction of paw volume on 3rd hour. METI 400mg/kg b.wt treated group showed highest percentage of inhibition when compared to standard and it was 71.43% at 3rd hour. Effect of METI on controlling paw volume was high when compared to reference drug. HETI seems to be less

effective among four extracts (55.19%) whereas ACTI has 61.24 and AQTI has 54.74%. of inhibition at 400 mg/kg body weight (Table 2-6 and graph 2).

The inflammatory activity based on the inflammation obtained to the hind paws oedema was measured by the plethysmography. The values are expressed in mean ± SEM and the experiment is significant based on P value and the P value is < 0.05. Values which are <0.01 are more significant. The values were tabulated in the order of increasing concentration of four extracts. These are compared with the positive control and normal one.

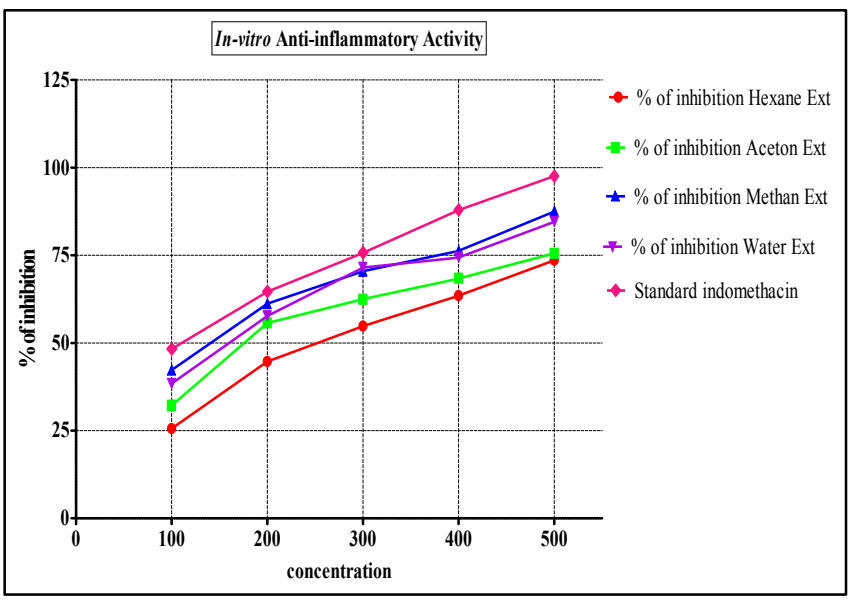
In the present study anti-inflammatory activity of different solvent extracts of *T. indicum* were investigated. Carrageenan analysis was selected because of its sensitivity towards inflammatory reaction and detected orally in acute phase of inflammation[18,19]. Carrageenan injected in intraplantar region leads to two different phases: initial phase up to two hours after injection which releases serotonin, histamine and bradykinin on vascular permeability, second phase has complement dependent activity leads to prostaglandin over production in tissue[19]. It also increased production of prostaglandins E_2 and nitric oxide (NO) observed in carrageenan challenged animals.

Trichodesma indicum was used in traditional medicine for anti-inflammatory activities; present data indicates that *T. indicum* has a significant immunomodulatory effect on different inflammatory responses. Different concentrations of *T. indicum* with different extracts possess moderate anti-inflammatory activity and it is dose dependent nature. Anti-inflammatory activity among different extracts has shown its variability towards compositional variation of individual extract.

Nitric oxide plays a vital role in chronic and acute inflammation on over production[20]. Nitric oxide increases vascular permeability, vasodilation and prostaglandins synthesis at the site of inflammation[21,22]. A good antioxidant extract have to inhibit nitric oxide to suppress inflammation. Interference of METI towards the inflammation was active in two phases. The percentage inhibition of paw oedema was 71.43% has shown deliberate active nature. AQTI also have significant inhibition along with METI. 400 mg/kg body weight concentrations of all extracts have substantial activity towards inflammatory reactions. However, treatment of these extracts has provided strengthened defence mechanism.

Table 1. *In-vitro* Anti-inflammatory activity of *Trichodesma indicum (L)*

S.No	Name of the Extract	IC $_{50}$ Value (µg/ml)
01.	Hexane Extract	280.70
02.	Acetone Extract	210.75
03.	Methanol Extract	133.55
04.	Aqueous Extract	159.72
05.	Indomethacin	97.52



Graph 1 *In-vitro* Anti-inflammatory activity of *Trichodesma indicum (L)*

Table 2 *In vivo* Anti-inflammatory Activity of *Trichodesma indicum (L)* Hexane Extract (HETI)

S. No	Drug	0 h	1st h	2nd h	3rd h	4th h	% inhibition at 3rd h
1	control (1%gum Acacia)	0.63 ± 0.03	1.64 ± 0.04	1.42 ± 0.03	1.54 ± 0.04	1.76 ± 0.04	
2	Indo methacin (10mg/Kg bwt)	0.59 ± 0.03	0.96 ± 0.06**	0.81± 0.05**	0.59 ± 0.03**	0.48 ± 0.05**	61.69
3	HETI (200 mg/kg bwt)	0.54 ± 0.06	1.35 ± 0.04*	1.12 ± 0.03*	0.78 ± 0.03*	0.64 ± 0.04*	49.35
4	HETI (400 mg/kg bwt)	0.61 ± 0.03	0.98 ± 0.04**	0.85 ± 0.05**	0.69 ± 0.04**	0.51 ± 0.03**	55.19
Values Expressed as Mean ±SEM (no of animals per group=6) animals *P<0.05 **P<0.01 ***P<0.001 compared to control (one way ANOVA followed by dunnett's multiple comparison test)							

Table 3 *In vivo* Anti-inflammatory Activity of *Trichodesma indicum (L)* Acetone Extract (ACTI)

S. No	Drug	0 h	1st h	2nd h	3rd h	4th h	% inhibition at 3rd h
1	control (1%gum Acacia)	1.05 ± 0.04	1.36 ± 0.05	1.64 ± 0.06	1.78 ± 0.07	1.89 ± 0.06	
2	Indo methacin (10mg/Kg bwt)	1.12 ± 0.03	0.96 ± 0.03***	0.85 ± 0.04***	0.64 ± 0.05***	0.6 ± 0.06***	64.04
3	ACTI (200 mg/kg bwt)	1.09 ± 0.05	1.02 ± 0.04**	0.91 ± 0.06**	0.76 ± 0.06**	0.73 ± 0.03**	57.30
4	ACTI (400 mg/kg bwt)	1.16 ± 0.06	0.98 ±0.04**	0.81 ± 0.03**	0.69 ± 0.03**	0.63 ± 0.05**	61.24
Values Expressed as Mean ±SEM (no of animals per group=6) animals *P<0.05 **P<0.01 ***P<0.001 compared to control (one way ANOVA followed by dunnett's multiple comparison test)							

Table 4 *In vivo* Anti-inflammatory Activity of *Trichodesma indicum (L)* Methanol Extract (METI)

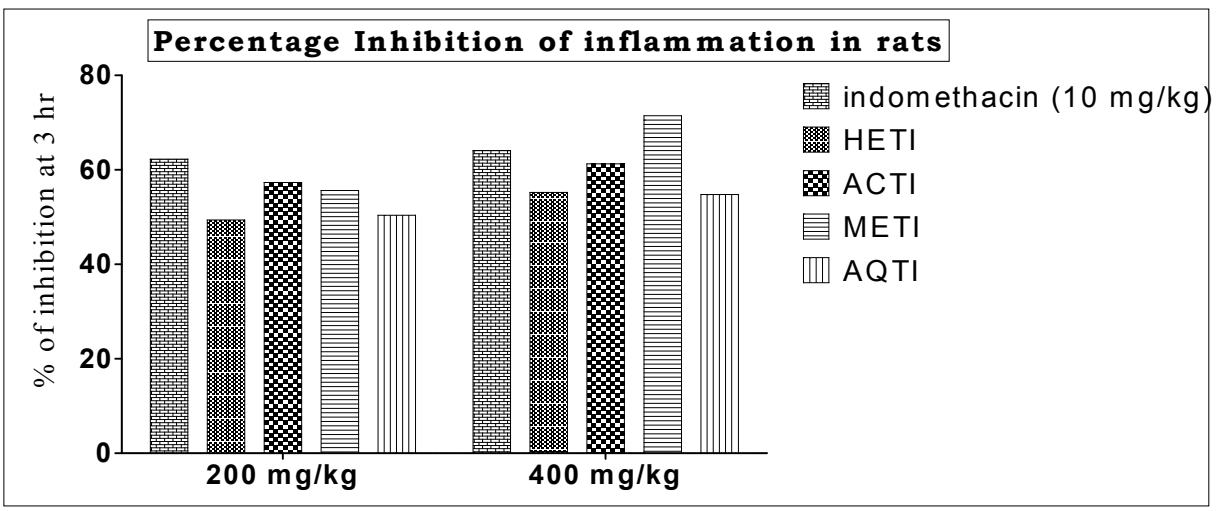
S. No	drug	0 h	1st h	2nd h	3rd h	4th h	% inhibition at 3rd h
1	control (1%gum Acacia)	1.52 ± 0.03	1.62 ± 0.03	1.79 ± 0.04	1.96 ± 0.04	2.24 ± 0.05	
2	Indo methacin (10mg/Kg bwt)	1.43 ± 0.05	1.21 ± 0.04**	0.96 ± 0.03**	0.74 ± 0.04**	0.69 ± 0.03**	62.24
3	METI (200 mg/kg bwt)	1.54 ± 0.06	1.32 ± 0.07**	1.05 ± 0.03**	0.87 ± 0.05**	0.83 ± 0.05**	55.61
4	METI (400 mg/kg bwt)	1.45 ± 0.05	1.12 ± 0.04**	0.84 ± 0.04**	0.56 ± 0.03**	0.53 ±0.03**	71.43
Values Expressed as Mean ±SEM (no of animals per group=6) animals *P<0.05 **P<0.01 ***P<0.001 compared to control (one way ANOVA followed by dunnett's multiple comparison test)							

Table 5 *In vivo* Anti-inflammatory Activity of *Trichodesma indicum (L)* Aqueous Extract (AQTI)

S. No	Drug	0 h	1st h	2nd h	3rd h	4th h	% inhibition at 3rd h
1	control (1%gum Acacia)	2.34 ± 0.03	2.46 ± 0.04	2.62 ± 0.05	2.74 ± 0.03	2.78 ± 0.04	
2	Indo methacin (10mg/Kg bwt)	2.29 ± 0.06	1.84 ± 0.06**	1.46 ± 0.05**	1.12 ±0.04**	1.08 ± 0.04**	59.12
3	AQTI (200 mg/kg bwt)	2.18 ± 0.05	1.94 ± 0.06**	1.56 ±0.03**	1.36 ± 0.04**	1.28 ± 0.05**	50.36
4	AQTI (400 mg/kg bwt)	2.25 ± 0.03	1.76 ± 0.06**	1.43 ± 0.05**	1.24 ± 0.06**	1.21 ± 0.05**	54.74
Values Expressed as Mean ±SEM (no of animals per group=6) animals *P<0.05 **P<0.01 ***P<0.001 compared to control (one way ANOVA followed by dunnett's multiple comparison test)							

Table 6 Comparative analysis of percentage inhibition at 3rd h

	HETI	ACTI	METI	AQTI	Indomethacin (10 mg/kg)
200 mg/kg b.wt	49.35	57.30	55.61	50.36	62.24
400 mg/kg b.wt	55.19	61.24	71.43	54.74	64.04



Graph 2 Comparative analysis of percentage inhibition at 3rd h

CONCLUSION

In summary, our results confirmed that *Trichodesma indicum* (L) exhibit significant anti-inflammatory property at all dose levels in both *in vitro* and *in vivo* analysis. The results have stated and suggest presence of bioactive components which may be worth for further elucidation.

AKNOWLEDGEMENT

The authors acknowledge to the University Grants Commission (UGC) Government of India, New Delhi, for funding this work and thanks to the Department of Biotechnology, Acharya Nagarjuna University, Guntur for supporting to carry out this work.

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How to cite this article:
K Narendra, DSD Suman Joshi, M Satya Prasad, KVN Rathnakar Reddi, J Swathi, KM Sowjanya, A. Krishna Satya. *In vitro* and *In vivo* anti-inflammatory efficiency of *Trichodesma indicum* (L.) leaf extracts. J Pharm Sci Innov. 2015;4(6):319-323 http://dx.doi.org/10.7897/2277-4572.04670

Source of support: University Grants Commission (UGC) Government of India, New Delhi, Conflict of interest: None Declared