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Original Article

Anti-inflammatory and analgesic activities of methanol extract of *Indigofera Trita Linn*

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ABSTRACT

An Inflammation is a major condition associated with various diseases. Rheumatoid arthritis is one of the challenging disorders associated with inflammatory conditions. Medicinal plants are the natural sources which have viewed as a fruitful and logic research strategy in the search of new antiinflammatory and analgesic drugs. The Present study was to evaluate the analgesic and anti-inflammatory activities of the methanol extract of *Indigofera trita* (MIT). Anti-inflammatory activity of the extract was evaluated by using carageenan induced Paw edema method. The analgesic activity of the extract was evaluated for its central and peripheral pharmacological actions by using Eddy's hot plate method and acetic acid induced writhing respectively the study was carried out using two different dose of 200 & 400 mg/kg orally. In this experimental model an inhibition of paw oedema significant from the treatment hour was observed. It is concluded that methanol extract of *Indigofera trita* have an, as well as acute and chronic anti-inflammatory activity.

Key Words: Anti-inflammatory, analgesic, *Indigofera trita*

INTRODUCTION

Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy [1]. Medicinal plants are the basic for the treatment of various disease [2]. Nearly 80% of people living in developing countries still depend on plant based traditional medicine for their primary health care and almost three-fourths of the herbal drugs used worldwide are derived from medicinal plant [3]. The plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [4]. Oxidative stress is thought to play an important role in the pathogenesis of inflammation not only through direct injurious effects, but also by involvement in molecular mechanism [5]. Inflammation is a disorder involving localized increases in the number of leukocytes and a variety of complex mediator molecules [6]. Prostaglandins are ubiquitous substance that indicate the modulate cell & tissue, response involved in inflammation their biosynthesis has also been implicated in the pathophysiology and connective tissue disorder, cancer & cardiovascular disease [7]. Medicinal plants are the natural source of biologically active compounds knowns as phytonutrients. Phytonutrients that have either defensive or disease preventive properties [8]. The research into plants with alleged folkoric use as pain revelers anti-inflammatory agents should therefore be viewed as a fruitful and logical research strategy in the search of new analgesis and antiinflammatory drugs.

Carrageenan induced inflammation is an acute test is widely used as a model for the evaluation of anti-inflammatory activity of drugs. Drugs which are used presently for the management of pain inflammatory conditions which are known side toxic effects. It will documented the use of non-steroidal anti-inflammatory drugs (NSAIDS) produce intestinal tract ulcers 30-50% of cases, it associated with 10,000 – 20,000 deaths per year in the U.S.[9].

Indigofero trita Linn, a family of Fabaceae in an under shrup widely distributed in India, Ceylon, South Africa and North Australia the plant was known as kattuavuri and punal murungai in Tamil. The entire plant is traditionally used for various ailments including Liver disorders and tumors[10]. It is found to be active against transplantable tumors the plant also possesses strong antioxidant and hepatoprotective activity [11]. Hence the present

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study was designed to investigate as an effort to develop safe and more effective drug for rheumatoid arthritis, *Indigofera trita* was chosen to evaluate its protective effect on experimentally induced arthritis by using various *in vitro* and *in vivo* models.

- To find out Analgesic activity of methanolic extract of *I. trita* by acetic acid induced writhing test in mice.
- To study the effects of Anti-inflammatory activity of methanolic extract of *I.trita* in Albino rats.
    - Carragenan induced rat paw edema
    - Hot plate method.

MATERIALS AND METHODS

Collection and Identification: *Indigofera trita* was collected from Ammapettai in Thanjavur District. The plant was authenticated by Dr. Jayaraman Director Plant anatomy & research centre west Thambaram Chennai (PAR/2015/3042). The Specimen was stored in our Lab.

Preparation of Plant Extract

Extraction: The entire plant was shade dried and pulverized. The coarse powder of 500gm packed in a soxhlet apparatus to continuous hot percolation, for 8 hours using 1.5 litres of methanol as a solvent. The extract was concentrated under vacuum and dried in a desiccators yield and 23.25g (6.7% w/w).

Animals: Male wistar albino rats (150-200g) and swiss albino mice (20-25g) were divided into four group of six animals used throughout the study. They were housed & in a controlled environment with standard laboratory diet and water *ad libitum*. The experiments were performed in accordance with the guidelines established by the Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Trichy, The of Registration number is CPCSEA/265.

Antiinfiammatory Activity

Carrageenan-induced rat paw edema: The rats were divided into four groups (n=6). The different groups were treated orally with MIT (200 & 400 mg/kg), Indomethacin (10mg/kg) and Vehicle control 5% gum acacia, (2ml/kg) of body weight. Administration of extract and drug was 1 hr prior to injection of 0.1ml of 1% freshly prepared suspension of carrageenan in normal saline in the left hind paw of the rat. The Paw volume was measured initially and then at 1, 2 and 3hrs after carageenan injection by using PlethySmometer[12].

The edema inhibitory activity was calculated according to the following formula.

Anti-inflammatory activity (%) = $1 - (V_T/V_C) \times 100$

V_T = respresent paw volume in drug treated animal

V_C = Paw Volume of Control group

Analgesic Activity

Acetic Acid Induced writing Test: The test was carried out in mice by using the method of koster et al.[13]. 0.6% v/v acetic acid (80mg/kg) induced by intraperitoneal injection, the writhes were counted. Two different dose of MIT (200 & 400 mg/kg) were administered orally to the group II and group III of six animals. Group I were served as control (5% gum acacia, 1ml/100g of body weight) and group IV animals received Aspirin 300 mg/kg. The extract and standard drug was administered 30min before chemical stimulus. The number of muscular contraction was counted over a period of 30min and it is expressed as writhing numbers.

Hot Plate Method: To evaluate parameters were the latency time for paw licking and jumping responses on exposure to hot plate surface maintained at room temperature $55^0C \pm 2$. The hot plate methods in rats were performed by Eddy and Leimbach[14]. The animals were divided into four groups of six animals. The animals were kept in the hot plate until it lifted one of its hind paws. Group I served as control (5% gum acacia, 1ml/100g body weight) group II & group III received MIT at dose of 200 & 400 mg/kg orally. Group IV received pentazocin at a dose of 5mg/kg. All the treatment were given 30min response were determined at 60,120 & 180 min.

RESULTS

The results of MIT against carrageenan – induced paw edema is show in Table–1 MIT (200 & 400 mg / kg) have significant ($P < 0.001$) reduction of rate paw edema at all assessment times in dose dependent manner. The extract showed maximum inhibition 53% at the dose of 400mg/kg after 3h of drug treatment in carrageenan – induced paw edema, whereas standard drug showed 59% of inhibition. The extract (200 & 400 mg / kg) dose dependent in reduce acetic acid induced writhing in mice. The result were showed in Table 2 and the reduction was statistically significant ($P < 0.01$) when compared to control. The effect of MIT (200 x 400 mg/kg) the animals were pretreated showed dose dependent increase in latency of response in the hot plate method. The results of increase in the latency responses were significant ($P < 0.01$) shoed in the Table. 3.

Table – 1 Effect of MIT on Carageenan induced rat paw edema.

Treatment	Dose	Paw Volume in ml				% inhibition after 3 hrs.
		0hrs.	1 hrs.	2hrs.	3hrs.	
Control (Saline)	2 ml	0.16 ± 0.001	0.24 ± 0.05	0.26 ± 0.003	0.35 ± 0.007	-
Indonethacin	10mg	0.12 ± 0.006	0.14 ± 0.004	0.16 ± 0.009	0.19 ± 0.130	41
MIT	200mg	0.14 ± 0.001	0.20 ± 0.08	0.21 ± 0.010	0.25 ± 0.007	49
MIT	400mg	0.12 ± 0.006	0.15 ± 0.011	0.18 ± 0.009	0.20 ± 0.004	43

N=6, P<0.001 Vs Control Date were analysed by one ANOVA Followed by Dunnett test.

Table. 2. Effect of MIT on Chemical Stimulus Induced Pain in Rats

Treatment	Dose / Kg	No. of Writing (20 min)	Percentage Inhibition
Control	-	79.8 ± 2.45	-
Aspirin	300 mg	26.5 ± 1.72	66.79
MIT	200 mg	63.5 ± 1.72	22.32
MIT	400 mg	54.6 ± 1.60	31.93

N=6, P < 0.01 Vs Control Date were analysed by one way ANOVA followed by Dunnet test.

Table 3. Effect of MIT on Thermic Stimulus Induced (Hot Plate Pain in Rats)

S. No	Treatment	Dose/kg	Reaction times in seconds of time (hr)			
			0hrs.	1 hrs.	2hrs.	3hrs.
1.	Control (Saline)	2 ml	2.3±0.14	2.32±0.30	2.44±0.15	2.34±1.3
2.	Pentazocin	5 mg	2.2±0.4	7.5±0.22	9.71±1.09	7.84±0.14
3.	MIT	200mg	2.5±0.22	5.2±0.21	8.05±0.74	6.8±1.09
4.	MIT	400mg	2.6±0.06	6.84±0.31	8.78±0.37	7.20±0.36

N=6, P<0.001 Vs Control Date were analysed by one way ANOVA

DISCUSSION

In recent times, many traditionally used medicinally important herbs were clinically reported for their anti-inflammatory potential by various investigations working on the same line, in the present study the anti-inflammatory and analgesic activity of traditional medicine *Indigofera trita* has been undertaken.

Edema which develops after carrageenan inflammation is a biphasic event. The edema maintained between the first & second phase is due to bradykinin like substances[15]. Based on this, it could be argued that the suppression of the First Phase may be due to inhibition of the release of early mediators, such as histamine & Serotonin, and the action in the second phase may be explained by an inhibition of Cyclooxygenase[16].

Besides in the carrageenan induced rat paw edema model, the production of prostanoids has been through the serum expression of Cox–2 by a positive feedback mechanism[17]. Therefore it is suggested that the mechanism of action of MIT may be related to prostaglandin synthesis inhibition.

It is known that non-steroidal anti-inflammatory drugs usually do not increase the pain threshold in normal tissue, whereas local anesthetics and narcotics[18]. However, the hot plate test was undertaken to verify if MIT would have any central analgesic effect. The results of MIT treated showed significant activity when compared to control group & nearly equal to the group treated with pentazocin (5mg /g). Hence, it is assumed that MIT has significant analgesic effect on the central nervous system.

In writhing test, the research group of Deraedt et al.[19] describe the quantification of Prostoglandins by radioimmunoassay in the peritoneal exudates of rats obtained after intraperitoneal injection of acetic acid. After injection of acetic acid in the first 30 min prostaglandins PGE 2α and PGF 2α is found in increased level.

Nevertheless, injection of acetic and it induce not only prostaglandins, but also of the sympathetic nervous system mediators[20-21]. Since MIT was effectively inhibiting the writhings in mice in dose dependent manner. The result was comparable with the group treated with aspirin. From the above discussion, the methanol extract of whole plant of *Indigotera trita* exhibited significant anti-inflammatory and analgesic activity.

Methanolic extract of *Indigofera trita* significantly diminished in a dose dependent way the induced paw edema in rats. The extracts were able to reduce the inflammation in acute and chronic phases. It is

concluded that high doses of 200 and 400mg/kg methanolic extract of *Indigofera trita* plant extract have moderate anti-inflammatory and analgesic activity. Methanolic extract of *Indigofera trita* as a novel and potential agent in the management of Pain, which are mediated by inhibition of various autocoids formation & release. Further studies to develop novel formulation with significant anti-inflammatory & analgesic potential can be used for the prevention or treatment of human diseases such as cancer, arthritis and diabetes mellitus. However the chemical constituents and mechanism responsible for the pharmacological activities remain investigated.

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