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PROTECTIVE EFFECT OF AQUEOUS EXTRACT OF *TINOSPORA SINENSIS* AGAINST FORMALDEHYDE INDUCED ARTHRITIS IN RATS

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ABSTRACT

The present study was aimed to assess the anti-arthritic nature of aqueous extract of *Tinospora sinensis* leaves (AETS) against formaldehyde induced arthritis in rats. The degree of inflammation was evaluated by hind paw swelling and increase in paw diameter. AETS showed significant changes in paw swelling, paw diameter and percent inhibition of paw volume. The results of the current investigation concluded AETS possess a significant anti-arthritic activity against formaldehyde induced arthritis model and justifying its therapeutic role in arthritic condition. The observed anti-arthritic activity may be due to the presence of phytoconstituents such as alkaloid, saponins and flavonoids.

INTRODUCTION

Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic, progressive, systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint destruction, which is responsible for the deformity and disability. The consequent morbidity and mortality has a substantial socio-economic impact. Epidemiology of the arthritis in female: male is 3:1 and the prevalence is 1% of the world population [1]. In human as well as in animal models, RA is characterized by a series of pathological processes of the joints, such as leukocyte infiltration, a chronic inflammation, pannus formation, and extensive destruction of the articular cartilage and bone. Although the exact cause of RA has not been elucidated in detail, pro-and anti-inflammatory cytokines seem to play an important role in the etiology of the disease. In particular, it was reported that the inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 play key roles in the inflammation and joint damages during the development of RA [2].

Tinospora sinensis (syn: *Tinospora malabarica*) is a plant that grows almost throughout India and other South East Asian countries and belongs to the family Menispermaceae. The stem of this plant has great therapeutic value traditionally in treating debility, dyspepsia, fever, inflammation, syphilis, ulcer, bronchitis, jaundice, urinary disease, skin disease and liver disease [3] and known for its adaptogenic and immunomodulatory properties [4]. The aqueous and alcoholic extracts of this species are reported to have many biological potential, such as anti-inflammatory, antidiabetic, hepoto protective, and immunomodulatory, and adaptogenic [5]. Previous phytochemical investigations have discovered that this species contains steroids, flavonoides and alkaloids. In India, cassava is used for the treatment of ringworm, tumor, conjunctivitis, sores and abscesses. The plant has been used in the Indian traditional medicines from time immemorial. It is associated with various important medicinal properties. Earlier findings suggest that presence of phytochemicals such as alkaloids, flavonoids, steroids are responsible for anti-arthritic

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activity [6]. So the present study was carried out to evaluate effect of aqueous extract of *Tinospora sinensis* leaves on formaldehyde induced arthritis in male wistar rats.

MATERIALS AND METHODS

Materials

Chemicals and Drugs

Diclofenac Sodium injection (Diclolab, BDH industries, mumbai), Formaldehyde (Poona chemical Ltd, Pune).

Instruments Used

Plethysmometer (UGO Basile, Italy), Verneir caliper (Malik tools, Mumbai), Oral feeding needle (BIK Industries, Mumbai).

Animals

Male Wistar rats (150-250 g) or female Swiss albino mice (20-25 gm) obtained from the Yash Farm and National Toxicological Centre, Pune, were used for study.

Housing conditions

Animals were maintained at a temperature of 25±1°C and relative humidity of 45 to 55 % under 12 hr light and 12 hr dark cycle. The animals had free access to standard food pellets, procured from Pranav Agro Industries Ltd., Sangli, India and water *ad libitum*.

Methods

Collection and Authentication of Plant Material

The leaves of *Tinospora sinensis* was collected from Bhor region of Maharashtra in the month of September-October 2011 and authenticated by Botanical Survey of India, Pune and herbarium voucher specimen No: BSI/WRC/Tech/2012/NVDAEM5.

Preparation of Aqueous Extract of *Tinospora sinensis* (AETS) leaves

Leaves of *Tinospora sinensis* were shade dried and coarsely powdered by using grinder mixer. The powdered material was macerated in sufficient quantity of distilled water with small quantity of chloroform to prevent fungal growth and kept for 3 days. During maceration it was shaken twice daily. On third day it was filtered and dried at 60 °C on water bath [7]. The extract was then preserved in the desicator and then used for phytochemical and pharmacological studies.

Phytochemical screening of the extract

AETS was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids [8].

Acute oral toxicity study (AOT)

Healthy adult swiss mice (20-30 gm) were subjected to acute oral toxicity studies as per Organization for Economic Co-operation and Development (OECD) guidelines (AOT-423). Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days.The changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity and behaviour pattern were noted.

Experimental Designs

Effect of aqueous extract of *Tinospora sinensis* in formaldehyde induced arthritis

Animals were randomly divided into five groups of six animal each (n=6). Rats were injected with 0.1 ml 2% (v/v) of formaldehyde solution in the planter surface of the left foot, on the first and third day of the test. Drug treatment was started from the initial day i.e. from the day of formaldehyde injection (0day) and continued till 10^{th} day. The rat paw volume and paw diameter was recorded daily by using following Plethysmometer and verneir caliper respectively [9,10].

STATISTICAL ANALYSIS

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using student t-test or one-way analysis of variance (ANOVA) followed by Dunnet's test and *P<0.05 and **P<0.01were considered to be statistically significant.

RESULT

Physical properties of AETS

Colour - Blackish Brown
Odour- Characteristic
Taste- Bitter
Appearance- Sticky
% Yeild- 10.23 %

Phytochemical screening of the extract

Phytochemical study of AETS showed the presence of various phytoconstituents like alkaloids, carbohydrates, glycosides, saponins, tannins, and flavonoids.

Acute oral toxicity (AOT) of AETS

According to OECD guidelines for acute oral toxicity at the dose of 2000mg/kg, animals in the group treated with AETS did not showed any symptoms of toxicity at this dose level and no mortality was observed during the 14 days of observational period. Hence, according to the guideline, the different doses of AETS selected present study for per oral administration were 200 mg/kg (Middle dose) and 400 mg/kg (Upper dose).

Table 1. Treatment Schedule in formaldehyde induced arthritis model

Group No	Treatment	Dose	Route of Administration
I	Normal	Distilled water 5 ml/kg	Per Oral.
II	Control	Formaldehyde (2%) 0.1ml	Sub Planter.
III	Diclofenac	Diclofenac 10 mg/kg	Intraperitoneally.
		Formaldehyde (2%) 0.1ml	Sub Planter.
IV	AETS I	AETS 200 mg/kg	Per Oral.
		Formaldehyde (2%) 0.1ml	Sub Planter.
V	AETS II	AETS 400 mg/kg	Per Oral.
		Formaldehyde (2%) 0.1ml	Sub Planter.

Table 2. Effect of AETS on Formaldehyde induced arthritis paw volume (ml)

Groups	Paw Volume (ml)			
	Day 0	**Day 3**	**Day 6**	**Day 10**
Normal	0.82 ± 0.09	0.82 ± 0.05	0.83± 0.09	0.84 ± 0.04
Control	0.94 ± 0.05	1.75 ± 0.13##	2.11 ± 0.12##	1.92 ± 0.10##
Diclofenac (10mg/kg)	0.92 ± 0.06	1.23 ± 0.10*	1.29 ±0.14**	1.05 ± 0.12**
AETS (200mg/kg)	0.92 ± 0.08	1.47 ± 0.19	1.53 ± 0.12**	1.34 ± 0.11**
AETS (400mg/kg)	0.97 ± 0.07	1.41 ± 0.12	1.45 ± 0.10**	1.24 ± 0.14**

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01. as compared with control (One-way ANOVA followed by Dunnet's test). ## indicates significant induction when compared with normal group.

Table 3. Effect of AETS on % Inhibition of Paw Volume

Groups	% Inhibition of Paw Volume			
	Day 0	**Day 3**	**Day 6**	**Day 10**
Control	-	-	-	-
Diclofenac (10mg/kg)	-	61.72%	69.23%	86.73%
AETS (200mg/kg)	-	32.09%	47.86%	57.14%
AETS (400mg/kg)	-	40.74%	55.55%	69.38%

Table 4. Effect of AETS on Formaldehyde induced arthritis Paw diameter (mm)

Groups	Paw diameter (mm)			
	Day 0	**Day 3**	**Day 6**	**Day 10**
Normal	7.35 ± 0.08	7.37± 0.10	7.38 ± 0.04	7.38 ± 0.07
Control	7.55 ± 0.36	16.50 ± 0.76##	19.67 ± 1.02##	17.50 ± 1.38##
Diclofenac (10mg/kg)	9.00 ± 0.44	12.66 ± 1.05	12.50±1.05**	10.17±0.70**
AETS (200mg/kg)	8.00 ± 0.25	13.67 ± 1.35	15.00 ± 1.06*	12.00 ± 0.93**
AETS (400mg/kg)	8.33 ± 0.21	13.00 ± 1.21	14.67±1.43**	11.67±0.88**

Values are expressed as mean ± SEM (n=6). *P<0.05, **P<0.01. as compared with control (One-way ANOVA followed by Dunnet's test). ## indicates significant induction when compared with normal group.

Effect of AETS on Formaldehyde induced arthritis paw volume (ml)

Sub planter injection of Formaldehyde (0.1ml) on 1st and 3rd day to the rat hind paw led to development of arthritis which reached a peak edema on 6th day of injection. Diclofenac (10mg/kg) treated group showed significant decreased in paw edema on 3rd (P<0.05), 6th (P<0.01) and 10th (P<0.01) day. AETS (200mg/kg) and AETS (400mg/kg) showed significant de decreased in paw volume on 6th and 10th day with P<0.01

Effect of AETS on Formaldehyde induced arthritis Paw diameter (mm)

Sub planter injection of Formaldehyde (0.1ml) on 1st and 3rd day to the rat hind paw led to increase in paw diameter which reached peak at 6th day of injection. Diclofenac (10mg/kg) treated group showed significant decreased in paw edema on 6th and 10th day with P<0.01. AETS (200mg/kg) and AETS (400mg/kg) showed significant decreased in paw edema on 6th and 10th day with P<0.01.

Fig 1. Effect of AETS on Formaldehyde induced arthritis paw volume (ml)

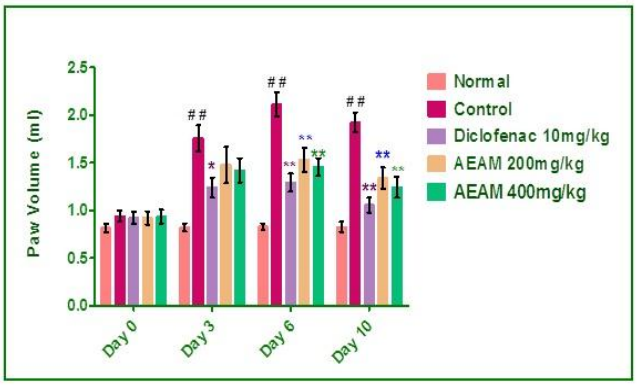


Fig 2. Effect of AETS on % Inhibition of Paw Volume

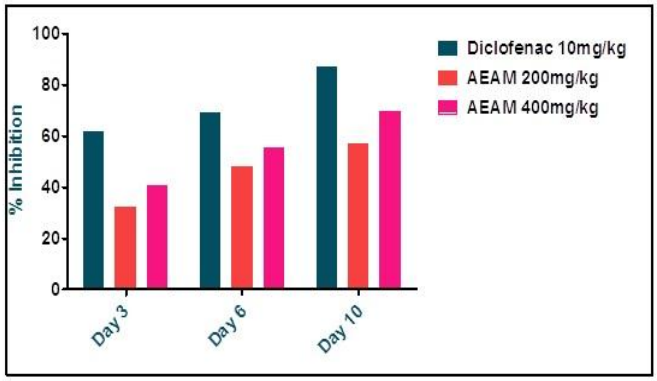
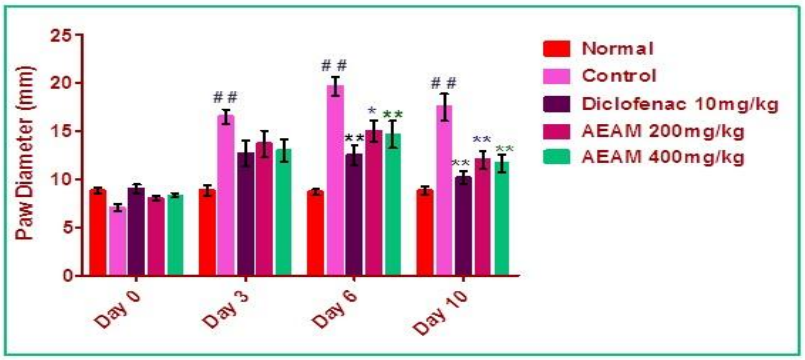


Fig 3. Effect of AETS on Formaldehyde induced arthritis paw diameter (mm)



Effect of AETS on Percent inhibition of paw volume

Animals treated with AETS 200mg/kg showed % inhibition of paw volume on the day 3 (32.09%), day 6 (47.86%) and day 10 (57.14%) whereas animals treated with AETS 400mg/kg showed % protection of paw volume on the day 3 (40.74%), day 6 (55.55%) and day 10 (69.38%). Diclofenac treated group showed % protection of paw volume on the day 3 (61.72%), Day 6 (69.23%) and day 10 (86.73%).

DISCUSSION

RA is a chronic inflammatory disease affecting about 1 % of the population in developed countries. The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and a pause in body weight gain; during the acute period, the hind and fore paw joint diameters increase. In chronic stages of the disease rats with arthritis are often relatively immobile due to the severity of paw swelling [10,11].

Eventhough various categories like immune suppressants, NSAIDs, steroidal anti inflammatory drugs are being used till now, but the potential side effects give a limitation for their use. Traditional medicines derived mainly from plants play major role in the management of arthritis as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for arthritic therapy [12]. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded. Formaldehyde induced arthritis is one of most commonly used acute model for assessing anti-arthritic potential of plant extract. The development of edema in the paw of the rat after injection of formaldehyde (0.1ml,2% w/v) is due to the release of histamine, serotonin and the prostaglandin like substances at the site of injection. Both histamine and prostaglandin are the key mediators in inflammatory hyperalgesia that is mediated through the activation of local pain receptors and nerve terminals producing hypersensitivity in the area of injury [13, 18]. Inhibition of paw edema and paw diameter observed in formaldehyde models may be due to the ability of the AETS to inhibit histamine, serotonin and the prostaglandin which are responsible for inflammation. In present study Diclofenac is used as standard drug. The Diclofenac is a NSAIDs acts by inhibition of prostaglandins (PGs) synthesis by blocking COX enzymes responsible for inflammation [14].

CONCLUSION

Our photochemical investigation revealed that the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, and flavonoids in AETS. Presence of wide range

of constituents indicates the good efficacy of this plant in various pathological disorders [15]. Saponins, steroids, alkaloids are known to inhibit articular swelling ,decrease arthritic index and regulate down the content of IL-IB and TNF-α in the inflammatory tissues of arthritic rats [16]. Beside these flavonoids has been reported to inhibit the cyclooxygenase enzyme thereby inhibiting prostaglandin synthesis which are responsible for development of arthritis. Pharmacological studies indicate that flavonoids and saponin have anti-inflammatory and antiarthritic activity [17-23]. Thus, in the light of above facts, it can be demonstrated that the AETS may serve as an effective anti-arthritic drug and the effect might be speculated due to phytochemicals such as saponins and flavonoids. This study warrants the investigation to isolate and identify the active principles and to investigate the exact mechanism of action of AETS against arthritis.

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