



IN-VIVO SCREENING OF *TINOSPORA SINENSIS* LEAVES FOR ITS ANALGESIC, ANTI-PYRETIC AND ANTI-INFLAMMATORY ACTIVITIES

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ABSTRACT

Previous phytochemical analysis of methanolic extract of *Tinospora sinensis* leaves (METS) has indicated the presence of steroids, flavonoids, glycosides and terpenoid types of compounds. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, prompted us to check *Tinospora sinensis* leaves for possible analgesic anti-inflammatory and anti-pyretic activities. The analgesic activity of METS was studied using tail immersion method in mice. While the efficacy of the METS (150 and 300mg/kg.p.o) was compared with pentazocaine 30 mg/kg. METS recorded 79.88 % inhibition with the highest dose and 81.21% with pentazocaine. The antipyretic activity of METS was studied in Brewer's yeast-induced pyrexia in rats. METS showed more significant activity. METS were also subjected to evaluate anti-inflammatory activity by carrageenan induced rat paw edema. Indomethacin (100mg/kg) was taken as standard drug. METS produced dose-dependent of oedema, which was comparable to Indomethacin and found to be 48.32% and 42.32 % inhibition in carrageenan induced rat paw edema model. Findings from the present study showed METS to possess central and peripheral analgesic activity, anti-inflammatory property similar to steroidal and non-steroidal agents as well as antipyretic effect.

INTRODUCTION

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 and known for its adaptogenic and immunomodulatory

properties. The aqueous and alcoholic extracts of this species are reported to have many biological potential, Such as anti-inflammatory, antidiabetic, immunomodulatory, hepatoprotective and adaptogenic. 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 [1-4]. The main part used is leaves. Hence, the present investigation is an attempt in this direction and includes evaluation of analgesic and anti-pyretic activity of aqueous and methanolic extracts.

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MATERIALS AND METHODS



Collection and authentication of plant material

The fresh leaves of *Tinospora sinensis* were collected in the Tirumala Hills and authenticated by Department of Pharmacognosy, Trinity College of Pharmaceutical Sciences, Andhra Pradesh and the voucher specimen was kept in the Trinity College of Pharmaceutical Sciences, A.P India.

Extraction of plant drug

The collected leaves were washed, shade dried and converted into moderately coarse powder by mechanical grinder. The powdered material was extracted with methanol by using soxhlet apparatus. The solvent was removed under reduced pressure which yields respective extracts in the form of semisolid mass.

Animals

The study was conducted on male Wister rats (175-200gm) housed in polypropylene cages under standard conditions of temperature ($22 \pm 2^\circ\text{C}$), relative humidity ($60 \pm 5\%$) and light (12h light/dark cycle) were used. They were fed with standard diet and water. The food was withdrawn 18 hours before the experiment but allowed free access of water. All animal experiments were carried out in accordance with the guidelines of CPCSEA. The experimental protocol is duly approved by the institutional ethical committee (reg.no.212/2012/apc).

Acute oral toxicity studies

Acute toxicity was carried out according to Organization of Economic Co-Operation and Development (OECD) no 425 guidelines and LD₅₀ values was estimated to be >5000mg/kg. Based on the results obtained from this study, the doses of further pharmacological studies were fixed to be 150 and 300mg/kg [5].

ANALGESIC ACTIVITY

Tail immersion method

Mice were divided into four groups each consisting of six mice. The treatment regimen was as follows [6]

1. Group I (Control): Vehicle (2ml/kg, p.o), 1% suspension of tween-80.
2. Group II (Standard): Pentazocaine (30mg/kg, p.o)
3. Group III (Test 1): METS(150mg/kg, p.o)
4. Group IV (Test 1): METS(300mg/kg, p.o)

The distal part of the tails of the animals was immersed in hot water maintained at 55. The time taken to withdraw the tail was noted as the reaction time. A cut-off time of 10s was maintained at 55C to prevent tissue damage. The reaction time was checked at 0, 15, 30, 45 and 60 min respectively after treatment.

ANTIPYRETIC ACTIVITY

Yeast induced pyrexia method

Rats were divided into four groups each consisting of six Rats. The treatment regimen was as follows [7]

1. Group I (Control): Vehicle (2ml/kg, p.o), 1% suspension of tween-80.
2. Group II (Standard): Paracetamol (20mg/kg, p.o)
3. Group III (Test 1): METS (150mg/kg, p.o)
4. Group IV (Test 2): METS (300mg/kg, p.o)

The test was performed in rats by injecting 10ml/kg, s.c, of the 10% aqueous solution of brewer's yeast to induce pyrexia. The rectal temperature of each animal was taken before and 24hr after the yeast injection using a digital clinical thermometer. Animals did not show a minimum increase of 0.7C in the temperature 24h after the yeast injection was discarded. The rectal temperature of each animal was given recorded at 0, 1, 2, 3, 4,5hr after treatment.

ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced rat paw edema

Rats were divided into four groups each consisting of six Rats. The treatment regimen was as follows [8]

1. Group I (Control): Vehicle (2ml/kg, p.o), 1% suspension of tween-80.
2. Group II (Standard): Indomethacin (20mg/kg, p.o)
3. Group III (Test 1): METS (150mg/kg, p.o)
4. Group IV (Test 2): METS (300mg/kg, p.o)

The drugs were given orally. After one hour, a sub plantar injection of 0.1ml of 1% carrageenan was administered in the right hind paw to all the three groups. The paw volume was measured plethysmographically at 0 min, 60 min, 120 min and 180 min. The average paw of swelling in a group of extract treated rats was compared with control group (treated with vehicle) and the positive control (Indomethacin).

Statistical analysis

The result were express as Mean \pm SEM. Statistical analysis was carried out using one way ANOVA followed by Student-t test.

RESULTS AND DISCUSSION

The results obtained showed that the methanolic extracts possess a significant analgesic effect on tail immersion model. The results were tabulated in table 1. This suggests that the analgesic effect of the extract may be peripherally mediated and it might be centrally acting.

The methanolic extract caused a better hypothermal activity against yeast-induced pyrexia in rats. The subcutaneous injection of yeast induces pyrexia by increasing the synthesis of prostaglandin and is used to screen agents for an antipyretic effect. The results were tabulated in table 2.

Carrageenan induced paw edema was taken as prototype of exudation phase of inflammation where



development of edema being described as biphasic. The initial phase which occurs between 0 and 2h after injection of carrageenan has been attributed to release of histamine, serotonin and bradykinin. Inflammation volume reaches its maximum approximately 3h post treatment after which it begins to decline. A more second phase is related to release of prostaglandin like substances. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action. The carrageenan induced paw edema model in rats is known to be sensitive to

cyclooxygenase inhibitors. Based on this report it was concluded that the inhibitory effect of METS on carrageenan induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Pre-treatment of rats with the extract (50-200 mg/kg) significantly inhibited the paw oedema induced by carrageenan when compared with the control group and 300 mg/kg produced a better efficacy comparable to Indomethacin-treated group. The results were tabulated in table 3.

Table 1. Analgesic activity of METS by tail immersion model in mice

Group	Treatment	Reaction time (S)	Latency (%)
I	Control (2ml/kg, p.o)	2.81±0.42	--
II	Standard(Pentazocaine:30mg/kg, p.o)	4.91±0.21	81.21
III	METS (150mg/kg, p.o)	3.01±0.51	49.34
IV	METS (300mg/kg, p.o)	4.7±0.42	79.88

Table 2. anti-pyretic activity of METS in yeast induced pyrexia in rats

Group	Dose (mg/kg)	Rectal Temperature in °C at time (hr)						
		18 hr after yeast injection	0	1	2	3	4	5
Control	2 ml/kg (p.o)	37.2±0.02	37.1±0.02	36.7±0.04	36.4±0.02	36.0±0.01	35.8±0.03	35.4±0.02
Paracetamol	20 (mg/kg.p.o)	37.1±0.02	36.8±0.08	36.5±0.01	36.2±0.07	36.0±0.03	35.5±0.06	35.0±0.01
METS	150 (mg/kg.p.o)	37.5±0.03	37.5±0.08	37.1±0.05	36.7±0.07	36.5±0.04	36.2±0.06	36.1±0.04
	300 (mg/kg.p.o)	37.3±0.02	73.0±0.01	36.6±0.08	36.1±0.03	35.9±0.06	35.3±0.01	35.1±0.02

Table 3. Anti-inflammatory effect of METS in carrageenan induced paw edema in rats

Groups	Treatment	% Increase in paw volume				% inhibition in paw volume
		0	60	120	180	
I	Control(2ml/kg, p.o)	29.41±1.21	65.32±1.31	95.24±1.42	102.24±2.1	--
II	Standard(Indomethacin 20mg/kg, p.o)	28.21±1.71	55.22±3.1	74.31±0.31	56.21±1.2	42.32
III	METS(150mg/kg, p.o)	25.42±0.62	44.21±2.3	65.32±0.21	49.23±2.1	60.23
IV	METS(300mg/kg, p.o)	27.31±2.12	53.21±2.1	71.11±0.21	54.31±1.2	48.32

Values are expressed as Mean± S.E., n=6 by students 't' test

CONCLUSION

Hence the presence of flavonoids in methanolic extract of *Tinospora sinensis* leaves; they might suppress the analgesic, pyrexia and inflammatory conditions.

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