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EVALUATION OF ANALGESIC ACTIVITY OF ETHANOLIC EXTRACT OF *TINOSPORA SINENSIS* LEAVES IN RATS

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ABSTRACT

Ethanolic extract of *Tinospora sinensis* leaves (EETS) were evaluated for analgesic activity using Tail flick test and Acetic acid induced Writhing test. EETS at 250 mg/kg and 500 mg/kg were treated for 7 days and compared with diclofenac sod. 10 mg/kg single dose administered on 7th day before one hour of taking result. EETS at 250 mg/kg and EETS 500 mg/kg treated group showed highest significant ($p<0.001$) increase in latency time in tail flick test and protected no of writhing at 25.27% and 32.74% respectable. Both were comparable with standard diclofenac sod. 10 mg/kg. in both test models. The results of the present study support the folklore use of this plant in pain.

Key Words: *Tinospora sinensis*, Analgesic activity, Tail flick test, Acetic acid induced Writhing test.

INTRODUCTION

In present time, plants are used to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as analgesic activity should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic drug. Assam is enriched with plant diversity and several plants have been used traditionally by Assamese people for therapeutic potentials. *Tinospora sinensis* of family Verbenaceae which is known as Nagol bhanga in Assamese is use traditionally for different pharmacological activities. 1 The root of the plant has reported to have analgesic and anti-inflammatory activity2, antibacterial3, hepatoprotective4, anti-oxidant5, anticancer 6 and anti-arthritic activity7.The plant leaves contain major active constituents like polyphenolics (hydrolysable tannins and flavonoids), terpenoids, saponins and flavanoids.5, 7 However, there is no systemic report has been published on analgesic activity of this plant leaves to prove its folkloric use. The present study aimed to evaluate analgesic activity of *Tinospora sinensis* leaves.

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MATERIAL AND METHODS

Plant material

Fresh leaves of the plant *Tinospora sinensis* were collected from Tirumala hills, Tirupati, Andhra Pradesh, Identification and authentification of the crude drug was carried out by K.Madhava chetty, Botony Department, S.V.University, Tirupati, Andhra Pradesh, India. The leaves were dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the leaves was defatted with petroleum ether (60-80) and subsequently extracted with ethanol in a soxhlet apparatus at 550C and extract was concentrated and stored in refrigerator till use. The percentage yield was 11.4%.

Preliminary Phytochemical analysis

The plant extract screened for the presence of various phytochemical constituents i.e. steroids, alkaloids, tannins, flavonoids, glycosides, etc by employing standard screening tests.8

Experimental Animals

Healthy adult male Wister rats weighing (150-200gm) were selected for the studies. Rats were housed in

polypropylene cages (3 animals per cage), maintained under standard laboratory conditions (i.e. 12:12hr light and dark sequence; at an ambient temperature of 25 +2 oC).The animals were fed with standard pellet diet and water *adlibitum).* Before performing the experiment the ethical clearance was obtained.

Acute oral toxicity studies

Acute oral toxicity study was carried out for ethanolic extract of *Tinospora sinensis* leaves using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No.423. The extract was found non toxic when studied with starting dose of 2000 mg/kg and studied for 14 days.

Experimental design

The adult male Wister rats Rats were divided into 4 groups of 6 each and studied for analgesic activity. The ethanolic extract of *Tinospora sinensis* leaves (EETS) were made suspended in 1% Carboxy methyl cellulose and treated for 7 days and on 7th day standard drug diclofenac sodium in 3mg/kg is given 1 hr before study. The volume administered was 1ml/100 gm body weight and given by using intra-gastric feeding needle.

Group I: Control; (Vehicle, 1% CMC, P.O) for 7 days)

Group II: Test Low dose, (EETS 250 mg/kg in 1% CMC, P.O) for 7days.

Group III: Test High dose, (EETS 500 mg/kg in 1% CMC, P.O) for 7days.

Group IV: Standard, (Diclofenec sod., 10 mg/kg, P.O in 1% CMC) 1 hr before study on 7th day.

Tail flick Test

Centrally acting Analgesic activity was assessed by tail flick model using analgesiometer.10-11 The instrument has a nichrome wire, which would be heated to the required temperature (550c) and maintained by means of heat regulators. The strength of the current passing through the naked nichrome wire was kept constant at 4 Amps. The rat was kept in a rat holder with only the tail portion protruding out. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting. A cut off time of 15 sec was planned to avoid any tissue damage in the animal. The reaction time for each group was measured at 30, 60, 90 and 120 minutes using analgesiometer on the 7th day after drug/extract administration.

Acetic acid induced writhing Test

Peripherally acting Analgesic activity was evaluated by Acetic acid induced writhing method.12-13 The animals are administered acetic acid (0.6%, 1ml/100g) intraperitoneally. After 60minutes of the administration of test and standard drug. Rats are placed individually into glass chamber and number of writhes is counted for 30minutes. A writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The percent inhibition was calculated.

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The data were analyzed by using Graph pad software version5 by one way analysis of variance (ANOVA). The test was followed by multiple Dunnett's test, p values less than 0.05 were considered as significance.

RESULT AND DISCUSSION

In this study analgesic activity of ethanolic extract of *Tinospora sinensis* leaves was evaluated by Tail flick method and Acetic acid induced writhing test. The phytochemical analysis of ethanolic extract of *Tinospora sinensis* leaves revealed the presence of alkaloids, steroids, flavanoids, carbohydrate, tannins, phenols etc. In the acute toxicity assay no deaths were observed or no stereotypical symptoms associated with toxicity, such as convulsion, ataxy, diarrhoea or increased diuresis thus the median lethal dose (LD50) was determined to be higher than the dose tested i.e. 2.0g/ kg b.w.

Effect of EETS on Tail flick test in rats

Effect of ethanolic extract of *Tinospora sinensis* leaves (EETS) at 250 mg/kg, 500 mg/kg and Diclofenec sod. 10 mg/kg and control vehicle were studied on Tail flick test in rats. The result were given in Table-1 and shown in Fig-1. The result showed significant ($p<0.05$) increase in latency time in EETS 250 mg/kg treated and EETS 500 mg/kg treated group showed highest significant activity ($p<0.001$) and these maintains for longer period of time. Whereas, standard drug diclofenac sod. 10 mg/kg showed most significant ($p<0.05$) activity at 60 min and reduced after 90 min. all test groups compared with Control using One way ANOVA followed by Dunnett's test.

Effect of EETS on Acetic acid induced writhing Test in rats

Effect of ethanolic extract of *Tinospora sinensis* leaves (EETS) at 250 mg/kg, 500 mg/kg and Diclofenec sod. 10 mg/kg and control vehicle were studied on acetic acid induced writhing in rats. The result were given in Table-2 and shown in Figure-2. The result showed significant ($p<0.01$) reduction in no of writhes in EETS 250 mg/kg treated group and % percentage protection was 25.27% and EETS 500 mg/kg treated group showed significant reduction($p<0.001$) and % percentage protection was 32.74% . Whereas, standard drug diclofenac sod. 10 mg/kg showed most prominent reduction ($p<0.001$) and % protection was 69.94%.

when all test groups compared with Control using One way ANOVA followed by Dunnett's test.

Drugs that act centrally inhibit pain produced by thermal stimuli14. In our present study ethanolic extract of *Tinospora sinensis* leaves (EETS) at both dose level showed significant analgesic activity. Although, this model is specific for centrally inhibited pain but in the present study, diclofenac also inhibited the pain produced by tail flick method where there are evidences that support that NSAID's also inhibit the pain induced by thermal stimuli [15-17]. In acetic acid induced writhing method, acetic acid causes an increase in peritoneal fluid level of prostaglandins involving in part peritoneal receptor 16 and inflammatory pain by capillary action17. It is widely used for analgesic screening and predominately involves induction of prostaglandins. In our present study ethanolic extract of *Tinospora sinensis* leaves (EETS) and standard drug diclofenec significantly reduced the no of writhing compared to the control groups. This suggests mechanism of analgesic effect of *Tinospora sinensis* leaves is probably due to a blockade of capillary permeability or release of endogenous substances like prostaglandins.

Table 1. Analgesic activity of ethanolic extract of *Tinospora sinensis* leaves in Tail flick test in rats

Treated Groups	Latency time (sec)			
	30 mins	60 mins	90 mins	120 mins
Control 1 % CMC	2.36±0.14	2.33±0.18	2.36±0.15	2.33±0.12
EETS 250/kg	3.60 ±0.15	3.71±0.22**	3.78±0.22*	3.77±0.22*
EETS 250/kg	4.76±0.01*	4.83±0.20**	4.88±0.20***	4.87±0.20***
Diclofenac sod. 10 mg/kg	2.86±0.02^{ns}	4.18±0.16*	4.07±0.18*	3.57±0.15*

Values are in Mean ± S.E.M (n=6); ns -Non Significant, *p<0.05, **p<0.01, ***p<0.001.

Table 2. Analgesic activity of ethanolic extract of *Tinospora sinensis* leaves in Acetic acid induced writhing in rats

Treatment	No. of Writhing	% Protection
Control, 1 % CMC	17.17± 1.50	-
EETS 250 mg/kg	12.83± 0.85**	25.27%
EETS 500 mg/kg	11.60± 0.85***	32.74%
Diclofenac sod. 10 mg/kg	5.16 ± 0.48***	69.94%

Values are in Mean ± S.E.M (n=6); ns -Non Significant, *p<0.05, **p<0.01, ***p<0.001

CONCLUSION

The results of the present study support the folklore use of this plant in pain. Thus, the results of this study confirmed the traditional uses, claiming that the ethanolic extract of plant *Tinospora sinensis* showed significant analgesic activity which is comparable to the standard drug Diclofenac sodium. Presently available synthetic analgesic agents are rapidly losing their therapeutic value. So conjoint the use of medicinal plants (naturally occurring analgesic agents) and standard drugs may prove useful in future medical practice. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent analgesic agent with a low toxicity and better therapeutic index.

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