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Screening of *tecoma stans* leaves fractions for analgesic and anti-inflammatory activities

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ABSTRACT

To test the analgesic and anti-inflammatory activity of different fractions of dried Tecoma stans leaves using various pain and inflammation models. The analgesic activity of T. stans was tested in mice with acetic acid-induced writhing and rats with the tail flick test. Cotton pellet-granuloma formation in rats was used to test the anti-inflammatory activity. T. stans was examined in five distinct fractions (FRI, FRII, FRIII, FRIV, and FRV) at doses of 20 and 40 mg/kg, p.o.When compared to the other fractions, the fractions FRI (40 mg/kg, p.o.) and FRIII (40 mg/kg, p.o.) were shown to be more efficacious (P0.01) in reducing cotton pellet granuloma development and acetic acid induced writhing. In the tail flick method, FRI (20 mg/kg, p.o.) and FRIII (20 mg/kg, p.o.) were likewise found to be more effective in increasing latency time. FRI and FRIII, two of the five fractions of T. stans leaves examined, have significant analgesic and anti-inflammatory properties against several types of inflammation and pain.

Keywords:*Tecoma stans*, granuloma formation, carrageenan, tail flick, acetic acid

INTRODUCTION

Despite progress within medical research during the past decades, the treatment of many serious diseases remains problematic. [1] Chronic inflammatory diseases remain one of the world's major health problems. [2] Currently, both steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs (NSAIDs) are used in the aid of inflammation. Steroids have an obvious role in the treatment of inflammatory diseases, but due to their toxicity, they can only be used over short periods except in very serious cases where the risks are acceptable. Prolonged use of NSAIDs is also associated with severe side effects, notably gastrointestinal hemorrhage. [3],[4] Inflammatory diseases are among the most common health problems treated with traditional remedies. Therefore, it is crucial to evaluate the potential of herbal remedies that might serve as leads for the development of potent drugs. A large number of Indian medicinal plants are attributed with various pharmacological activities owing to their different class of phytochemicals.

Tecoma stans (common name yellow bell) also known as yellow trumpet bush belongs to the family bignoniaceae. It is an ornamental plant. It is an erect, branched, sparingly hairy or nearly smooth shrub two to four meters in height. The leaves are opposite, odd-pinnate, Up to 20 centimeters in length with 5 to 7 leaflets. The leaflets are lanceolate to oblong- lanceolate, 6 to 13

centimeters long, pointed at both ends and toothed on the margins. Trumpet shaped flowers are yellow faintly scented and borne in short, dense, terminal clusters. The calys is green. 5 to 7 millimeters long and 5 toothed. Flowering can begin as early as April and continue in to fall. The flowers are followed by 6-inch-long, tan pods that are filled with small, papery winged seeds.9

Leaves of *Tecoma stans* contain the alkaloids tecomin and tecostamine are potent hypoglycaemic agent when given intravenously. Anthranilic acid is responsible for the anti-diabetic activity. Roots are powerful diuretic and vermifuge10. *Tecoma* is not a toxic because this plant is used in latine America as a remedy for diabetes and moreover for feeding cattle and goats in mexico11. The preliminary phytochemical screening of methanolic extract of flower extract of *Tecoma stans* showed the presence of flavaniods, phenol, alkaloids, tannins, steroids, triterpenes, anthraqunones and saponins etc. [5-10] A study has, therefore, been carried out to investigate the analgesic and anti-inflammatory activity of different fractions of extract of *T. stans* leaves.

MATERIAL AND METHODS

Collection and Authentication of Plant Materials

The leaves of *T. stans* were collected from local region of Nashik, India, in the month of July 2020. The plant material was identified and authenticated by Prof. P.G.Diwakar, Botanical survey of India, Pune.

Preparation of *T. stans* Fractions

The extraction was carried out using petroleum ether followed by alcohol, then it was allowed to evaporate slowly in shallow dish and resinous mass was discarded. For column chromatography, neutral alumina was first activated at 150 0 C for 3 hrs in an oven. After cooling, slurry was prepared in benzene; it was poured in glass column and set aside for 2 hrs. The residue of petroleum ether extract was dissolved in minimum volume of benzene and it was mixed thoroughly with neutral alumina. It was air dried and charged into the column. The elution was carried out first with benzene (FRI) and successively eluted with ethyl acetate (FRII). The dried alcoholic extract was separated into water soluble and water insoluble portion. Water soluble portion was shaken vigorously with methanol yielded a gelatinous precipitate (FRIII). The water insoluble part was dissolved in minimum volume of absolute alcohol and column chromatography was carried out with benzene (FRIV) and ethyl acetate (FRV).

All the fractions were prepared fresh prior to the administration in 0.5% w/v gum acacia.

Experimental Animals

Albino rats of Wistar strain (150-200 g) and Swiss albino mice (25-30 g) of either sex were used in the entire study. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2°C; relative humidity 60-70%) in a 12 h light-dark cycle. The animals were fed with standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during the experimental hours. The experimental protocol was approved by Institutional Animal Ethics Committee.

Phytochemical Investigation

Preliminary phytochemical tests for fractions were performed using specific reagents through standard procedure. [11]

Tail flick latency period in rats

Male rats of 125-150 g were divided into six groups containing five animals in each group. A tail flick response was evoked by placing each rat tail over the wire heated electrically, using analgesiometer (Space Scientific, Nashik, India). The intensity of heat was adjusted so that baseline tail flick latency averaged 3-4 sec in all animals. Cut off time was 15 sec in order to avoid injury to tail. The fractions FRI, FRIII, and reference standard ibuprofen were administered orally in their respective doses 1 hr prior to the test. [12]

Acetic acid induced writhing in mice

The writhing syndrome was elicited by intraperitoneal injection of acetic acid (0.1ml of 0.6% solution) and numbers of writhes displayed from 5 to 20 min were recorded. [13] The fractions FRI, FRIII, and reference standard ibuprofen were administered orally in their respective doses 30 min prior to the test. [14]

Granuloma formation induced by cotton pellet in rats

Male rats of 125-150 g were divided into seven groups containing five animals in each group. The cotton pellet weighing 50±1 mg was sterilized in an autoclave (Lab hosp, Mumbai, India) handled with sterile instrument. The pellet was inserted in each animal on the back. Control group received vehicle. Group II, III, IV and V were treated with FRI (20 and 40 mg/kg, p.o) and FRIII (20 and 40 mg/kg, p.o) whereas group VI and VII were treated with reference standard i.e. hydrocortisone (30 mg/kg, p.o) and ibuprofen (40 mg/kg, p.o) for consecutive six days. [15],[16] The animals were sacrificed on seventh day and cotton pellet along with granuloma

mass were collected; it was weighted and dried at 60 0 C. Results of the assay were calculated as % inhibition of dry weight of granuloma formation by using the formula: 100 (A-B)/A, where, A= gain in dry weight of control pellet (mg), B= gain in dry weight of drug treated (mg).

STATISTICAL ANALYSIS

Results have been indicated in terms of mean±SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) with Dunnett's multiple comparisons test using GraphPad In Stat version 5.00, GraphPad Software, CA, USA. The level of significance was set at $P < 0.05$.

RESULTS

Phytochemical investigation

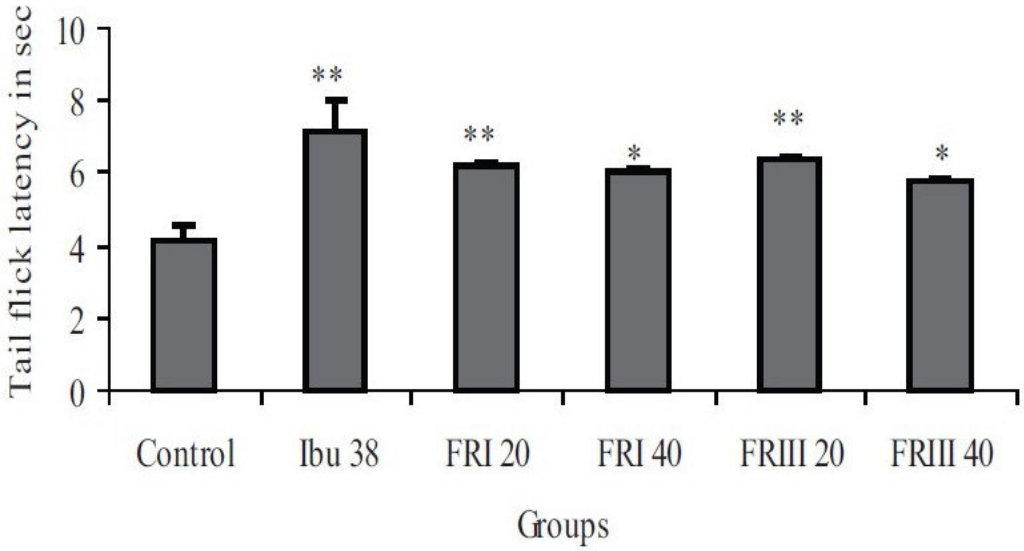
Preliminary phytochemical analysis revealed the presence of different phytochemicals present in different fractions of *T. stans* plant [Table 1].

Table 1: Phytochemical analysis of different fractions of *T. stans* +, Positive test; -, Negative test

Test	FR I	FR II	FR III	FR IV	FR V
Carbohydrates	+	+	+	-	-
Proteins	+	-	+	-	-
Amino acids	+	-	+	-	-
Tannins And Phenols	-	+	+	+	+
Glycosides	+	+	+	-	-
Saponins	-	+	+	+	+
Flavanoids	-	-	-	-	-
Alkaloids	-	-	-	-	-
Steroids	+	-	+	+	-

Effect of *T. stans* fractions on tail flick latency period and acetic acid induced writhing in mice

Treatment of FRI and FRIII (20 mg/kg, p.o.) significantly inhibited nociception in rats by 19.26% and 20.92%, respectively. Whereas, FR I and FRIII (40 mg/kg, p.o) significantly inhibited pain perception by 17.5% and 15.29%, respectively. Ibuprofen treatment (40 mg/kg, p.o) significantly inhibited pain perception by 27.92 % ($P<0.01$). [Figure 1].



Data were expressed as mean ± SEM (n=5) and analyzed using one way analysis of variance (ANOVA) followed by Dunnet's test and differences between means were considered as significant at *P<0.05 and **P<0.01 Control-0.5% gum acacia FRI-*T. stans* benzene fraction. FRII-*T. stans water soluble alcoholic fraction.*

Figure 1: Effect of *T. stans* fractions on Tail flick latency period

Table 2 shows the effect of different fractions of *T. stans* against acid induced writhing in mice. It was observed that mice treated with FRI 20 (31.89%) and FRIII 20 (36.20%) shows significant ($P < 0.01$) protection compared to control group, however, FRI 40 (44.17%) and FRIII 40 (46.50%) was found to be more significant ($P<0.01$) in protecting acetic acid induced writhing compared to control group. Ibuprofen showed 56.13% protection against acetic acid induced writhing in mice.

Table 2: Effect of *T. stans* fractions in acetic acid induced writhing in mice

Treatment (mg/kg)	Number of writhing	% Inhibition
Control	60.1±2.48	--
Ibuprofen (40)	26.3±0.91	56.13
FRI (20)	41±1.13	31.88
FRI (40)	33.5±2.91	44.17
FRII (20)	38.3±1.12	36.20
FRII (40)	32.1±1.95	46.50

Data analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± SEM (n=5) followed by Dunnet's test and differences between means were considered as significant at *P<0.05 and **P<0.01 Control-0.5% gum acacia FRI-*T. stans* benzene fraction. FRII-*T. stans water soluble alcoholic fraction.*

Effect of T. stans fractions on cotton pellet granuloma formation in rats

Treatment with FRI and FRIII (40 mg/kg, p.o) to rats showed a significant ($P<0.01$) inhibition in the weight of cotton pellet compared to control group and the percentage inhibition was found to be 38.74 and 40.30, respectively. Treatment with the reference standard i.e. hydrocortisone (30 mg/kg, p.o) and ibuprofen (40 mg/kg, p.o) also showed significant inhibition in cotton pellets granuloma formation as compared to control group [Table 4].

Table 4: Effect of *T. stans* fractions on cotton pellet granuloma formation in rats

Treatment (mg/kg)	Average weight of cotton pellet	Average weight of cotton pellet with granuloma	% Inhibition
Control	50±0.01	127. ±4.98	-
FRI (20)	50±0.01	84±1.60	33.55
FRI (40)	50±0.01	77.4±0.91	38.74
FRII (20)	50±0.01	82±2.54	35.14
FRII (40)	50±0.01	75.3±4.25	40.30
Hydrocortisone (30)	50±0.01	74±2	41.39
Ibuprofen (40)	50±0.01	65.1±6.86	48.27

Data analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± SEM (n=5) followed by Dunnet's test and differences between means were considered as significant at *P<0.05 and **P<0.01 Control 0.5% gum acacia FRI-*T. stans* benzene fraction. FRII-*T. stans water soluble alcoholic fraction.*

DISCUSSION

In the present study, analgesic and anti-inflammatory effects of different fractions of *T. stans* were tested in different experimental models of pain and inflammation. The animal models for assessing analgesic activity viz. non-narcotic model like acetic acid induced writhing and narcotic model like tail flick method were used. In acetic acid induced abdominal constriction acetic acid causes inflammatory pain by inducing capillary permeability and release of arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism. [17-19]

Results from the study revealed that the intensity of antinociception of fractions of *T. stans* treated groups was higher than the control group in acetic acid induced writhing in mice. The mechanism of analgesia by fractions of *T. stans* could probably be due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of ibuprofen and other NSAIDs. The most common test to test narcotic drugs is the tail flick method. This test is based on phasic stimulus of high intensity. The nociceptive experience is short lasting and it is well accepted that agonist of μ opioid receptors producing analgesia in acute pain models. [20] Therefore, it is believed that substances effective in

tail flick exert their effects predominantly through μ opioid receptors. The fractions of *T. stans* showed increased latency for tail flick which suggest that the analgesic activity may in part be mediated by opioid receptors. Therefore, fractions of *T. stans* exhibited analgesic activity in all the animal models of nociception used in this study possibly exerted their effect through diverse mechanism that may involve both central and peripheral pathways.

In cotton pellet induced granuloma, the test fractions produced significant anti-inflammatory activity at the dose of 20 and 40 mg/kg which might be due to the presence of various active constituents in the leaves of *T.stans*. Phytochemical investigations on *T. stans* have revealed the presence of various phytoactive constituents such as glycosides, sterols, tannins, amino acids, campesterol, isofucosterol, stigmasterol, and lupeol. Leaf extract of *T. stans* had been shown to contain high amounts of tannins, phenols, triterpenoids, glucosides, and sterols. [21-24], Analgesic and anti-inflammatory effects of flavonoids, steroids, and tannins have been reported earlier. [25],[26] Deleterious effects of excessive releases of nitric oxide (NO) have been implicated in tissue damage and inflammation. Tannic acid and polyphenols have been shown to be potent inhibitors of NO synthetase activity and NO production independent of their antioxidant activity. [27] Therefore, the analgesic and anti-inflammatory properties observed might thus be related in part to the tannin content of this plant. Hence, the analgesic and anti-inflammatory effects produced by these fractions may be attributed individually or collectively to the tannins and steroids.

CONCLUSION

The results presented here demonstrate that *T. stans* fractions inhibit pain and inflammation with an interesting analgesic and anti-inflammatory activity profile similar to other compounds of this kind previously described. The results confirm that *T. stans* collected at Nashik, India, has great value as a source of tannins and polypenols compounds with analgesic and anti-inflammatory properties.

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