



ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

Phytochemical and pharmacological evaluation of *Tragia cannabina* for anti-inflammatory activity

Raju Venkategowda Hosahally¹, Ganapaty Seru², *Prasanna Shankarrao Sutar³, Vijaya Gopalachar Joshi³, Kishori Prasanna Sutar³, Asif Abdulrahiman Karigar³

¹Department of Pharmacognosy, Visveswarapura Institute of Pharmaceutical Sciences, Bangalore, Karnataka, India

²Department of Pharmaceutical Sciences, Andhra University, Vishakapatnam, India

³Department of Pharmacognosy, Maratha Mandal's College of Pharmacy, Belgaum-590016, Karnataka, India

ABSTRACT

The present study investigates the anti-inflammatory activity of methanolic and chloroform extracts of *Tragia cannabina*. The whole plant of *Tragia cannabina* was extracted with methanol and chloroform by using Soxhlet apparatus. The effect of both extracts of *Tragia cannabina* was studied on carrageenan induced paw edema. The methanolic extract decreased the edema induced in hind paw. The percentage inhibition of paw edema was maximum with methanolic and chloroform extracts of *Tragia cannabina* at 300mg/kg body weight and has showed significant anti-inflammatory activity. It has been concluded that both the methanolic and chloroform extracts of *Tragia cannabina* showed significant anti-inflammatory activity comparable to that of reference standard Ibuprofen.

Key Words: Methanolic extract, chloroform extract, anti-inflammatory activity, carrageenan, Ibuprofen, Euphorbiaceae.

INTRODUCTION

The major screens for biological activities of plant extracts have been carried out in the search for new anticancer, antiviral and anti-inflammatory drugs (Balandrin *et al.*, 1993). The development of the rapid screening tests now in use in industry has meant that many more plants can be evaluated for a wide range of biological activities (Neil *et al.*, 1993). Unfortunately the results of such tests do not necessarily reach the public domain and are kept in locked industrial files (Cragg *et al.*, 1993). There still remains an urgent need to develop new clinical drugs and this can be exemplified by the numerous diseases which result from the malfunction of the central nervous system, e.g. Alzheimers and Parkinsons disease, epilepsy, migraine, pain, schizophrenia, sleeping disorders (Jardin *et al.*, 1980; Kingston *et al.*, 1993). Natural products already have a proven track record for central nervous system

activities, e.g. caffeine, codeine, morphine, nicotine, reserpine and it is possible that there are further such drugs still to be found from nature (Wall *et al.*, 1993; Kinghorn *et al.*, 1993). The Euphorbiaceae is the one of the largest family of dicotyledons, and also has significant economic importance. The family has a cosmopolitan distribution with 300 genera and about 7500 species. *Tragia* is one of the largest genus of Euphorbiaceae. It contains trees, shrubs (or) herbs and used as anti-inflammatory, anti-diabetic and anti oxidant. *Tragia* species are rich in steroids, flavonoids, alkaloids and tannins. The literature survey revealed that the methanolic extract of *Tragia cannabina* posses anti hyperglycemic property on Streptozotocin –Induced Diabetic rats (Vaiyapuri *et al.*, 2007). In view of the reported medicinal properties and the presence of a various chemical constituents the author selected *Tragia cannabina*, to study the chemical examinations and anti-inflammatory activity (Kinghorn *et al.*, 1993; Kirtikar *et al.*, 2002).

*Corresponding Author:

Prasanna Shankarrao Sutar, Assistant Professor
Department of Pharmacognosy
Maratha Mandal's College of Pharmacy
#1007 opposite Police parade ground, Subash nagar
M.M.Extension, Belgaum-590016, Karnataka, India
E-mail: prs2005@rediffmail.com

MATERIALS AND METHODS

Plant material was collected around the local area Mandya and was authenticated (RRCBI No. 3716, dated 30-03-2007) by Dr. Gajendra Rao, Survey Officer, Regional Research Institute, Bangalore. The whole plant of *Tragia cannabina* (1.2kg) were air dried, powdered in willy mill and extracted successively with petroleum ether, chloroform and methanol (3lit × 2 each) and concentrated under vacuum to the corresponding residues:

Petroleum ether extract residue - 8g

Chloroform extract residue - 9g

Methanolic extract residue - 15g

The Chloroform and Methanolic extracts of *T. cannabina* were evaluated for anti-inflammatory activity using Ibuprofen (10mg/kg) as reference standard. The activity was tested on carrageenan induced paw oedema in rats.

Preliminary Phytochemical Evaluation of *Tragia cannabina*

The whole plant of *Tragia cannabina* (1kg) was extracted by soxhlet extractor with methanol (3 liters) and concentrated under vacuum to get the residue of 15 gm. The residue was dissolved in methanol and this dark brown residue was examined on thin layer chromatography, which showed 3 prominent spots in Petroleum ether: benzene (1:1) solvent system with Rf values 0.6, 0.4 and 0.1 (Wagner *et al.*, 2002).

The methanolic extract showed 3 spots with chloroform: benzene (1:1) with Rf values 0.47, 0.16 and 0.26 and showed two prominent spots in Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27) solvent system with Rf values 0.75, and 0.76.

Then the extract was tested for various chemical constituents like steroids, alkaloids, tannins flavonoids, carbohydrates and glycosides.

Test for Tannins

- 0.1gm of the chloroform extract was dissolved in methanol in a test tube and 2 drops of ferric chloride was added. An olive green color appeared which indicated the presence of tannins.
- 0.1gm of the methanolic extract was dissolved in methanol in a test tube and 2 drops of ferric chloride was added. An olive green color ap-

peared which indicated the presence of tannins (Kokate, 1994).

Test for Sterols and Triterpenoids

- 0.1gm of the chloroform extract was dissolved in chloroform in a test tube and 2 drops of acetic anhydride was added. It was boiled and 1 drop of concentrated sulphuric acid was added after cooling. A green color appeared which indicated the presence of sterols.
- 0.1gm of the chloroform extract was dissolved in chloroform in a test tube and 2 drops of concentrated sulphuric acid was added. It was allowed to stand for some time until red color appeared, which indicated the presence of sterols (Peach, 1995).

Tests for Flavonoids

- 0.1gm of the chloroform extract was dissolved in methanol in a test tube with few fragments of magnesium ribbons and 1 drop of hydrochloric acid. A crimson red color appeared which indicated the presence of flavonoids.
- 0.1gm of the chloroform extract was dissolved in methanol in a test tube with 0.5g of zinc dust and 1ml of concentrated hydrochloric acid. A red color appeared which indicated presence of flavonoids (Pulok, 2002).

Tests for Alkaloids

- 0.1gm of the methanolic extract was dissolved in methanol in a test tube. 1ml of potassium bismuth iodide was added. A reddish brown precipitate appeared which indicated the presence of alkaloids.
- 0.1gm of the methanolic extract was dissolved in methanol in a test tube and 1ml of saturated picric acid was added. A yellow precipitate appeared which indicated the presence of alkaloids (Brain, 1975).

Test for Carbohydrates

- 0.1gm of the methanolic extract was dissolved in methanol in a test tube and 1ml of alcoholic α -naphthol and 0.2 ml concentrated sulphuric acid were added. A purple to violet color ring was not appeared which indicated the absence of carbohydrates.
- 0.1gm of the methanolic extract was dissolved in methanol in a test tube and 1ml of Benedict's

Table 1: Anti inflammatory activity of *Tragia cannabina* extracts on carrageenan induced paw oedema in rats.

Sl. No.	Treatment	Dose	Volume of mercury displaced in ml at various time intervals in hours					
			0	1	2	3	4	5
1	Control	0.1ml 1% W/V	0.36 ± 0.01	0.54±0.02	0.66± 0.01	0.78± 0.04	0.86± 0.03	0.88± 0.05
2	Ibuprofen	10 mg/ kg	0.36± 0.01	0.37±0.02***	0.38±0.01***	0.38±0.03***	0.39±0.01***	0.39±0.04***
3	Chloroform Extract	100 mg/ kg	0.36± 0.01	0.52±0.01*	0.62±0.02*	0.72±*0.01	0.76±0.01*	0.77±0.01*
4	Chloroform Extract	300 mg/ kg	0.36±0.01*	0.51±0.03*	0.62±0.05*	0.70±**0.04	0.72±0.03**	0.73±0.02**
5	Methanolic Extract	100 mg/ kg	0.36±0.01	0.52±0.01*	0.62±0.02*	0.78±0.01*	0.72±0.03*	0.72±0.04*
6	Methanolic Extract	300 mg/ kg	0.36± 0.01	0.47±0.02**	0.50±0.05**	0.52±0.03**	0.54±0.01***	0.55±0.04***

Significance of difference in control and extracts treated groups were determined by one way analysis of variance (ANOVA)

*** P<0.001 **P<0.01 and * P<0.05 are significant

reagent (alkaline sodium cupric citrate) was added. The solution was boiled in water bath. No brick red precipitate appeared which, indicated the absence of carbohydrates (Jean, 1999).

Test of Glycosides

200mg of methanolic extract was dissolved in methanol in a test tube and boiled with 5ml of dilute Sulphuric acid for 2 minutes. It was filtered and the filtrate was neutralized with 5% sodium hydroxide. 0.1ml Fehling's solution A and B were added to the solution and was heated on a water bath for 2 minutes. No brick red color was formed which indicated the absence of glycosides (Khandelwal, 2002).

Chemical Tests and thin layer chromatographic studies of *T. cannabina* revealed the presence of tannins, flavonoids and alkaloids.

Evaluation of Anti-Inflammatory Activity

In this method, 0.5mg of 1% w/v carrageenan in saline was injected into the footpad of rat subcutaneously, to induce oedema. The thickness of paw was measured before and after carrageenan injection at hourly intervals up to 5 hrs with plethysmometer (Vogel, 2002). Albino rats of either sex weighing between 200-250g were selected,

housed under standard temperature (23±1°C) and divided into 6 groups of six animals each.

Group I served with normal saline (5ml/kg, p.o).

Group II was given Ibuprofen (10mg/kg) remaining groups received 100mg and 300mg of methanolic and chloroform extract of *Tragia cannabina* 0.1ml of carrageenan (1%) in normal saline (0.9%) was injected into sub-planter area of right hind paw for all the groups. All the drugs were administered orally one hour prior to carrageenan injection. After the administration of carrageenan, volume of rats paw was measured at 0 hour and at hourly intervals up to 5th hour by volume displacement method, using plethysmometer.

The anti-inflammatory activity was expressed as:

$$\text{Percentage inhibition of oedema} = 100 - \left[1 - \left(\frac{V_t}{V_c} \right) \right]$$

where V_t and V_c are volume of carrageenan-injected paws of drug treated group and control group respectively. The results were tabulated and data is statistically analyzed using one-way ANOVA followed by Dunnet's test and results were presented in the table 1 and 2.

Table 2: Percentage inhibition of *Tragia cannabina* extracts on carrageenan induced paw oedema in rats.

Sl. No.	Treatment	Dose	% Inhibition at various time intervals					
			0	1	2	3	4	5
1	Control	0.1ml 1% W/V	00	00	00	00	00	00
2	Ibuprofen	10 mg/ kg	00	31.44	42.43	51.29	54.66	55.69
3	Methanolic Extract	100 mg/ kg	00	3.71	6.07	12.83	16.28	17.05
4	Methanolic Extract	300 mg/ kg	00	12.97	24.43	33.33	37.21	37.5
5	Chloroform Extract	100 mg/ kg	00	3.71	4.55	7.7	11.63	12.5
6	Chloroform Extract	300 mg/ kg	00	5.5	6.07	10.26	16.28	17.05

RESULTS AND DISCUSSION

Chloroform extract of *Tragia cannabina* at dose level 100mg/kg and 300mg/kg, the percentage inhibitions of paw volume were found to be at 1st hour is 3.71%, 2nd hour is 4.55%, 3rd hour is 7.7%, 4th hour is 11.63 % and 5th hour is 12.5%. The % inhibition of paw oedema was more at 5th hour and at 1st hour is 5.5%, 2nd hour is 6.07 %, 3rd hour is 10.26%, 4th hour is 16.28 % and 5th hour is 17.05%. The % inhibition of paw oedema was more at 5th hour respectively.

Methanolic extract of *T. cannabina* at a dose level of 100mg/kg and 300mg/kg the percentage inhibitions were found to be 1st hour is 3.71%, 2nd hour is 6.7%, 3rd hour is 12.83%, 4th hour is 16.28 % and 5th hour is 17.05%. The percentage inhibition of paw at 1st hour is 12.97%, 2nd hour is 24.43%, 3rd hour is 33.33%, 4th hour is 37.21 % and 5th hour 37.5%. The percentage inhibition of paw oedema was maximum at 5th hour. Chloroform and methanolic extracts showed good anti-inflammatory activity comparable to standard. The chloroform and methanolic extracts showed significant anti-inflammatory activity comparable to that of reference standard Ibuprofen.

Carrageenan induced oedema is mediated by release of histamine and 5HT followed by the prostaglandin kinin and have been frequently used to assess the anti-inflammatory effects of natural products. The chloroform and methanolic extracts may act by reducing the effect of inflammatory mediators.

CONCLUSION

It was demonstrated by animal model studies that methanolic and chloroform extracts of *Tragia cannabina* reduced inflammation at a dose of 100mg/kg body weight and 300mg/kg body weight. Further this study indicates chloroform and methanolic extracts showed significant anti-inflammatory activity comparable to that of reference standard.

ACKNOWLEDGEMENT

Authors are thankful to the Rajiv Gandhi University of Health Sciences Bangalore, Karnataka, India and Visveswarapura Institute of Pharmaceutical Sciences, Bangalore Karnataka, India for providing facilities to carry out the research work.

REFERENCES

- Balandrin, M.F., Kinghorn, A.D. and Farnsworth, N.R. (1993). Human Medicinal agents from Plants, ACS Symposium Series 534, Washington; DC, pp. 2.
- Brain, K.R., and Turner, T.D. (1975). The Practical Evaluation of Phytopharmaceuticals, 155.
- Cragg, G.M., Boyd, M.R., Cardellina, J.H., Grever, M. (1993). Human Medicinal Agents from Plants, ACS Symposium Series 534, Washington, DC, pp. 80.
- Jardine, I. (1980). Anticancer Agents Based on Natural Product Models., Academic press, New York, pp. 319.
- Jean Brunteon. (1999). Phytochemistry of Medicinal plants, pp. 225-369.
- Khandelwal, M. (1995). Practical Pharmacognosy. Nirali Publications, pp. 140-143.
- Kinghorn, A.D and Balandrin, M.F. (1993). Human Medicinal Agents from Plants, ACS Symposium Series 534, Washington; DC, pp. 48.
- Kingston, D.G.I. (1993). Human Medicinal Agents from Plants, ACS Symposium Series 534, Washington, DC, pp. 48.
- Kirtikar K.R., Basu B.D. (2002). Indian Medicinal Plants, 3, 2190-2191.
- Kokate C.K., Purohit A.P., Gokhale S.B. (1994) "Practical Pharmacognosy", 2, 54-60.
- Neill, M.O., Lewis, J.A. (1993). Human Medicinal Agents from Plants, ACS Symposium Series 534, Washington; DC, pp. 48.
- Peach, K., and Tracey, M., V., (1995). Modern methods of Plant analysis. 3, pp. 467-478.
- Pulok, K., Mukherjee, (2002). Quality control of Herbal Drugs, pp. 540-542 .
- Vaiyapuri, S., Akalanka, D., B., Jayakar, (2007). Antihyperglycemic property of *Tragia cannabina* in Streptozocin induced diabetic rats. Journal of Medicinal Food. 10(2): 361-365. [\[DOI\]](#) PMID:17651075
- Vogel, H.G., (2002), Drug discovery and evaluation, pp. 755-769. [\[DOI\]](#)
- Wagner, H., Bladet, S., Zgainski, E.M., (1994). Plant drug analysis- A TLC Atlas, Springer Verlag Berlin Heidelberg New York, pp. 163-192.
- Wall, M.E., and Wani, M.C., (1993). Human Medicinal Agents from Plants, ACS Symposium Series 534, Washington; DC, pp. 48.