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Evaluation of Anti-inflammatory and Analgesic Activity of Root Extracts of *Tragia bicolor miq*

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Abstract
Inflammation is a protective local response attempt by the organisms to remove the injurious stimuli and to initiate the healing process. It is a body defense mechanism in order to eliminate or limit the spread of of necrosed cell or tissue. Progressive destruction of the tissue would compromise the survival of the organism. Inflammation can also lead to a host of diseases such as hay fever, Atherosclerosis, Rheumatoid arthritis. Tragia bicolor belonging to family Euphorbiaceae is commonly used in the Ayurvedic system of medicine. The aqueous and alcoholic root extracts was evaluated for its *in-vivo* anti-inflammatory activity by carrageenan induced paw edema in wistar albino rats (Plethysmometer). Also evaluated for the analgesic activity by Eddy's hot plate method and 0.6% acetic acid induced writhing response models of swiss albino mice. The oral administration of the aqueous and alcoholic root extracts at a dose of 200mg/kg and 400mg/kg were showed a significant anti-inflammatory and analgesic activities. The extracts have showed significant decrease in paw edema volume as compared to the untreated vehicle control group. The extracts caused an inhibition on writhing response induced by 0.6% acetic acid and the Eddys hot plate method also proved the analgesic activity. These effects may be due to the potentiation of its inhibitory effect on the synthesis and release of various mediators.

Keywords
Tragia bicolor; Anti-inflammatory effect; Analgesic activity; Diclofenac sodium; Pentazocine.

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INTRODUCTION:
Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants.[1] Inflammation is a protective attempt by the organisms to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by microorganism, inflammation is one of the responses of organism to the pathogen. Without inflammation wounds and infections would never heal. Similarly, progressive destruction of the tissue would compromise the survival of the organism. The enzyme phospholipase A_2 is known to be responsible for the formation of mediators like prostaglandins, leukotrienes. Phospholipase A_2 converts phospholipids into archidonic acid in the

cell membrane which is then rapidly metabolized by cyclooxygenase to propstaglandins, a component that induces pain and inflammation.[2]Vasodilation and its resulting increased blood flow causes the redness (rubor) and increased heat (calor). Increased permeability of the blood vessels results in an exudation(leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling (tumor). Some of the released mediators such as bradykinin increase the sensitivity to pain (hyperalgesia, dolor).[3] The loss of function is probably the result of a neurological reflex in response to pain. There are various species of *Tragia* available which showed the significant anti diabetic, anti-inflammatory, analgesic and antioxidant activities. Therefore, the present study has been planned to evaluate the anti-inflammatory and analgesic activity of aqueous and alcoholic root extracts of the plant *Tragia bicolor miq.,* [4,5]

MATERIALS AND METHODS:

Procurement of experimental animals:

Swiss albino mice (20-25gm) and Wister rats (150-200gms) of either sex or of approximate same age used in present studies were procured from listed suppliers of Sri Venkateshwara enterprises, Bengaluru, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bengaluru) and water *ad libitium.* all the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. OECD/OCDE guideline for testing of chemicals.

Acute oral toxicity – acute toxic class method 423. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No.-01/2013/IAEC/VMCP.) after scrutinization. The animals received the drug treatment by oral gavage tube.

Phytochemical screening:

The phytochemical examination of the selected extracts showed the presence of various constituents. From that ethanolic and aqueous extracts showed maximum phytoconstituent especially flavonoids, tannins and phenolic compounds. The alcoholic and aqueous root extracts of *Tragia bicolor miq.* yielded 4.64 % and 7.30% w/w respectively. The phytochemical screening of the alcoholic and aqueous root extracts *Tragia bicolor miq.* showed the presence of flavonoids, carbohydrates and glycosides, phenolic compounds and tannins.

Standard drugs:

Pentazocine and Diclofenac sodium was used for experiments. All chemicals and solvents are of analytical grade.

Acute oral toxicity studies [6]:

The alcoholic and aqueous root extracts of *Tragia bicolor miq* did not showed any lethal effect on the animals up to the doses of 2000 mg/kg and the animals were observed for further 14 days for any sign of delayed toxicity. The LD_{50} value considered as 2000mg/kg. So, the ED_{50} dose 200mg/kg.

Statistical analysis:

The results are presented as mean ± SEM one-way analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparison where used for statistical evaluation**.** p values less than 0.05 were considered as significance.

ANTI-INFLAMMATORY ACTIVITY [7,8,9]:

1. CARRAGEENAN INDUCED PAW EDEMA:

Animals:

Animals	:	Wistar albino rat
Weight	:	150-180g
Animals per group	:	6
Number of groups	:	7

Experimental design for carrageenan induced paw edema model:

Group I : Vehicle control 1% Tween 20 and 1% Normal saline (Dose 10ml/kg).
Group II : Animal treated with 1% carrageenan (Dose 0.1ml/kg).
Group III : Animal treated with Diclofenac Sodium (Dose 50mg/kg)
Group IV : Animal treated with alcoholic extract of *T. bicolor* (Dose 200mg/kg)
Group V : Animal treated with alcoholic extract of *T. bicolor* (Dose 400mg/kg)
Group VI : Animal treated with aqueous extract of *T. bicolor* (Dose 200mg/kg)
Group VII : Animal treated with aqueous extract of *T. bicolor* (Dose 400mg/kg)

Experimental procedure:

Carrageenan induced paw edema is a classical model for determination of acute phase inflammation. The rat paw edema was provoked by sub plantar injection of 1% carrageenan in 0.9% saline in right hind paw. The hind paw volume was measured by dipping the foot in plethysmometer up to the lateral malleolus (Winter et. al. 1962). The initial paw edema was measured and recorded; it was considered as 0-hour reading. The drug or test substance like 1% Tween-20 and DMSO (vehicle, control), Diclofenac Sodium and various extracts were administered orally 60 minutes before administration of carrageenan. 0.1 ml of 1% w/v of carrageenan in saline was injected into right hind paw of rats. The hind paw volume was measured at 1hour interval up to 3 hours of experiment.

The difference between paw volume at various interval indicated the edema volume due to inflammation. The percentage inhibition produced by the drug and extracts were calculated by following formula,

Percentage inhibition of paw edema (%) = Control – Treated**/**Control X 100

ANALGESIC ACTIVITY [10]:

Method 2:

Analgesic activity of alcoholic and aqueous extracts of *Tragia bicolor miq* against 0.6%v/v acetic acid induced writhing in Swiss albino mice.

Animals:

Animals	:	Swiss albino mice
Weight	:	25-30g
Animals per group	:	6
Number of groups	:	6

Experimental design for study of analgesic activity of alcoholic and aqueous extracts against 0.6%v/v acetic acid induced writhing in Swiss albino mice:

Group I : Animal treated with 0.6% v/v acetic acid (Dose 10ml/kg).
Group II : Animal treated with Pentazocine (Dose 5 mg/kg).
Group III : Animal treated with alcoholic extract of *T. bicolor* (Dose 200mg/kg).
Group IV : Animal treated with alcoholic extract of *T. bicolor* (Dose 400mg/kg).
Group V : Animal treated with aqueous extract of *T. bicolor (*Dose 200mg/kg).
Group VI : Animal treated with aqueous extract of *T. bicolor (*Dose 400mg/kg).

Experimental procedure:

The method used in this test has been described by *Koster et al,* The total number of writhings following intra-peritoneal administration of acetic acid solution 0.6%v/v (10 ml/kg) was recorded over a period of 30 min, starting 5 min after acetic acid injection. Immediately after the algic compound injection, each animal was placed in a transparent observation cage and the number of writhes per mouse was counted for 10 minutes. The writhing activity consists of a contraction of the abdominal muscles together with a stretching of the hind limbs. The mice were treated with the alcoholic and aqueous extract of *Tragia bicolor miq.* (200 and 400 mg/kg), or vehicle (Tween 20 (1%)) or standard drug (pentazocine, 5 mg/kg), 30 min before administration of acetic acid. The number of writhings and stretching was recorded and permitted to express the percentage of protection.]. The percentage of inhibition was calculated using the following ratio: **(Control – treated) × 100/control.**

RESULTS and DISCUSSION: -

The phytochemical screening of the alcoholic and aqueous root extracts *Tragia bicolor miq.* showed the presence of flavonoids, carbohydrates and glycosides, phenolic compounds, and tannins. The alcoholic and aqueous root extracts of *Tragia bicolor miq* did not showed any lethal effect on the animals up to the doses of 2000 mg/kg. Alcoholic extract of Tragia bicolor miq 200 mg/kg showed better anti-inflammatory activity by reducing the edema volume compared to 400 mg/kg. While comparing aqueous extract and alcoholic extract, aqueous extract produced anti-inflammatory activity, as shown in table 1. Table no.2 shows percentage of protection against writhing response, while comparing both extracts, aqueous extract 400 mg/kg produced better activity P <0.001***. Also, while comparing high dose and low dose high dose produced better inhibition of inflammation.

Table No.1 Anti-inflammatory effect of alcoholic and aqueous root extracts of *Tragia bicolor miq*. on paw edema volume in wistar rats.

Groups	Treatment	Dose	Edema volume (ml)		
			1 hour	2 hours	3 hours
I	Vehicle control	10ml/kg	0.21±0.01	0.24±0.02	0.18±0.02
II	Carrageenan control	0.1ml of 1%w/v	0.60±0.03	0.92±0.03	0.86±0.01
III	Diclofenac sodium(standard)	50mg/kg	0.38±0.02***	0.58±0.03***	0.44±0.02***
IV	Alcoholic extract of *Tragia bicolor miq*	200mg/kg	0.44±0.01**	0.64±0.04**	0.58±0.02**
V	Alcoholic extract of *Tragia bicolor miq*	400mg/kg	0.48±0.02**	0.78±0.03**	0.70±0.03**
VI	Aqueous extract of *Tragia bicolor miq*	200mg/kg	0.42±0.03**	0.68±0.04**	0.54±0.02**
VII	Aqueous extract of *Tragia bicolor miq*	400mg/kg	0.44±0.04***	0.53±0.04***	0.51±0.04***

Values are expressed as mean± S.E.M. (n=6).
***, compared with carrageenan treated group.**
*****= p<0.001, **=p<0.01, *=p<0.05.**

Table 2: Analgesic activity of alcoholic and aqueous extracts against 0.6%v/v acetic acid induced writhing response in Swiss albino mice:

Groups	Treatment	Dose(mg/kg)	Mean number of writhing response (in 10 minutes)	Percentage protection
I	Control (0.6%v/v acetic acid)	10ml/kg	48±1.2	-
II	Pentazocine (standard)	5mg/kg	10±0.5***	79.16%
III	Alcoholic extract of *Tragia bicolor miq*	200mg/kg	24±0.5**	50%
IV	Alcoholic extract of *Tragia bicolor miq*	400mg/kg	18±0.6***	62.5%
V	Aqueous extract of *Tragia bicolor miq*	200mg/kg	22±0.5**	54.16%
VI	Aqueous extract of *Tragia bicolor miq*	400mg/kg	16±0.6***	66.66%

Values are expressed as mean± S.E.M.(n=6).
***, compared with acetic acid treated group.**
*****= p<0.001, **=p<0.01, *=p<0.05.**

CONCLUSION

It was concluded that the root extracts of *Tragia bicolor miq* had significantly inhibited carrageenan induced paw edema in rat. Based on these reports it can be inferred that the inhibitory effect of the alcoholic and aqueous root extracts of *Tragia bicolor miq.*on carrageenan induced inflammation in rat could be due to inhibition of enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. The result showed that the aqueous extract possessed slightly better anti-inflammatory activity than alcoholic extract and more and less equipotent to the positive control after about 60 minutes of treatment and inhibited paw edema in a dose dependent manner. The alcoholic and aqueous root extracts of *Tragia bicolor miq* also exhibited analgesic activity in rodents. The extracts were found to significantly increase the basal reaction time in mice. Based on reports it was concluded that the aqueous extract possessed slightly better analgesic activity than alcoholic extract. The alcoholic and aqueous root extracts of *Tragia bicolor miq* reduces the writhing response induced by acetic acid (0.6%v/v). Acetic acid causes the pain and algic reaction by liberating endogenous substance including histamine, bradykinin, prostaglandins, which stimulates pain. The extracts might inhibits the synthesis and release of these endogenous substance.The results of the study showed the presence of flavonoids, tannins, phenolic compounds may be responsible for the anti-inflammatory and analgesic activity. Further In-vivo and *In-vitro* investigation are required to prove the mechanism of action of the plant extract and isolation of active substance responsible for its biological action are necessary.

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REFRENCES

1. Domaj MI, Glassco W, Aceto MD and Martin BR. Antinociceptive and pharmacological effects of metanicotina, a selective nicotine agonist. *J. Pharmacol. Exp. Ther*. (1999) 291: 390-398.
2. Farshchi A, Ghiasi G, Malek Khatabi P and Farzaei Hossein NA. Antinociceptive Effect of Promethazine in Mice. *Iran. J. Basic Med. Sci.* (2009) 12: 140-145.

3. Hunskaar S and Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. *Pain* (1987) 30: 103-114.
4. Dhara A K, V Subha, T Sen, S Pal, A K Nag Chaudhri. Preliminary studies on the anti-inflammatory and analgesic activity of *Tragia involucrate linn*. Journal of ethnopharmacology., 2000; 72(1-2): 265-268.
5. Dhara A K, S Pal, A K Nag Chaudhri. Psychopharmacological studies on *Tragia involucrate linn*. root extract. Phytother., 2002;16(4): 326-330.
6. OECD Health Effects Testing Guidelines, OECD/OCDE guideline for testing of chemicals acute oral toxicity – acute toxic class method 423, december 200;1-page no. 223 and 234.
7. R V Hosahallyet al., Phytochemical and pharmacological evaluation of *Tragia cannabinna* for anti- inflammatory activity. International current pharmaceutical Journal. 2012; 1(8): 213-216.
8. S K Kulkarni. Handbook of experimental pharmacology; Vallabh Prakashan: 2014; 124-128.
9. G. Ramakrishnan, J. Joshua allan, Krishna Goudar, A. Amit. Comparitive evaluation of anti-inflammatory activity of different extracts of *Boswellia serrata* in wistar albino rats. International journal of Pharmatech Research, 2011; 3(1): 261-267.
10. M Alimuzzaman, Muniruddin Ahmed. *Analgesic Activity of Tragia involucrate.* Dhaka University Journal of Pharmaceutical Sciences. 2005; 4(1): 236-243.