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Analgesic and Anti-Inflammatory Effects of Methanolic Extraction of *Flaveria trinervia Leaves*

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Abstract

This study investigated the analgesic and anti-inflammatory effects of the Methanolic leave extract of *Flaveria trinervia.* Analgesic activity was evaluated using acetic acid induced writhing and eddy's hot plate while anti-inflammatory actions were investigated using the carrageenan induced hind paw edema tests. The analgesic and anti-inflammatory activities of Methanolic extraction of *flaveria trinervia leaves* were significant ($P < 0.001$). The maximum analgesic effect was observed at 120 min at the dose of 400mg/kg (i.p.) and analgesic and Anti-inflammatory activity was compared with standard Diclofenac sodium (5 mg/kg). Methanolic extract of *flaveria trinervia* leaves has shown significant analgesic and anti-inflammatory activities at the dose of 400 mg/kg and was comparable with corresponding standard drugs. The activity was attributed to the presence of phytoconstituents in the tested extract.

Keywords: *Flaveria trinervia,* acetic acid induced writhing, eddy's hot plate, carrageenan induced hind paw edema

INTRODUCTION

Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Rig-Veda (2500–1800 BC) offers the earliest depiction of healing properties of medicinal plants, while Charka Samhita and Sushruta Samhita unfold elaborately on several medicinal herbs[1-4]. At present, the application of plant derived natural products in the synthesis of drugs becomes a drift. Hence, there is a requirement to update data on the properties, uses, effectiveness, and safety of medicinal plant products[5, 6]. Most of the drugs used at present for anti-inflammatory and analgesic effects are synthetic in nature and prolonged use of which causes severe side effects and exhibit toxic effects[7]. In this regard, a novel possibility of evaluating herbs in pain therapy arises. Plants still remain a vase unexploited resource of structurally novel molecules that can aid in the development of novel drugs.

Pain according to the International Association for the Study of Pain (IASP) is unpleasant, sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage[8].

Inflammation is a protective mechanism by the body to remove the harmful stimuli such as pathogens, damaged cells or irritants and initiate the healing process in the body. Acute inflammation is the initial response of the body which begins within seconds to minutes after a tissue injury caused by the harmful stimuli. Acute inflammation will turn chronic if injurious foreign substances persist for longer time and cannot be removed by the body[9].

Flaveria trinervia is a species of flowering plant in the aster family known by the common names clustered yellowtops, speedyweed, and yellow twinstem. It is native to parts of the Americas, including the southeastern and southwestern United States (Florida, Texas, Arizona, New Mexico), most of the Bahamas, Mexico, Belize, and parts of the Caribbean, especially Cuba, Cayman Islands, Jamaica, Hispaniola, Puerto Rico and Barbados. It is also known in many other places as an introduced species and often a noxious weed, such as in Hawaii[10].

The present study was to evaluate the analgesic and anti-inflammatory effects of Methanolic extraction of *flaveria trinervia leaves*

MATERIALS AND METHODS

Plant collection and authentication

Leaves of flaveria *trinervia* were obtained from the local places of Tirupati, AP. And the Plant was authenticated by Dr. K. Madhava Chetty, M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, and Andhra Pradesh.

Extraction by Maceration

Fresh leaves were washed with water to get rid of contaminants like dirt and other impurities and were shade-dried. These dried leaves were ground and sieved to get a uniform, coarse powder. Powdered plant material was weighed (1Kg) and is immersed in Methanol and kept for maceration for a period of 7 days with occasional stirring. On the 8th day, the solvent was filtered by pressing with a muslin cloth and was evaporated in a rotary evaporator at 40^{o}C. The resultant extract was put in a desiccator to remove any ethanol left in it. The dried Methanolic extract of Flaveria *Trinervia*. (MEFT) was packed in an air-tight bottle and put in a dry place for further studies[11].

Qualitative evaluation of phytoconstituents

The MEFT was screened for the presence of various phytoconstituents like carbohydrates, flavonoids, polyphenolic compounds, saponins, tannins, triterpenoids, etc[12].

ANIMAL STUDIES:

Toxicity studies

Albino rats (200-200gm) of either sex were selected and segregated in to 8 groups of 6 animals each. Single dose of methanolic extract of poly herbal formulation, starting from the minimal dose of 50mg/kg up to 3000mg/kg

administered orally. The drug treated animals were observed carefully for its toxicity signs and mortality. From the maximum dose, 1/5th and 1/10th of the concentration was considered as therapeutic dose for further study.

Animals

Albino Rats and Mice of either sex and of approximate same age used in the present studies were procured from Central Animal facility, CMR college of Pharmacy, Hyderabad, India. The animal was fed with standard pellet diet and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours in darkness and light. The animals were acclimatized to the laboratory condition for a one week before starting the experiment.

Analgesic activity

Acetic Acid Induced Writhing Method

This study was carried out using acetic acid induced abdominal writhing reflex pain model. Thirty five mature mice were randomly divided into 4 groups (1-4) of 6 mice per group, fasted for 12 hours and treated as follows, Group 1 (control group) received 10ml/kg normal saline, group 2 (standard) received 30mg/kg of Diclofenac sodium; groups 3 and 4 received 200 and 400mg/kg of MEFT respectively using gastric gavage. One hour after drug and extract administration, 0.6% glacial acetic acid (10ml/kg) was administered intraperitoneally (I.P) to all the mice to induce abdominal contortions or writhings. The analgesic effect was assessed in each mouse for 30, 60,120 minutes and recorded[13].

Eddy's Hot Plate Method: (Thermal stimulus):

In the hot plate method albino mice (18-28) were divided into four groups each consisting of six animals. All the animal selected for the studied were under gone the normal basal reaction time and then separated as different groups like Group I served as a control (received vehicle), Group II served as a standard (received Diclofenac sodium 30mg/kg) while the Group III and IV received the MEFT (200 and 400mg/kg respectively). All animals were lowered onto the surface of a hot plate (50±1.00ºC) enclosed with cylindrical glass and the time for the animal to jump or lick the fore limb was noted as the reaction time (RT). Cut off time in the absence of a response was 15 sec to prevent the animals from being burnt. The observations were made before and after administration of respective drugs at 30 min, 60 min, 120 min, and at the end of 180 min[14].

Anti inflammatory activity

Carrageenan induced hind paw edema:

Albino rats of either sex weighing 150-200 grams were divided into four groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline 0.5 ml/kg), Group II – Diclofenac sodium (30 mg/kg, p. o.), Group - III and IV - MEFT (200 mg/kg and 400 mg/kg, p. o.). All the drugs were administered orally. Diclofenac sodium served as the reference standard anti inflammatory drug. After one hour of the administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rat and the right hind paw was served as the control. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at the end of 0 min., 60min., 120min., 180min.and 240min. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied[15].

RESULTS

The MEFT with two selected doses i. e 200 and 400mg/kg have exhibited significant increase percentage of inhibition in writhing method in mice at different time intervals. Results were shown in **Table 1.** Diclofenac sodium (30mg/kg) was used as standard and it has significantly increased the percentage inhibition of writhing by 90.7±4.3% at the end of 2hr which was found to be a time dependent effect. During first 30 min of the study MEFT with medium and high dose the % of inhibition of writhing were 56.3±5.56, and 61.5±5.65%. During 1hr of the study MEFT with medium and high dose the % of inhibition of writhing was 65.6±5.78 and 74.3±6.74% respectively which was time dependent effect. During 2hr of the study MEFT with medium and high dose the % of inhibition of writhing was 80.8±3.62 and 87.7±5.45% and the results were tabulated.

The MEFT with two selected doses i.e. 200 and 400mg/kg have exhibited significant increase in reaction time in Eddy's hot plate method in mice at different time intervals. Results were tabulated in **Table 2**. Diclofenac sodium (30mg/kg) was used as standard and it has significantly increased the reaction time at the end of 1st and 2nd h which was found to be a time dependent effect. During 30min of the study MEFT with medium and high dose the reaction time was 4.53±1.23, 4.53±1.54 seconds. During 60min of the study MEFT with medium and high dose the reaction time was 5.75±1.32, 5.32±1.55 seconds respectively which was time dependent effect. During 120min of the study MEFT with medium and high dose the reaction time was 8.46±1.65, 8.32±1.12 seconds respectively. During 180min of the study MEFT with medium and high dose the reaction time was 7.57±1.25, 7.45±1.13seconds. Diclofenac sodium showed a significant reaction time during 30min and 60min whereas test groups have shown during 60 and 180min.

The MEFT in 200 mg/kg and 400mg/kg have exhibited a significant reduction in paw edema volume in Carrageenan induced paw oedema in rats at different time intervals. Results are tabulated in **Table 3**. Diclofenac sodium (30 mg/kg) was used as standard reference and it has significantly reduced paw oedema volume which was found to be a time dependent effect. Diclofenac showed maximum response at 4th hour which was found to be 91.43%. At 4th hour MEFT showed significant reduction in oedema volume, 400mg/kg showed the maximum reduction in oedema volume i.e. 90%.

Table 1: Acetic acid induced writhing test in mice:

Treatment	Dose	Number of writhing (Mean± SEM)			% inhibition of writhing		
		30 min	60 min	120 min	30 min	60 min	120 min
Control	-	26.56± 3.62	27.56± 4.35	35.83± 4.1	-	-	-
Standard (Diclofenac sodium)	30 mg/kg	13.23± 1.33**	8.46± 1.02**	3.66± 1.2**	64.7± 3.2	76.8± 2.6	90.7± 4.3
MEFT	200mg/kg	17.26± 2.62**	13.23± 1.65**	9.32± 5.52**	56.3± 5.56	65.6± 5.78	80.8± 3.62
MEFT	400mg/kg	15.44± 3.44**	10.76± 1.32**	6.65± 1.43**	61.5± 5.65	74.3± 6.74	87.7± 5.45

Values indicate mean± SEM (ANNOVA test followed by Dunnet's *t*- test), Control n =6: comparison with control group

Table 2: Analgesic effect of MEFT in Eddy's hot plate method in mice

Groups	Treatment	Hot plate reaction time (Sec)			
		30 min	60 min	120 min	180 min
Control	Formalin (1%w/v)	2.51± 0.318	2.39± 0.38	2.15± 0.36	2.17± 0.35
Standard	Diclofenac sodium (30mg/kg)	3.57± 0.47	6.05± 1.14**	9.94± 0.45**	7.95± 0.32**
MEFT	200mg/kg	4.53± 1.23	5.75± 1.32	8.46± 1.65**	7.57± 1.25**
MEFT	400mg/kg	4.53± 1.54	5.32± 1.55*	8.32± 1.12**	7.45± 1.13**

Values are expressed in mean ± SEM, where n = 6, Significant at P < 0.05*, 0.01** and 0.001***, compared to control group

Table 3: Carrageenan induced paw edema in rats

Groups	Edema volume (ml)				
	0 hr	1hr	2hr	3hr	4hr
Control	0.18 ± 0.06	0.25 ± 0.09	0.30 ± 0.07	0.33 ± 0.07	0.36 ± 0.06
Diclofenac sodium(30mg/kg)	0.16 ± 0.03	0.27 ± 0.05*	0.20± 0.04*	0.18 ± 0.04*	0.11 ± 0.03*
MEFT 200mg	0.17± 0.021	0.28± 0.042	0.24± 0.08**	0.20± 0.05**	0.17± 0.05
MEFT 400mg	0.16 ± 0.05	0.26 ± 0.06*	0.22 ± 0.05*	0.18 ± 0.07*	0.13 ± 0.03*

Data presented as mean ± S.E.M. n=6, *** *P*< 0.001, ** *P*< 0.01, * *P*< 0.05, Compared with control group, followed by ANOVA followed by Post hoc test (Dunnett's't' test)

DISCUSSION

The present study depicts the analgesic activity of *flaveria trinervia* Methanolic leaf extract in both the methods centrally acting hot plate model and peripherally acting acetic acid induced writhing model. All the extracts protected mice against both hot and chemical induced noxious stimuli. Intra-peritoneally administered acetic acid, caused elevated levels of prostaglandins PGE2 and PG-$F_{2\alpha}$ in peritoneal fluid. The abdominal constriction in mice was associated with sensitization of peritoneal nociceptors by the prostaglandins[16,17].

The analgesic effects exhibited by the extracts may be associated with the inhibition of prostaglandins level.

The hot plate model has been employed extensively for the screening of compounds exhibiting analgesia by central mechanism, where elevation in pain threshold of mice towards heat is determined. It is well known fact that, the response (paw licking, jumping) by mice to noxious thermal stimuli in hot plate method is supra-spinally mediated response[18]. The analgesic effect exhibited by the extracts in hot plate test could be due to their interaction with various receptors present in supra-spinal sites.

Carrageenan-induced edema is commonly used experimental model for evaluation of acute inflammation in animals. Carrageenan, when injected into the sub-plantar region of rat's paw, produces inflammatory reaction (edema) which is visible within 30 min. The presumptive mechanism of action of carrageenan-induced edema has been found to be biphasic. The early phase (1–2 h) is due to the liberation of serotonin, histamine and bradykinin, while the second phase is attributed to the release of prostaglandins[19, 20]. All the extracts exhibited maximum inhibition in paw volume at 3h, suggesting that the extracts could possibly have an inhibitory effect on the release of prostaglandins at the second phase.

CONCLUSION

In conclusion, our results reveal that the extracts of leaves of *Flaveria trinervia* Methanolic leaf extract exhibited significant analgesic and anti-inflammatory activities. These findings validated the claim for the traditional use of this plant in the treatment of pain and inflammatory ailments. Further research work is needed to segregate the active constituents from the active extract exhibiting significant analgesic and anti-inflammatory activities. In addition to this, research regarding the mechanism responsible for these activities is also required which will guarantee its clinical worth.

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CONFLICTS OF INTEREST

The authors declare that they have no Conflicts of interest.

ETHICAL APPROVA

The experiment protocols were approved by Institutional Animal Ethics committee after securitization (**IAEC No: CPCSEA/1657/IAEC/CMRCP/COL-19/67**).

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