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# EVALUATION OF ANALGESIC ACTIVITY OF ETHANOLIC EXTRACT OF MOMORDICA TUBEROSA LEAVES IN EXPERIMENTAL ANIMALS

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## ABSTRACT

Pain is a disabling accompaniment of many medical conditions. It is direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer. Momoradica tuberosa has been recognized as traditional medicine for the treatment of different diseases. **Objectives**: The aim of the present study was to evaluate the analgesic activity of ethanolic extract of Momordica Tuberosa leaves (EEMTL) in experimental animals. **Methods:** The study was designed to evaluate the peripheral and central analgesic activity of EEMTL (250mg/kg, 500mg/kg body weight p.o) by using 0.7% acetic acid induced writhing test and radiant heat tail flick method in mice and rats respectively. Aspirin is used as the standard drug. The mean reaction time and number of writhings were measured. **Results:** EEMTL significantly decreased the number of writhing and produced 60.89% of inhibition at 500mg/kg bw($p<0.0001$)which was comparable with that of the standard drug aspirin ($p<0.0001$) in writhing test. It also showed increase in the mean reaction time in tail-flick method ($p<0.0001$) at all the doses at $1^{st}$,$2^{nd}$ and $3^{rd}$ hr. EEMTL produced significant tail flick latency and percentage inhibition of writhing response at all the doses in contrast to control group. **Conclusion:** EEMTL has significant peripheral and central analgesic activity.

**KEYWORDS:** Momoradica tuberosa, pain, analgesic, writhing, tail flick.

## INTRODUCTION

The task of medicine is to preserve and restore health and to relieve suffering. Understanding the pain is essential to both these goals. Pain is an unpleasant sensation no doubt, but it is a protective mechanism for the body that occurs whenever tissues are actually or potentially damaged and it causes the individual to react and to remove the pain stimulus.[1] With many pathological conditions, tissue injury is the immediate cause of pain and this result in the release of various chemical mediators which are assumed to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulus.

Pain is dual in nature, it is both sensational and emotional.[2] The pathophysiology of pain involves two components, peripheral nociception and central mechanism. There are two classes of pain- integumental pain and visceral pain.[3]

Drugs which are used presently for the management of pain are either steroidal like corticosteroids or nonsteroidal like Aspirin. These drugs possess more adverse and toxic effects.[4] Opiates cause physical dependency, tolerance and addiction while NSAIDs usually cause gastrointestinal disorders. Therefore research to discover other alternatives to treat pain is crucial. On the contrary many medicines of plant origin has been used since ages without any side effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and more economical analgesic drugs.

Therefore, the present study was designed to investigate the peripheral and central analgesic effect of the ethanolic extract of Momordica tuberosa leaves in mice using acetic acid induced writhing test and in rats using tail flick method respectively.

Momordica Tuberosa (synonyms- Luffa tuberosa, Momordica cymbalaria) belongs to the family Cucurbitaceae commonly known as Momordica cymbalaria.

### Plant Profile

Family : Cucurbitaceae
Latin name : Momordica Tuberosa
Synonyms : English - Momordica
Hindi - Kakrol
Kannada - Karchikai
Tamil - Athalkkai
Sanskrit - Kaarali kanda

The plant is originating in tropical regions of India and South East Asia. It is perennial climber available during the monsoon season and is found in south Indian states of Karnataka, Andhra Pradesh, Madhya Pradesh, Maharashtra and Tamil Nadu as a weed. It has slender, scandent, branched, striate stem. The leaves are orbicular, reniform in outline deeply chordate at the base, sparsely hairy. The roots are woody, tuberous and perennial.[5]

It contains several phyto-constituents such as sterols, saponins, triterpenoids, cardiac glycosides, flavanoids, carbohydrates.[5]

The medicinal properties of various parts of Momordica Tuberosa are testified. It possess antidiabetic, hypolipidemic[6], antiovulatory, abortifacient[7], antidiarrhoeal[8], anticonvulsant[9], antioxidant, hepatoprotective[10], nephroprotective[11], antidepressant[12], antiulcer[13] properties.

## MATERIALS AND METHODS

### *Plant material*

Fresh green leaves of Momordica Tuberosa popularly known as kasarakai were obtained in sufficient quantity from suburban places of Raichur in the month of august 2014. They were carefully washed to remove dust particles and other foreign materials and dried in shaded area and it was authenticated by Mr. Harish. B.S. (Asst. Prof, Medicinal and Aromatic Crops) and the specimen (Voucher number: SNMC/Pharma 007), is preserved for reference in the department herbarium of Pharmacology, SNMC Bagalkot.

**Figure-1. Momoradica tuberosa leaves.**

***Preparation of Plant extract***

The leaves of the plant were dried under shade for a period of 2 weeks. The dried leaves were milled to a fine powder. The material was extracted with 80% ethanol using soxhlet extraction apparatus and it was evaporated to dry at 60°C. Dried leaves (20 g) of Momordica Tuberosa leaves yielded 4 g of crude extract. The solid residues were stored in airtight container and preserved in the refrigerator at −20°C.[14] From this stock, fresh preparations were obtained whenever required.

**Figure – 2 Extraction of Momordica tuberosa leaves using Soxhlet appartus**

***Phytochemical analysis***

Preliminary phytochemical studies of ethanolic extract of Momordica Tuberosa leaves revealed the presence of flavanoids, triterpenoids, steroids and carbohydrates.[5]

***Acute oral toxicity study***

The acute toxicity studies were conducted according to OECD 423 guidelines. The ethanolic extract of Momordica tuberosa leaves found to be non toxic up to 2000 mg/kg.[15]

**Experimental animals**

All the animals were procured from the Central Animal house, S. N. Medical College, Bagalkot. Wistar albino rats of either gender weighing 150-250 g and Swiss albino mice of either gender weighing 20 to 25 g were selected for the experiment. Pregnant rats/mice, animals with an infection, animals with injuries, deformities were excluded from the study. Prior to and during study, all the animals were maintained under standard animal house conditions at 12:12 hrs dark: light cycle, at temp 25±2°C, 35-60% humidity and other micro

and macro environment conditions as suggested by Committee for the Purpose of Control and Supervision of Experiment on Animals(CPCSEA). All animals were housed in a polypropylene cage covered with a stainless steel wire mesh and a paddy husk bed, with adequate provision for feed and water. All the animals were maintained on standard laboratory diet (VRK Nutritionals, Pune) and water was provided *ad libitum*.

The study was started after getting the Institutional Animal Ethics Committee approval (IAEC/SNMC Reg No.829/AC/04/CPCSEA).

**Evaluation of peripheral analgesic activity**

***Acetic acid induced writhing test***

Swiss albino mice were divided into 4 groups of 6 each.

Group I: 0.9% Normal saline (control)

Group II: Aspirin 100 mg/kg bodyweight (standard)

Group III: EEMTL 250 mg/kg bodyweight

Group IV: EEMTL 500 mg/kg bodyweight

All the groups received drugs by the oral route. Abdominal constrictions were induced by 0.7 % v/v glacial acetic acid solution (10 ml/kg, I.P.) in mice pre-treated with normal saline or aspirin. The number of abdominal writhing were measured over 20 min after the injection of acetic acid. Results were expressed as percentage inhibition of abdominal constrictions with respect to control. The same procedure was repeated with EEMTL at a dose of 250 and 500 mg/kg.

**Evaluation of central analgesic activity**

***Radiant heat tail flick method***

Wistar albino rats were divided into 4 groups of 6 each.

Group I: 0.9% Normal saline (control)

Group II: Aspirin 300 mg/kg bodyweight (standard)

Group III: EEMTL 250 mg/kg bodyweight

Group IV: EEMTL 500 mg/kg bodyweight

All the drugs were given orally. After ½ hr,1 hr, 2 hrs, 3 hrs the tail flick response was carried out and the reaction time was measured by placing the distal 1/3rd of the tail about 1 cm from the radiant heat source of the analgesiometer. The time taken by the animal to withdraw the

tail was taken as the reaction time. Cut off time was kept as 20-30 sec. The animals showing reaction time of >20-30 sec were excluded from the study.

**Statistical Analysis**

The stastical data were presented as Mean ± SEM and results were analyzed using One way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. For all the tests 'p' value of 0.05 or less was considered as statistical significance.

## RESULTS

***Acute oral toxicity study***

No adverse effect or mortality was detected in Swiss albino mice at 2g/kg of EECO by using five animals. All the animals were alive, healthy and active during the observational period of 14 days. So the LD 50 was considered as >2000mg/kg.

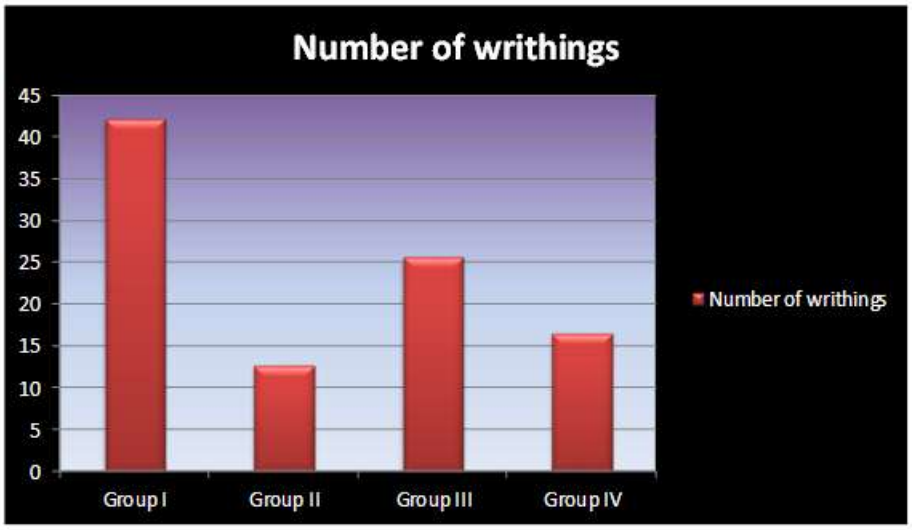
***Peripheral analgesic activity***

The results of the peripheral analgesic activity of EEMTL on acetic acid induced writhing test in albino mice were depicted in Table-1 and Graph -1. EEMTL at a dose of 250 mg and 500 mg /kg body weight produced 39.29% and 60.87% reduction in writhing response and results were highly significant when compared to control. EEMTL at a dose of 500 mg /kg body weight showed 60.87% ($p<0.0001$) of inhibition of writhing response which is comparable to standard drug Aspirin (69.95%, $p<0.0001$).

**Table -1: Number of writhings and percentage inhibition of acetic acid induced writhing test.**

| Groups | M ± SEM | Percentage of Inhibition(%) |
|---|---|---|
| Group I (Control) | 42.17 | - |
| Group II(Standard 300 mg/kg) | 12.67 | 69.95% |
| Group III (EEMTL 250 mg/kg) | 25.60 | 39.29% |
| Group IV( EEMTL 500 mg/kg) | 16.5 | 60.87% |

***when compared with control; *p<0.05, **p<0.01, ***p<0.0001. All the values are expressed as mean ± Standard Error of mean (n=6).***




**Graph 1 showing number of writhings**

## CENTRAL ANALGESIC ACTIVITY

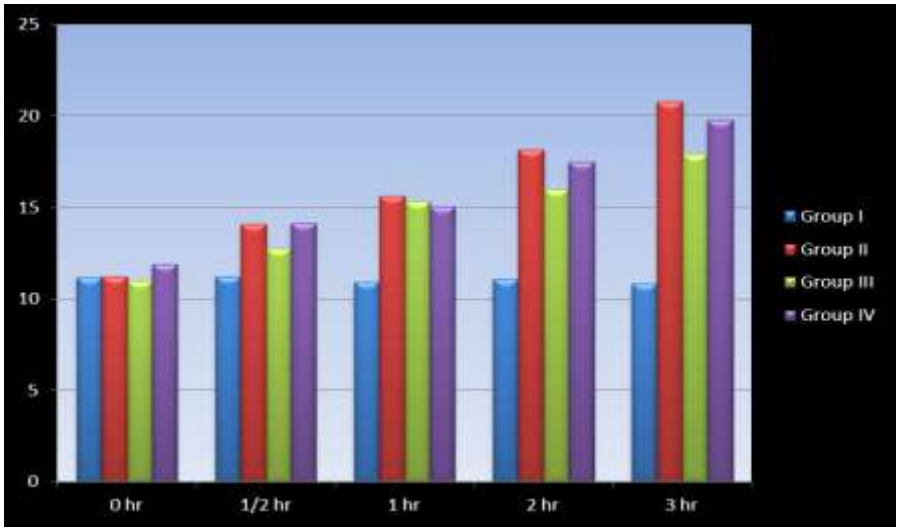
***Radiant heat tail flick method***

The results obtained from tail flick response were shown in Table-2 and Graph-2. At 0 hr, there was no difference between mean reaction time (MRT) of different groups. The group I(control) shown the MRT of (10.82 ± 0.29 sec ) at 3rd hour. Group III and Group IV showed significant increase in MRT (12.74± 0.33, 14.15±0.46; $p<0.05$, $p<0.0001$) at ½ hr respectively in comparison to control. EEMTL at dose 250mg and 500mg /kg body weight showed highly significant increase in MRT (15.35 ± 0.28, 18.16 ± 0.77, 17.93 ± 0.44; 15.02 ± 0.48, 17.49 ± 0.23, 19.75 ± 0.21 ,$p<0.0001$) at 1st, 2nd,3rd hr respectively which are comparable to standard drug aspirin(MRT 15.61 ± 0.77, 18.16 ± 0.77, 20.76 ± 0.52 $p< 0.0001$)

**Table-2: Mean Reaction time (seconds) in Tail-flick method.**

| GROUPS | 0 hr M±SEM | ½ hr M±SEM | 1 hr M±SEM | 2 hr M±SEM | 3 hr M±SEM |
|---|---|---|---|---|---|
| **Group I** Control | 11.19± 0.20 | 10.95 ± 0.35 | 10.94 ± 0.31 | 11.09 ± 0.19 | 10.82 ± 0.29 |
| **Group II** Standard Aspirin(300 mg/kg) | 11.23± 0.96 | 14.10 ± 0.63 | 15.61 ± 0.77*** | 18.16 ± 0.77*** | 20.76 ± 0.52*** |
| **Group III** EEMTL (250mg/kg) | 10.98± 0.44 | 12.74± 0.33* | 15.35 ± 0.28*** | 16.01 ± 0.28*** | 17.93 ± 0.44*** |
| **Group IV** EEMTL(500mg/kg) | 11.89± 0.44 | 14.15±0.46*** | 15.02 ± 0.48*** | 17.49 ± 0.23*** | 19.75 ± 0.21*** |

**Post-hoc test: when compared with control ; *p<0.05, **p<0.01, ***p<0.0001. All the values are expressed as Mean ± SEM (n=06). SEM=Standard error of mean.**




**Graph-2: Mean Reaction time at different hours.**

## DISCUSSION

This study is the first report regarding analgesic activity of EEMT leaves. Anti nociceptive models like acetic acid induced writhing test and radiant heat tail flick method were used to evaluate analgesic activity of EEMTL. The results of the present study shown that EEMTL produced significant analgesic activity against chemical and thermal models of nociception in mice and rats.

Acetic acid induced writhing test is a model for visceral pain. Several chemicals such as phenylquinone, aceticacid could induce writhing reflex in laboratory animals. Intra peritoneal injection of 0.7% glacial acetic acid produced writhing by activating the chemosensitive nociceptors.[16] Acetic acid produces nociception by liberating endogenous substances like serotonin, bradykinin, histamine, prostaglandins which may stimulates sensory nerve endings.[17] Therefore EEMTL might be inhibiting synthesis or release of these endogenous substances.

Even though writhing test is very sensitive, it may give false positive results, so tail flick test was conducted to confirm and study the analgesic property in EEMTL. The significant increase in pain threshold produced by EEMTL at 250 mg, 500 mg and aspirin in radiant heat tail flick model suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems.[18] The analgesic effect produced by the EEMTL may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes and other endogenous substances that are key

mediators in pain. Preliminary qualitative phytochemical screening of ethanolic extract of Momordica Tuberosa leaves revealed the presence of flavanoids, triterpenoids, steroids and carbohydrates.

Flavanoids are known to target prostaglandins which are involved in pain perception.[19] The presence of flavanoids in EEMTL may be responsible for its analgesic activity.

## CONCLUSION

The present research has shown that ethanolic extract of Momoradica tuberosa leaves has notable peripheral and central analgesic activity. Further pharmacological analysis of the extract is needed to isolate and characterize the active ingredient responsible for its analgesic effect.

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