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Analgesic and anti-Inflammatory activity of methanolic extract of leaves of *Quisqualis indica*

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Abstract

The present study was designed to evaluate the analgesic and anti-inflammatory activity of methanolic extract of *Quisqualis indica* (MEQI) leaves in rodents. The peripheral analgesic activity of methanolic extract of *Quisqualis indica* leaves (MEQI; 200 and 400 mg/kg) was studied using acetic acid-induced writhing test and central analgesic activity of MEQI was studied using hot-plate method in mice. Anti-Inflammatory activity of MEQI (200 and 400 mg/kg) was studied in carragenin induced paw edema in rats. MEQI significantly decreased the writhing movements in acetic acid-induced writhing test and increased the reaction time in hot-plate test in mice. MEQI also showed significant anti-Inflammatory activity in carragenin induced paw edema by reducing paw volume in rats. From our study, we concluded that MEQI has potential analgesic and anti-inflammatory activities.

KeyWords: Analgesic, Antiinflammatory, *Quisqualis indica.*

1. Introduction

Pain has been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Failure to relieve pain is morally and ethically unacceptable [1]. Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [2]. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs either possess well known side and toxic effects or very expensive to develop [1]. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations without or with minimal adverse effects[3]. As a part, this study seeks to assess analgesic and anti-inflammatory activitiy of methanolic extract of *Quisqualis indica* in rodents. *Quisqualis indica* (Combreteceae) is a strong climber, ligneous vine that can reach from 2.5 meters to up to 8

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meters. It is commonly known as Rangoon creeper. It is indigenous in Africa, Indo Malaysian region and cultivated all over India.

2. Materials and Methods

2.1Preparation of Quisqualis indica leaf extract

Quisqualis indica leaves were collected from kapalitheertham forest Andhra Pradesh, India. The plant was identified by Dr. K. Madhava Chetty, Asst. Professor, Botany Department, Sri Venkateshwara University, Tirupathi, Andhra Pradesh, India. The shade-dried leaves of *Quisqualis indica* were powdered and then 500 g of powder was extracted with methanol by heating under reflux. The methanolic extract was concentrated under reduced pressure to a semisolid mass of 48.59 g (yield 9.7%, w/w) which was stored in a closed bottle and kept in a refrigerator at temperature below 4°C until tested. For in vivo studies, the concentrated methanolic extract of *Quisqualis indica* (MEQI) was administered orally by using normal saline as a vehicle. The freshly prepared solution of MEQI was used in each experiment.

2.2 Chemicals

All the drugs used in this study were of pharmaceutical grade and chemicals were of analytical grade. Carragenin was supplied by Sigma Chemicals Company, St. Louis, USA.

2.3 Experimental animals

Swiss albino mice and Wistar rats procured from Mahaveer Enterprises, Hyderabad were used in the study. The animals were kept in polypropylene cages and maintained on balanced diet with free access to clean drinking water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to the experimental session. The animals were divided into different groups each consist of six animals were fasted overnight prior to the experiments. The protocol of the study has been approved by ethical committee of the institute.

2.4 Acute oral toxicity of MEQI [4]

Methanolic extract of *Quisqualis indica* was orally administered as a single dose to groups of mice (n = 6) at different concentrations (50, 100, 200, 500, 1000 and 2000 mg/kg, *p.o.*). These animals were observed for a 24-h period, and then for 14 days.

Evaluation of Analgesic activity of MEQI

2.5 Acetic acid induced abdominal writhing response [5,6]

Peripheral analgesic activity of MEQI was tested using acetic acid induced abdominal writhing (muscular contraction causing pain) test. Swiss albino mice were divided into four groups (n = 6). Group I received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and number of writhing reflexes were noted for the period of 30 minutes. Group II received aspirin (100 mg/kg b.w. p.o.), Group III and IV received MEQI at the doses of 200 mg/kg and 400 mg/kg b.w., p.o. respectively. 30 min after aspirin and MEQI administration, group II, III and IV received acetic acid (1% v/v, 10 ml/kg b.w., i.p.) and number of writhing reflexes were noted for the period of 30 min.

2.6 Hot Plate Method [6]

Central analgesic action of MEQI was assessed by eddy's hot-plate method. Mice were placed on a hot plate maintained at 55 ± 1°C and the reaction latency (in seconds) for licking of hind paw or jumping noted. The mice which reacted within 15 sec (cut-off period to avoid damage to paw) and which did not show large variation when tested on four separated occasions were selected for study. Four groups of mice (n = 6) were used in this study. Group I recieved normal saline (5 ml/kg b.w. p.o.). Where as group II, III and IV were treated with morphine sulphate (10 mg/kg b.w. p.o.), MEQI (200 and 400 mg/kg b.w. p.o.) respectively. Recordings were

taken before treatment and at 30, 60, 90,120 min after treatment.

Evaluation of Anti inflammatory activity of MEQI

2.7 Carragenin induced paw edema [7]

The rats were divided into four groups containing six rats in each group. 0.1 ml of 1.0% carragenin in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The MEQI was administered to the rats 1 h before carragenin injection. Different groups were treated as follows:

Group I: Carragenin (0.1 ml of 1.0% carragenin/rat to the sub plantar region).

Group II: Carragenin + Indomethacin (10 mg/kg b. w., p. o.)

Group III and IV: Carragenin + MEQI (200 mg/kg and 400 mg/kg b. w., p. o. respectively).

The paw volume was measured initially and at 1, 2, 3 and 4 h after carragenin injection, using Plethysmograph, and was compared with control rats

The percent inhibition of edema as calculated for each group with respect to its vehicle-treated control group. The anti-inflammatory activity was calculated by using the formula

$$\frac{A-B}{A} X\ 100$$

Where A and B denote mean increase in paw volume of control and drug-treated animals respectively.

2.8 Statistical analysis

Results are expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The results were considered statistically significant when $P < 0.05$, $P < 0.01$, $P < 0.001$.

3. Results

3.1 Acute oral toxicity

No toxic symptoms and mortality was observed and the extract was found to be safe upto 2000 mg/kg.

3.2 Analgesic activity

Acetic acid induced abdominal writhing response

The effect of different doses (200 and 400 mg/kg) of MEQI on acetic acid induced writhing response were shown in table 1. In this test both the doses i.e. 200 and 400 mg/kg of *Quisqualis indica* significantly suppressed the number of writhings in dose dependent manner and the inhibition was 32.9% and 71.3% with 200 and 400 mg/kg respectively. The standard drug, aspirin, produced better reduction than the extract.

3.3 Eddy's hot plate method

Pretreatment with methanolic extract of *Quisqualis indica* leaves significantly increased the reaction time (time taken by the animal to react to thermal pain) at all time points, but the effect was maximum at 60 and 90 min after administration. Both the doses i.e. 200 and 400 mg/kg of MEQI showed significant activity. The analgesic effect of higher dose of MEQI was comparable with standard morphine sulphate and these results were shown in table 2.

3.4 Antinflammatory activity

Carragenin induced paw edema

The effect of different doses (200 and 400 mg/kg) of methonolic extract of *Quisqualis indica* leaves against carragenin induced paw edema were shown in table 3. In this model both the doses of MEQI reduced paw volume at all the time points but the effect was significant at 3rd hour with 200 mg/kg, where as the anti inflammatory effect with 400 mg/kg was significant at 3rd and 4th hours of post treatment.

Table 1: Showing the number of writhings (for 30 min) and % inhibition

Sl. No.	Treatment	Dose (mg/kg)	Number of Writhings	% Inhibtion
1	Acetic acid 1% v/v	10 ml/kg	102.33±2.21	—
2	Acetic acid + Aspirin	100	18.83±4.40***	81.6
3	Acetic acid + MEQI	200	68.66±6.33**	32.9
4	Acetic acid + MEQI	400	29.33±4.33***	71.3

Values are expressed as mean ± SEM; n=6,

***P*<0.01, ****P*<0.001 when compared with control group.

Table 2: Showing the reaction time for paw licking/ jumping response at different time intervals (min)

Sl.no	Treatment	Dose (mg/kg)	Reaction time (sec) at different time intervals			
			30 min	60 min	90 min	120 min
1	Control	5 ml/kg	4.33±0.21	4.83±0.22	3.83±0.22	3.5±0.22
2	Morphine Sulphate	10	8.83±0.40***	9.66±0.33***	9.33±0.33***	5.66±0.42*
3	MEQI	200	5.33±0.33	6.33±0.21**	5.83±0.21**	3.83±0.16
4	MEQI	400	6.83±0.33**	8.83±0.30***	8.66±0.33***	4.83±0.21

Values are expressed as mean ± SEM; n=6

**P*<0.05, ***P*<0.01, ****P*<0.001 when compared with control

4. Discussion and Conclusion

In our present study, acetic acid induced writhing test is used to evaluate the peripheral analgesic effect of extract and the algesic response of acetic acid is thought to be mediated by prostaglandin pathway[8]. In this method MEQI at both dose levels (200 and 400 mg/kg) produced antinociception against chemical (acetic acid) induced pain stimuli in mice at various time points of post treatment. Therefore, it may be inferred that the inhibitory effect of the compound could be due to the inhibition of prostaglandin pathway. The hot plate test is considered to be selective for centrally acting analgesics like opioid compounds in several animal species. Hence this method was selected to screen central analgesic activity of MEQI[9]. Methanolic extract of *Quisqualis indica* at both dose levels (200 and 400 mg/kg) produced antinociception against thermal induced pain stimuli in mice at various time points of post treatment. The effect observed was statistically significant. The intensity of analgesic activity of high dose (400mg/kg) of methanolic extract of *Quisqualis indica* dose was similar to that of standard drugs used in the study. Carragenin-induced rat paw edema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The development of odema in the paw of the rat after the injection of carragenin is due to release of histamine, serotonin and prostaglandin like substances. In case of the time course of edema development in carragenin induced paw edema model in rats is generally two phases are found. The first phase, which occurs between 0 to 2.5 h of

Table 3: Showing the difference in paw edema of mean±SEM and % reduction in edema volume at different time intervals

			Difference in Paw edema volume (ml)							
			After 1st hour		After 2nd hour		After 3rdhour		After 4th hour	
Sl.No	**Treatment**	**Dose mg/kg**	**MEAN ±SEM**	**% ROV**	**MEAN ±SEM**	**% ROV**	**MEAN ±SEM**	**% ROV**	**MEAN ±SEM**	**% ROV**
1	Control	—	0.165±0.01	—	0.495± 0.02	—	0.65±0.02	—	0.85± 0.036	—
2	Indomethacin	10	0.14±0.01	15.32	0.32±0.03*	35.85	0.14±0.02***	79.12	0.19±0.016***	78.5
3	MEQI	200	0.15±0.01	09.09	0.43±0.015	13.65	0.49±0.01*	25.27	0.68±0.019	20.85
4	MEQI	400	0.15±0.02	9.09	0.39±0.02	21.71	0.32±0.02**	51.42	0.485±0.04**	43.8

Values are expressed as mean ± SEM; n=6
*P<0.05, **P<0.01, ***P<0.001 when compared with vehicle treated group

injection of the phlogistic agent, has been attributed to the release of histamine or serotonin. The second phase of inflammatory reaction which is measured at 3h is caused by the release of bradykinin, protease, prostaglandin and lysosome[1]. In our present study, pretreatment with MEQI at two dose levels produced reduction in paw volume at all time points but significant inhibition was observed after 3rd and 4th hours of post treatment. This interferres that the extract may be acting by inhibiting second phase mediators. From our results we concluded that aqueous extract of *Quisqualis indica* possess centrally and peripherally mediated analgesic properties and anti-inflammatory activity. The analgesic and anti-inflammatory effects were in dose dependent manner and comparable with respective standard drugs.

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