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Research Article

EVALUATION OF ANALGESIC ACTIVITY OF AQUEOUS EXTRACT OF LEAVES OF *SOLANUM MELONGENA LINN* IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: The present study was aimed to find out the central and peripheral analgesic activity of aqueous leaf extract of *Solanum melongena Linn* (AESML) in experimental animals.

Methods: Preliminary phytochemical analysis of AESML was performed by chemical tests. The central analgesic activity was measured by tail flick method in Wistar rats and peripheral analgesic activity was measured by acetic acid induced writhing in Swiss albino mice. Aspirin was used as the standard drug in the dose of 300 mg/kg b.w. in tail flick method and 100 mg/kg b.w. in acetic acid induced writhing test.

Results: Phytochemical analysis showed the presence of flavonoids, alkaloids, glycosides, saponins, tannins, sterols, carbohydrates, and resins. The aqueous extract was administered at 100 mg/kg, 200 mg/kg, 400 mg/kg b.w. All the doses showed significant central and peripheral analgesic activity ($p<0.001$), which is comparable to that of the standard drug aspirin.

Conclusion: AESML leaves has both central and peripheral analgesic activity.

Keywords: *Solanum melongena Linn*, Aqueous extract, Analgesic activity.

INTRODUCTION

Pain is one of the most common reasons for which an individual seeks the advice of a physician. Sherrington described pain as, "The physical adjunct of an imperative protective reflex" [1]. Pain is defined by the International Association for the study of pain as, "an unpleasant sensory and emotional experience associated with actual (or) potential tissue damage (or) described in terms of such damage" [2]. Pain is an emotional component, which varies from person to person and in the same person, from time to time. Unrelieved acute pain can cause chronic pain and long standing pain can cause anatomical and even genetic changes in the nervous system [3].

Traditional medicine is widely used around the world and valued for a number of reasons. At the international conference on traditional medicine for South-East Asian countries in February 2013, the WHO Director-General, Dr. Margaret Chan, stated that "traditional medicines, of proven quality, safety, and efficacy, contribute to the goal of ensuring that all people have access to care." For millions of people, herbal medicines, traditional treatments, and traditional practitioners are the main source of health care, and sometimes the only source of care [4].

With the easy availability of the analgesic drugs we are now facing a new era of people presenting with symptoms of analgesic abuse. With the development of more and more synthetic drugs which have their unique adverse effects, it is high time that attention should be turned to the possible remedies that may be found among indigenous herbal plants. This has accelerated the global effort to harvest those medicinal plants that have substantial beneficial effects with least adverse effects.

Solanum melongena Linn. (Garden egg) is a culinary vegetable, which has been in use in the Indian medicinal system since antiquity. Various parts of the plant are used in the treatment of inflammatory condition, cardiac debility, neuralgia, ulcer in nose, and cholera. It also has analgesic, antipyretic, anticonvulsant, hypolipidemic activity. The plant can also be used in bronchitis and asthma [5].

The aqueous extracts of leaves of *S. melongena Linn*. (AESML) was used for the present study with the objectives of evaluation of the central analgesic activity in Wistar albino rats by using tail flick method and evaluation of the peripheral analgesic activity in Swiss albino mice by using writhing test.

METHODS

Chemicals

Soluble form of aspirin was obtained from Nice, Cochin. In this study aspirin was taken as standard drug. It belongs to salicylates (non-steroidal anti-inflammatory drugs). Acetic acid is used to induce writhings in mice. It was obtained from Nice, Cochin. 10 ml/Kg of 0.7% v/v acetic acid was used to induce writhings. 0.7% v/v acetic acid was prepared by adding 0.7 ml of acetic acid in 100 ml of distilled water. The solution was prepared freshly before each experiment.

Plant materials

Fresh leaves of *S. melongena Linn*. were collected from the rural areas of Bagalkot district, Karnataka in the month of March 2011. The plant identity was authenticated by Botanist Prof. Jadimath. The fresh leaves were dried in shade and powdered in the food processor. About 500 g of the powdered sample was boiled in hot water for 30 minutes and allowed to cool. After which it was filtered using a piece of white cotton gauze. The filtrate was aliquoted into small containers and was evaporated to dry at room temperature producing a greenish yellow color solid residue (yield 13% w/w). The solid residues were stored in the air tight container and preserved in the refrigerator at 4°C. From this stock, fresh preparation was obtained whenever required (Voucher number: SNMC/Pharma/002).

Animals

A total of 30 Wistar albino rats of either sex weighing 150-250 g were used for tail flick method, 30 Swiss albino mice of either sex weighing 20-30 g were used for writhing test, and five Swiss albino mice of either sex weighing 20-30 g were used for acute oral toxicity study. All the animals were obtained from the Animal house, Department

of Pharmacology, S Nigalingappa Medical College, Bagalkot. Pregnant rats, animals with infection, injuries, deformities were excluded from this study. All the animals received standard laboratory diet, reverse osmosis water and *ad libitum*.

Acute oral toxicity study

It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (up and down procedure). All the five mice were administered 2000 mg/kg of AESML orally and observed continuously for a period of 14 days, every hourly for 24 hrs, and every day for 14 days for its movements, grooming activity, exploring activity, writing reflex, eye movements, and convulsion etc. [6].

Qualitative phytochemical analysis of plant extracts

The *S. melongena* leaf extract was analyzed for flavonoids, alkaloids, glycosides, saponins, tannins, proteins and aminoacids, sterols, carbohydrates, anthraquinones, acidic compounds, and resins [7].

Acetic acid induced writhing

This test is used to identify the peripheral analgesic activity. After 12 hrs fasting 30 healthy Swiss albino mice of either sex weighing 25-30 g were randomly divided into five groups of six animals each. Group I received 0.5 ml of normal saline (NS) (control group), Group II received 100 mg/kg of aspirin (standard group) [7]. Group III, IV, V received AESML in the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w. respectively (test groups). All the drugs were given orally. After 1 hr all the animals received 10 ml/kg of 0.7% v/v acetic acid injection intraperitoneally. Number of writhings were counted between 5 and 20 minutes after acetic acid injection [8].

Tail flick method

This method is used to screen the central analgesic activity. The test was carried out in healthy Wistar rats. A total of 30 animals weighing 150-250 g were randomly divided into five groups of six animals each after 12 hrs fasting. Group I received 0.5 ml of NS (control group), Group II received 300 mg/kg of aspirin (Standard group) [9], Group III, IV, V received AESML in the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w. respectively (test groups). All the drugs were given orally. After ½ hr, 1 hr, 2 hrs, and 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. The time taken by the animal to withdraw the tail was taken as the reaction time. Cut off time was kept as 20-30 seconds. The animals showing reaction time of >20-30 were excluded from the study [10].

Statistical analysis

All the data were analyzed using one-way ANOVA followed by *post-hoc* test and the results were expressed as mean±standard error mean.

RESULTS

Acute oral toxicity study

No adverse effect or mortality was detected in Swiss albino mice at 2 g/kg of AESML 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 >2000 mg/kg.

Phytochemical analysis

The extract was almost pasty in nature with characteristic smell. It was greenish yellow in color, basic in nature. Percentage of yield of the aqueous extract was 13% w/w. Table 1 represents the qualitative analysis of various phytochemicals present in the extract. The AESML contains flavonoids, alkaloids, glycosides, saponins, tannins, sterols, carbohydrates (1.5%), and resin.

Peripheral analgesic activity

Acetic acid induced writhing test

Table 2 shows the analgesic activity of the AESML on acetic acid induced writhing test in albino mice (Graph 1).

Table 1: Qualitative phytochemical analysis of aqueous extract of leaves of *S. melongena* Linn

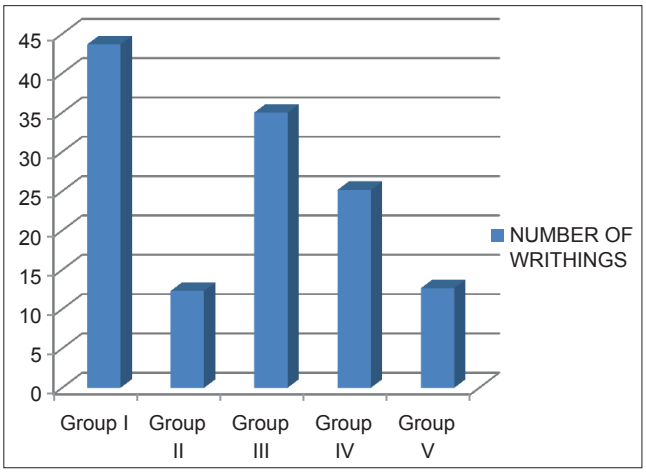
Phytochemicals	Constituents
Flavonoids	Present
Alkaloids	Present
Glycosides	Present
Saponins	Present
Tannins	Present
Proteins and aminoacids	Not present
Sterols	Present
Carbohydrates	Present
Anthraquinones	Not present
Resins	Present

S. melongena: Solanum melongena

Table 2: Number of writhings and percentage of inhibition of acetic acid induced writhing

Group	Dose	Number of writhings (10-20 minutes)	Percentage of inhibition
Group I	0.5 ml NS	43.67±0.98	
Group II	Aspirin 100 mg/kg	12.33±0.49***	71.8
Group III	SM 100 mg/kg	35.00±0.73***	19.9
Group IV	SM 200 mg/kg	25.17±0.98***	42.4
Group V	SM 400 mg/kg	12.67±0.67***	70.9

Post-hoc test : When compared with control, *p<0.05, **p<0.01, ***p<0.001. All the values are expressed as mean±SEM (n=6). SEM: Standard error mean



Graph 1: Number of writhings

All the doses of (100 mg/kg, 200 mg/kg, 400 mg/kg) AESML showed highly significant reduction in a number of writhings (p<0.001). The test drug at the dose of 100 mg/kg, 200 mg/kg, and 400 mg/kg showed 19.9%, 42.4%, and 70.9% inhibition of writhing movements compared with control group. The standard drug Aspirin showed 71.8% inhibition of writhing movements. The number of writhing was significantly reduced in both the test and standard groups. The peripheral analgesic activity of test drug at 400 mg/kg is comparable to that of standard drug aspirin 100 mg/kg (p<0.001).

Central analgesic activity

Tail flick method

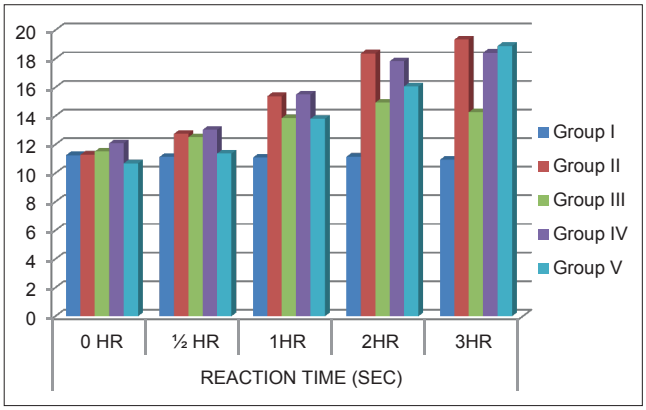
Table 3 shows the analgesic activity of the AESML on the tail-flick method in Albino rat (Graph 2).

There was no significant difference between mean reaction time of different groups (p>0.05) at 0 hr. The control group showed the

Table 3: Reaction time (seconds) in tail flick method

Group	Dose	Reaction time (seconds)				
		0 hr	½ hr	1 hr	2 hrs	3 hrs
Group I	0.5 ml of NS	11.26±0.31	11.14±0.32	11.09±0.36	11.15±0.25	10.94±0.39
Group II	Aspirin 300 mg/kg	11.29±0.55	12.75±0.49*	15.38±0.72***	18.37±0.32***	19.34±0.18***
Group III	SM 100 mg/kg	11.52±0.42	12.51±0.47	13.85±0.40***	14.93±0.21***	14.26±0.61***
Group IV	SM 200 mg/kg	12.09±0.49	13.03±0.96*	15.50±0.46***	17.82±0.37***	18.42±0.42***
Group V	SM 400 mg/kg	10.68±0.52	11.37±0.38*	13.8±0.66***	16.06±0.57***	18.89±0.50***

Post-hoc test: When compared with control; *p<0.05, **p<0.01, ***p<0.001. All the values are expressed as mean±SEM (n=6). SEM: Standard error mean, NS: Normal saline, SM: *Solanum melongena*



Graph 2: Mean reaction tine at different hours

mean reaction time of 10.9467±0.39119 seconds at 3rd hr. Test groups showed increase in the reaction time significantly after 1 hr in the doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg body weight, per orally compared to the control group, (mean reaction time 13.86±0.41 seconds, 15.50±0.47 seconds, 13.81±0.67 seconds, p<0.001, p<0.001, p<0.001).

Test drug at the dose of 400 mg/kg body weight, per orally showed highly significant increase in reaction time (mean reaction time 18.89±0.50 seconds, p<0.001) at 3rd hr which is comparable to that of the standard drug aspirin 300 mg/kg body weight, per orally (mean reaction time 19.35±0.19 seconds, p<0.001).

DISCUSSION

Present study shows the analgesic activity of AESML in Swiss albino mice and Wistar albino rats.

S. melongena is an important food crop grown for its large pendulous purple or white fruit. Besides having many traditional uses, it has important pharmacological actions like hypolipidemic, anti-asthmatic, analgesic and antipyretic activities [11].

Phytochemical study of AESML possess flavonoids, alkaloids, glycosides, saponins, tannins, sterols, carbohydrates (1.5%), fixed oils, and resin.

Various methods for the experimental evaluation of analgesic activity have been described. They are all based upon the change which occurs in the response of the experimental subject to a painful stimulus after dosage with an active compound. Various animal species, as well as man, have been used as an experimental subject, and a variety of stimuli have been employed.

Peripheral analgesic activity was evaluated by using writhing test in mice according to the method of Koster *et al*. (1959) [12-14]. The extracts derived from leaves of *S. melongena Linn*. exhibited significant analgesic activity in albino rats by inhibiting acetic acid induced writhing, which is a model of visceral pain. Intraperitoneal injection of acetic acid produces pain through activation of chemosensitive nociceptors [14] or irritation of the visceral surface, which lead to the liberation of histamine, bradykinin, prostaglandins and serotonin [15,16].

Acetic acid-induced writhing is a highly sensitive and useful test for analgesic drug development but not a selective pain test as it gives false positive result with sedatives, muscle relaxants [16].

The test drug at the dose of 400 mg/kg b.w. produced 12.67±0.67 writhing movements in 20 minutes duration. The percentage of protection from writhing with test drug at 400 mg/kg was 70.9%. Mutalik *et al*. found the number of writhings and percentage of protection from writhing with the dried juice of *S. melongena Linn*. was 54.66±4.71 and 33.06% respectively. Ashish *et al*. found the number of writhings and percentage protection of writhing with the hydro alcoholic root extract of *S. melongena* was 18±0.34 and 61.34% respectively. In the present study standard drug aspirin produced 12.33±0.49 writhings and 71.8% of protection at the dose of 100 mg/kg [11,17].

The results obtained with the test and standard drugs were significant when compared to the control. The test drug, however, was found to be equally effective as that of standard drug aspirin (100 mg/kg body weight); and more effective than the juice of dried leaves of *S. melongena*, and hydro alcoholic root extract of *S. melongena Linn*. in increasing the pain threshold.

Although the writhing response test is very sensitive it has poor specificity in analgesic screening tail flick test was conducted to confirm and study the possible analgesic mechanism of *S. melongena Linn.*

Central analgesic activity was evaluated by using the tail flick test which is considered to be a spinal reflex induced by heat according to Schumacher *et al*. (1940), and Wolff *et al*. (1940) [18,19], but could also involve higher neural structures (central analgesic activity) [20].

In the tail flick method, a mean of reaction time of control was 10.94±0.391 seconds at 3rd hr. Standard drug aspirin at the dose of 300 mg/kg body weight showed the mean reaction time of 19.34±0.18 seconds at 3rd hr and test drug in the dose of 400 mg/kg showed the mean reaction time of 18.89±0.50 seconds at 3rd hr which is comparable to that of standard drug. AELSM at a dose of 200 mg/kg, 400 mg/kg showed significant activity from 30 minutes and 100 mg/kg showed significant activity from 1st hr onwards.

The leaves of *S. melongena Linn.* contains flavonoids, alkaloids and tannins [10]. In an earlier study, the alkaloid extract of *S. melongena* Linn was found to produce significant analgesic effect [21]. Various flavonoids, both glycosides and aglycones were previously reported as having potent anti-inflammatory and analgesic activity. It is suggested that some flavonoids blocks both cyclooxygenase and lipoxygenase pathway of the arachidonate cascade at high concentration, while at low concentration only lipoxygenase pathway is blocked [22]. There are few reports on the role of tannins in analgesic and anti-inflammatory activity [23]. Previous studies suggested that alkaloids also involve in analgesic action [24]. In the present study the analgesic activity of *S. melongena Linn*. might be attributed to the presence of flavonoids (solanoflavone), tannins, and alkaloids.

CONCLUSION

From the present study, we come to the conclusion that the aqueous extract from *S. melongena Linn.* possess both peripheral and central analgesic activity in experimental animals. The present study also substantiates the traditional use of *S. melongena Linn.* for the treatment of various inflammatory ailments. The plant can be recommended for further studies to isolate the active ingredients.

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