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Evaluation of Anti-Inflammatory Activity of Aqueous Extract of Leaves of *Solanum Melongena Linn*. in Experimental Animals

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ABSTRACT

Introduction: Aqueous extract of leaves of *Solanum melongena Linn* was investigated for its anti-inflammatory activity.

Materials and Methods: Acute oral toxicity study according to OECD425 guidelines was done to find out the LD50 of test drug. Carrageenan induced paw oedema method in Wistar Albino rats were used in this study. Aspirin in the dose of 300mg/kg was used as the standard drug and three doses of aqueous extract of leaves of *Solanum melongena L*. (100mg/kg, 200mg/kg, 400mg/kg b.w.) was used as the test drug. The results were measured at 1st h, 3rd h, and 5th h after the carrageenan injection.

Results: In acute oral toxicity study none of the animals died at the dose of 2000mg/kg. Aqueous extract of *Solanum melongena Linn* leaf in the dose of 200mg/kg showed significant anti-inflammatory activity ($p < 0.05$) at 3rd hr and highly significant anti-inflammatory activity ($p<0.001$) at 5th hr; in the dose of 400 mg/kg, test drug showed $p<0.01$ at 3rd and $p<0.001$ at 5th hr and in the dose of 100mg/kg it showed significant ($p<0.05$) anti-inflammatory activity at 5th hr. In doses of 200mg/kg and 400 mg/kg of aqueous extract of *S. melongena* L showed the percentage of inhibition of 42.62% which is less than the standard drug aspirin which showed 64.5% inhibition.

Conclusion: Aqueous extract of leaves of *Solanum melongena Linn* has anti-inflammatory activity.

Keywords: Anti-inflammatory activity, LD 50, *Solanum melongena*

INTRODUCTION

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals, or microbiologic agents. Inflammation is the body's effort to inactivate or destroy the invading organism, remove irritants and set the stage for tissue repair. When healing is complete, the inflammatory process usually subsides. Persistent or over inflammation leads to tissue damage. Proinflammatory cytokines like TNF-α, IL-6, and IL-1β are produced in large quantities during inflammation by macrophages and monocytes that stimulate the cellular responses via increasing prostaglandins (PGs) and reactive oxygen species [1].

There is currently a worldwide upsurge in the use of herbal preparation and the active ingredient isolated from medicinal plants in health care. Plant based drugs have been used worldwide in traditional medicines for the treatment of various diseases. Approximately 60% of worldwide population still relies on medicinal plants for their primary health care [2].

With the easy availability of the analgesic and anti-inflammatory drugs like non steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, we are facing a new era of people presenting with symptoms of analgesic abuse and its adverse effects like gastric discomfort, gastric erosion, hypersensitivity reactions, muscular weakness, precipitation of diabetes mellitus, increased susceptibility to infection [1]. 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 is an economic flowering plant belonging to the family Solanaceae. The family contains 75 genera and over 2000 species [3]. Members are mostly herbaceous plants, and the fruit is berry and seeds have large endosperm and are grown mainly for food and medicinal purposes [4].

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 [3], anti-inflammatory activity [5]. The plant can also be used in bronchitis and asthma [3].

With this background, this study was conducted with the objective of evaluation of the acute anti-inflammatory activity of aqueous extract of leaves of *Solanum melongena Linn* in Wistar albino rats.

MATERIALS AND METHODS

Animals

All the animals were obtained from the Animal house, Department of Pharmacology, S N Medical College, Bagalkot. The study was started after getting the Institutional Animal Ethics Committee approval (IAEC/SNMC Reg No 829/AC/04/CPCSEA). Five Swiss albino mice of either sex weighing 25-30g were selected for the acute oral toxicity study. Thirty wistar albino rats of either sex weighing 150-250g were selected for the anti-inflammatory experiment.

Pregnant animals, animals with infection, injuries, deformities were excluded from the study. All the rats were divided into 5 groups of 6 animals each. All the animals received standard laboratory diet, water and ad libitum.

Chemicals

Kappa type of carrageenan was used to induce paw oedema. Carrageenan (kappa) was obtained from Titan biotech Ltd. Mumbai. 0.1ml of 1% w/v carrageenan was used to induce paw oedema. 1% w/v carrageenan was prepared by dissolving 1gm of carrageenan in 100ml of normal saline.

S.No	Acute toxic signs	Findings
1	Weight changes	Absent
2	Changes in Skin & Fur	Absent
3	Changes in eyes	Absent
4	Changes in Mucous membrane	Absent
5	Respiratory rate	Normal (156/min)
6	Heart rate	Normal (480/min)
7	Blood pressure	Normal (Systole -157 mmHg by non-invasive method)
8	Rectal temperature	38.5^{0}C (temperature probe)
9	Tremors	Absent
10	Convulsion	Absent
11	Salivation	Absent
12	Diarrhoea	Absent
13	Lethargy	Absent
14	Sleep	Normal
15	Coma	Absent

[Table/Fig-1]: Findings of Acute oral toxicity study

Group	Dose	Paw Volume (ml)			Percentage of Inhibition (5th h)
		I H	3H	5H	
Group I	0.5 ml NS	2.5 ±0.18	2.7 ± 0.17	3.1± 0.03	0%
Group II	Aspirin 300mg/kg	2.1± 0.07	1.1 ± 0.08	1.0±0.08***	64.5%
Group III	SM 100mg/kg	2.9± 0.27	2.5± 0.18	2.5 ± 0.18*	18.03%
Group IV	SM 200mg/kg	2.2± 0.10	2.2± 0.10*	1.7± 0.11***	42.6%
Group V	SM 400mg/kg	2.0± 0.08	1.8±0.16**	1.7± 0.11***	42.62%

[Table/Fig-2]: Carrageenan induced paw oedema\
* p<0.05, **p<0.01, ***p<0.001. All the values are expressed as Mean ± SEM (n=6)

Preparation of leaves extract

Five kg of fresh leaf of *S.melongena Linn* were collected from the rural areas of Bagalkot district, Karnataka, India in the month of March 2011. The plant identity was authenticated by Botanist Prof. Jadimath and the voucher specimen number was given (Voucher number: SNMC/Pharma/002). The fresh leaves were air dried and powdered in the food processor. About 500 g of powdered sample was boiled in hot water for 30min and allowed to cool. After which it was filtered using a piece of white cotton gauze. The filtrate 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 refrigerator at 4^{0}C. From this stock, fresh preparations were obtained when ever required [6].

Phytochemical analysis

The aqueous leaf extract of *Solanum melongena* was qualitatively analysed for Flavonoids, Alkaloids, Glycosides, Saponins, Tannins, Proteins and Amino acids, Sterols, Carbohydrates, Fixed oils, Anthraquinones, and Resins [7,8].

Acute oral toxicity study

It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (Up and down procedure). Five animals were randomly selected, marked to permit individual identification, and kept in their cages for at least five days prior to dosing to allow for acclimatization to the laboratory conditions. All the animals were fasted for 12 h prior to experiment. Maximum volume of 1ml/100g of test drug solution was administered orally. Limit test was used for aqueous extract of *Solanum melongena* leaf because it is known to be non-toxic. Food was administered 3-4 h after the administration of test substance. First, one animal was administered 2000mg/kg of aqueous extract of *Solanum melongena Linn* leaf and the animal was observed at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for a total of 14 d. The animal was observed for its changes in skin, fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Attention was directed to tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. After 24h, remaining four animals received the test compound and was observed for14 d [9].

Carrageenan induced paw oedema: The test was carried out in healthy Wistar albino rats of either sex weighing 150-250 g. After 12 h fasting, 30 animals were randomly divided into 5 groups of 6 animals each. Group I received 0.5 ml of normal saline (control group), Group II received 300mg/kg of aspirin (standard group) [10], Group III ,IV & V received aqueous extract of *Solanum melongena Linn* leaves in the doses of 100 mg/kg, 200mg/kg and 400mg/kg b.w. respectively (test groups). All the standard and test drugs were given orally 1 h before the injection of 0.1ml of 1% w/v carrageenan in normal saline solution intra-dermally in the left hind paw. Fluid displacement in Plethysmometer [11] was measured at 1st, 3rd, and 5th h after carrageenan injection [12] and the percentage of inhibition was calculated by the formula: % inhibition = 100 (1-Vt/Vc) [13].

STATISTICAL ANALYSIS

All the data were analysed by using One-way ANOVA followed by Post Hoc Test. The results were expressed as Mean ± SEM and p<0.05 was considered as significant.

RESULTS

Phytochemical Analysis

The extract was almost pasty in nature with a characteristic smell. It was greenish yellow in colour, alkaline (pH- 7.6) in nature. Percentage of yield of the aqueous extract was 13% w/w. The aqueous extract of *Solanum melongena* contains flavonoids, alkaloids, glycosides, saponins, tannins, sterols, carbohydrates (1.5%), fixed oils, and resin. The extract does not have proteins, amino acids and Anthraquinones.

Acute Oral Toxicity Study

No adverse effect or mortality was detected in Swiss albino mice at 2g/kg of aqueous extract of *Solanum melongena Linn* by using five animals. All the animals were alive, healthy and active during the observational period of 14 d. There were no significant weight changes. So the LD 50 was considered as >2000mg/kg. [Table/Fig-1] shows the findings of acute oral toxicity study.

[Table/Fig-2] shows the anti-inflammatory activity of the aqueous leaf extract of *Solanum melongena Linn* in Wistar albino rats by Carrageenan induced paw oedema method.

Control group has shown maximum oedema around 3 hours after carrageenan injection. 'Mean paw volume' for control group was 3.05 ± 0.03 ml, which was considered as 100% oedema in the present study.

Test drug in the doses of 200mg/kg, 400mg/kg body weight, per orally produced highly significant inhibition of paw volume compared to control group (mean paw volume is 1.75 ± 0.11 ml, 1.75 ± 0.11 ml ; p<0.001, p<0.001) at 5th hourly. At the dose of 100 mg/kg body weight, aqueous extract of *Solanum melongena Linn*. showed significant reduction in mean paw volume (2.50 ± 0.18 ml, p<0.05 and 18.03% inhibition of paw volume) at 5th h. Test drug showed highly significant reduction in paw volume in the doses of 200mg/kg and 400mg/kg equally with 42.62 % of inhibition of paw volume which is less than that of aspirin 300mg/kg body weight (mean paw volume 1.08 ± 0.08 ml, p<0.001 and 64.5 % of inhibition of paw volume).

DISCUSSION

The present study shows the acute toxicity study and anti-inflammatory activity of aqueous extract of leaves of *Solanum melongena Linn* in Swiss albino mice and Wistar albino rats. *Solanum 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, antiasthmatic, analgesic, anti-inflammatory and antipyretic activities [13,14].

Phytochemical study shows the aqueous extract of *Solanum melongena Linn* possess flavonoids, alkaloids, glycosides, saponins, tannins, sterols, carbohydrates (1.5%), fixed oils, and resin. According to acute oral toxicity study LD50 was considered as more than 2000mg/kg b.w. Carrageenan-induced hind paw oedema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility [15,16].

Carrageenan-induced oedema is a biphasic response. The first phase is mediated by the release of histamine, serotonin, and kinins whereas the second phase is related with the release of prostaglandin and slow reacting substances which peaks at third hour [17,18].

In this method the mean paw volume was found to be 3.05 ± 0.03 ml in control group. Test drug at the dose of 200mg/kg body weight and 400 mg/kg body weight showed the percentage of inhibition of 42.6%. Percentage of inhibition of mean paw volume of test drug at the dose of 400mg/kg was less than that of the standard drug aspirin 300mg/kg body weight (64.5%). Dadi et al., used different extracts to find out the anti-inflammatory activity of roots of *Solanum melongena*. In that study Dadi et al., used 500mg/kg of aqueous extract which showed 74% of inhibition of paw oedema in Wistar rats at 4th h after carrageenan injection [13]. In another study Han et al., used the water extract of *Solanum melongena* which inhibited PAR-2 mediated myeloperoxidase activity and tissue necrosis factor-α [19].

The leaves of *Solanum melongena Linn* contain flavonoids, alkaloids, tannins and sterols. Various flavonoids, glycosides and aglycones were previously reported as having potent anti-inflammatory activity. Pourmotabbed et al., suggested that some flavonoids blocks both cyclooxygenase and lipoxygenase pathway of the arachidonate cascade at high concentration, while at low concentration only lipoxygenase pathway [20]. Salerno L et al., found out flavonoids present in 50% aqueous and 50% ethanol extract of skin of *Solanum melongena* fruit contains anti-oxidant property [21]. Also there are few reports on the role of tannins in anti-inflammatory activity [22]. In the present study the anti-inflammatory activity of *Solanum melongena Linn* might be due to the presence of flavonoids and tannins.

CONCLUSION

From the present study we come to the conclusion that the extract of *S.melongena Linn* possesses anti-inflammatory 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 the further studies to isolate the active ingredients.

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