

EVALUATION OF DIURETIC AND ANTI-INFLAMMATORY PROPERTY OF ETHANOLIC EXTRACT OF *SOLANUM SURATTENSE* IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Aim: in the present study, *Solanum Surattense* plant was selected to investigate its Diuretic and Anti-inflammatory effect in experimental animal models.

Materials and Methods: the dried leaf powder was subjected to successive Soxhlet extraction with ethanol. The ethanolic extract of *Solanum Surattense* was administered to experimental rats orally at doses 500 mg/kg, 1000mg/kg to investigate diuretic activity by using standard methods. Furosemide (20mg/kg) was used as positive control. The Diuretic activity was evaluated by measuring Urine output, sodium and potassium concentration, conductivity and pH. For anti-inflammatory study, 500mg/kg, 1000mg/kg of ethanolic extract of *Solanum Surattense* was given orally in carrageenan induced rat paw edema models. Aspirin (300mg/kg) was used as standard drug for comparison

Results: ethanolic extract of *Solanum Surattense* was shown significant Diuretic effect at dose of 1000mg/kg when compared with 500mg/kg, by increasing total urine volume and levels of sodium, potassium, and chloride but there was no significant change in the conductivity and pH. The same extract 1000mg/kg; have shown significant anti-inflammatory effect almost equal to ibuprofen when compared with 500mg/kg due to inhibition of various inflammatory mediators.

Conclusion: in the dose dependent manner, ethanolic extract of *Solanum Surattense* have showed significant Diuretic, and Anti-inflammatory effect when compared with their respective standard and control drugs. The present study provides a quantitative basis for explaining the folk use of *Solanum Surattense* as a Diuretic and Anti-inflammatory agent.

Keywords: Diuretic, Inflammation, *Solanum Surattense*, Furosemide, Aspirin

INTRODUCTION

Diuretics are the drugs that commonly increase the urine volume. Various diuretics act by different mechanisms to increase excretion of sodium and water from the body. Diuretics can enhance the rate of urination that can be used in the treatment of various CVS abnormalities, renal dysfunction and in edematous states. Drugs which are in use presently for the management of pain and inflammatory condition are either narcotics e.g. Opioids or non-narcotics e.g. salicylates and corticosteroids e.g. Hydrocortisone. All of these drugs possess well known side and toxic effects. More over synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries, people have been trying to alleviate and treat disease with different plant extracts and formulations [1]. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [2]. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethanobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. The plant *Solanum Surattense* belongs to *solanaceae* family.[3] *Solanum Surattense* is a thorny shrub widely distributed in waste places, along railway lines, on road sides as weed. Plant occurs wild throughout India, Sri Lanka, South-East Asia, Malaysia and tropical Australia.[4] An ingredient of an Ayurvedic drug *Arkadhi* a remedy for bronchitis, dengue and fever. The major

constituents are Alkaloid Solasodine, Solanine, Steroids, flavonoids, saponins or organic acids, Carpesterol, Diasogenin, Carbohydrate, F&Os, Proteins, Lignin, and Cutin. Amino acid, Tannin, Cal-Oxalate. It is used traditionally as anti-inflammatory, Diuretic, astringent, anti-asthmatic, agent. Also used in bronchitis, constipation, ulcer, epilepsy, skin disorders, cardiac disorders and in dropsy [5,6]. According to literature available of this plant, the present study (Diuretic plus Anti-inflammatory) was undertaken to investigate its Diuretic and Anti-inflammatory properties by using experimental animal models.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Solanum Surattense* were collected during July-August from Kanchipuram surrounding areas in Chennai. The plant medicinal properties were identified and authenticated by Dr.Ramnath, Head of the department of Biotechnology, Osmania university college.AP. The literature was collected from the book-Indian Medicinal Plants by Vaidyaratnam P.S. Varier vol-5(164) [7].

Preparation of alcohol extract

The dried fine powder of leaves of the *Solanum Surattense* was weighed on balance 30g, and taken into the sac like cloth material and placed in the Soxhlet basket. 300 ml of ethyl alcohol was taken as solvent into the Soxhlet flask. Cold tap water must flow through the inlet and outlet of the condenser. The Soxhlet apparatus kept running for 24hours for proper extraction. The extract laden solvent falling from the Soxhlet basket is dark in color and it becomes clearer, that indicates the extraction process is finished. At the end of the extraction process the solvent is then evaporated and the remaining mass is measured.[8]

Table 1: The percentage yields are calculated as Mg per Grm dried powder.

S. No.	Weight in gm.		Percentage Yields
	Solvent	Dry powder of <i>Solanum Surattense</i>	
1.	Ethyl Alcohol-300ml	30gm.	23.33%

The yield of the ethyl alcohol extract is 23.33%. The extract was suspended in 2ml of 2% Gum acacia and used for the oral administration.

Toxicity study

The acute oral toxicity study was conducted according to the OPPTS (office of prevention, pesticide and toxic substances) Up and Down procedure.[9]

Chemicals

All the drugs and materials used for this study are of pharmacopeia grade. Furosemide (E.Merck), Aspirin (Dr.Reddy's) and gum acacia-2%, Normal saline, carrageenan (Sigma) were purchased from the local supplier.

Experimental animals

Swiss albino Rats weighing 150-200g male and female rats were supplied from Srinath Agencies, Chennai, India. They were randomly distributed into groups and housed in cages (6/cage) and kept under standard conditions at 26±2°C and relative humidity 44-56% and 10 hours light: 14 hrs dark cycles each day for 1 week before and during the experiments. All animals were fed the standard rodent pellet diet and libitum. This study was cleared by institutional animal ethical committee according to CPCSEA guidelines.

Experimental design

The animals used for the experiment were divided into 4 groups for each model, 6 rats for each group.[10] Food and water was withdrawn 14 hr before starting the work.

Diuretic activity

Wister albino rats of either sex weighing 150-200 Gms were divided into 4 groups of 6 animals each. The animal was fasted for 16 hrs, deprived of food and water. All the animals received priming dose of normal saline 25ml/kg before giving test drugs. The **Group-I** served as control, received the vehicle only (4% gum acacia 1ml. /100g p.o). **Group-II** received the standard drug of Furosemide 20 mg per kg body weight in a normal saline. The other two **Groups-III, IV** received product containing Ethanolic extract of *Solanum Surattense* dose of 500mg/ kg, 100mg/kg respectively suspended in normal saline. All the substances were orally administered by gavage using intragastric cannula. Dose volume was completed with physiological saline solution up to a total constant administration volume of 40ml/kg. As described in the guidance used [11,12]. Immediately after the respective treatment the animals placed in fabricated metabolic cages and Urine was collected in a measuring cylinder containing mineral oil up to 6 hrs. Mineral oil in measuring cylinder prevents evaporation of urine. The bladder was emptied by pulling base of tail of each rat [13]. During this period no food and water was made available to animals. Then the volume of urine and Na+, K+ and Cl- were estimated for assessing diuretic activity. Sodium and Potassium concentrations were determined by Flame Photometer and Clconcentration was estimated by titrated with AgNO₃

solution (0.17N) using 2 ml of Ferric alum solution as indicator [14]

Diuretic Index = Mean urine volume of test/Mean urine volume of control.

Lipschitz value = Mean urine volume of test/Mean urine volume of standard.

Anti-inflammatory activity

The animals were divided into 4 groups (n=6). **Group-I** served as control, received the vehicle only (4% gum acacia 1ml. /100g p.o). **Group-II** served as standard, received Aspirin (300mg/Kg p.o). **Group-III and Group-IV** served as test, received Extract of *Solanum Surattense* preparations low dose 500mg/kg, high dose 1000mg/kg respectively.

Carrageenan induced paw edema

The test was used to determine the anti-inflammatory activity of the *Solanum Surattense* by the method of Winter et al (1962) [15]. The animals pre-treated with Extract of SS and Aspirin 1 hour before were injected with 0.1ml of 1% Carrageenan solution into the sub-plantar region of the right hind paw. Paw volume was measured by displacement of the mercury column in a plethysmometer immediately after Carrageenan application at 0 hr and at the end of 3 hr after the stimulus. Reduction in the paw volume compared to the control group animals was considered as anti-inflammatory response.

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 Dennett's test. The

Results were considered statistically significant when P< 0.05.

RESULTS AND DISCUSSION

J). Effect of *Solanum Surattense* Extract on Urine Volume and Electrolytes

In acute toxicity study, all the animals were found to be alive healthy after 48 hrs. This indicates that, the extract dose (up to 1000mg) used in the study was safe. The results obtained in diuretic study were depicted in table: 2 & 3.

From the results it can be observed that ethanolic extract of *Solanum Surattense* have significant diuretic activity by increasing urine output (60%) and increased excretion of sodium, potassium and chloride levels (P<0.01) when compared to control. This effect was dose dependent manner. 1000mg/kg (54%) was shown better effect than 500mg/kg (38%), but less effective than standard drug-Furosemide (78%) drug. The plant *Solanum Surattense* contains steroidal compounds, flavonoids, and saponins. These chemical constituents might be responsible for diuretic activity. Several previous studies have revealed that these agents have diuretic property. The effect may be produced by stimulation of regional blood flow or initial vasodilatation or by inhibiting tubular reabsorption of water and electrolytes.[16,17]

Table 2: Effect of *Solanum Surattense* (SS) Extract on Urine volume

Groups	Dose(mg/kg).p.o	Mean Urine volume(ml)	Urine pH	Diuretic Index(t/c)	Lipschitz value
Control	4% Gum acacia	4.62±0.15	7.23±0.18	-----	-----
Standard	Furosemide (20mg/kg)	8.41±0.13***	7.40±0.15	1.654	1.00
Test-1	Extract of SS (500mg/kg)	6.45±0.23***	6.12±0.22	1.254	0.66
Test-2	Extract of SS (1000mg/kg)	7.15±0.25***	7.32±0.18	1.268	0.86

Each value is the Mean±SEM. for 6 rats *P<0.05, **P<0.01, ***P<0.0001 compared with control.

Table 3: Effect of *Solanum Surattense*(SS) Extract on Urinary Electrolytes.

Groups	Dose(mg/kg).p.o.	Na ⁺ (mEq/lit)	K ⁺ (mEq/lit)	Cl ⁻ (mEq/lit)
Control	4% Gum acacia	60±2.15	11.5±0.23	55±1.23
Standard	Furosemide (20mg/kg)	96±3.12	14.5±0.25	110±2.32
Test-1	Extract of SS (500mg/kg)	69±2.32	11.6±0.23	49±1.42
Test-2	Extract of SS (1000mg/kg)	74±2.08	12.1±0.56	66±1.02

Each value is the Mean±SEM. for 6 rats *P<0.05, **P<0.01, ***P<0.0001 compared with control.

II). Effect of *Solanum Surattense* Extract on Inflammation

The Extract of *Solanum Surattense* preparation on Carrageenan induced paw edema in rats is shown in table 4. The results obtained indicates that the extract of *Solanum Surattense* found to have significant (p<0.001) anti-inflammatory activity in rats. The Extract at the test doses 500mg/kg, 1000mg/kg reduced the edema induced by Carrageenan by 76, 74% respectively at the end of 3rd hour, whereas standard drug showed 78% of inhibition as compared to the control group (no inhibition). It is well known that Carrageenan

induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2hr after Carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3-4h after Carrageenan injection), kinin and prostaglandins are involved[18]. Our results revealed that administration of Extract of *Solanum Surattense* inhibit the edema starting from the first hour and all phases of inflammation in dose dependent manner which is probably inhibition of different aspects and chemical mediators of inflammation.

Table 4: Effect of *Solanum Surattense* (SS) Extract on Carrageenan induced rat paw edema at '0' hr and at the end of '3' hr

Groups	Dose (Mg/Kg)	Paw Volume (ML)		% Inhibition
		0 Hr	3hr	
Control	4% Gum acacia	1.014±0.001	0.759±0.095	0
Standard	Aspirin(300mg/Kg)	0.750±0.005*	0.164±0.012***	78
Test-1	Extract of SS (500mg/kg)	1.145±0.053	0.178±0.011***	76
Test-2	Extract of SS (1000mg/kg)	1.157±0.021	0.318±0.029***	57

Each value is the Mean±SEM. for 6 rats *P<0.05, **P<0.01, ***P<0.0001 compared with control.

CONCLUSION

From the results explained above, it can be concluded that the ethanolic extract of *solanum Surattense* having significant diuretic activity by increasing the total urine output and increased excretion of sodium, potassium, chloride levels when compared with control group animals but lesser than standard drug Furosemide. The same extract also showed anti inflammatory activity more effectively in acute inflammatory models with low toxicity and better therapeutic index and compared with standard drug aspirin.

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