Research Article

Phytochemical and Pharmacological Evaluation of Fruits of *Solanum indicum* Linn.

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ABSTRACT

The present study focused on the preliminary phytochemical screening and pharmacological evaluation of the dried fruit extract of *Solanum indicum* Linn. on experimental animal models of a variety of ailments viz. pain, fever, inflammation and CNS complication. The acute toxicity study as per OECD guidelines 425 revealed the drug to be safe till a dose of 2000 mg/kg. Adult Wistar albino rats (180-200 gm) of either sex were used for the study and subdivided into four (4) groups (n=6). The extract was evaluated for its analgesic, anti-inflammatory, antipyretic and CNS depressant activity. Statistical analysis was done through one way ANOVA followed by post hoc Tukey's multiple comparison tests. Phytochemical screening of MeOH extract revealed the presence of flavonoids, steroid, tannin, glycosides and saponins among other constituents. The main findings depicted that the crude MeOH extract of the dried fruits of *Solanum indicum* Linn. Exhibited statistically significant analgesic ($P \leq 0.05$), antipyretic ($P \leq 0.05$), analgesic ($P \leq 0.05$) and CNS depressant activity ($P \leq 0.05$) on the established animal models. Experimental evidences lead to the hypothesis that MeOH extract of *Solanum indicum* Linn. fruit possesses pharmacological activity which satisfies the claim of traditional medicinal uses.

Keywords: Analgesic, Antipyretic, Anti-inflammatory, CNS depressant activity, *Solanum indicum* Linn. Fruit.

INTRODUCTION

Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported from various plants. In many cases the people claim the good benefit of certain natural or herbal products. However, pre-clinical and clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim.[1]

Solanum indicum Linn. (Synonym: *Solanum anguivi*) belongs to the family Solanaceae commonly known as Byakur, Guta begun, Kata begun, Brihati, Indian Night shade etc. It is a bushy herb containing prickly spikes in the stem and available throughout the India and all over the tropical and subtropical regions of the world.[2,5] The fruits are edible and traditionally used to treat various diseases. The different parts (fruits, leaves, roots) of this plant used by the traditional practitioners in the treatment of loss of appetite and anorexia, blood disorders, rhinitis, cough, asthma, sore throat and hiccup, sexual disorders, abdominal pain and worm infestation, pain and fever, inflammation, insomnia, urinary complications, cardiac weakness etc. It has been reported earlier, fruits and roots of this plant contains wax, fatty acids, alkaloid solanine and solanidine, disogenin, lanosterol, β-sitosterol, solasornine, solamargine and solasidine etc.[2-5]

However, the medicinal properties have not been properly reported in previous studies. This led to the present team to investigate into the preliminary phytochemistry and pharmacological action of the crude methanolic extract (ME) of fruits of *Solanum indicum* Linn.

MATERIALS AND METHODS

Drugs & Chemicals used

Brewer's Yeast (Sigma, USA), Carrageenan (Sigma, USA), carboxy methyl cellulose (Fisher Scientific), Aspirin, Diclofenac Sodium, Diazepam, Paracetamol. All the APIs were collected from State Drug Testing Laboratory, Gurkhabasti, Agartala, Tripura (W) as a gift sample. All the solvents and reagents were purchased from Rankem Lab., Okhla Industrial area, New Delhi were analytical grade and used without further purification.

Source of plant material, extraction & preparation of test sample

The fresh fruits (berries) of *Solanum indicum* Linn. were collected from the local market (Lake Chowmuhani Bazar, Agartala, West Tripura) and authenticated (Voucher specimen No. SI001/RIPSAT/TU/2012) by Prof. B. K. Datta, Taxonomist, Plant Taxonomy & Biodiversity Research

Laboratory, Department of Botany, Tripura University (A Central University).

The fresh fruits (berries) of *Solanum indicum* Linn. were dried under sunlight and powdered in a hand mill. 300 gm of dried fine powder (# 40 mesh size) was treated with petroleum ether (60-80) for 24 hours to remove the fatty materials and was extracted with MeOH in a Soxhlet apparatus exhaustively at 40-45°C temperature for 12 hours. The crude MeOH (Methanolic) extract was concentrated under reduced pressure in vacuum to get a semisolid residue (48 gm). This semisolid residue of MeOH extract (ME) has been used for further experiments.

Phytochemical screening

The crude MeOH extract obtained was subjected to different qualitative chemical tests for the identification of various phyto-constituents. Different qualitative tests like tests for alkaloids, steroids, flavonoids, saponins, reducing sugars, tannins, gums, amino acids and anthraquinones etc. were performed according to the established procedure.

Selection of Experimental Animals

Adult Albino rats (Wistar strain) of either sex with weighing 180–200 gm were used for screening of Pharmacological activity. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals had access to laboratory chow and water *ad libitum*. The experimental protocols were approved and a written permission from Institutional Animal Ethical Committee (Regd. No.-1006/ac/06/CPCSEA, 2006, from Ministry of Environment & Forests, Govt. of India) has been taken to carry out and complete this study.

Acute Toxicity Study [6, 7]

The acute toxicity was determined as per the OECD guideline no. 425 (OECD guideline 425, 2000). Immediately after dosing, the animals were observed continuously for the first 4 hours for any behavioral changes. Thereafter, they were kept under observation up to 14 days after drug administration to find out the mortality if any. It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, the dose of 250 and 500 mg/kg was arbitrarily selected for the study.

Evaluation of Analgesic activity by radiant tail flick method [8-10]

The central analgesic activity was determined by radiant heat tail-flick method in rats. Tail-flick latency was assessed by using the analgesiometer (INCO, India). Animals of either sex were divided into four (4) groups containing six in each group for this experiment. The animals of different groups were treated with drugs (test extract 250 mg/kg and 500 mg/kg, Aspirin 100 mg/kg as standard, p.o.) and normal saline (1 ml p. o.) as control. The time taken by rats to withdraw (flick) the tail was taken as the reaction time. The animals were subjected to the same test procedure at +30, +60 and +120 min after the treatment.

Evaluation of Antipyretic activity by Brewer's yeast induced pyrexia method [9, 11]

Albino rats of either sex were divided into four groups containing six in each group for this experiment. The antipyretic activity was evaluated using Brewer's yeast induced pyrexia in Wistar Albino rats. All the animals were injected subcutaneously with 15% Brewer's yeast suspended in 0.5% carboxy methyl cellulose in normal saline. After 18 hours of yeast induction rectal temperature of all the animals was recorded. All the treatments (Control, standard drug and test drugs) were administered to different respective groups after 19 hours of induction of pyrexia. 10 ml/kg (p.o) of normal saline was administered to the control groups of animals and Paracetamol at dose of 150mg/kg (p.o) was administered to standard group of animals. The test extract was administered at a dose of 250 mg/kg and 500 mg/kg (p.o) of body weight to the respective groups of animals. Rectal temperature was recorded by clinical thermometer upto 4 hrs (20, 21, 22, and 23 hour) after drug administration.

Evaluation of Anti-inflammatory activity by Carrageenan induced paw oedema method [12-14]

Anti-inflammatory activity of the extract was evaluated by carrageenan induced paw oedema method. Animals of either sex were divided into four (4) groups containing six in each group for this experiment. After acclimatization to the laboratory environment the test rats were treated with both test extract (250 and 500 mg/kg), Diclofenac Sodium (1 mg/kg) and normal saline (1 ml p.o) as control. All the treatments were given orally to the respective groups. After 30 min. of drug treatment right hind paw of all the animals were injected with 0.1 ml of 1% Carrageenan suspension in normal saline. Paw volume was measured by using mercury plethysmometer at 0, 2, 4, 6 and 24 h after induction of edema. Inhibition of edema was calculated from the difference in paw volume between control and extract treated rats as:

$$\text{Percent inhibition} = [(V_c - V_t)/V_c] \times 100$$

Where V_c is average increase in paw volume of control rats and V_t is average increase in paw volume of treated rats.

Evaluation of CNS depressant activity by locomotion inhibition method [15]

Adult Wistar albino rats of 180 – 200 gm body weight were used. The spontaneous locomotor activity of each rat was recorded individually for 10 min using Actophotometer (INCO, India) before the drug administration. After that, test drugs (250 and 500 mg/kg

p.o) and standard diazepam 0.5 mg/kg (p.o) were administered and control group was treated with normal saline 10 ml/kg (p.o). Number of movements of each animal after drug administration was observed in Actophotometer at 1, 2, 3 hour intervals respectively.

Statistical analysis

Data are analysed by one way ANOVA followed by Tukey's multiple correlation test. Comparison was made with control and the significance level was considered at $P \leq 0.05$. All the data were analyzed using Graph Pad Prism 5.0 software.

RESULTS AND DISCUSSION

Phytochemical screening

Extract showed positive reactions for flavonoids, steroid, saponins, anthraquinones, reducing sugars, tannins but exhibited negative response for gums, amino acids and alkaloids.

Acute oral toxicity study

Oral acute toxicity study revealed that the herbal extract (Methanolic extract) did not show any untoward effect till dose of 2000 mg/kg (p. o).

Analgesic activity by radiant tail flick method

Table 1: Analgesic activity of *Solanum indicum* Linn. fruits MeOH extract

Tail Flick Reaction Time after Drug administration				
Treatment	**Basal (0 hour)**	**30 min**	**60 min**	**120 min**
Control (NS)	2.16 ± 0.4	2 ± 0.25	2.16 ± 0.13	2.5 ± 0.22
ME (250 mg/kg)	2.19 ± 0.4	4.66 ± 0.21*	4.83 ± 0.46*	5 ± 0.44*
ME (500 mg/kg)	2.22 ± 0.3	5.23 ± 0.36*	5.83 ± 0.32*	5.83 ± 0.11*
Aspirin(100 mg/kg)	2.25 ± 0.3	3.83 ± 0.69*	4.5 ± 0.14*	3.16 ± 0.31*

Each value represents mean ±SEM of 6 rats, *p value ≤0.05

Antipyretic activity by Brewer's yeast induced pyrexia method

Table 2: Antipyretic activity of *Solanum indicum* Linn. fruits MeOH extract

Rectal Temp. (^{0}C) before and after drug administration						
Treatment	**Rectal Temp. after Yeast induction**					
	Basal 0 h	**19 h**	**20 h**	**21 h**	**22 h**	**23 h**
Control (NS)	37.42 ± 0.15	39.63 ± 0.19	39.35 ± 0.61	39.26 ± 0.13	39.31 ± 0.03	39.28 ± 0.05
ME (250 mg/kg)	37.31 ± 0.44	39.23 ± 0.12*	38.43 ± 0.18*	38.18 ± 0.15*	38.03 ± 0.33*	37.61 ± 0.08*
ME (500 mg/kg)	37.38 ± 0.61	39.13 ± 0.24*	36.76 ± 0.12*	36.45 ± 0.17*	37.73 ± 0.14*	37.31 ± 0.04*
Paracetamol (150 mg/kg)	37.28 ± 0.34	39.21 ± 0.14*	35.88 ± 0.14*	36.56 ± 0.25*	37.6 ± 0.18*	37.63 ± 0.09*

Each value represents mean ±SEM of 6 rats, *p value ≤0.05

Anti-inflammatory activity by Carrageenan induced paw oedema method

Table 3: Anti-inflammatory activity of *Solanum indicum* Linn. fruits MeOH extract

Time after pholgistic (Oedema induction) agent administration (volume displaced in ml)					
Treatment	**0 h**	**2 h**	**4 h**	**6 h**	**24 h**
Control (NS)	0.46 ± 0.005	0.86 ± 0.01	1.7 ± 0.08	1.86 ± 0.08	1.99 ± 0.01
ME (250 mg/kg)	0.45 ± 0.005	0.82 ± 0.01	1.4 ± 0.01	0.99 ± 0.005	0.69 ± 0.005*
ME (500 mg/kg)	0.46 ± 0.005	0.78 ± 0.01	1.2 ± 0.01	0.83 ± 0.004*	0.57 ± 0.009*
Diclofenac Sodium (1 mg/kg)	0.45 ± 0.005	0.69 ± 0.01	0.99 ± 0.004	0.78 ± 0.007*	0.54 ± 0.005*

Each value represents mean ±SEM of 6 rats, *p value ≤0.05

CNS depressant activity by locomotion inhibition method

Table 4: CNS depressant activity of *Solanum indicum* Linn. fruits MeOH extract

Actophotometer Score after drug administration				
Treatment	**0 h**	**1 h**	**2 h**	**3 h**
Control (NS)	195.66 ± 9.24	197.33 ± 12.12	198.63 ± 13.76	196.5 ± 11.4
ME (250 mg/kg)	197.5 ± 12.13	66.83 ± 2.40*	33.83 ± 1.76*	48 ± 1.73*
ME (500 mg/kg)	199.33 ± 11.62	52.03 ± 2.60*	21.66 ± 2.31*	29.33 ± 2.19*
Diazepam (0.5 mg/kg)	203.13 ± 3.38	62.1 ± 1.86*	6.16 ± 0.58*	5.33 ± 0.88*

Each value represents mean ±SEM of 6 rats, *p value ≤0.05

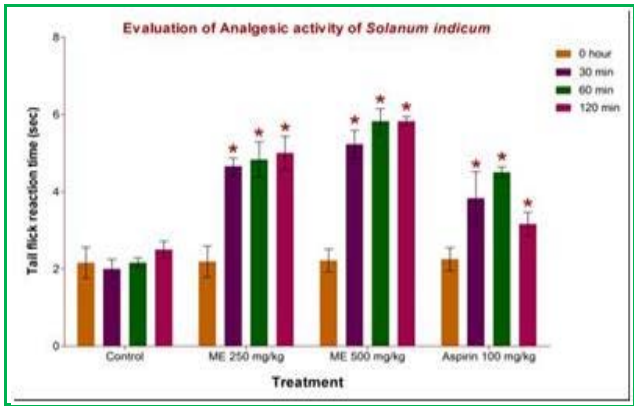

Figure 1: Graph depicting tail flick reaction time after drug administration of the crude methanolic extract of *Solanum indicum* for screening of analgesic activity. *P<0.05 treated groups vs. control group.

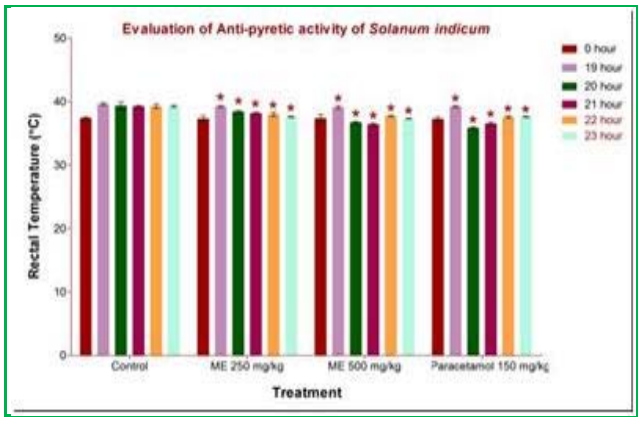

Figure 2: Graph showing the comparable antipyretic effect of the different study groups reflected through rectal temperature over time and drug concentration. *P<0.05 treated groups vs. control group.

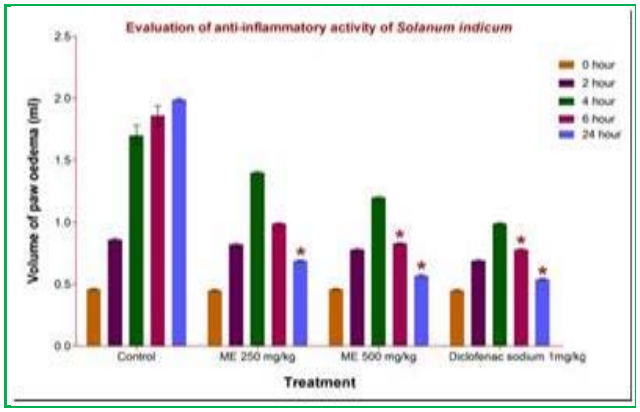

Figure 3: Graph showing comparison of dose and time dependant anti-inflammatory activity across treatment groups after oedema induction. *P<0.05 treated groups vs. control group.

The analgesic activity of MeOH fruit extract of S. indicum was found to be significant ($p \leq 0.05$) with respect to the control group as well as the reference drug Aspirin (100mg/kg) in increasing the latency period. Highest activity exhibited by extract (ME 500 mg/kg) at 60 min. (5.83±0.32) after drug administration whereas, Aspirin showed less efficacy (4.5±0.14) after same time interval as test drug. The antipyretic activity of the MeOH extract was assessed by the Brewer's yeast induced pyrexia method exhibited significant ($p \leq 0.05$) antipyretic effect compared to the control and standard group in a dose and time dependant manner. Results exhibited marked reduction of rectal temperature in treated groups (38.43±0.18 for ME 250mg/kg, 36.76±0.12 for ME 500mg/kg and 35.88±0.14 for Paracetamol at 20 hour) after yeast induction and treatment. MeOH extract of S. indicum fruit exhibited comparable anti-inflammatory activity on Wistar rats in comparison to the reference drug Diclofenac sodium (1mg/kg) after 6 hours of treatment, showed (46.77% for ME 250 mg/kg, 53.37% for ME 500 mg/kg and 58.06% for Diclofenac sodium) inhibition of paw oedema. CNS depressant activity of MeOH extract was found to be significantly ($p<0.05$) better than the standard drug diazepam (0.5 mg/kg) in case of ME 500 mg/kg after 1 hour of treatment (52.03±2.60) . The results of all the extracts including the standard drug are compared with the result produced by control and it was considered as significant as $P<0.05$. All the data were presented as mean ± SEM and analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

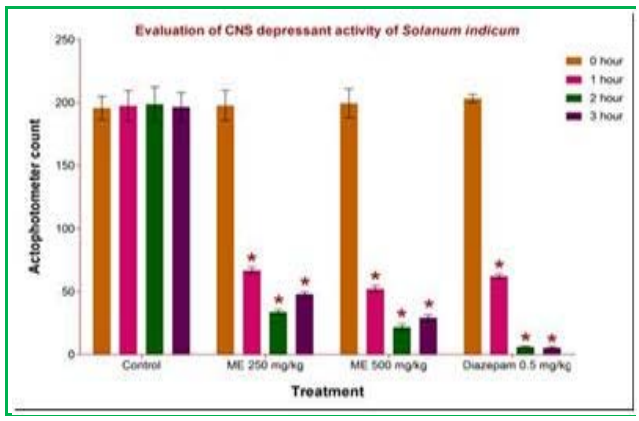

Figure 4: Graph showing the comparable CNS depressant effect of the different study groups reflected through locomotor inhibition over time. *P<0.05 treated groups vs. control group.

CONCLUSION

In the light of all these above mentioned experimental evidences lead to hypothesize that the fruit extract of *Solanum indicum* Linn. indeed possesses significant analgesic, antipyretic, anti-inflammatory and CNS depressant activity as depicted in the animal model. However, further studies using larger sample size may be warranted to corroborate the present experimental findings. From the present study findings it is amply evident that the traditional therapeutic claims of the fruits of *Solanum indicum* Linn. fruit has some scientific basis to it.

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REFERENCES

1. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L, Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts, Afr J Tradit Complement Altern Med, 8(1), 2011, 1-10.
2. Chopra RN, Nayer SL, Chopra IC, Glossary of Indian Medicinal Plants, PID, CSIR, New Delhi, 1992, 229.
3. Bhattacherya AS, Chiranjivi Banaushadhi, Ananda Publishers, Kolkata, Vol. II, 3rd reprint, 1982, 292- 297.
4. Bhakta T, Common Vegetables of the Tribals of Tripura. Tripura Tribal Research Institute, Agartala, Tripura, India. 2004, pp. 44, 46, 51, 70.
5. Kirtikar KR, Basu BD, Indian Medicinal Plants, 2nd edn., Vol. II, International Book Publication Distribution, Dehradun, India, 1975, pp. 1755-1757.
6. OECD Guideline 425, Acute Oral Toxicity up and-Down Procedure in OECD Guidelines for the Testing of Chemicals, Organization for Economic Cooperation and Development, Paris, OECD. 2001.
7. Prabu PC, Panchapakesan S, Raj CD, Acute and Sub-Acute Oral Toxicity Assessment of the Hydroalcoholic Extract of Withania somnifera Roots in Wistar Rats, Phytother. Res. 27, 2013, 1169–1178.
8. Vogel HG, Drug Discovery and Evaluation Pharmacological Assays, 2nd ed., Springer, New York, 2002, p. 720, 759, 772.
9. Ghosh MN, Fundamentals of experimental pharmacology, 2nd edition. Scientific book Agency, Calcutta, 2005, 152-154.
10. Gharate M, Kasture V, Evaluation of anti-inflammatory, analgesic, antipyretic and antiulcer activity of Punarnavasava: an Ayurvedic formulation of Boerhavia diffusa, Orient Pharm Exp Med, 13, 2013, 121–126.
11. Evaluation of antipyretic potential of Jussiaea suffruticosa L. extract in rats. Murugesan T, Mandal SC, Bhakta T, Das J, Pal M, Saha BP. Phytomedicine, 7(3), 2000, 231-234.
12. Bhakta T, Mukherjee PK, Saha K, Pal M, Saha BP, Evaluation of Anti-inflammatory effects of Cassia fistula (Leguminosae) Leaf extracts o rats, Journal of Herbs, Spices & Medicinal Plants, 6(4), 1996, 67-72.
13. Bhattachrya KR, Mehta RK, Srivastavaa PN, A simple method for recording anti-inflammatory effects on rat paw oedema, Indian J. Phhysiol and pharma, 21, 1997, 399-400.
14. Vittalrao AM, Shanbarg T, Kumarik M, Bairy KL, Shenoycrgg S, Evaluation of anti-inflammatory and analgesic activity of alcoholic activity of Kaempferia galanga in rats, Indian J Physiol Pharmacol, 55(1), 2011, 13–24.
15. Parvathi M, Ravishankar K, Evaluation of Antidepressant, Motor Coordination and Locomotor Activities of Ethanolic Root Extract of Clitoria ternatea, Journal of Natural Remedies, 13(1), 2013, 19-24.

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