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Research Article

Analgesic, Anti-Inflammatory and Anxiolytic Activity Evaluation of Methanolic Extract of *Solanum Surattense* Leaf in Swiss Albino Mice Model

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ABSTRACT
The aim of the study to investigate the analgesic, anti-inflammatory and anxiolytic effect of the methanolic extract obtained from *Solanum surattense* leaves in mice to authenticate the scientific base of traditional use. Methanol were used to extract the phytochemicals. Separation of compounds was done through gravity column chromatography and Observation of TLC Plate for methanol extract was performed with Naked Eye, Under UV light and applied iodine vapor depending on R_f value for the marking of Fluorescent spots. Methanol extract of *Solanum surattense* leaves was selected for analgesic, anti-inflammatory and anxiolytic activity. Analgesic and anti-inflammatory were measured by acetic acid-induced writhing inhibition test and xylene induced ear edema test. Open field test (OFT), Elevated plus maze (EPM), Hole cross test and Hole board test were the screening tests used to assess the anxiolytic activity on mice. The crude dried methanol extract was prepared in doses of 100 and 200 mg / kg body weight administered orally to the mice. Extract of *Solanum Surattense* statistically showed noticeable analgesic as well as anti-inflammatory activity against control. But this extract did not ensure anxiolytic and tranquilizing activity except at high doses(2x) on total ambulation criteria during open field test and at low doses (1x) on close arm in elevated plus maze test against control. The report from studies hints the traditional uses of this plant as analgesic and anti-inflammatory. Further investigation including specific compound isolation of extract can claim the scientific basis as analgesic and anxiolytic agent.
Key words: SSE (*Solanum Surattense* extract), Analgesic, Anxiety, elevated plus-maze (EPM), open field test (OFT), and Diazepam

INTRODUCTION

The use of natural source based medicine is spreading all over the world especially in the developing countries such as Bangladesh, India, China, and the Middle East. About 25% of the prescribed drugs in the world are of plant origin [1]. Near about 80% people depend on traditional plant-based drugs for their primary health care demand in the developing countries [2]. There is a broad range of plant parts possessing a variety of pharmacological activities. Recent popular plant-derived drugs reflect its recognition of the validity of many traditional claims regarding the values of natural source in health care [3]. For quality evaluation of traditional medicines, phytochemical screenings are mainly concerned. Now a days, secondary plant metabolites with previously unknown pharmacological activities have been extensively investigated as source of medicinal agents [4]. According to WHO, medicinal plants are the best and the only affordable and accessible source of primary health care for rural people, a variety of new herbal drugs, especially in the absence of access to modern medical facilities. Though considerable progress has been achieved in medical science during the last few decades, management of adverse effects still remains a challenge for medical community. Therefore, in order to determine the potential use of herbal medicine, it is important to emphasize the study of medicinal plants that found in folklore [5].

Solanum surattense is a very prickly perennial herb, woody at the base, stem somewhat zigzag with numerous branches, ovate or elliptic leaves, purple flowers in few-flowered extra-axillary cymes and yellow or white fruits, grows in aimost all district in Bangladesh. It belongs to Solanaceae family. Common name of *Solanum surattense* is Kontikari in Bangladesh. It was one of the ten roots, the Dhasamoola. Solanaceae is a large plant family containing two thousand and three hundred species, nearly half of which belong to a single genus, *Solanum*. There are herbs, shrubs or small trees under this genus. This family comprises a number of plants widely known for the presence of variety of natural products of medicinal

significance. Solanum species are widely used in folk medicine [6-8].

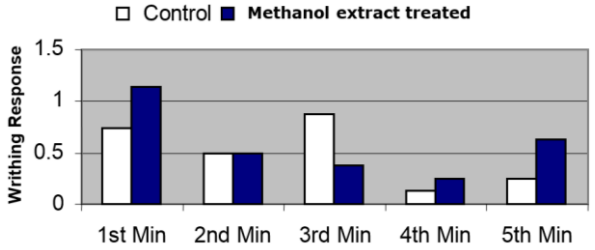

Figure 1: Graphical Presentation of the effect of Methanol extract (100mg/Kg) in the Acetic Acid Induced writhing test.

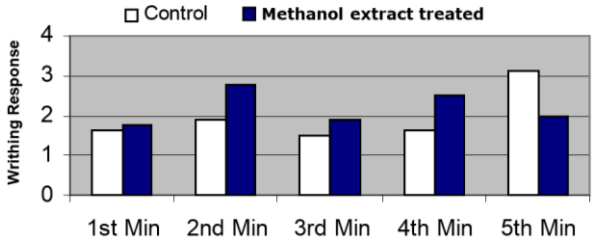

Figure 2: Graphical Presentation of the effect of Methanol extract (200mg/Kg) in the Acetic Acid induced writhing test.

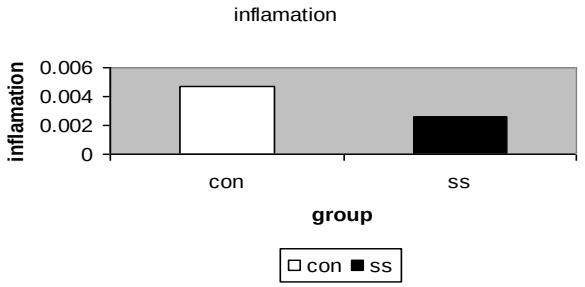

Figure 3: Graphical presentation of the effect of SS (100mg/Kg) on the Xylene treated anti-inflammatory test.

It is a rich source for several classes of compounds such as alkaloids [9], steroids [10] and phenolic compounds [11]. Many species of genus *Solanum* are used in the folk medicine of different countries, such as hypoglycemic [12], hepatoprotective and hepatotonic [13-14], laxative, appetizer, cardiotonic [15], antispasmodic, treatment of renal pain and epilepsy [16]. There are some plants of solanaceae family which possess anxiolytic and tranquilizing activity. These are- *Datura wrightii, Datura metalloids, Datura Stramonium,Withania simonii, Withania coagulens, Withania somnifera, Mandragora autumnalis,Mandragora officinarum,Mandragora turcomanica, Solanum lycopersicum* etc.

solanum surattense plant has many traditional uses in Ayurveda. *Solanum surattense* is used in asthma, cough, bronchspasm, sore throat, constipation, as an effective expectorant, diuretic and analgesic. Specifically, Stem, flowers and fruits are employed in cough, asthma and pains in chest, being used in the form of a decoction. They are prescribed for relief in burning sensation in the feet accompanied by vesicular watery eruptions. Leaves are applied locally to relieve pain. The juice of berries is used in sore throat. Like roots, seeds are also administered as an expectorant in asthma and cough. The plant is credited with diuretic properties and is used to cure dropsy. Its juice is mixed with whey and ginger and given in fevers. The juice of the leaves, mixed with black pepper, is used in rheumatism. The plant contains sterols, alkaloids and glycosides and also contains quercetin glycoside, apigenin, sitosterol and carpesterol. Fruits contain steroidal glyco-alkaloids, solasonine, solanargine, solasurine, solanocarpine, solanine-S and alkaloidal base (produced on hydrolysis of respective glycol-alkaloids), solanidine-S and solasodine. Seeds contain solanocarpine. The plant contain 1% of alkaloids,which is a source for cortisone and sex hormone preparations [6, 8,17-18].

The medicinal value of drug plants is due to the presence of some chemical substances in the plant tissues which produce a definite physiological action on the human body. No documented scientific reference was found on experimental evaluation of the analgesic, neuropharmacological, antidiarrheal, and cytotoxic effect of *Solanum surattense* . Therefore, to prove its common uses, the present study was performed to evaluate the analgesic, anti-inflammatory and neuro-pharmacological activities of methanolic leaf extract of *Solanum surattense* available in Bangladesh.

MATERIALS AND METHOD

Sample: Leaves of *Solanum surattense*

*Chemicals used for study:*methanol (CH_3-OH), 0.6% Acetic acid, Xylene were collected from local Pharmaceutical Company upon request.

Equipments used for study: Electronic Balance, Whattman filter paper no.1, rotary evaporator, Beaker, Funnel, Measuring Cylinder, Pipette and Pipette Pump, Spatula, Glass Rod, Stand and Clamp.

Collection of the plant material: Solanum surattense leaves has been collected from Ashulia, Dhaka from road side. It has been identified by Md. A. Rahim, Herbarium Assistant, Department of Botany, Jahangirnagar University.

*Preparation of methanol extract:*The collected leaves of *Solanum surattense* were thoroughly washed with water and sun-dried for 10 days. The dried leaves were pulverized with a locally fabricated grinding machine at the department of Pharmacology in Jahangirnagar University and were stored in an airtight container. The dried and coarsely powdered plant material (250 g) was soaked in 1000 ml methanol as solvent for 96 hours in a beaker, every 12 hours of interval the mixture was stirred through glass rod. The solvent was filtered through Whattman filter paper no.1 and evaporated (temperature 50-60 °C, lower pressure, rpm 120) using rotary evaporator (IKA, Germany) to get dried extract (11.6% w/w). The dry extract was kept in a refrigerator until use.

Experimental animals: Swiss albino male mice (20-40 gm body weight) were purchased from the Animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The animals were kept under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2 °C and 12 hr

light/dark cycle) in the animal house of the Department of Pharmacy, Jahangirnagar University, were used for the pharmacological experiment. They were kept for two weeks prior to experimentation for adaptation with the laboratory conditions. Animals were fasted overnight with free access to water prior to each experiment and the time interval between the tests was two weeks. The mice were fed with standard food (ICDDR, B formulated) and water *ad libitum*. The experimental protocols were approved by the Animal Experimentation Ethics Committee (AEEC) of East West University and Jahangirnagar University.

Chemical Investigation: Separation of chemical compounds in methanol extract of *Solanum surattense* was done through gravity column chromatography and TLC. Methanol and Ethyl Acetate were used as solvent. First, 55 gram silica gel was weighed and 200 ml solvent system (20ml ethyl acetate+ 180 ml methanol) was prepared. Then silica gel and solvent system were mixed properly in a beaker to prepare slurry for packing the column. Slurry was poured into a column with constant stirring and allowed to settle the silica into the column which known as column packing. Methanol extract of *Solanum surattense* (leaves) was in liquid form, that's why sample was taken into a beaker and some amount of silica gel was added into a beaker to produce powder form sample which facilitated the separation procedure.

Then powder form of sample was introduced upon a packed silica gel into the column. 300 ml solvent system was prepared and slowly introduced into a column using separating funnel. Separating funnel used as a solvent reservoir and solvent was introduced sideway into the column to facilitate proper separation. Finally 9 different fractions of ethyl acetate and methanol (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9) were collected from the column and observed by using TLC plate where color band produced. Naked Eye, UV light and iodine vapor were applied separately for noticing the marking spots of fluorescence depending on R_f value. R_f is the ratio of distance from start to centre of substance spot and distance from start to solvent spot. Different fractions were collected into a different beaker with proper identification label.

Pharmacological investigation

*Extract used:*Methanol extract of *Solanum surattense* leaves was selected for biological and pharmacological activity study.

Analgesic activity (Acetic Acid Induced Writhing Test): The peripheral analgesic activity of the extract was evaluated using acetic acid induced writhing inhibition method in mice. In this method, mice were randomly divided into two groups, each consisting of 08 animals. For this experiment, each group received a particular treatment i.e control, sample extract. Muscular contraction was induced by the intraperitonial injection of 0.6% acetic acid (AA) (0.25ml/animal). The test preparations were administered orally 30 minutes before the intraperitonial injection of acetic acid. Mice were cased individually to count number of writhes (painful muscular contraction) after 15 minutes of AA injection for 5 minutes. The average number of writhes and the percent protection were calculated and then compared between the animals of the experimental groups and the animals of the Control group. Full writhing was not always accomplished by the animals; the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhings were taken as one full writhing. Analgesic activity was expressed as writhing inhibition (%) and was calculated for each animal using the following formula: Writhing inhibition (%) = {(Wc – Ws)/Wc}100 Where, Wc is the mean number of writhings of the control and Ws is the mean number of writhings of the test sample. [19-20]

*Anti-inflammatory activity (Xylene induced ear edema test):*Mice were divided into groups of 10 mice each. After 30 min of the i.p. injection of the extract (100 mg/Kg), xylene (0.03 ml) was applied to the anterior and posterior surfaces of the right ear. Mice were sacrificed 2 h after xylene application and both ears were removed. Circular sections of both treated and untreated ears were taken using a 7 mm diameter cork borer and weighed. The difference in weight between left untreated ear sections and right treated ear section was calculated [21].

Anxiolytic activity:

Treatment Schedule: The anxiolytic activity was examined by using the Elevated Plus Maze, open field test (OFT), hole cross and hole board test. The animals were divided in to four groups, with each group consisting of 03 mice. First group receives normal saline, second group received diazepam (1 mg/kg body weight), third and fourth groups received plant extract (100 and 200 mg/kg body weight)

*Sample and control preparation for the tests:*Methanol extract was weighed in a beaker and added distilled water to it for maintaining the desired concentration of sample (3ml). For the pharmacological experiment, the liquid was administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. For the pharmacological study the drug was administered per oral route at two different doses of 100 mg/kg and 200 mg/kg body weight.

*Open-Field Test:*The open field test is one of the tests used to observe general motor activity, exploratory behavior and measures of anxiety. The open field area was made of plain wood and consisted of a square area (45 cm ×45 cm ×20 cm). The floor had a square sheet of wood (45 cm ×45 cm) with the surface divided into sixteen small squares. Mice were divided into four groups of 3 mice and treated similarly as described in hole cross test. About 30 min after treatment, mice of both the control and treated groups were placed individually in the center of the open field and behavioral activities were videotaped for 5 min. Subsequently, hand operated counters and stopwatches were used to score the following behavioral parameters for a period of 5 min. (1) Total Ambulation, (2) Total Ambulation in Center region, (3) Total standing up behavior and (4) Total Emotional Defecation were monitored.[22]

Elevated Plus Maze: The anxiolytic activity of the extract was evaluated using the elevated plus-maze (EPM) test. The EPM test apparatus consisted of two open arms (16×5 cm each) and two enclosed arms (16×5×12 cm each) that

were extended from a common central platform (5×5 cm). The maze was elevated to a height of 40 cm above floor level. Thirty minutes after the oral treatment with the control, diazepam and *Solanum surattense* extract, each mouse was placed individually on the central platform facing towards an open arm.

The numbers of open and enclosed arms entries, plus the time spent in open and enclosed arms, were recorded for a 5-min test period. An entry into an arm was defined when the mouse had all four paws in the arm. For each mouse, total exploratory activity (number of entries in both arms) and other ethologically derived measures such as grooming (itching of the face by the front legs), rearing (vertical movement against the side and/or end of the walls), and stretch-attend postures (exploratory posture in which the body is stretched forward then retracted to the original position without any forward locomotion) were also determined. All types of animal behavior were recorded using a digital video camera located above the maze [23].

*Hole cross method:*The method was adopted as described by Takagi *et al*. (1971). A wooden partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the centre of the cage. The number of passage of a mouse through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the extract [24].

Hole board test: The study was conducted using a wooden board measuring 20 cm by 40 cm with sixteen evenly spaced holes. Thirty minutes after treatment, the mice were placed singly on the board and the number of times the mice dipped their head into the holes at the level of their eyes during a five minute trial period was counted using a tally counter. The mice were placed and released singly in the centre of the board, facing away from the observer. The number of holes explored and the duration of each of the explorations was shown in real time to the observer and at the end of the experiment (usually 5 min) all the information was stored in a file for post experiment study. After each trial the apparatus was wiped clean to remove traces of the previous assay. A decrease in the number of head-dips, the time spent during head-dipping reveals the sedative behavior of experimental mice [25-26].

Statistical analysis: All data were presented as mean ±SD (Standard deviation) and were analysed by one-way ANOVA. The groups treated with extracts were compared with the respective vehicle (control) group. Unpaired "t" tests were done for statistical significance tests. SPSS (Statistical Package for Social Science) for WINDOWS (Ver. 12) was applied for the analysis of data. p = 0.05 was taken to be the level of significance. Note: *(< 0.05) =Significant, ** (< 0.01) = Highly Significant, *** (< 0.001) = Very Highly Significant.

Table 1: The effect of Methanol extract (100 and 200 mg/kg body weight) in the Acetic Acid Induced Writhing Test

Group	1st Min		2nd Min		3rd Min		4th Min		5th Min	
	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg
Control (n=8)	0.75±0.620	1.63±0.420	0.50±0.327	1.88±0.693	0.88±0.441	1.50±0.598	0.13±0.125	1.63±0.498	0.25±0.164	3.13±0.479
Methanol extract treated (n=8)	1.13±0.666	1.75±0.959	0.50±0.327	2.75±1.236	0.38±0.375	1.88±0.833	0.25±0.164	2.50±0.707	0.63±0.398	2.0±0.681
t- test (P value)	0.687	0.907	1.000	0.547	0.402	0.720	0.554	0.329	0.486	0.198

Table 2: The effect of Methanol extract (100 & 200mg/kg) in the Acetic Acid Induced Writhing Test from from 01 to 05 minutes study period

Group for 100mg/Kg body weight extract.	PARAMETER	
Male mice	Min 0-5	% Protection
Control (n=8)	2.51±1.677	-115.139%
Methanol extract treated (n=8)	2.89±1.93	
Group for 200mg/Kg body weight extract.	PARAMETER	
Male mice	Min 0-5	% Protection
Control (n=8)	9.77±2.688	-111.361%
Methanol extract treated (n=8)	10.88±4.416	

Table 3: Tabular presentation of the effect of SS (100mg/Kg) on the Xylene treated anti-inflammatory test

Group	*Inflammation*
Ctrl (n=10)	.00470 ±0.000955
SSE (100mg/Kg) (n=10)	0.00270±0.000600
t-test (P value)	0.079

Table 4: The effect of Methanol extract (100 and 200mg/kg body weight) on Ambulation in the open field test (mice ambulation in center)

Group	Min 30		Min 60		Min 120		Min 180		Min 240	
	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg
Control (n=6)	1.00± 0.516	1.17±0. 749	0.50± .342	0.50±0. 342	0.17± 0.167	0.50±.5 00	0.17± 0.167	0.17±0. 167	0.17± 0.167	0.00±.0 00
Methanol extract treated (n=6)	0.83± 0..307	0.67±0. 422	0.67± 0.211	0.50±.5 00	1.50± 0.563	0.00±.0 00	0.67± 0.422	0.00±.0 00	00± 0.000	0.00±0. 000
t- test (P value)	0.787	0.574	0.687	1.000	0.064	0.363	0.309	0.363	0.363	1.000

Table 5: The effect of Methanol extract (100mg/kg and 200 mg/Kg body weight) on Ambulation in the open field test (total ambulation)

Group	Min 30		Min 60		Min 120		Min 180		Min 240	
	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg
Control (n=6)	66.17± 4.498	34.83± 12.384	48.67± 10.171	26.00± 11.027	28.50± 9.258	14.67± 8.143	10.50± 5.807	8.33±2. 333	9.50± 5.045	13.83± 6.665
Methanol extract treated (n=6)	77.67± 6.184	27.83± 12.973	54.67± 10.531	20.17± 12.534	49.67± 7.205	4.33±2. 445	34.67± 12.085	1.00±.5 16	14.50± 4.703	2.67±2. 290
t- test (P value)	0.164	0.704	0.691	0.734	0.101	0.252	0.102	0.025*	0.485	0.144

Table 6: The effect of Methanol extract (100 and 200mg/kg body weight) on Ambulation in the open field test (emotional defecation)

Group	Min 30		Min 60		Min 120		Min 180		Min 240	
	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg
Control (n=6)	0.17±0. 167	0.00±.0 00	0.00± 0.000	0.00±0. 000	0.00± 0.000	0.17±0. 167	0.33± 0.333	0.00±.0 00	0.00± 0.000	0.00±0. 000
Methanol extract treated (n=6)	0.00± 0.000	0.00±0. 000	0.00± .000	0.17±0. 167	0.00± .000	0.00±0. 000	0.33± 0.333	0.00±0. 000	0.17±0.1 67	0.00±0. 000
t- test (P value)	0.363	1.000	1.000	0.363	1.000	0.363	1.00	1.000	0.363	1.000

Table 7: The effect of Methanol extract (100 and 200mg/kg body weight) on Ambulation in the open field test (mice standing)

Group	Min 30		Min 60		Min 120		Min 180		Min 240	
	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg
Control (n=6)	6.67± 1.82	4.33±1. 961	5.33± 2.305	2.67±1. 745	5.83± 2.81	0.83±0. 833	1.50± 0.619	0.33±0. 333	0.83± 0.654	0.83±0. 654

Methanol extract treated (n=6)	14.67± 2.124	5.33±3. 051	12.33± 1.892	1.83±1. 014	10.50± 2.217	0.00±0. 000	6.83± 2.509	0.17±0. 167	3.17± 0.749	0.17±.0 167
t- test (P value)	0.170	0.788	0.410	0.688	0.222	0.363	0.660	0.664	0.410	0.347

Table 8: The effect of Methanol extract (100 and 200mg/kg body weight) on Ambulation in the Hole Cross test

Group	Min 30		Min 60		Min 120		Min 180		Min 240	
	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg	100 mg	200 mg
Control (n=6)	7.83±1. 352	5.00±1. 095	8.50±1. 408	4.83±1. 046	6.33±1. 542	4.50±1. 057	5.00±.8 16	7.33±2. 171	6017±1. 537	5.83±1. 778
Methanol extract treated (n=6)	6.67±1. 229	5.83±1. 222	7.50±.7 19	5.50±1. 544	9.33±1. 406	5.50±1. 607	6.50±1. 522	4.50±1. 432	7.00±1.5 28	5.83±1. 046
t- test (P value)	0.538	0.623	0.541	0.728	0.181	0.614	0.406	0.301	0.709	1.000

Table 9 : The effect of Methanol extract (100 and 200 mg/kg) on Ambulation in the Hole Board test (Total mice ambulation)

Group	Min 30		Min 60		Min 120		Min 180		Min 240	
(n=6)	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg
Frequency for Control	20.67± 1.406	43.17±1 .973	46.17±2 .774	23.83±7 .705	23.50±3 .713	34.67± 4.499	24.83±3. 114	24.50±6 .677	29.50±5 .334	20.83±6 .685
Frequency for extract	22.50± 1.578	64.50±6 .438	41.17±4 .861	43.33±8 .417	37.83±7 .622	33.83± 10.467	39.33±5. 863	39.00±9 .508	34.83±8 .658	28.67±7 .719
t-test (P value)	0.390	0.010 *	0.393	0.118	0.122	0.944	0.54	0.240	0.611	0.461

Table 10: The effect of Methanol extract (100 & 200mg/kg body weight) on Ambulation in the Hole Board test (Head deeping)

Group	Min 30		Min 60		Min 120		Min 180		Min 240	
(n=6)	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg
Frequency for Control	8.00±2. 978	6.17±2. 040	5.50±1. 607	5.83±2. 626	4.00±1. 528	7.00±2. 805	2.67±0.9 55	3.67±1. 229	7.67±2. 305	2.83±0. 946
Frequency for extract	6.33±2. 044	7.67±1. 606	6.00±2. 324	12.17±2 .937	3.00±1. 770	6.33±1. 944	4.50±1.5 65	8.83±2. 548	6.00±2. 324	6.00±1. 897
t-test (P value)	0.654	0.576	0.863	0.139	0.678	0.849	0.341	0.098	0.622	0.166

Table 11: The effect of Methanol extract (100 & 200mg/kg body weight) on Ambulation in the Elevated Plus Maze test (mice position in close arm)

Group	Min 0		Min 60		Min 120		Min 180		Min 240	
(n=6)	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg
Frequency for Control	13.67± 1.498	15.17±1 .537	14.17±1 .621	10.33±2 .974	12.83±2 .651	4.83±2. 400	8.17±1.7 01	0.67±0. 333	11.00±1 .317	0.5±.22 4
Frequency for extract	12.17± 0.601	16.33±2 .155	6.67±2. 974	7.67±3. 537	5.17±2. 040	2.17±0. 792	5.50±2.2 32	0.17±0. 167	3.67±2. 140	0.67±0. 333

t-test (P value)	0.386	0.669	0.059	0.577	0.045 *	0.316	0.364	0.209	0.015 *	0.687

Table 12: The effect of Methanol extract (100 & 200mg/kg body weight) on Ambulation in the Elevated Plus Maze test (mice position in open arm)

Group	Min 0		Min 60		Min 120		Min 180		Min 240	
(n=6)	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg
Frequency for Control	00±00	08±02	00±00	04±01	01±00	00±00	02±02	00±00	01±01	00±00
Frequency for extract	02±01	07±02	00±00	08±06	00±00	00±00	03±03	03±03	00±00	00±00
t-test (P value)	0.093	0.810	1.00	0.582	0.084	1.00	0.800	0.363	0.175	1.00

Table 13 : The effect of Methanol extract (100 & 200mg/kg body weight) on Ambulation in the Elevated Plus Maze test (mice staying time in open arm)

Group	Min 0		Min 30		Min 60		Min 120		Min180	
(n=6)	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg	100mg	200mg
Stay time for Control (Sec)	0.00±0.00	2.50±0.563	0.33±0.211	1.33±0.558	0.50±.224	0.17±0.167	0.33±0.333	0.00±0.00	0.33±0.211	0.00±0.00
Stay time (Sec) for extract	0.83±0.401	3.17±1.014	0.17±0.167	2.17±1.515	0.83±0.833	0.00±0.00	1.00±0.816	0.00±0.00	0.00±0.000	0.00±0.00
t-test (P value)	0.093	0.578	0.549	0.617	0.707	0.363	0.467	1.00	0.145	1.00

RESULT AND DISCUSSION

Separation of chemical entity: From TLC analysis we found that maximum spots are colored. From these findings we can say that the compounds present in the spots may contain chromophore. Among these spots some are yellow which indicate that nitro groups may be present in the compound which is imparting the yellow color and so on. In presence of Iodine vapor some spots changed their color which indicates that there may be presence of un-saturation in the compound.

Analgesic activity: According to the tabular and graphical presentation of the effect of Methanol extract (100mg/kg, 200mg/kg) of *Solanum surattense* on the Acetic Acid Induced Writhing Test utilizing Male mice, the analgesic activity is statistically insignificant. Methanol extract (100mg/kg) treated male mice exerted an increase in writhing response compared to the control group from the initial 1st min to 5th min. except 3rd min. At the dose of 200mg/kg, Methanol extract treated male mice showed an increased response compared to the control group from the 1st to the 4th min. But all of the results were statistically insignificant. The percent of protection by Methanol extract was-

-115.139% (100 mg/kg) (pain perception increased)
-111.361% (200 mg/kg) (pain perception increased)
(Table 1,2 and Figure-1 & 2).

Anti-inflammatory activity: In the table we can see the p value, here the p value is less then 0.1 it is noticeable effect in anti-inflammatory activity. So we can predict that the leaf extract may have anti-inflammatory activity. The graph sown two sample of the test that is control (only xylene) and drug (Xylene and extract-100mg/Kg) test group. In graph the X axis is inflammation produced by xylene in mice ear and the Y axis contain the group of mice (Xylene and extract). In case of control group the xylene produced inflammation at a certain level but in case of drug group the xylene produced significantly less amount of inflammation at the same dose. So we can assume that the leaf extract of *Solanum sarattense* may have anti-inflammatory activity (Table 3 and Figure-3).

Anxiolytic activity

*Open Field Test:*The experiment was carried out to get a clear picture of the effect of the drugs under consideration on the pattern of movement and behavior. This experiment presents with a different and more complex environment to explore.

Total Ambulation: SSE treated male mice at dose 100 mg/Kg levels exerted overall increase in ambulation. But none of the results were statically significant. But at dose 200 mg/Kg, exerted overall decrease in ambulation compare with the control group. The decreasing effect at min 180 (p=0.025*) was significant statically. None other results were significant statically (Table 4).

Total Ambulation in Center region: SSE treated male mice at dose levels 100 mg/Kg exerted overall increase in total movement in the center region except at min 30 & min

240 While at dose level 200 mg/kg total ambulation in the center region had decreased. But no statically significant result were found (Table 5)..

Total standing up behavior: At dose 100 mg/Kg SSE standing was overall decreased compare with the control group. Similarly at dose 200 mg/kg in all through out the experimental period, SSE treated mice exerted a decrease in the standing up behavior except at min 60 in comparison to that of control group. Therefore, statistically significant out put was found (Table 6).

Total Emotional Defecation : SSE at doses (100 mg/Kg and 200 mg/Kg) showed very less response in emotional defecation all through out the 240 min study with the comparison of the corresponding control group. At 100 mg/kg (only exception being at min 30 emotional defecation decreases and at min 240 increases. At other doses (200 mg/kg) SSE emotional defecation were similar for both. None of the results were statistically significant(Table 7).

Elevated Plus Maze Test: This experiment was carried out to confirm whether any benzodiazepine like anxiolytic activity is present in the extract or not.

Staying time: At dose 100 mg/Kg SSE showed overall decrease in the time spent in open arm except 180 min while at 200 mg/kg, SSE treated mice showed no difference in the time spent in open arm from 30 min onwards till the end of 240 min study period in comparison with the control group. None of the results were statistically significant (Table 13).

Close arm position: At dose 100 mg/Kg, SSE treated mice showed a decrease in total movement in close arm from 30 min onwards till the end of 240 min study period in comparison with the control group. The results of 180 min *(p=0.045*)* and 240 min *(p=0.015*)* were significant. On the other hand

at dose 200 mg/Kg, SSE treated mice showed also a decrease except at 240 min of total movement from 30 min to 240 min of 4 hours study periods in comparison with the control group in Close arm position from. Results from 200 mg/Kg dose at 240 min were inconclusive and mysterious (Table 11).

Open arm position: At doses of 100 mg/Kg, SSE treated mice showed an overall decrease except at 240 min in total movement in open arm from 30 min onwards till the end of 240 min of study period in comparison with the control group. While at dose 200 mg/Kg, SSE treated mice showed no difference in total movement in open arm position in comparison with the control group (Table 12).

Hole cross test: SSE treated male mice at two dose levels (100 mg/kg - except at min 30 and min 60, 200 mg/kg- except at min180) exerted overall increase in hole cross activity. Although there were overall increases in the response none of the results were statistically significantly different from the corresponding control animals (Table 8).

Hole Board test :

Ambulation: SSE treated group, at dose 100 mg/Kg , showed an overall decrease in ambulatory activity except min 30 and min 60. But none of the results were statistically significant. At dose 200mg/kg SSE treated group showed overall increase in ambulatory activity in all through out the experimental study period when compared to the corresponding control group. when treated with dose 200 mg/kg at min _30 *(p=0.01*)* increase was noted at statically significant level(Table 9).

Head Dipping: At dose 100mg/Kg, SSE treated group showed an overall decrease in head dipping activity except min180 and min 240, in all through out the experimental study period when compared to the corresponding control group.

At dose 200mg/Kg, overall increasing effects were denoted except min 120.The results were not statistically significant (Table 10).

CONCLUSION

The objective of this study was to find out the chemical compounds present and evaluate analgesic, anti-inflammatory and anxiolytic activity against traditional claim. We just were able to detect some chromophore and nitro functional groups with unsaturation. In pharmacological view we found that methanol extracts of *Solanum surattense* leaf have noticeable anti-inflammatory activity. In case of anxiolytic activity, methanol extract of *Solanum surattense* dose not ensure anxiolytic effect except at high doses(2x) on total ambulation criteria during open field test and at low doses (1x) on close arm in elevated plus maze test . Unfortunately due to lack of instruments facilities and other constraints we could not reach in our goal properly. If pure compounds could be isolated and any standard drug could be used for comparison with the extract then the effect can be judge authentically. However, other parts such as extract of root and stem can be analyzed for pharmacological action against standard.

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