



Evaluation of activities of *Solanum nigrum* fruit extract

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Abstract

The present study was designed to evaluate the analgesic, anti-inflammatory and antimicrobial activities of ethanolic extract of fruits of *Solanum nigrum* plant belonging to Solanaceae family. The anti-inflammatory activity of the extract was evaluated by using Carrageenan-induced rat paw edema while analgesic activity of the extract was evaluated for its central and peripheral pharmacological actions by using Eddy's hot plate and acetic acid induced writhing respectively. The study was carried out using doses of 100, 250 & 500 mg/kg orally. The extract showed significant analgesic and anti-inflammatory activity at the dose of 500 mg/kg ($P < 0.01$) as compare to standard drug Diclofenac sodium (50 mg/kg). The plant extract significantly inhibited the *S. aureus* and *B. subtilis* (Gram +ve) at all the tested concentrations (100, 75, 50 and 25mg/ml) as compare to standard drug Ciprofloxacin (20 µg/ml) whereas the extract failed to show inhibitory effect against *E. coli* and *P. aeruginosa* (Gram -ve) at a concentration of 25mg/ml. The extract also showed significant inhibitory effect against *C. albicans* at all concentrations except at 25mg/ml as compare to standard drug Amphotericin B (100µg/ml).

Keywords: *Solanum nigrum*, Anti-inflammatory, Analgesic, Antimicrobial, Fruit extract.

Introduction

Pain and inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases. A number of natural products are used in various traditional medical systems to treat relief of symptoms from pain and inflammation [1].

Solanum nigrum Linn. (Solanaceae) is commonly known as 'Black nightshade'. The plant has been extensively used in traditional medicine in India and other parts of world to cure liver disorders, chronic skin ailments (psoriasis and ringworm), inflammatory conditions, painful periods, fevers, diarrhea, eye diseases, hydrophobia etc [2]. The phytochemical studies revealed the plant contains glycoalkaloids (solanine, solamargine, solanigrine and solasodine), steroidal glycosides (β -solamargine, solasonine and α,β -solansodamine), steroidal saponins (diosgenin), steroidal genin (gitogenin), tannin and polyphenolic compounds. Mature fruits are low in alkaloid (solanine) content [3-5]. The fruit of *S. nigrum* is reported to have antiulcer, antioxidant and antitumor promoting agent in rats [5-7]. The fruit of *S. nigrum* has been reported in the ancient Indian medicinal literature with beneficial effects in inflammation, tuberculosis, diuretics etc. [8]. Though the plant has great potential for anti-inflammatory, analgesic and antimicrobial activity, nobody has not been yet documented these activities on fruits of this plant. So, in this study we have attempted to investigate the analgesic, anti-inflammatory and antimicrobial activities of *Solanum nigrum* (SN) fruit extract.

Materials and Methods

All the strains used for these studies were procured from Microbial Type Culture Collection, IMTECH, Chandigarh, India. All the standard drugs (Ciprofloxacin, Amphotericin B and Diclofenac sodium) were obtained from various chemical units – E. Merck India Ltd. and S. D. Fine Chem. Ltd. (India).

The fruits of *Solanum nigrum* were collected from local areas during the month of November 2008. The plant got identified and authenticated by Department of Botany, Kurukshetra University, Kurukshetra, Haryana, (India) and a voucher specimen of the sample (Sr. No. KUK/IPS/2008/SN-107) has deposited in the Herbarium collection at Department. The fruits were cleaned and dried in the shade, then powdered to 40 mesh and stored in an airtight container.

Wistar rats weighing 180-200 gram and Swiss albino mice weighing 25-30 gm were obtained from Haryana Agriculture University, Hisar, Haryana (India). The animals were housed in Animal house, Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra (Haryana) in polycarbonate cages, in a room maintained under controlled room temperature $22 \pm 2^{\circ}\text{C}$, relative humidity 60 -70% and provided with food and water *ad libitum*. All the experimental procedures and protocols used in the study were reviewed by the Institutional Animal Ethics Committee (Register Number: 562/02/a/CPCSEA) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India.

Preparation of Extract

Dried fruits powder (500 gram) was divided in three parts, treated each three times with fresh ethanol (1000 ml) separately for 48 h. The ethanolic extracts thus obtained were combined, filtered and distilled on a water bath. The last traces of the solvent were evaporated under reduced pressure in rotatory evaporator (Heidolph Laborota 4011 digital). The yield of the ethanolic extract was 1.75 % w/w. Pharmacological studies were carried out by suspending a weighed amount of the extract in normal saline (95 ml): tween 80 (5 ml) ratio.

Determination of antimicrobial activity

A total of four bacterial and one fungal strain were selected on the basis of their clinical importance in causing diseases in humans. Two Gram positive bacteria - *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121) and two Gram negative - *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) and a yeast *Candida albicans* (MTCC 227) were chosen for evaluation of antimicrobial activity. All the test microorganisms were maintained on Nutrient Agar except *C. albicans*, which was maintained on Malt Yeast Extract Agar and stored at 4°C.

Various concentrations (100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml) of the extract of the fruits of *S. nigrum* were evaluated for antimicrobial activity by agar well diffusion method [9]. All the microbial strains were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/ml [10]. 20ml of agar media was poured into each petri plate and plates were swabbed with 100 µl inocula of each test microbial strain and kept for 15 min. for adsorption. Wells of 8mm diameter were punched into seeded agar plates and loaded with a 100 µl volume with different concentrations of extracts reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 hrs. Antimicrobial activity was evaluated by measuring the diameter of inhibition zone with zone reader (Hi Antibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin (bacteria) and Amphotericin – B (fungi) used as positive control. The experiment was carried out in triplicate and mean of the diameter of inhibition zones was calculated.

Acute toxicity test

Acute toxicity tests were performed according to OECD – 423 guidelines (acute toxic class method) [11]. Swiss mice (n = 6) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. The ethanolic extract of *S. nigrum* suspended in normal saline:tween 80 (95:5) was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was observed in 5/6 or 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in less than four mice out of six animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was then repeated with higher doses such as 100, 300 and 1500 mg/kg.

All studies were carried out by using five groups of ten animals each for both anti-inflammatory and analgesic activity.

Group I received normal saline : tween 80 (p.o),
Group II received Diclofenac sodium (50 mg/kg i.p.),
Group III received SN extract (100 mg/kg p.o.),
Group IV received SN extract (250 mg/kg p.o.) and
Group V received SN extract (500 mg/kg p.o.).

Anti-inflammatory activity (Carrageenan-induced paw edema)

The anti-inflammatory activity of ethanolic extract of *S. nigrum* using carrageenan-induced paw edema was studied according to Winter *et al* [12]. Thirty minutes after

administration of test and standard drugs, 0.1 ml of 1% w/v of carrageenan suspension in normal saline was injected to all animals in the left hind paw (plantar region). The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer (model 7140, Ugo Basile, Italy). The measures were determined at 0 h (before carrageenan injection) and 30, 60, 90 and 120 minutes after drug treatment. The anti-inflammatory effect of ethanolic extract was calculated by the following equation:

$$\text{Anti-inflammatory activity (\%)} = (1 - V_t/V_c) \times 100$$

Where V_t represents the paw volume in drug treated animals and V_c represents the paw volume of control group of animals.

Analgesic activity

Acetic acid-induced abdominal writhing test

The test was performed as described by Collier *et al* [13]. Nociception was induced by an intraperitoneal (i.p.) injection of acetic acid 1.0%, 0.1 ml/10g body weight. Mice were treated with the standard drug (Diclofenac sodium) and extracts of SN (100, 250 and 500 mg/kg, orally) 30 min before acetic acid injection. The number of stretching or writhing was recorded from 5 minutes to 15 minutes.

Hot-plate test

The hot-plate was used to measure response latencies according to the method described by Eddy and Leimbach (Eddy and Leimback, 1953), with minor modifications [14]. The paws of mice are very sensitive to heat at temperature, which are not damaging the skin. The response is in the form of jumping, withdrawal of the paws or the licking of the paws. The animals were placed on Eddy's hot plate kept at a temperature of $55 \pm 0.5^\circ \text{C}$. A cut off period of 15 sec, was observed to avoid damage of the paw. Reaction time and the type of response were noted using a stopwatch. The latency was recorded before and after 30, 60, 90 and 120 min of both test and standard. Average reaction times were then calculated and the percentage variation calculated using following relation:-

$$\% \text{ Inhibition} = [(\text{Before treatment} / \text{after treatment}) - 1] \times 100$$

Statistical analysis

All data were represented as mean \pm S.E.M. and as percentage. Results were statistically evaluated using Dunnett's *t*- test. $P < 0.01$ was considered significant.

Results and Discussion

Acute toxicity test

S. nigrum fruit extract did not produce any mortality even at the dose of 1500 mg/kg, p.o. All the doses (5, 50 and 300 mg/kg, p.o.) of *S. nigrum* were thus found to be non-toxic. On the basis of above results, three doses (100, 250, 500 mg/kg, p.o.) of *S. nigrum* were selected for further pharmacological studies.

Carrageenan-induced rat paw edema

The anti-inflammatory effect of the ethanolic extract of *S. nigrum* is shown in Table 1. The extract at the doses of 500 mg/kg p.o. showed significant results ($P < 0.01$) as compared to Diclofenac sodium (50 mg/kg) and caused a significant inhibition in paw edema volume.

Table 1. Anti-inflammatory activity of ethanolic extract of fruits of *S. nigrum*

Treatment	Dose (mg/kg) p.o.	Change in Edema vol (ml)			
		30 min	60 min	90 min	120 min
Control Normal Saline (95): tween 80 (5)	--	0.40±0.01	0.62±0.01	0.87±0.02	1.06±0.02
Standard (Diclofenac Sodium)	50 i.p.	0.30±0.7 (25) ^a	0.40±0.02 (32)	0.50±0.07** (42)	0.63±0.01** (40)
SN Extract	100	0.38±0.05 (5)	0.58±0.05 (6)	0.80±0.03 (8)	0.94±0.01 (11)
	250	0.38±0.8 (5)	0.47±0.06 (24)	0.73±0.02 (16)	0.99±0.03 (6)
	500	0.31±0.05 (22)	0.53±0.01 (14)	0.57±0.1** (34)	0.74±0.01** (30)

N = 10, values are mean ± SEM. The data were analyzed by one way ANOVA followed by Dunnett's *t*-test. ** $p < 0.01$ compared to control group. ^a Values in the parenthesis represents % inhibition.

Acetic acid-induced writhing test

The results of acetic acid induced writhing test of the ethanolic extract of *S. nigrum* is shown in Table 2. The ethanolic extract at the doses of 100, 250 and 500 mg/kg p.o. caused an inhibition on the writhing response induced by acetic acid. The maximal inhibition of the nociceptive response was achieved at a dose of 500 mg/kg ($P < 0.01$).

Table 2. Effect of Ethanol Extract of *S. nigrum* on Chemical Stimulus (Writhing Test) Pain in mice

Treatment	Dose (mg/kg) p.o.	No. of wriths	Inhibition (%)
Control Normal Saline (95): tween 80 (5)	--	23.16±0.5	--
Standard (Diclofenac Sodium)	50 i.p.	4.167±0.4**	82
SN Extract	100	17.8±0.4*	26
	250	13.6±1.3**	43
	500	10.8±1.0**	56

N = 10, values are mean ± SEM. The data were analyzed by one way ANOVA followed by Dunnett's *t*-test. * $p < 0.05$ compared to control group, ** $p < 0.01$ compared to control group.

Hot plate test

The results of hot plate test of ethanolic extract of *S. nigrum* is shown in Table 3. The oral dose of fruit extract at 500 mg/kg ($p < 0.01$) elicited a significant analgesic activity as evidenced by increase in latency time on comparison with negative control at the end of 30, 60, 90, 120 min. The increase in latency time was found in a dose dependent manner.

Table 3. Effect of Ethanol Extract of *S. nigrum* on Thermal Stimulus Induced (Hot Plate) Pain in mice

Treatment	Dose (mg/kg) p.o.	Time (min)			
		30	60	90	120
Control Normal Saline (95): tween 80 (5)	--	1.5 ± 0.2	1.8±0.3	1.9±0.3	2.0±0.3
Standard (Diclofenac Sodium)	50 i.p.	4.1 ± 0.4 ^{**} (63.41) ^a	5.8±0.4 ^{**} (68.96) ^a	6.6±0.2 ^{**} (71.21) ^a	4.6±0.2 ^{**} (56.52) ^a
SN Extract	100	1.7±0.4 (11.76)	2.1±0.4 (14.28)	3.1±0.4 (38.70)	2.5±0.4 (20)
	250	2.2±0.1 (31.81)	2.8±0.2 (35.71)	2.3±0.3 (17.39)	2.7±0.3 (25.92)
	500	2.6±0.3 (42.30)	4.9±0.2 ^{**} (63.26)	3.5±0.2 ^{**} (45.71)	2.9±0.2 (31.03)

N = 10, values are mean ± SEM. The data were analyzed by one way ANOVA followed by Dunnett's *t*-test. ^{**} $p < 0.01$ compared to control group, ^a Values in the parenthesis represents % inhibition.

Antimicrobial activity

The ethanolic extract of *Solanum nigrum* inhibited all the tested bacterial and fungi strains as shown in Table 4. The results showed that the increase in concentration of extract increased the zone of inhibition against all the tested microbial strains. From the results, ethanolic extract of *S. nigrum* was found to inhibit the Gram positive bacteria at all the tested concentrations (100mg, 75mg, 50mg and 25mg) whereas Gram negative bacteria did not showed any activity at a concentration of 25mg/ml. SN extract showed good activity against the tested microorganisms except for *P. aeruginosa*, which showed low activity as compare to other tested microorganisms. SN extract was found to be most effective against *S. aureus* showing the maximum zone of inhibition (25.6mm) followed by *B. subtilis* (24.3mm) whereas incase of Gram negative bacteria, ethanolic extract was found to be most effective against *E. coli* showing the maximum zone of inhibition (18.6mm) followed by *P. aeruginosa* (13.6mm). The activity of the extract against *S. aureus* and *B. subtilis* was comparable to that of standard drug ciprofloxacin. Interestingly, the ethanolic extract showed high activity against *C. albicans* with a zone of inihition (15.6mm) than that of standard drug Amphotericin B (13.6mm).

The abdominal contraction response induced by glacial acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. Intraperitoneal administration of acetic acid causes an increase in peritoneal fluids of PGE2 and PGF2 α involving in part, peritoneal receptors and produces analgesia by inducing capillary permeability and liberating the noxious

endogenous substances including serotonin, histamine, prostaglandin and bradykinin that sensitize pain nerve endings.

Table 4. Antimicrobial activity of Ethanolic *S. nigrum* extract

Micro-Organisms	Diameter of growth of inhibition zone (mm) ^a						
	Extract concentration (mg/ml)				Control		
	100	75	50	25	Ciprofloxacin (20 µg/ml)	Amphotericin B (100 µg /ml)	DMSO
<i>S. aureus</i>	25.6	22.3	20.6	15.6	26.3	ns	-
<i>B. subtilis</i>	24.3	24	21.3	1.6	25.6	ns	-
<i>E. coli</i>	18.6	14.3	12.3	-	25	ns	-
<i>P. aeruginosa</i>	13.6	13.3	11.6	-	23.3	ns	-
<i>C. albicans</i>	15.6	15.6	13.3	-	ns	13.6	-

- No activity, ns –not studied

^a Values, including diameter of the well (8mm), are means of three replicates

It has been suggested that acetic acid stimulates the vanilloid receptor (VRI) and bradykinin β_2 receptor in the pathway comprising sensory afferent C-fibres. Therefore, the observed activity is because of the interfering the synthesis or release of those endogenous substances or desensitization of the nerve fibers involved in pain transmission pathway [15,16].

Nociception reaction towards thermal stimuli is a well validated model for detection of opiate analgesic as well as several type analgesic drugs from spinal origin.

The most widely used test for screening of anti-inflammatory agents is carrageenan induced edema in the rat hind paw [12]. The development of edema in the paw of the rat after injection of carrageenan is believed to be biphasic event. The initial phase has been attributed to the release of histamine and serotonin and the second phase is due to the release of prostaglandin like substance [17].

SN extract showed variable activity against all the tested microbial strains. It was observed that the zone of inhibition varies from one organism to another at different concentrations. According to Prescott [18], the activity of antimicrobial agent is concentration dependent. Among the Gram positive and negative bacteria tested, Gram positive bacteria were more susceptible to the extracts. These results are in accordance with the earlier reports indicating that plant extract are most active against Gram positive bacteria than that of Gram negative bacteria [19]. The activity of extracts was comparable to those of standard antibiotics in case of Gram positive bacteria and found to be more active, in case of fungi (*C. albicans*).

Conclusion

All these data obtained by the study show that the ethanol leaves extract of *S. nigrum* as a novel and potential agent in the management of inflammation and pain which are probably mediated via inhibition of various autocoids formation and release. SN extract

showed broad spectrum activity against the tested bacteria and in case of *C. albicans* it showed activity greater than the standard drug, so it can also be used for the treatment of candidiasis. Based on the results obtained our findings have confirmed folklore use of this plant as analgesic, anti-inflammatory and as antimicrobial agent.

Several types of bioactive compounds, isolated from *S. nigrum* could be linked to the SN extract observed activities. For example, the glycoprotein has been shown to stimulate apoptosis partly via reduction of nitric oxide (NO) in HCT -116 cells and NO has been associated with analgesic and anti-inflammatory activities [20]. The observed antibacterial and antifungal activities of *S. nigrum* species probably may be due to presence of steroidal alkaloid glycosides [21-22].

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