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An investigation to evaluate the analgesic and central nervous system depressant activities of *Solanum nigrum* (Linn.) in Homoeopathic potencies in experimental animal models

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ABSTRACT

Background and Objective: In Homoeopathy, *Solanum nigrum* is clinically used in the treatment of ergotism, meningitis, irritation during dentition and some of the symptoms of neurological disorders but its Central Nervous System (CNS) potential has not been explored experimentally yet. Therefore, a preliminary study was conducted with an objective to evaluate the analgesic and CNS depressant effects of homoeopathic potencies of *S. nigrum* in experimental animal models.

Materials and Methods: The study was conducted in Wistar albino rats using a hot plate, ice plate and Randall–Selitto assay for analgesic; rota-rod and open field test for CNS depressant activities. The different potencies (3X, 6X, 12X and 30C) of *Solanum nigrum* were administered orally (0.5 ml/rat/day) for 30 days and response was assessed after 30 minutes of drug administration on 10th, 20th and 30th day.

Results: The result shows that all the four potencies of *Solanum nigrum* has increased the latency time required to raise and lick the paws for thermal sensation on hot plate test and for cold sensation on ice plate test and also increased the degree of threshold pressure to mechanically induced pain on Randall–Selitto assay but depressed the motor coordination and locomotor activities.

Conclusion: The result obtained from this preliminary study suggests that homoeopathic preparation of *Solanum nigrum* in different potencies possess analgesic and CNS depressant activities. Further detailed investigations are required for its possible human use.

Keywords: Albino rats, Analgesic, Central nervous system depressant activity, Motor coordination, Neurological disorders, Solanum nigrum

INTRODUCTION

Solanum nigrum commonly known as Kakmachi grows naturally throughout India. *Solanum nigrum* is an erect herb that grows about 30–45 cm in height[1] and demonstrates a diversity of therapeutic properties. It has been extensively used traditionally to treat various ailments such as pain, inflammation and fever.[2] In

ethnomedicine, the plant is used as a remedy for cough, asthma, nasal catarrh, ophthalmic disorders, skin disorders, giddiness and nervous disorders.[3] The plant is also used in oriental systems of medicine as a diuretic and an antitumorigenic agent.[4]

Solanum nigrum possesses various compounds that are responsible for diverse activities. Its major active components are glycoalkaloids, glycoproteins, and polysaccharides. It also contains polyphenolic compounds such as gallic acid, catechin, protocatechuic acid, caffeic acid, epicatechin, rutin and naringenin.[5]

The fruit of *Solanum nigrum* has been found to possess antiulcer and antitumor effects in rats.[6] The extracts of plant exhibit antinociceptive, anti inflammatory[7] and antipyretic activity in rats[8] whereas its aqueous extract show anticonvulsant property in chicks, mice and rats.[9] Ethanol extract of *Solanum nigrum* has been reported to possess hepatoprotective activity against CCl_4-induced hepatic damage in rats.[10] The ethanol extract of the fruit is also shown to possess potential Central Nervous System (CNS) depressant action.[11] Crude extract of *Solanum nigrum* leaves has been found to possess antioxidant effect in rats.[12]

In Homoeopathy, *Solanum nigrum* in different potencies used for treating ergotism, tetanic spasms and stiffness of whole body with mania, meningitis, irritation during dentition, chronic intestinal toxemia and other neurological disorders[13]. But the scientific evidence for its use in the treatment of neurological disorders as used in ethnomedicine was lacking. The present study was, therefore, carried out to assess the analgesic and CNS depressant activities of the homoeopathic potencies (3X, 6X, 12X and 30C) of *Solanum nigrum* in experimental animal models. The positive results in animal models, if obtained, can be made more meaningful by conducting human trials to prove its therapeutic effects on human brain.

MATERIALS AND METHODS

Collection of Plant Material

The whole plant of *Solanum nigrum* Linn. (Family: Solanaceae) was collected from Nilgiris hills, Tamil Nadu, India during the month of July and taxonomically identified/authenticated by the Botanist at Survey of Medicinal Plants Collection Unit, Udagamandalam, Tamilnadu.

Preparation and Selection of Medicines

Homoeopathic potencies of *Solanum nigrum* in 3X, 6X and 12X dilutions in Decimal Scale and 30C dilution in Centesimal Scale were prepared according to the standard procedures mentioned in Homoeopathic Pharmacopoeia of India and supplied by M/S. Bahola Laboratories, Puducherry, India from a single batch of whole plant.[14] The 3X, 6X, 12X and 3C dilutions of *Solanum nigrum* were selected in this study because these potencies are commonly prescribed by Homoeopathic practitioners for their clinical uses.[13]

Animals

Adult Wistar albino rats (120–140 g) procured from National Centre for Laboratory Animal Sciences, Hyderabad were used in the study. They were housed in polypropylene cages (47 cm × 34 cm × 20 cm) under standard laboratory conditions (12/12 hour, light/dark cycles and room temperature 25°C ± 2°C) and allowed free access to food and water *ad-libitum*. The animals were acclimatized to laboratory conditions for a period of 10 days before initiation of the study.

Experimental Design

The study protocol was approved by the Institutional Animal Ethics Committee (Reg. No. 383/01/a/CPCSEA), Department of Zoology, Osmania University, Hyderabad, where the study was conducted. A total of 180 rats were taken and divided into five batches (one batch for each study) of 36 each which were further divided into six groups of six each. The animals were accustomed to respective test procedures initially by subjecting them for test trials for three subsequent observations at 10-minute intervals on each day for 3 days before giving them any drug treatment. The test potencies (3X, 6X, 12X and 30C) of *Solanum nigrum* and vehicle (91.5% alcohol used as vehicle for preparation of different potencies of test drug) were diluted with distilled water in the ratio of 1:4 and kept as stock solutions. The animals were fed with 2 ml stock solution of different potencies (3X, 6X, 12X and 30C) of *Solanum nigrum* that is, 0.5 ml (drug)/rat/day for 30 days. Vehicle and saline-treated rats were also maintained simultaneously along with drug-treated animals. The response of the drug was measured after 30 minutes of drug administration on 10th, 20th and 30th day. Reading taken just before administration of the drug/vehicle/saline on first day of the study was considered as the initial control value in the same group for comparison.

Analgesic activity

Analgesic activity was studied by (i) hot plate (ii) ice plate and (iii) Randall–Selitto methods

Hot plate method

The hot plate latency assay was performed as per the method of Eddy *et. al*.[15] 30 minutes after the administration of drug, vehicle or saline, the rats were gently placed individually on a hot plate maintained at a constant temperature of 55°C ± 2°C and recorded the latency time (s). The time taken by the animals to lick the fore or hind paw or jump out of the hot plate was considered as latency (reaction) time. The reaction time noted just before administration of the drug, vehicle or saline on day 1 first day of the study was considered as the initial control values for comparison. A cut-off reaction time of 15 seconds was chosen in order to avoid physical injury to the animal.[16]

Ice plate method

Ice plate test was carried out as described by Sundaram *et al.*[17] After 30 minutes of drug, vehicle or saline administration, all rat, one at a time was gently placed on the ice cubes (0–4°C) filled in a transparent container (20 cm × 20 cm × 20 cm) and covered with a plastic cover. The rat was visualized through the transparent wall and latency time (s) taken to lick the fore or hind paws to cold sensation was noted. The latency time of animal to cold sensation taken just before administration of drug, vehicle or saline on day first day of the study was considered as the initial control values for comparison. A cut-off reaction time was set at 15 seconds in order to avoid tissue damage to the animals due to frozen temperature.

Randall-Selitto method

The analgesic activity of the drug against mechanically induced pain was measured by Randall–Selitto assay.[18] After 30 minutes of drug, alcohol or saline administration, the rats were gently held in the hand. Afterward, the paw of the right foot of the rat was placed on the rubber base of the apparatus and pressure (in pounds; expressed in grams) was applied either on 2^{nd}–3^{rd} or 3^{rd}–4^{th} metatarsal region through a pointed tip and increased gradually until vocalization elicited which was considered as threshold pressure to mechanical induced pain. Threshold pressure to mechanical induced pain taken on first day just before the administration of drug, alcohol or saline was considered as the initial control values for comparison.

Central Nervous System depressant activity

Central Nervous System depressant activity was performed using (i) Rota-rod and (ii) open field methods

Rota-rod Test method

Motor coordination and grip strengths were measured using the automated rota-rod apparatus.[19] The rats capable of remaining on the rota-rod for 60 second or more, in three successive trials were selected for the study. After 30 minutes of drug, vehicle or saline administration, the rats were gently placed on the rotor with the body axis perpendicular to the rotor's long axis with the head directed opposite to the direction of rotating rod (5 rpm) and the fall-off time from the rod was noted for each rat. The control grip strengths of the rats were measured on first day just before administration of the drug, vehicle or saline for comparison.

Open field test method

For recording the locomotor activity of the rats, the Open Field Test was used.[20] The apparatus (96 cm × 96 cm × 6 cm) was made up of the wooden box and divided in to 36 equal squares which were painted alternatively with black and white colors. At the time of the experiment, it was illuminated with low-intensity diffuse light (40 W) placed at a height of 100 cm. The rats were placed gently in the center of the apparatus one after another and the number of squares crossed in 5 minutes was recorded before and 30 minutes after drug, vehicle or saline administration. The floor of the box was cleaned after every trial.

Statistical Analysis

The data were expressed as mean ± standard error of the mean. The difference between mean values of groups were statistically analyzed by student's' t- test. $P < 0.05$ were considered significant.[21]

RESULTS

Analgesic Activity

Hot plate test

Table 1 represents the data for the analgesic effect of different potencies (3X, 6X, 12X and 30C) of *Solanum nigrum*. The latency time required to raise and lick the hind paw for thermal stimulus was more or less same (3.38–3.67 seconds) in untreated (on first day before administration of drug/vehicle) and saline-treated animals when measured on different days of the experiment. On the other

hand, the animals treated with different potencies of *Solanum nigrum* and vehicle at a dose of 0.5 ml/rat/day showed increase in the latency time (5.22–5.88 seconds) to thermal stimulus when measured after 30 minutes of drug/vehicle administration on 10th day. The difference in the increase in latency time to hot sensation was significant ($P < 0.05$) with those groups which were treated with 3X and 6X potencies of *Solanum nigrum*. Thereafter, the increase in the duration of latency time tapered off gradually on 20th day and 30th day of treatment [Figure 1].

Table 1: Analgesic effect of *Solanum nigrum* (0.5 ml/rat/day) on a hot plate test

Groups	Latency time to pain response during treatment in seconds			
	Initial	10th day	20th day	30th day
Control (saline)	3.45±0.52	3.53±0.34	3.46±0.36	3.38±0.39
Vehicle (91.5% alcohol)	3.59±0.42	5.22±0.47	4.48±0.42	4.01±0.41
S. nigrum 3X	3.61±0.37	5.88±0.38*	4.59±0.32	4.05±0.40
S. nigrum 6X	3.65±0.48	5.46±0.44*	4.59±0.32	4.05±0.33
S. nigrum 12X	3.67±0.47	5.42±0.49	5.49±0.50	4.25±0.38
S. nigrum 30C	3.58±0.46	5.24±0.44	4.47±0.52	4.19±0.38

Values are mean±SEM. *Significantly different at $P<0.05$. *S. nigrum: Solanum nigrum*; SEM: Standard error of the mean

Table 2: Analgesic effect of *Solanum nigrum* (0.5 ml/rat/day) on ice plate test

Groups	Latency time to pain response during treatment in seconds			
	Initial	10th day	20th day	30th day
Control (saline)	5.91±0.45	6.09±0.37	5.80±0.55	6.04±0.43
Vehicle (91.5% alcohol)	5.85±0.51	7.76±0.56	7.36±0.48	6.44±0.37
S. nigrum 3X	6.00±0.50	8.06±0.52*	6.92±0.49	6.34±0.49
S. nigrum 6X	6.01±0.42	8.09±0.50*	7.72±0.50	6.34±0.44
S. nigrum 12X	5.94±0.52	7.56±0.52	6.89±0.51	6.18±0.37
S. nigrum 30C	5.99±0.51	7.31±0.41	7.21±0.44	6.17±0.44

Values are mean±SEM. *Significantly different at P<0.05. *S. nigrum: Solanum nigrum*; SEM: Standard error of the mean

Ice plate test

Table 2 represents the data for the analgesic effect of different potencies (3X, 6X, 12X and 30C) of *Solanum nigrum*. The latency time required to raise and lick the hind paw for cold sensation was more or less same (5.80–6.09 seconds) in untreated and saline-treated animals when measured on different days of the experiment whereas *Solanum nigrum* in different potencies and vehicle at a dose of 0.5 ml/rat/day had significantly increased the latency time (7.31–8.09 seconds) on 10th day which was gradually tapered off on 20th day and 30th day of treatment. The difference was significant ($P < 0.05$) only with those rats treated with 3X and 6X when compared to initial latency time taken just before administration of drug on first day of the study [Figure 2].

Randall-Selitto assay

The results of the analgesic effect of different potencies of *Solanum nigrum* are presented in Table 3. The quantum of threshold pressure required to elicit vocalization to applied mechanical pain was more or less same (131.33–133.66 g) on first day before administration of drug, vehicle or saline and 30 minutes after the administration of saline

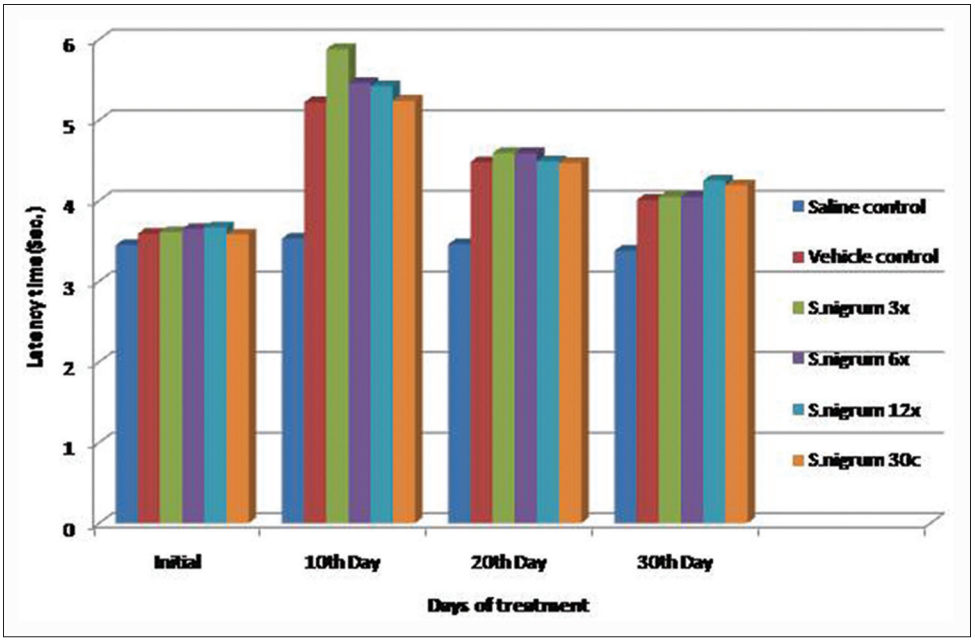

Figure 1: Analgesic effect of *Solanum nigrum* on hot plate test

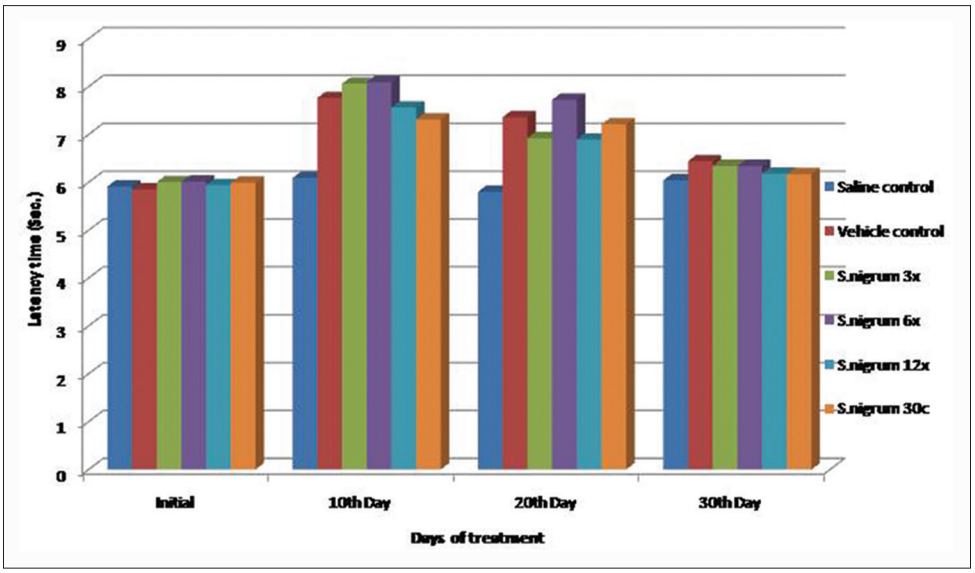

Figure 2: Analgesic effect of *Solanum nigrum* on ice plate test

on different days of experimentation. There was an increase in the quantum of applied threshold pressure (145.00–148.66 g) required to elicit vocalization to mechanical pain when measured 30 minutes after the administration of different potencies (3X, 6X, 12X and 30C) of *Solanum nigrum* and vehicle at a dose of 0.5 ml/rat/day on 10th day. The difference was significant ($P < 0.05$) only with those rats treated with 3X and 30C potencies when compared to that of normal saline-treated rats. Afterward, the increase in the quantum of threshold pressure required to elicit vocalization to applied mechanical pain tapered off gradually on 20th day and 30th day of experiments [Figure 3].

Central Nervous System depressant activity

Rota-rod test

The results of different potencies (3X, 6X, 12X and 30C) of *Solanum nigrum* on motor coordination activity of rats are summarized in Table 4. Untreated rats and rats administered with saline stayed on the rotating rod on an average of 49.07–51.55 seconds whereas, rats treated with different potencies of *Solanum nigrum* and vehicle at a dose of 0.5 ml/rat/day fell between 32.81 and 38.05 seconds when they were subjected to test on 10th day of experiment after 30 minutes of drug administration. The decrease in grip strength was significant ($P < 0.05$) only in those rats treated with 3X and 6X potencies of *Solanum nigrum*. Afterward, there was a progressive increase in the grip strength of drug/vehicle treated rats as they stayed comparatively for more time on the rotating rod when tested on 20th and 30th day of experiment though stay on rotating rod was still less than that of control initial values observed in the same group on first day just before administration of drug or with that of saline administered rats [Figure 4].

Table 3: Analgesic effect of *Solanum nigrum* (0.5 ml/rat/day) on Randall–Selitto test

Groups	Pressure on rat paw in gram applied during treatment			
	Initial	10th day	20th day	30th day
Control (saline)	132.00±4.55	132.66±3.89	131.33±3.75	133.33±4.40
Vehicle (91.5% alcohol)	133.66±3.71	145.66±4.33	142.33±4.33	138.33±4.73
S. nigrum 3x	132.33±3.38	147.33±4.05*	145.00±3.78	140.00±4.72
S .nigrum 6x	132.00±4.00	145.00±4.61	144.66±4.33	141.00±4.66
S .nigrum 12x	133.66±3.84	145.66±4.66	146.66±3.75	140.00±4.16
S. nigrum 30c	133.00±3.51	148.66±3.17*	147.66±3.75	140.33±5.33

Values are mean±SEM. *Significantly different at $P<0.05$. *S. nigrum: Solanum nigrum*; SEM: Standard error of mean

Table 4: Behavioral effect of *Solanum nigrum* (0.5 ml/rat/day) on rota-rod test

Groups	Grip strength used during treatment in seconds			
	Initial	10th day	20th day	30th day
Control (saline)	51.53±2.84	51.55±2.39	49.97±2.61	50.70±3.45
Vehicle (91.5% alcohol)	49.07±3.64	35.47±3.61	43.93±2.33	47.43±2.44
S.nigrum 3x	50.31±3.47	32.81±2.71*	39.92±2.90	47.74±2.46
S. nigrum 6x	50.69±2.57	33.72±3.17*	39.50±3.55	43.91±2.09
S. nigrum 12x	50.33±3.21	38.05±3.61	42.79±2.70	45.29±3.27
S. nigrum 30c	50.59±3.26	37.46±3.65	39.71±2.65	46.68±3.72

Values are mean±SEM. *Significantly different at $P<0.05$. *S. nigrum: Solanum nigrum*; SEM: Standard error of mean

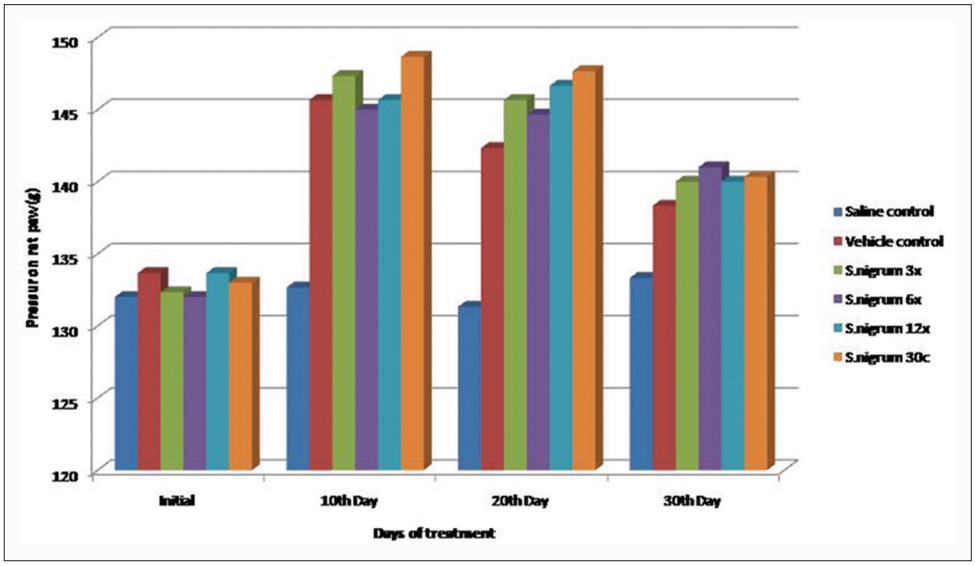

Figure 3: Analgesic effect of *Solanum nigrum* on Randall–Selitto test

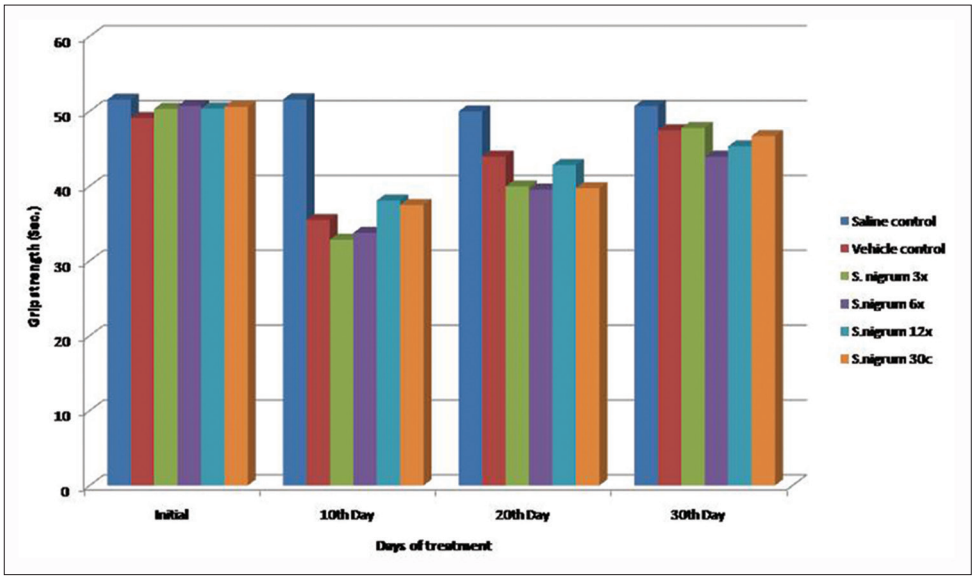

Figure 4: Behavioral effect of *Solanum nigrum* on rota-rod test

Open field test

The results of different potencies (3X, 6X, 12X and 30C) of *Solanum nigrum* and vehicle on locomotor activity of rats are presented in Table 5. Control untreated rats and rats administered normal saline crossed on an average of (63.00–65.66) squares in 5 minutes. On the other hand, there was a decrease (46.00–47.33 squares in 5 minutes) in locomotor activity of rats administered *Solanum nigrum* and vehicle at a dose of 0.5 ml/rat/day when tested after 30 minutes of drug administration on 10th day of experiment. The decrease in locomotor activity was significant ($P < 0.05$) only with those rats treated with 6X and 12X potencies of *Solanum nigrum* when compared to initial latency time taken just before administration of drug on first day of the study [Figure 5].

DISCUSSION AND CONCLUSION

Solanum nigrum is used as homoeopathic drug to treat various ailments including many symptoms of neurological disorders clinically, but has not garnered the attention to verify its use experimentally.[15] Therefore, the present preliminary study was carried out for the first time with an objective to assess the analgesic activity using hot plate, ice plate and Randall–Selitto assays and CNS depressant activities using Rota-rod Test (for motor coordination) and Open Field Test (for locomotor activity) of

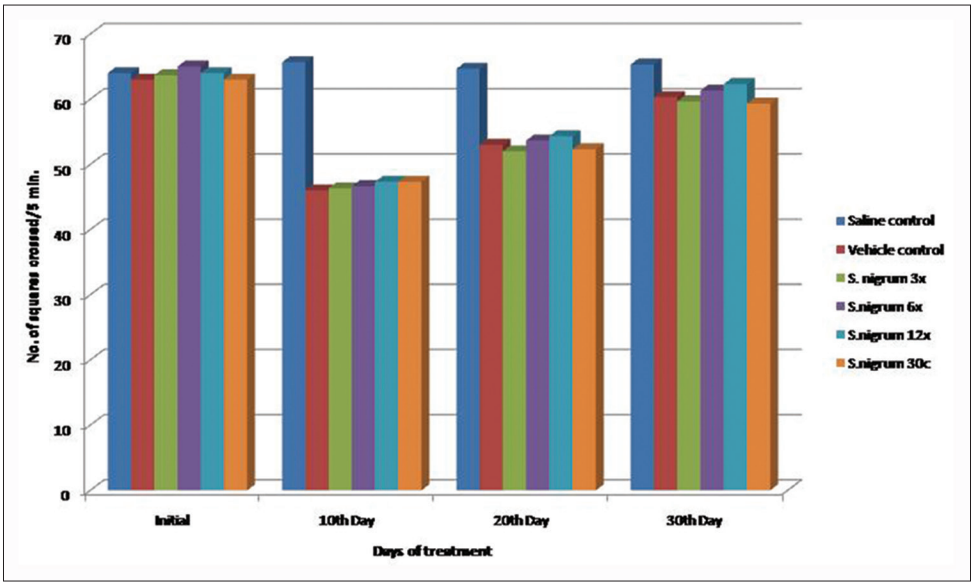

Figure 5: Behavioral effect of *Solanum nigrum* in open field test

Table 5: Behavioral effect of *Solanum nigrum* (0.5 ml/rat/day) in open field test

Groups	Number of squares crossed during treatment in 5 minutes			
	Initial	10th day	20th day	30th day
Control (saline)	64.00±3.51	65.66±4.70	64.66±3.52	65.33±4.80
Vehicle (91.5% alcohol)	63.00±4.00	46.00±4.93	53.00±3.21	60.33±4.17
S. nigrum 3x	63.66±4.33	46.33±4.66	52.00±4.93	59.66±3.92
S. nigrum 6x	65.00±3.60	46.66±3.75*	53.66±3.84	61.33±3.71
S. nigrum 12x	64.00±3.05	47.33±4.25*	54.33±4.66	62.33±4.25
S. nigrum 30c	63.00±4.35	47.33±4.25	52.33±3.38	59.33±3.28

Values are mean±SEM. *Significantly different at $P<0.05$. *S. nigrum: Solanum nigrum*; SEM: Standard error of mean

homoeopathic potencies of *Solanum nigrum* (3X, 6X, 12X and 30C) in albino rats.

The data for the analgesic effect showed that all the four (3X, 6X, 12X and 30C) potencies of *Solanum nigrum* has increased the latency time required to raise and lick the hind paw for thermal sensation on hot plate assay and for cold sensation on ice plate assay and also increased the quantum of threshold pressure to mechanical induced pain on Randall–Selitto assay when measured 30 minutes after the administration of different potencies (3X, 6X, 12X and 30C) of *Solanum nigrum* on 10th day. These effects were gradually tapered off on 20th day and 30th day of treatment.

The data obtained for CNS depressant effect showed that different potencies (3X, 6X,12X and 30C) of *Solanum nigrum* has significantly decreased motor co-ordination on Rota-rod Test and locomotor activity in Open Field Test. The depressant effect was the maximum on 10th days and slowly decreased thereafter very similar to that of analgesic response. The level of significance varied not only between the potencies of the drug but also between different sets of experiments as compared to the values of saline group or initial values taken just before administration of drug on first day of the study.

Increased in the latency time to noxious thermal stimulus and/or cold sensation, and increased in the quantum of threshold pressure to mechanically induced pain on drug treatment are the clear indication that the drug possess analgesic effect.[22] Similarly, decreased motor coordination and locomotor activity of the drug is the sign of CNS depression.[23] Wearing off the analgesic and depression on prolonged and continuous use of the drug may be either due to decreased sensitivity of the CNS or due to increased metabolizing enzymatic activity in the liver.

The present preliminary study demonstrates that the Homoeopathic potencies (3X, 6X, 12X and 30C) of *Solanum nigrum* possess analgesic and CNS depressant effect. However, further detailed investigations are needed for its possible clinical use.

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प्रायोगिक जंतु मॉडलों में सोलेनम नाइग्रम (Linn.) की होम्योपैथिक शक्तिमत्ताओं की वेदनाहर तथा केंद्रीय तंत्रिका तंत्र अवसादक सक्रियता का मूल्यांकन करने हेतु अन्वेषण

सार

पृष्ठभूमि व उद्देश्य: होम्योपैथी में सोलेनम नाइग्रम का प्रयोग अर्गट रोग, तंत्रिका शोथ व दंतविन्यास के दौरान जलन तथा तंत्रिकीय विकारों के कुछ लक्षणों की चिकित्सा में प्रयोग होता है परंतु इसकी केंद्रीय तंत्रिका तंत्र संभाव्यता को अभी तक प्रायोगिक रूप से नहीं खोजा गया है। अतः प्रायोगिक जंतु मॉडलों में सोलेनम नाइग्रम की होम्योपैथिक क्षमताओं के वेदनाहर तथा केंद्रीय तंत्रिका तंत्र (केंद्रीय तंत्रिका तंत्र) अवसादक प्रभावों का मूल्यांकन करने के उद्देश्य से पहली बार वर्तमान प्रारंभिक अन्वेषण आरंभ किया गया।

सामग्री व विधि: विस्टर एल्बीनो चूहों पर वेदनाहर के लिए उष्ण पट्टिकाएँ, बरफ पट्टिका तथा रैंडाल; सेलिटो आमापन; केंद्रीय तंत्रिका तंत्र अवसादक सक्रियता के लिए घूर्णी छड़ तथा खुला क्षेत्र परीक्षण का उपयोग कर अध्ययन किया गया। सोलेनम नाइग्रम की विभिन्न शक्तिमत्ताओं (3x, 6x, 12x तथा 30सी) को 30 दिनों तक प्रत्येक चूहे (0.5 मि.ली.) को मुख द्वारा पिलाया गया और चिकित्सा के 10वें, 20वें तथा 30वें दिन औषधि देने के 30 मिनट बाद अनुक्रिया का आकलन किया गया।

परिणाम: परिणाम दर्शाता है कि सोलेनम नाइग्रम की सभी चारों शक्तिमत्ताओं से उष्ण पट्टिका परीक्षण में तापीय संवेदन हेतु और बरफ पट्टिका परीक्षण में शीत संवेदन हेतु पंजों को उठाकर चाटने के लिए आवश्यक प्रसुप्ति समय में वृद्धि हुई और रैंडाल- सेलिटो आमापन पर यांत्रिक प्रेरित पीड़ा हेतु देहली दाब की मात्रा में भी वृद्धि हुई परंतु प्रेरक समन्वय तथा चलन सक्रियता में कमी आई है।

निष्कर्ष: इस प्रारंभिक अन्वेषण से प्राप्त परिणाम इंगित करते हैं कि सोलेनम नाइग्रम की विभिन्न शक्तिमत्ताओं में वेदनाहर तथा केंद्रीय तंत्रिका तंत्र अवसादक सक्रियता होती है। वर्तमान कार्य एक प्रारंभिक प्रयास था। मनुष्यों में इसके संभावित उपयोग हेतु और विस्तृत अन्वेषण आवश्यक है।

शब्दकोश: होम्योपैथिक औषधि, सोलेनम नाइग्रम, वेदनाहर, केंद्रीय तंत्रिका तंत्र अवसादक सक्रियता, एल्बीनो चूहे।