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Ameliorative effects of standardized extract from *Trigonella foenum-graecum* L. seeds on painful peripheral neuropathy in rats

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ABSTRACT

Objective: To evaluate the effects of the standardized extract of fenugreek (*Trigonella foenum-graecum* L. Family: Leguminasae) seed (IND01) in animal models of peripheral neuropathy. **Methods:** IND01 was prepared from fenugreek seeds and standardized by high performance liquid chromatography to a marker compound, trigonelline. The effects of daily oral administration of IND01 (50, 100 and 200 mg/kg) were studied in rats after partial sciatic nerve ligation (PSNL) and sciatic nerve crush injury (SNCI) during 30-days period. The measurements on thermal hyperalgesia (TH), motor function test (MFT) score and motor nerve conduction velocity (MNCV) were recorded. **Results:** IND01 offered sustained protection against TH and deranged MFT scores in both models from 7-day onwards. Fifteen days of daily oral administration of IND01 restored MNCV reduction in rats with SNCI but not with PSNL. **Conclusions:** IND01 was found to be effective in rat models of painful peripheral neuropathy.

1. Introduction

Peripheral neuropathy is a problem with the nerves that can produce pain, loss of sensation, and an inability to control muscles. Complaints of pain, sensation of "pins and needles," numbness, and tingling are frequent in the distribution of the affected nerve. Peripheral neuropathy, associated with diabetes mellitus (DM), neurotoxic chemotherapy, human immunodeficiency virus/antiretroviral drugs, alcoholism, nutrient deficiencies, heavy metal toxicity, and other etiologies, results in significant morbidity[1]. Conventional pain medications primarily mask symptoms and have significant side effects and addiction profiles. At the same time, widening body of research indicates medicine from natural source may offer significant benefit to this patient population.

Trigonella foenum-graecum L. (Fabaceae), also known as fenugreek, is one of the oldest medicinal plants, and has a long history of medical uses in Ayurvedic and Chinese traditional medicine. There are several reports concerning the anti-inflammatory, antipyretic and antinociceptive effects of the fenugreek in traditional medicinal literature[2] and use of fenugreek for relieving skin inflammation is approved by many regulatory agencies *e.g.* German Commission E[3]. Fenugreek leaves were reported to be potent anti-inflammatory[4] and analgesic[5] properties in animal models of inflammation and pain respectively. These effects are attributed to alkaloid content of fenugreek[6].

Trigonelline (N-methylnicotinic acid) is a major alkaloid of fenugreek seeds along with other constituents like 4-hydroxyisoluecine, and galactomannans[7,8]. Trigonelline is shown to be potent antidiabetic[9] and antioxidant[10] compound. Therefore, fenugreek extract standardized to bioactive marker compound, trigonelline can prove to be beneficial in management of neuropathic pain.

The fenugreek seed extract (IND01) has been shown to be effective in animal models of DM[7,11,12]. Moreover, its major alkaloid, trigonelline, showed anti-diabetic effects in animal models and patients[13] of DM. However, IND01 or trigonelline, is not yet evaluated in management of neuropathic pain. Therefore, the objective of the present work was to standardize the fenugreek extract (IND01) for the marker compound, trigonelline and to evaluate its effects in the animal models of peripheral neuropathic pain.

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2. Materials and methods

2.1. Animals

Male Wistar rats (150-200 g) were purchased from National Toxicology Centre, Pune. During the experiment, rats were housed at standard housing condition like temperature of (25 ±1)℃, relative humidity of 45%-55% and 12 h light: 12h dark cycle. Rats had free access to food pellets (Navmaharashtra Chakan Oil Mills Ltd., Sangli, India) and tap water *ad libitum* during the experiments. All experiments are in accordance with ethical guidelines for investigations of experimental pain in conscious animal. Research protocol was approved by Institutional Animal Ethics Committee of Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune.

2.2. Chemicals

Pyridoxine hydrochloride (HCl) (Sigma-Aldrich, USA) and anaesthetic ether (TKM Pharma, Hyderabad, India) were purchased from respective vendors. All other chemicals were of analytical grade. The seeds collected in the areas of State of Rajasthan in India during November 2006 were used in the preparation of test compound, IND01. The seeds were authenticated by Dr. A. M. Mujumdar, Agharkar Research Institute, Pune, India and voucher specimen was deposited at that Institute.

2.3. Extraction and standardization

The IND01 is an *Trigonella foenum-graecum* seed extract and prepared by reported procedure[14]. Briefly, Fenugreek seeds (1 000 g) purchased locally were flaked to a thickness of 5 mm, loaded in an extractor and water: alcohol (30:70) was circulated in the extractor at room temperature for 8 h. The extract was drained and distilled to remove alcohol. The marc was extracted with hexane (5 L) to remove oil and other lipids. The oil free water extract was passed through strong acid cation exchange resin in gel (500 mL). The resin was thoroughly washed with de-ionized water to remove adhering sugars and other contaminants. The column was eluted with 10% aqueous ammonia. The solution was concentrated to remove ammonia and water. The resulting powder (IND01) was made free flowing using spray drying process and used for further study (Yield: 9.0 g, 0.9% w/w).

The standardization of IND01 was done on the marker compound, trigonelline by HPLC as follows: 50 mg of IND01 powder was dissolved in 100 mL demineralized water with stirring. The solution was filtered through Whatman filter paper (#41) and injected in HPLC system. Similarly, the preparation of reference standard of trigonelline HCl (Sigma-Aldrich, USA, ≥ 98.0%) was prepared with 10 mg dissolved in 100 mL of demineralized water. The HPLC conditions were as follows: Model - JASCO LC 2000 with UV-2075; Column: Reverse phase C-18 column L1 (250 mm × 4.6 mm, particle size of 5 μm); Detector: UV-VIS; Mobile phase: trifluroacetic acid (60) with water: acetonitrile mixture (40). Injection volume: 20 μL, Method time: 30 mins, Flow rate: 1.5 mL/min; Detector : UV (at 254 nm). The area under the curve (AUC) was recorded from the resultant chromatogram and content of marker compound (trigonelline HCl) was calculated. Solutions of IND01 were freshly prepared in distilled water and was administered as mg per kg basis for further evaluation in experimental animals.

2.4. Induction of mononeuropathy caused by partial sciatic nerve ligation (PSNL) in rats

The neuropathic pain was produced in rats by PSNL according to reported procedure[15]. On baseline (day 0), the rats were divided into following groups of 6 rats each(Ⅰ) vehicle control (Ⅱ) sham control (Ⅲ) PSNL control and (Ⅳ) IND01 (50 mg/kg, *p.o.*) (Ⅴ) IND01 (100 mg/kg, *p.o.*) and (Ⅵ) IND01 (200 mg/kg, *p.o.*) treated (Ⅶ) pyridoxine HCl (100 mg/kg, *p.o.*) group. In rats of group-Ⅲ, Ⅳ, Ⅴ, Ⅵ and Ⅶ, the right sciatic nerve was exposed and underwent partial injury with the help of silk suture. In rats of group Ⅱ (sham control), exposure of nerve was conducted but PSNL was not done. After surgical procedures, the wounds were aseptically closed with sutures. In rats of group Ⅰ (vehicle control), no surgical procedure was performed. Sciatic nerve of left leg was kept untouched in all rats. Rats were treated with antibiotics and returned to their cages and allowed to recover after surgery for 2 days. After recovery, rats from group Ⅰ, Ⅱ and Ⅲ were treated with once daily administration of distilled water for next 30 days. Rats from group Ⅳ, Ⅴ, Ⅵ and Ⅶ received once a day treatment of IND01 (50 mg/kg, *p.o.*), IND01 (100 mg/kg, *p.o.*), IND01 (200 mg/kg, *p.o.*) and pyridoxine HCl (100 mg/kg, *p.o.*) respectively for next 30 days.

2.5. Induction of mononeuropathy caused by sciatic nerve crush injury (SNCI) in rats

SNCI was induced in rats according to reported method[16] and its modification[17]. The rats were divided into following groups of 6 rats each (Ⅰ) vehicle control (Ⅱ) sham control (Ⅲ) SNCI control and (Ⅳ) IND01 (50 mg/kg, *p.o.*) (Ⅴ) IND01 (100 mg/kg, *p.o.*) and (Ⅵ) IND01 (200 mg/kg, *p.o.*) treated groups (Ⅶ) pyridoxine HCl (100 mg/kg, p.o.). In rats of group-Ⅲ, Ⅳ, Ⅴ, Ⅵ and Ⅶ, the right sciatic nerve was exposed at high thigh level under ether anaesthesia and aseptic conditions and was crushed using a aneurysm clip twice for 30 s with an interval of 60 s between. In rats of group Ⅱ (sham control), the sciatic nerve was explored but not crushed performed. After surgical procedures, the wound closure and recovery procedure, treatments and measurements were as per repeated as performed in PSNL model.

2.6. The measurements

The parameters of the pain related parameters were recorded daily in the morning (10 am to 12 pm) and doses were administrated immediately afterwards. Measurement of thermal hyperalgesia (TH) was done using principle of Eddy and Leimbach using hot-plate method[17]. A plastic cylinder was used to confine the rats on the electrically heated surface of the hot-plate analgesiometer (Ugo Basile, Versace, Italy) which was maintained at (55.0±0.5)℃. The rats were individually placed on the heated plate and the time was recorded when either licking of hind paw or jumping (pain latency) occurs. Time for pain latency was recorded on day 0, 2, 7, 14, 21, 28 following treatment. A cut off time of 22 seconds was adopted to avoid tissue injury.

Motor functions test (MFT) were measured by scoring

spontaneous gait and hind paw posture at three 5-minutes (300 sec.) interval (total-15 min) by reported method[18]. The rats were placed in the plexiglass chamber and allowed to acclimatize before the observation period. Each rat was individually observed for three 5-minutes (300 sec.) interval (total-15 min). The positions of the lesioned hind paw were graded between 0-5. Each rat was scored 3 times and mean was calculated. The observations were made on day 0, 2, 15 and 30. Based on the grading, the MFT score was determined as per following formula: MFT score = [1(T1) + 2(T2) + 3(T3) + 4(T4) + 5(T5)] / 300. MFT is a measure of Pain. More the MFT score, more is the pain.

Measurement of motor nerve conduction velocity (MNCV) was performed in rats on 15th day after PSNL or SNCI as per reported method[18,19]. Rats were anesthetized and hairs form dorsal side of rats paws were shaved with hair remover cream. The paws of rats were cleaned using a moist cotton plug. MNCV was recorded by stimulating the sciatic and tibial nerves at sciatic and tibial notch respectively. The recording specification: stimulator (Weltronics India), pulse (0.1 ms square wave), with a pair of monopolar needle electrodes (1.0-1.5 mA, 2.0 mV/D). Responses were recorded from the plantar muscles using data acquisition system (Biopac, Santa Barbara, CA, USA). MNCV was determined using following formula: MNCV (m/s) = [Distance between the sciatic and tibial stimulation points (in m)]/ [latency for sciatic (in s) -latency for tibial (in s)].

2.7. Statistical analysis

Data for TH was expressed as mean pain latency in paws (in s) ± SEM and was analyzed by two-way repeated measures ANOVA followed by *post hoc* Bonferroni test for comparison of groups. MFT and MNCV data was expressed as mean parameter value (in s) ± SEM. The data for MFT was analysed by two-way repeated measures ANOVA followed by Bonferroni test for comparison of groups. MNCV data was analysed by one-way ANOVA followed by Dunnett's multiple comparison test for comparison of groups.

3. Results

3.1. Standardization

At wavelength of 254 nm, the HPLC of sample of IND01 showed presence of the marker compound, trigonelline HCl, at retention time of 1.750 with AUC = 14 704.3 (Figure 1). The reference standard of trigonelline HCl showed peak at retention time 1.775 min with AUC = 15 922.2 (Figure 1). The concentration of trigonelline in the sample of IND01 was found to be 18.1%.

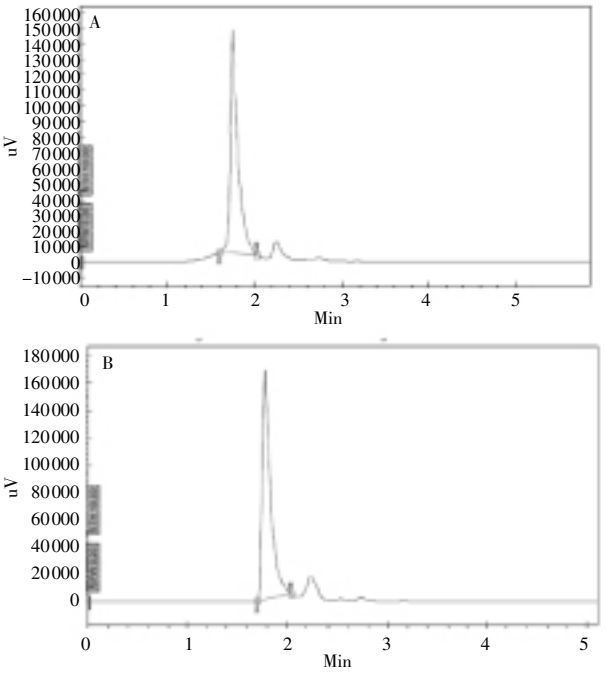

Figure 1. The HPLC fingerprint of (A) IND01 (retention time = 1.750) and (B) reference standard (retention time = 1.775) at wavelength of 254 nm for detection of the marker compound, trigonelline HCl.

3.2. Effect on TH in Hot-plate test after PSNL in rats

No significant change was observed in mean paw withdrawal latencies in normal and sham control rats during the study period (Table 1). PSNL in rats resulted in significant ($P < 0.001$) decrease in mean paw withdrawal latencies on day-2 till day-14 as compared with normal or sham control rats confirming TH (Table 1). The reduction in paw withdrawal latencies were significantly reversed by daily treatment of IND01 (50, 100 or 200 mg/kg, p.o.) and pyridoxine HCl (100 mg/kg) on day-2 days and onwards (Table 1).

Table 1
Effect of IND01 on mean pain latency (sec.) against TH in hot-plate test after PSNL in rats.

Group	Mean pain latency (sec) ± SEM			
	Day 0	Day 2	Day 8	Day 14
Normal	10.02 ± 0.44	10.37 ± 0.35	9.62 ± 0.42	10.65 ± 0.34
Sham control	9.93 ± 0.35	7.63 ± 0.37	9.17 ± 0.15	10.40 ± 0.22
PSNL control	10.43 ± 0.30	3.28 ± 0.15$^{\#\#\#,\$\$\$}$	3.67 ± 0.13$^{\#\#\#,\$\$\$}$	3.82 ± 0.15$^{\#\#\#,\$\$\$}$
IND01 (50) + PSNL	9.63 ± 0.39	3.80 ± 0.25	4.03 ± 0.14	4.52 ± 0.17
IND01 (100) + PSNL	10.13 ± 0.39	4.30 ± 0.25*	8.03 ± 0.14***	9.52 ± 0.17***
IND01 (200) + PSNL	10.43 ± 0.30	4.98 ± 0.11***	8.10 ± 0.08***	8.75 ± 0.18***
Pyridoxine HCl (100) + PSNL	10.00 ± 0.56	4.53 ± 0.18**	8.68 ± 0.11***	9.92 ± 0.14***

Data was expressed as mean pain latency in paws with PSNL (in seconds) ± SEM for six rats per group. Data was analyzed by two-way repeated measures ANOVA followed by *post hoc* Bonferroni test for comparison of groups. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ as compared with normal rats. $^{\$\$\$}P < 0.001$ as compared with sham control group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with PSNL control group.

3.3. Effects on MFT score after PSNL in rats

No significant change was observed in MFT scores (spontaneous gait and hind paw posture) in normal and sham control rats during the study period (Table 2). PSNL increased the MFT score significantly ($P < 0.001$) compared with normal or sham control rats (Table 2). In PSNL rats, MFT score was significantly increased on day-2 (2.92) and reached to 2.98 (day-15) and 3.02 (day-30) indicating more pain. PSNL-induced increase in MFT score was prevented by daily treatment of IND01 (100 and 200 mg/kg) for 15 days onwards whereas pyridoxine HCl (100 mg/kg) showed significant ($P < 0.01$) prevention of MFT score on day-2 and onwards. Daily treatment of IND01 (50 mg/kg, *p.o.*) did not alter the MFT score as compared to PSNL control rats (Table 2).

3.4. Effect on MNCV after PSNL in rats

The MNCV in normal and sham control rats was found to be 53.14 and 46.89 m/s respectively as observed on day-15 of the study (Table 2). PSNL control rats showed MNCV of 20 m/s, which is significantly ($P < 0.001$) less as compared with normal or sham control rats (Table 2) indicating neuropathy induction. Treatment of IND01 (50, 100 or 200 mg/kg) for 15 days did not change MNCV as compared with PSNL control rats (Table 2). On 15-days of treatment, pyridoxine (100 mg/kg, *p.o.*) showed MNCV of 31.25 m/s, which was significantly ($P < 0.01$) more compared with MNCV of PSNL control rats (Table 2).

3.5. Effect on TH in hot-plate test after SNCI in rats

The mean paw withdrawal latencies in normal rats were in the range of 10.97 to 11.87 s and that of sham control rats were 9.55 to 11.92 s during the study period (Table 3). SNCI in rats resulted in significant ($P < 0.001$) decrease in mean paw withdrawal latencies on day-2 onwards compared to normal or sham control rats confirming TH (Table 3). Mean paw withdrawal latencies after daily treatment of IND01 (100 and 200 mg/kg) or pyridoxine (100 mg/kg) were significantly ($P < 0.001$) more compared to mean paw latencies of SNCI control rats on 7-days of treatment (Table 3). IND01 (50 mg/kg, *p.o.*) group showed mild and significant ($P < 0.05$) increase in paw withdrawal latencies on 14-days treatment (Table 3).

3.6. Effect on MFT score after SNCI in rats

No significant change was observed in MFT scores (spontaneous gait and hind paw posture) in normal and sham control rats during the study period (Table 4) indicating induction of neuropathy. SNCI increased the MFT score significantly ($P < 0.001$) as compared with normal or sham control rats (Table 4). In SNCI rats, MFT score was increased from 2.92 (on day-2) to 2.98 and 3.03 (on day-15 and day-30) respectively. SNCI-induced increase in MFT score was prevented by daily treatment of IND01 (50, 100 and 200 mg/kg) or pyridoxine HCl (100 mg/kg) for 15 days onwards. On

Table 2
Effect of IND01 on MFT score and MNCV after PSNL in rats

Group	MFT score± SEM				MNCV (m/s) Day 15
	Day 0	Day 2	Day 15	Day 30	
Normal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	53.14 ± 4.01
Sham control	0.00 ± 0.00	0.27 ± 0.05	0.09 ± 0.03	0.10 ± 0.02	46.89 ± 1.58
PSNL control	0.00 ± 0.00	2.92 ± 0.08$^{###, \$\$\$}$	2.98 ± 0.20$^{###, \$\$\$}$	3.02 ± 0.07$^{###, \$\$\$}$	20.00 ± 0.77$^{###, \$\$\$}$
IND01 (50) + PSNL	0.00 ± 0.00	3.06 ± 0.06	2.99 ± 0.04	3.19 ± 0.04	22.20 ± 0.89
IND01 (100) + PSNL	0.00 ± 0.00	2.91 ± 0.11	1.99 ± 0.04***	1.99 ± 0.04***	22.95 ± 0.89
IND01 (200) + PSNL	0.00 ± 0.00	2.71 ± 0.11*	1.98 ± 0.06**	1.98 ± 0.06***	23.75 ± 0.96
Pyridoxine HCl (100) + PSNL	0.00 ± 0.00	2.63 ± 0.06**	1.09 ± 0.03***	1.09 ± 0.03***	31.25 ± 1.42**

Data was expressed as mean parameter value (in seconds) ± SEM for six rats per group. Data for MFT was analyzed by two-way repeated measures ANOVA followed by *post hoc* Bonferroni test for comparison of groups. Data for MNCV was analyzed by one-way ANOVA followed by post hoc Dunnett´s multiple comparison test for comparison of groups. $^{###}$ $P < 0.001$ as compared with normal rats. $^{\$\$\$}$ $P < 0.001$ as compared with sham control group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared with PSNLcontrol group.

Table 3
Effect of IND01 on mean pain latency (sec.) against thermal hyperalgesia in hot-plate test after SNCI in rats

Group	Mean pain latency (sec) ± SEM			
	Day 0	Day 2	Day 14	Day 28
Normal	11.87 ± 0.48	11.68± 0.49	10.97 ± 0.34	11.40 ± 0.54
Sham control	11.92 ± 0.33	9.55 ± 0.22	11.40 ± 0.37	11.38 ± 0.35
SNCI control	11.92 ± 0.28	4.97 ± 0.19$^{###,\$\$\$}$	5.23 ± 0.07$^{###,\$\$\$}$	4.78 ± 0.20$^{###,\$\$\$}$
IND01 (50) + SNCI	11.83 ± 0.34	4.67 ± 0.17	6.42 ± 0.20*	5.98 ± 0.18*
IND01 (100) + SNCI	12.13 ± 0.34	4.97 ± 0.17	8.72 ± 0.20***	10.98 ± 0.18***
IND01 (200) + SNCI	12.22 ± 0.31	4.95 ± 0.18	8.72 ± 0.20***	10.98 ± 0.18***
Pyridoxine HCl (100) + SNCI	12.42 ± 0.37	4.95 ± 0.12	9.98 ± 0.35***	11.85 ± 0.30***

Data was expressed as mean pain latency in paws (in seconds) ± SEM for six rats per group. Data was analyzed by two-way repeated measures ANOVA followed by *post hoc* Bonferroni test for comparison of groups. $^{###}$ $P < 0.001$ as compared with normal rats. $^{\$\$\$}$ $P < 0.001$ as compared with sham control group. * $P < 0.05$, *** $P < 0.001$ as compared with SNCI control group.

day-2, neither IND01 (50, 100 or 200 mg/kg) nor pyridoxine HCl (100 mg/kg) showed change in MFT score as compared with SNCI control rats (Table 4).

3.7. Effect on MNCV after SNCI in rats

The MNCV in normal and sham control rats was found to be 52.89 and 47.58 m/s respectively on day-15 of the study (Table 4). SNCI control rats showed significantly ($P < 0.001$) reduction in MNCV (14.58 m/s) as compared with normal or sham control rats (Table 4) indicating induction of neuropathy. MNCV of rats treated with daily oral treatment of IND01 (50, 100 or 200 mg/kg) or pyridoxine HCl (100 mg/kg) was significantly ($P < 0.01$) more as compared with MNCV of PSNL control rats (Table 4).

Table 4
Effect of IND01 on MFT score and MNCV after SNCI in rats.

Measurements after SNCI	Mean MFT score± SEM				MNCV (m/s) Day 15
	Day 0	Day 2	Day 15	Day 30	
Normal	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	52.89 ± 2.14
Sham control	0.00 ± 0.00	0.27 ± 0.05	0.09 ± 0.03	0.10 ± 0.02	47.53 ± 2.21
SNCI control	0.00 ± 0.00	2.92 ± 0.08$^{\#\#\#,\$\$\$}$	2.98 ± 0.20$^{\#\#\#,\$\$\$}$	03 ± 0.07$^{\#\#\#,\$\$\$}$	14.58 ± 0.48$^{\#\#\#,\$\$\$}$
IND01 (50) + SNCI	0.00 ± 0.00	3.08 ± 0.15	2.66 ± 0.04**	3.33 ± 0.09**	22.92 ± 1.60**
IND01 (100) + SNCI	0.00 ± 0.00	3.1 ± 0.04	1.83 ± 0.09***	1.36 ± 0.04***	30.92 ± 1.60***
IND01 (200) + SNCI	0.00 ± 0.00	3.11 ± 0.14	1.50 ± 0.08***	1.12 ± 0.02***	32.01 ± 1.40***
Pyridoxine HCl (100) + SNCI	0.00 ± 0.00	2.98 ± 0.09	1.40 ± 0.03***	1.20 ± 0.04***	35.73 ± 2.19***

Data was expressed as mean parameter value (in seconds) ± SEM for six rats per group. Data for MFT was analyzed by two-way repeated measures ANOVA followed by *post hoc* Bonferroni test for comparison of groups. Data for MNCV was analyzed by one-way ANOVA followed by post hoc Dunnett's multiple comparison test for comparison of groups. $^{\#\#\#}P < 0.001$ as compared with normal rats. $^{\$\$\$}P < 0.001$ as compared with sham control group. ns – not significant, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with SNCI control group.

4. Discussion

PSNL and SNCI are most commonly used models to induce neuropathy and correlated well to clinical situations[20]. In the present study, PSNL and SNCI showed progressive thermal analgesia (decrease in mean paw withdrawal latencies) in rats. On daily oral treatment for 7 days, IND01 (100 and 200 mg/kg) protected the rats (with PSNL and SNCI) from TH and loss of motor function.

The deficiency of pyridoxine HCl (Vitamin B6) is known to cause peripheral neuropathy. Vitamin-B6 supplementation is proved to alleviate peripheral neuropathic pain in many studies in laboratory animals[21, 22] and patients[23, 24]. Therefore, we used pyridoxine HCl as positive control in our study. Pyridoxine HCl showed good neuroprotection in our study against PSNL and SNCI models of mononeuropathy in rats which is in line with our earlier finding[21].

TH is caused by the ectopic discharge, release of cytokines from the inflammatory cells around the injured nerve and changes in the sensory pathways to the spinal cord and brain[25]. Therefore, the results of present study indicated modulation of sensory pathway or inhibition of cytokine release as one of the probable mechanism of IND01 against TH in peripheral neuropathy. The behavioural symptoms, which develop simultaneously, include hyperalgesia, spontaneous pain, abnormal gait and weight bearing on the opposite side of the operated limb[26]. These symptoms have been reported to progress within 1-2 days post nerve insult[26]. In the present study, IND01 (100 and 200 mg/kg) showed time-dependant reduction of MFT score from day-7 onwards.

Neuropathy is resulted in structural changes in peripheral nerves. These changes lead to endoneural edema, increased intraneural pressure, decreased blood flow to nerves, ischemia, and finally Wallerian-like axonal degeneration and finally decrease in MNCV[27]. IND01 in all tested doses (50, 100 and 200 mg/kg) restored the MNCV of rats with neuropathy induced by SNCI but not by PSNL, probably by of sciatic nerve restoration property of fenugreek[28] or neurite outgrowth promoting property of trigonelline[29].

Tissue damage, inflammation or injury of the nervous system is cardinal cause of chronic neuropathic pain. In the past, potent antiinflammatory effects of fenugreek extract were attributed to alkoloidal constituents[6]. Therefore, marker compound, trigonelline, a major alkaloid of fenugreek seeds, is probably responsible for protection against neuronal inflammation in the present study. Fenugreek extract is reported to be potent analgesic agent through seroterngic and purinergic (P2X)[30] receptors involvement. P2X receptors plays an important role in the modulation of spinal and peripheral nociceptive transmission following the development of nerve or tissue injury and inflammation[31]. Therefore, the analgesic effects of IND01 in the present study against neuropathic pain can be attributed to serotonergic and purinergic pathways.

Oxidative stress, triggered by vascular abnormalities and associated microangiopathy, in the nerve is a key step in a cascade of changes leading to nerve damage[32] and leads to neuropathic pain. Free radicals and cytokines, that are responsible for cell damage, are released from neutrophils. The increased oxidative stress then further aggravates the nerve damage. Therefore, neuroprotection observed in the present study is in line with the existing reports of vital organ protection shown by fenugreek seed extract[33, 34] through antioxidant mechanism[35].

In conclusion, the present investigation suggested the usefulness of IND01 against neuropathic pain. Our results can prove to be vital step towards development of medicinal agent in management of peripheral neuropathic diseases including diabetic neuropathy, a long-term complication of DM. However, further studies involving evaluation of IND01 against neuropathic pain in diabetic animals and patients are suggested to substantiate such claim.

Conflict of interest statement

We declare that we have no conflict of interest.

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