



Received on 10 March, 2015; received in revised form, 05 May, 2015; accepted, 15 July, 2015; published 01 October, 2015

## A STUDY OF PREGABALIN, TRAMADOL, THEIR COMBINATION AND *NIGELLA SATIVA* IN NEUROPATHIC PAIN IN RATS

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### Keywords:

Anti-cancer drug, chemotherapy induced neuropathy, cisplatin, *Nigella sativa*, neuropathic pain, pregabalin, tramadol

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**ABSTRACT:** *Nigella sativa* (NS) is a commonly used herb in Asian and African countries. Extensive experimental studies have been done to shed light on its hepatoprotective, immunomodulator, antioxidant, antimicrobial and several other useful pharmacological properties. However, there is dearth of evidence to evaluate the effect of *Nigella sativa* extract in neuropathic pain. In this study we have used neuropathic pain model of Wistar rats using intraperitoneal cisplatin injection twice weekly for 4.5 weeks. Animals were divided into six groups of six rats each. Ethanolic extract of *Nigella sativa*, pregabalin, tramadol or combination of pregabalin and tramadol were given orally in Wistar rats in which neuropathy had been induced by cisplatin i.p. injections for 4.5 weeks. Single dose of drugs were given and pain assessment was done by soft touch, crude touch, Eddy's hotplate and tail flick analgesiometer at 0, 30, 60, 90, 120 and 240 minutes. *Nigella sativa* showed significant analgesic effect on cisplatin induced neuropathic pain in all the experiments however pregabalin and tramadol showed better efficacy.

**INTRODUCTION:** Pain is the most common reason for physician consultation worldwide. Little less than 30% of the patients, who were treated at a primary care practice, had some kind of medically defined pain problem, requiring the attention of a general physician.

Pain is usually the natural consequence of tissue injury. Pain motivates the individual to withdraw from damaging situations, to protect a damaged body part while it heals, and to avoid similar experiences in the future.

There are 3 functions of pain

- (i) Prevent further injury
- (ii) Teach us what not to do
- (iii) Limit our activity

Two general types of pain based on its time course viz. acute pain and chronic pain. Acute pain is typically defined as a pain response due to tissue damage that lasts less than 6 months. Chronic pain lasts more than 6 months and can be recurrent, progressive, or constant.

The International Association for Study of Pain (IASP) defines pain as: "The unpleasant sensory and emotional experience of actual or potential tissue damage or an experience expressed in such terms"<sup>1</sup>. It can last from a few seconds to many weeks but it usually goes away when normal healing occurs. Acute pain is self-limiting and

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serves a protective biological function by acting as a warning of on-going tissue damage. Associated psychological symptoms are minimal and are usually limited to mild anxiety.

### Neuropathic pain:

Neuropathic pain affects 6%–8% of the general population and has a great impact on the patients' quality of life and disability<sup>2</sup>. Subjects across neuropathic pain conditions exhibited high pain levels, which were significantly associated with poor function, compromised health status and sleep, and increased anxiety and depression<sup>3</sup>. Various conditions can affect nerve and may cause neuropathic pain. These include Prolapsed Intervertebral Disc (PIVD), trigeminal neuralgia, post herpetic neuralgia (Pain following Shingles), diabetic neuropathy, phantom limb pain following amputation, multiple sclerosis, pain following chemotherapy and pain due to alcoholism and vitamin deficiencies.

Neuropathic pain is a common type of chronic nonmalignant pain that comes from nerve problems. Neuropathic pain is defined by IASP as: "Pain initiated or caused by a primary lesion or dysfunction in the nervous system". It can be caused by the lesion of the peripheral or central nervous system or both<sup>4</sup>.

First-line therapy includes analgesic, then neuroactive agents. Several agents also have been used with varying degree of success in the treatment of neuropathic pain including nonsteroidal anti-inflammatory drugs (NSAIDs), topical agents, opioid analgesics, antiarrhythmics, N-methyl D-aspartate (NMDA) antagonists, antiepileptics and antidepressants (both tricyclic antidepressants and serotonin reuptake inhibitors)<sup>5</sup>. Drugs commonly used are opioids (Tramadol, Buprenorphine, Fentanyl, and Morphine etc.), Antidepressants (Venlafaxine, Duloxetine etc.) and Anti-convulsants (Gabapentin, Pregabalin)<sup>6</sup>. Neuropathic pain is often difficult to treat, because it is resistant to many medications and adverse effects associated with effective medications.

### Why *Nigella sativa*?

Among the promising medicinal plants, *Nigella sativa*, also known as Black seeds and Black

cumin, has been called the "Blessed Seed" for its miraculous curing ability. The results of extensive pharmacological studies justify the broad, traditional therapeutic value of Black Seeds. These studies found Black Seed to have analgesic, antilipemic, post coital contraceptive, diuretic and antihypertensive, bronchodilator and calcium antagonist, histamine release inhibitor, hepatoprotective, antihelminthic, antifungal, antimicrobial (against a wide range of organisms), and anticancer activities<sup>7</sup>. Its many uses have earned *Nigella* the Arabic approbation 'Habbatul barakah', meaning the seed of blessing.

*Nigella sativa* (NS) is a commonly used household herb, vernacularly known as Kalaunji. Extensive experimental studies have been done to shed light on its hepatoprotective potency, as an immunomodulator, antioxidant, antimicrobial agent<sup>8, 9, 10</sup>. *Nigella sativa* and its active constituent thymoquinone (TQ) have shown protective effect against diabetic peripheral neuropathy due to oxidative stress<sup>11</sup>. Histologic evaluation of the tissues in diabetic animals treated with TQ and especially NS showed fewer morphologic alterations. Myelin breakdown decreased significantly after treatment with NS and TQ.

The ultra structural features of axons also showed remarkable improvement<sup>12</sup>. *Nigella sativa* oil and TQ produce antinociceptive effects through indirect activation of the supraspinal  $\mu(1)$  - and kappa-opioid receptor subtypes<sup>13</sup>. Black cumin seed essential oil (BCSEO) was found to produce a significant analgesic effect in acetic in acetic induced writhing, formalin and light tail flick tests.

However, there is dearth of evidence to evaluate the effect of *Nigella sativa* (NS) extract in neuropathic pain. To the best of our knowledge no study has been carried out on *Nigella sativa* in comparison to other drugs in case of neuropathic pain. Hence we consider it worthwhile to evaluate its analgesic activity in animal model of neuropathic pain in comparison to clinically used drugs.

### Pregabalin:

Pregabalin effectively relieved neuropathic pain and prevented the conversion of acute pain to

chronic pain<sup>14</sup>. Pregabalin is commonly used in the treatment of diabetic peripheral neuropathy, though it may reduce pain, but may not reduce the oxidative stress. Pregabalin improves sleep, quality of life, and daily living abilities. Along with its efficacy in particular neuropathic pain conditions, pregabalin's safety led it to be one of the first pharmacotherapies considered for the management of neuropathic pain<sup>15</sup>.

Adverse effects associated with pregabalin (like dizziness, depression, loss of appetite, decrease or change in vision etc.) limits its use in neuropathic pain syndrome. Thus we need to discover new medications with similar or better efficacy but less adverse effects.

#### **Tramadol:**

The acute analgesic effect of tramadol has been extensively investigated. Opioids and tramadol are only considered second/third line, alone or in combination with first line drugs (National Institute for Health and Clinical Excellence, UK.NICE clinical guideline 173, 2013).

Adverse effects associated with tramadol (like agitation, anxiety, constipation, cough etc.) limits its use in neuropathic pain syndrome. Thus we need to discover new medications with similar efficacy but less adverse effects.

To the best of our knowledge there is no study to show whether pregabalin, tramadol or their combination is better for the treatment of neuropathic pain.

### **MATERIALS AND METHODS:**

#### **Collection of *Nigella sativa* seeds:**

Seeds of *Nigella sativa* were procured from Organic India Pvt. Ltd.-Lucknow, Uttar Pradesh, India and authenticated by a botanist at National Botanical Research Institute, Lucknow. Sample of seeds (voucher specimen number pharm/39/13) has been placed in museum of department of pharmacology, Era's Lucknow Medical College and Hospital

#### **Extraction method:**

Seeds of *Nigella sativa* were thoroughly washed in distilled water and dried in shade. The seeds were

grounded to powder with the help of mortar and pestle, 500 g of powder was soaked in 1.5 litre of 99% ethanol (analytical grade) in a closed container at room temperature for 7 days with periodic stirring with a sterile glass rod. After 7 days it was filtered with the help of Whatman's filter paper no.1 and the filtrate transferred in a petri dish and left in shade for 3 days to allow evaporation of ethanol. The extract so obtained was brown in colour and had a characteristic smell. It was then weighed in electronic weighing balance and was 50 g in weight (10% w/w). The extract was transferred in sterile tubes and was stored at 4<sup>0</sup>C for further use.

**Drugs and chemicals:** Doses were according to previous studies.

**Cisplatin:** Manufactured by Cipla. Dose 2 mg/kg<sup>16</sup>.

**Pregabalin:** Manufactured by Sun Pharma. Dose – 30mg/kg<sup>17</sup>.

**Tramadol:** Manufactured by Biochem Pharmaceutical Industries Ltd Dose - 20 mg/kg<sup>18</sup>.

***Nigella sativa*:** Dose - 500mg/kg, 1000mg/kg<sup>19</sup>.

#### **Animals:**

Adult male Wistar rats (weighing 100-150gm) obtained from CDRI (The Central Drug Research Institute) were used. The animals were housed in polycarbonate cages in a room with a 12 hour day – night cycle, temperature of 22° C ± 2°C and humidity of 45%–64%. Animals were fed with a standard pellet diet and water ad libitum. All studies were carried after prior permission of Institutional Animal Ethics Committee. Ethical guideline for animal care and animal experimentation by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) were followed.

**Sample size:** According to previous study<sup>20</sup> sample size is calculated using the formula –

Power of study = 80%

Sample size:  $n = 5 + (10\% \text{ data loss}) = 6$  in each group

**Statistical Analysis:**

Observations of different groups were compared using ANOVA along with Post-HOC Dunnett's T3test or Chi square test, as appropriate.

All analysis were done using SPSS 16.0 Version.  $P < 0.05$  was considered as significant.

**Study design:**

In this experimental study pain assessment was performed before starting experiments and cisplatin i.p. injections were given for 45 weeks and at 5 weeks before administering drug pain assessment was done (**Fig. 1**). After drug administration pain assessment was done at 30, 60, 90, 120 & 240 minutes.

**Cisplatin induced neuropathy:**

Neuropathic pain was induced using anticancer drug cisplatin as per the method of Mansour et al. (2013)<sup>16</sup>. Adult rats were treated with i.p. injection of cisplatin (2 mg/kg) twice weekly (a total of nine injections in 4.5 weeks). Each group was administered their respective drugs after induction of neuropathic pain followed by pain assessment was carried out to detect and quantify neuropathic pain.

Drugs were administered in dissolved form in distilled water (vehicle) orally.

**Group 1** - Control - distilled water (DW).

**Group 2** - Pregabalin (30 mg/kg).

**Group 3** - Tramadol (20mg/kg).

**Group 4** - Pregabalin + tramadol (30 mg/kg + 20 mg/kg).

**Group 5** - Ethanolic extract of *Nigella Sativa* (500mg/kg).

**Group 6** -Ethanolic extract of *Nigella Sativa* (1000 mg/kg).

**Pain Assessment:**

In groups 1 to 6 at 5 week, after induction of neuropathy pain assessment was done at 0, 30, 60, 90, 120 & 240 minutes after administration of single dose of test drugs. Following tests were carried out to detect and quantify neuropathic pain<sup>16, 21</sup>.

**1 Eddy's hotplate:** It was done according to the method of Hogan et al (2004)<sup>21</sup>. Animal was placed on the hotplate and observed for either paw licking or jumping reaction. The reaction time was recorded by stop-watch. Cut-off time was set at 30 seconds to avoid any further injury.

**2 Tail flick method (Analgesiometer):** It was done according to the method of Mansour Set al (2013)<sup>16</sup>. Animal was placed into restrainer and leaving the tail exposed outside the restrainer. The time of tail flick is measured and recorded. The cut-off time was set-up 30 seconds to avoid any further injury.

**3 Soft/ Light touch:** It was done according to the method of Hogan et al (2004)<sup>21</sup>. An 8-mm-wide Camel brush was stroked longitudinally along the center of the paw. The response was scored as either none or positive if the paw was removed.

**4 Crude touch with needle:** It was done according to the method of Hogan et al (2004)<sup>21</sup>. The point of a 23-gauge spinal anesthesia needle was applied to the center of the paw with enough force to indent the skin but not to puncture it. The response was scored as either none or positive if the paw was removed.

**RESULTS:**

Pain assessment at 5 weeks – after induction of neuropathic pain.

**Effect of *Nigella sativa*, pregabalin & tramadol on cisplatin induced neuropathy in rats.**

**Reaction time in seconds on Hotplate at 5 weeks**  
**Table 1** shows mean reaction time on hotplate apparatus at 0 minute was similar in all groups ( $P = 0.918$ ) & ranged between  $2.60 \pm 0.28$  to  $2.73 \pm 0.31$  seconds. All drugs caused an increased in reaction time at 30, 60, 90, 120 & 240 minutes. Significant difference were seen among different groups at 60 minutes ( $P = 0.002$ ).

Rats given pregabalin, tramadol or their combination showed significantly higher reaction time as compared to control. At 90 minutes also pregabalin, tramadol or their combination showed higher reaction time compared to control. *Nigella sativa* 1000 mg/kg group also exhibited



significantly higher reaction time which was statistically significant to control group at 90, 120 & 240 minutes ( $P \leq 0.001$ , 0.000 & 0.000 respectively).

*Nigella sativa* 500 mg/kg and *Nigella sativa* 1000 mg/kg groups showed significant difference as compared to pregabalin+tramadol group, which

means *Nigella sativa* 500 mg/kg and *Nigella sativa* 1000 mg/kg groups were not similar to pregabalin+tramadol group at 60, 90, 120 & 240 minutes.

Pregabalin and tramadol groups did not show significant difference as compared to pregabalin+tramadol group.

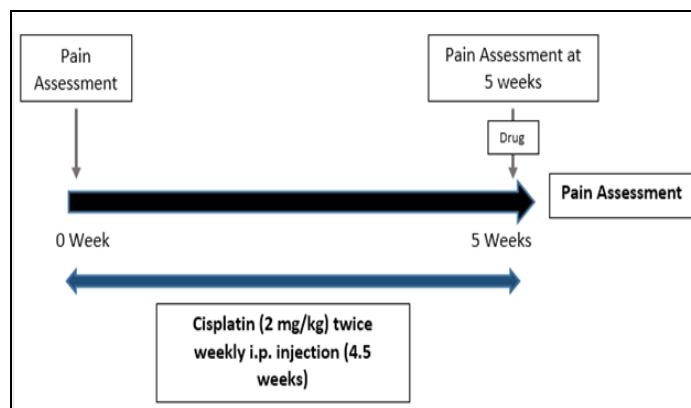


FIG. 1: SCHEMATIC DIAGRAM SHOWING CISPLATIN INDUCED NEUROPATHY AND PAIN ASSEMENT

TABLE 1: EFFECT OF *NIGELLA SATIVA*, PREGABALIN & TRAMADOL ON CISPLATIN INDUCED NEUROPATHY IN RATS. REACTION TIME IN SECONDS ON HOTPLATE AT 5 WEEKS

Groups (n=6)	0 minute Mean $\pm$ SD	30 minutes Mean $\pm$ SD	60 minutes Mean $\pm$ SD	90 minutes Mean $\pm$ SD	120 minutes Mean $\pm$ SD	240 minutes Mean $\pm$ SD
Control (DW)	2.68 $\pm$ 0.20	2.73 $\pm$ 0.20	2.68 <sup>#</sup> $\pm$ 0.17	2.66 <sup>#</sup> $\pm$ 0.15	2.61 <sup>#</sup> $\pm$ 0.19	2.56 <sup>#</sup> $\pm$ 0.12
<i>N. sativa</i> (500mg)	2.65 $\pm$ 0.314	2.68 $\pm$ 0.32	2.71 <sup>#</sup> $\pm$ 0.33	2.80 <sup>#</sup> $\pm$ 0.30	2.92 <sup>#</sup> $\pm$ 0.33	3.01 <sup>#</sup> $\pm$ 0.32
<i>N. sativa</i> (1000mg)	2.68 $\pm$ 0.12	2.82 $\pm$ 0.11	2.99 <sup>#</sup> $\pm$ 0.075	3.18 <sup>#</sup> $\pm$ 0.075	3.56 <sup>#</sup> $\pm$ 0.137	3.83 <sup>#</sup> $\pm$ 0.19
Pregabalin (30 mg/kg)	2.60 $\pm$ 0.28	3.15 $\pm$ 0.26	3.66 <sup>*</sup> $\pm$ 0.22	4.10 <sup>*</sup> $\pm$ 0.18	4.43 <sup>*</sup> $\pm$ 0.25	4.56 <sup>*</sup> $\pm$ 0.25
Tramadol (20 mg/kg)	2.73 $\pm$ 0.31	3.10 $\pm$ 0.24	3.51 <sup>*</sup> $\pm$ 0.20	3.97 <sup>*</sup> $\pm$ 0.14	4.20 <sup>*</sup> $\pm$ 0.22	4.33 <sup>*</sup> $\pm$ 0.26
Pregabalin +Tramadol (30+20 mg/kg)	2.60 $\pm$ 0.14	3.16 $\pm$ 0.26	3.86 <sup>*</sup> $\pm$ 0.33	4.25 <sup>*</sup> $\pm$ 0.37	4.46 <sup>*</sup> $\pm$ 0.34	4.58 <sup>*</sup> $\pm$ 0.32
ANOVA						
F	0.28	5.07	27.22	56.31	57.92	66.83
P	0.918	0.002	0.002	0.000	0.000	0.000

\*  $P < 0.05$  in comparison to Control

#  $P < 0.05$  in comparison to Pregabalin +Tramadol (One way ANOVA)

### Effect of *Nigella sativa*, pregabalin & tramadol on cisplatin induced neuropathy in rats. Reaction time in seconds on Tail flick at 5 weeks

Table 2 shows mean reaction time on tail flick apparatus at 0 minute was similar in all groups ( $P = 0.015$ ) & ranged between 2.11 $\pm$ 0.011 to 2.38 $\pm$ 0.13 seconds. All drugs caused an increased in reaction time at 30, 60, 90, 120 & 240 minutes. Significant difference were seen among different groups at 30, 60 minutes ( $P \leq 0.015$  & 0.000 respectively).

Rats given pregabalin, tramadol or their combination showed significantly higher reaction time as compared to control. At 60 minutes also pregabalin, tramadol or their combination showed

higher reaction time compared to control ( $P \leq 0.000$ , 0.000 & 0.000 respectively). *Nigella sativa* 1000 mg/kg group exhibited significantly higher reaction time which was statistically significant as compared to control group at 90, 120 & 240 minutes ( $P \leq 0.001$ , 0.000 & 0.000 respectively).

*Nigella sativa* 500 mg/kg and *Nigella sativa* 1000 mg/kg showed significant difference as compared to pregabalin+tramadol group. NS 500 and *Nigella sativa* 1000 mg/kg groups were not similar to pregabalin+tramadol group at 60, 90, 120 & 240 minutes. Analgesic activity of *Nigella sativa* was not similar to pregabalin, tramadol or their combination.

Pregabalin and tramadol groups did not show significant difference as compared to pregabalin+tramadol group.

**TABLE 2: EFFECT OF NIGELLA SATIVA, PREGABALIN & TRAMADOL ON CISPLATIN INDUCED NEUROPATHY IN RATS. REACTION TIME IN SECONDS ON TAIL FLICK AT 5 WEEKS**

Groups (n=6)	0 minute	30 minutes	60 minutes	90 minutes	120 minutes	240 minutes
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Control (DW)	2.25±0.15	2.30±0.17	2.32 <sup>#</sup> ±0.15	2.27 <sup>#</sup> ±0.15	2.25 <sup>#</sup> ±0.10	2.28 <sup>#</sup> ±0.11
<i>N. sativa</i> (500mg)	2.33±0.12	2.38±0.15	2.53 <sup>#</sup> ±0.17512	2.68* <sup>#</sup> ±0.17	2.83* <sup>#</sup> ±0.18	2.95* <sup>#</sup> ±0.14
<i>N. sativa</i> (1000mg)	2.25±0.55	2.35±0.08	2.55 <sup>#</sup> ±0.14	2.78* <sup>#</sup> ±0.13	3.20* <sup>#</sup> ±0.21	3.65* <sup>#</sup> ±0.19
Pregabalin (30 mg/kg)	2.11±0.11	2.60±0.09	3.48*±0.29	4.16*±0.10	4.53*±0.12	4.63*±0.18
Tramadol (20 mg/kg)	2.38±0.13	2.81±0.18	3.41*±0.08	3.95*±0.14	4.20*±0.22	4.30*±0.24
Pregabalin +Tramadol (30+20 mg/kg)	2.33±0.15	3.02*±0.19	3.68*±0.19	4.08*±0.11	4.31*±0.040	4.45*±0.12
ANOVA						
F	3.40	22.07	63.30	222.31	201.19	181.31
P	0.055	0.000	0.000	0.000	0.000	0.000

\* P < 0.05 in comparison to Control

# P < 0.05 in comparison to Pregabalin +Tramadol (One way ANOVA)

**Effect of *Nigella sativa*, pregabalin, tramadol on cisplatin induced neuropathic pain in rats on crude touch at 5 weeks.**

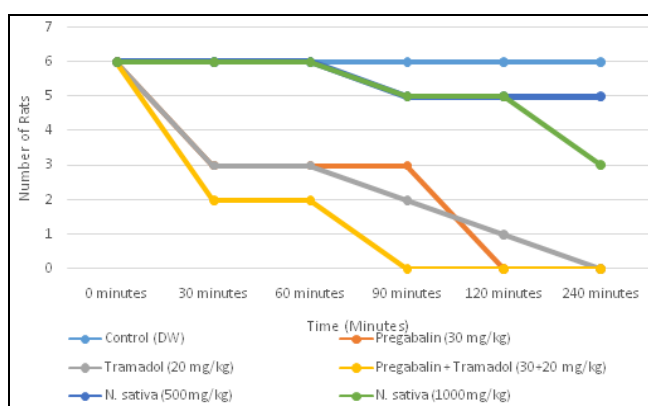
Table 3 & Fig. 2 show that at 5 weeks after drug administration number of rats in pregabalin, tramadol, pregabalin+tramadol groups responding to crude touch reduced significantly at 30, 60, 90, 120 & 240 minutes.

*Nigella sativa* 500 mg/kg and *Nigella sativa* 1000 mg/kg group showed delayed response as compared to pregabalin, tramadol and pregabalin+tramadol groups. *Nigella sativa* 1000 mg/kg group showed higher anti-nociceptive activity than *Nigella sativa* 500 mg/kg group but lesser than that in pregabalin, tramadol or pregabalin+tramadol groups.

**TABLE 3: EFFECT OF NIGELLA SATIVA, PREGABALIN, TRAMADOL ON CISPLATIN INDUCED NEUROPATHIC PAIN IN RATS ON CRUDE TOUCH AT 5 WEEKS. NUMBER OF RATS RESPONDING TO CRUDE TOUCH SHOWN**

Groups (n=6)	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes	240 minutes
Control (DW)	6	6	6	6	6	6
Pregabalin (30 mg/kg)	6	3	3	3	0	0
Tramadol (20 mg/kg)	6	3	3	2	1	0
Pregabalin + Tramadol (30+20 mg/kg)	6	2	2	0	0	0
<i>N. sativa</i> (500mg/kg)	6	6	6	5	5	5
<i>N. sativa</i> (1000mg/kg)	6	6	6	5	5	3
P	1.000	0.013	0.000	0.000	0.000	0.000

\*P value <0.05 in comparison to control (Chi-Square Test)



**FIG. 2: EFFECT OF NIGELLA SATIVA, PREGABALIN, TRAMADOL ON CISPLATIN INDUCED NEUROPATHIC PAIN IN RATS ON CRUDE TOUCH AT 5 WEEKS**

### Effect of *Nigella sativa*, pregabalin, tramadol on cisplatin induced neuropathic pain in rats on soft touch at 5 weeks.

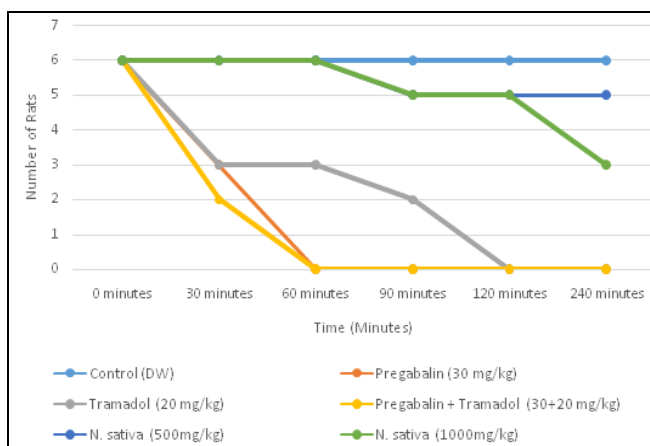
Table 4 & Fig. 3 show that at 5 weeks after drug administration number of rats in pregabalin, tramadol, pregabalin+tramadol groups responding to soft touch reduced significantly at 30, 60, 90, 120 & 240 minutes.

*Nigella sativa* 500 mg/kg and *Nigella sativa* 1000 mg/kg group showed delayed response as compared to pregabalin, tramadol and pregabalin+tramadol groups. *Nigella sativa* 1000 mg/kg group showed better anti-nociceptive activity than *Nigella sativa* 500 mg/kg group but lesser than in pregabalin, tramadol or pregabalin+tramadol groups.

**TABLE 4: EFFECT OF NIGELLA SATIVA, PREGABALIN, TRAMADOL ON CISPLATIN INDUCED NEUROPATHIC PAIN IN RATS ON SOFT TOUCH AT 5 WEEKS. NUMBER OF RATS RESPONDING TO SOFT TOUCH SHOWN**

Groups (n=6)	0 minutes	30 minutes	60 minutes	90 minutes	120 minutes	240 minutes
Control (DW)	6	6	6	6	6	6
Pregabalin (30 mg/kg)	6	3	0	0	0	0
Tramadol (20 mg/kg)	6	3	3	2	0	0
Pregabalin + Tramadol (30+20 mg/kg)	6	2	0	0	0	0
<i>N. sativa</i> (500mg/kg)	6	6	6	5	5	5
<i>N. sativa</i> (1000mg/kg)	6	6	6	5	5	3
P	1.000	0.013	0.000	0.000	0.000	0.000

\*P value <0.05 in comparison to control (Chi-Square Test)



**FIG.3: EFFECT OF NIGELLA SATIVA, PREGABALIN, TRAMADOL ON CISPLATIN INDUCED NEUROPATHIC PAIN IN RATS ON SOFT TOUCH AT 5 WEEKS.**

**DISCUSSION:** In our study *Nigella sativa* increased reaction time of rats with cisplatin induced neuropathic pain on hotplate as well as tail flick apparatus showing its analgesic effect through spinal and supraspinal pathways. *Nigella sativa* oil and thymoquinone produce antinociceptive effects which was blocked by naloxone, naloxonazine, norbilitorphimine and naltridone & oil produced antinociceptive effect through indirect activation of the supraspinal  $\mu$  (1) - and kappa ( $\kappa$ ) - opioid receptor subtypes<sup>13</sup>. However, in a study they failed to demonstrate reversal of analgesic activity of steam distilled essential oil of Irani black cumin seed by naloxone<sup>22</sup>. It was reported that *Nigella sativa* polyphenols did not produce a significant

analgesia on tail flick test in mice which showed lack of activity on spinal opioid receptor and failure of naloxone to reverse analgesia on formaline test. However, failed to observe analgesic effect of *Nigella sativa* polyphenols in tail flick test. This suggest that active components other than polyphenols in *Nigella sativa* ethanolic extract may be responsible of their analgesic effect on spinal level<sup>8</sup>.

*Nigella sativa* showed better analgesic activity at higher dose which was earlier reported<sup>23</sup> in therapeutic effects of thymoquinone (active constituent of *Nigella sativa*) in a dose-dependent

manner in a model of neuropathic pain following an experimentally applied spinal cord injury.

Anti-inflammatory and analgesic action of *Nigella sativa* on carrageenan induced paw oedema. It also produced significant increase in the hot plate reaction time in mice indicating analgesic effect<sup>24</sup>. Several studies<sup>8, 19, 25</sup> have demonstrated analgesic activity of *Nigella sativa* through peripheral mechanism including cyclooxygenase enzyme<sup>26</sup>. In our study, we can predict that beside analgesic activity at spinal and supraspinal levels there may be peripheral mechanisms showing anti-inflammatory activity at nociceptive pathways acting on spinal cord causing decreased release of inflammatory mediators<sup>27</sup>.

Ethanol extract of *Nigella sativa* possessed significant analgesic activity in mice. The active ingredients and components obtained in each of these extracts/oils also differ from each other. Thymoquinone has remained one of the main components in almost all of these extracts/oils<sup>13, 22, 28</sup>. But whether thymoquinone alone or some other active agents are also responsible for analgesic effect, is still unclear. Thymoquinone is reported to inhibit the generation of thromboxane A<sub>2</sub> and leukotriene B<sub>4</sub>, thus suggesting an inhibitory effect on both the cyclo-oxygenase and lipo-oxygenase pathway<sup>29</sup>.

Tramadol (20 mg/kg) showed an acute analgesic effect & also effects of repeated administration of tramadol on partial sciatic nerve ligation-induced neuropathic pain in rats<sup>18</sup>. This suggested tramadol has both  $\mu$ -opioid receptor-mediated acute analgesic and  $\alpha_2$ -adrenoceptor-mediated preventive and alleviative effects on neuropathic pain, and the latter is due to  $\alpha_2$ -adrenoceptor-mediated inhibition of astrocytic activation. This means tramadol has better analgesic activity on long term treatment as compared to short term treatment. This might be the reason tramadol is reserved for long term treatment of neuropathic pain as given in nice clinical guideline 173, (2013).

Pregabalin versus tramadol for postoperative pain management in patients undergoing lumbar laminectomy, in which pregabalin showed statistically significant analgesic effects compared

to placebo, but the effect was found to be less prevalent compared to tramadol<sup>30</sup>. In our study we could not find any statistical difference between analgesic effects of pregabalin and tramadol in cisplatin induced neuropathic pain in rats. However, pregabalin & tramadol combination showed early onset of response on all tests (hotplate, tail flick, crude touch & soft touch) but no additive and/ or synergistic effect was found.

Our study was limited by short duration of treatment, assessment & use of only 2 doses of test drug. A longer duration of treatment may have revealed better neuroprotective effect with *Nigella sativa* & other drugs. Repeated administration of tramadol showed better analgesic activity in neuropathic pain in rats as compared to short treatment<sup>18</sup>. A gradual increase in reaction time was seen in *Nigella sativa* group up to 240 minutes. Further assessment may have revealed better results comparable to standard drugs. Since *Nigella sativa* 1000 mg/kg group showed better efficacy than *Nigella sativa* 500 mg/kg group, a third dose of *Nigella sativa* extract could have confirmed its dose dependent activity.

Clinical implication of *Nigella sativa* can be in preventing & curing neuropathy. Further studies are required to elucidate its active principles & mechanism of action followed by clinical studies to advocate its clinical use as a dietary supplement as well as therapeutic agent.

**CONCLUSION:** There was no significant difference in tramadol and pregabalin analgesic activity. Also, pregabalin and tramadol combination did not show significant difference from pregabalin and tramadol alone. The acute effects of a single dose of *Nigella sativa* is inferior to those of pregabalin or tramadol which show better analgesic activity independently. Combination of pregabalin & tramadol does not confirm additional analgesic effect.

Further studies are required to advocate clinical use of *Nigella sativa* in neuropathic pain.

**ACKNOWLEDGEMENT:** I would like to thank organizers of national conference entitled 'Novel Tools and Treatment Approaches in Health Care



System' for selecting my paper for oral presentation, organized at Faculty of Pharmacy, Integral University, Lucknow on 3<sup>rd</sup> March 2015.

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### How to cite this article:

Tewari SN, Salman MT, Thadani S, Singh S and Ahmad A: A Study of Pregabalin, Tramadol, Their Combination and *Nigella Sativa* in Neuropathic Pain in Rats. Int J Pharm Sci Res 2015; 6(10): 4406-14. doi: 10.13040/IJPSR.0975-8232.6(10).4406-14.

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