Research Article

Ameliorative effect on neuropathy due to anti-cancer drug using herbal extract – An experimental study of *Nigella sativa*

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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, immunumodulator, 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 5 weeks along with daily oral ethanolic extract of *Nigella sativa* in test group and distilled water in control group. In our study we found significant reduction in neuropathy and neuropathic pain due to anti-cancer drug cisplatin by oral administration of ethanolic extract of *Nigella sativa* which was confirmed by histopathological analysis of sciatic nerve and pain assessment tests.

Keywords: Amelioration, anti-cancer drug, cisplatin, Experimental study, Herbal extract, *Nigella sativa*, neuropathic pain, Neuroprotective, Neuropathy.

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INTRODUCTION

Neuropathic pain affects 6%–8% of the general population and has a great impact on the patients' quality of life and disability (Torrance 2006; Bouhassira2008). Neuropathic pain is a common side effect of many anti-cancer drugs eg. Platinum analogues like cisplatin, oxaliplatin. 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 (Schaefer *et al.*. 2014). 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. Anti-cancer drugs are known to cause neuropathy due to their adverse effect which is dose limiting adverse effect of anti-cancer drugs which was used to make animal model of neuropathic pain in this study.

Why *Nigella sativa*?

Nigella sativa(NS) is a commonly used herb in Asian and African countries. 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,

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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 (Paarakh 2010) . Its many uses have earned Nigella the Arabic approbation 'Habbatulbarakah', 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 immunumodulator, antioxidant, antimicrobial agent (Ghannadi *et al.*. 2005; Chatterjee 2000; Ostapowicz *et al.*. 2002). *Nigella sativa* and its active constituent thymoquinone (TQ) have shown protective effect against diabetic peripheral neuropathy due to oxidative stress (Hamdy 2009). 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 ultrastructural features of axons also showed remarkable improvement (Kanter 2008).*Nigella sativa* oil and TQ produce antinociceptive effects through indirect activation of the supraspinalμ(1) - and kappa-opioid receptor subtypes (Abdel-Fattah *et al.*. 2000). 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.

MATERIAL 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 4^0C for further use.

Drugs and chemicals: Dose was according to previous studies.

Cisplatin – Manufactured by Cipla. Dose (2 mg/ kg) (Mansour *et al.*. 2013).

Nigella sativa- Dose - 500mg/ kg/ day *(Islam et al.. 2013*).

Animals: Adult male Wistar rats (weighing 100-150gm) were 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 22º C ± 2ºC 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, Era's Lucknow Medical College. 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 (*Zbarcea et al.. 2011*) sample size is calculated using the formula -

$$\mathbf{n = (\sigma_1^2 - \sigma_2^2)\,(Z_\alpha - Z_\beta)^2 / d^2}$$

Where $\sigma_1 = 6.87$

$\sigma_2 = 2.58$

$d = 9$

Difference in means considered to be significant

Type I error $\alpha = 5\%$

Type II error $\beta = 20\%$

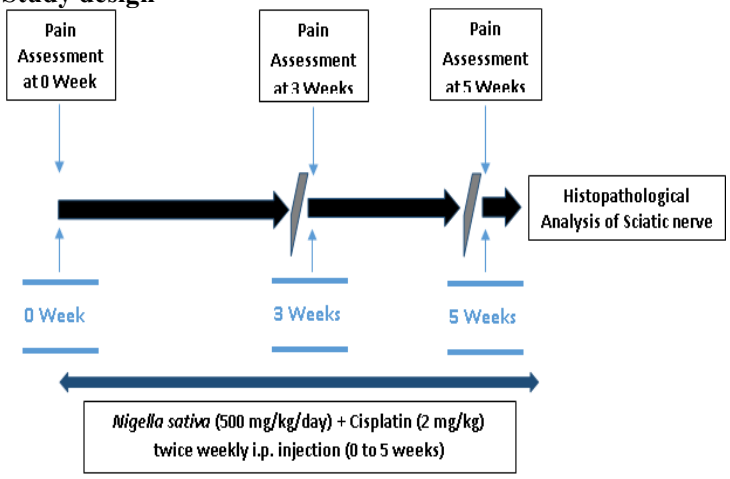
Power of study = 80%

Sample size: n = 5 + (10% data loss) = 6 in each group

Statistical Analysis

Observations of different groups were compared using independent t-test and ANOVA along with Post-HOC Dunnett's T3 test. All analysis were done using SPSS 16.0 Version. $P<0.05$ was considered as significant.

Study design



Cisplatin induced neuropathy

This was carried out for evaluation of the analgesic effects of drugs on the neuropathy induced by cisplatin. Neuropathic pain was induced using anticancer drug cisplatin as per the method of *Mansour et al.. (2013)*. Adult rats were treated with i.p. injection with cisplatin (2 mg/kg) twice weekly on Monday and Thursday (a total of nine injections in 5 weeks).Each group was administered their respective drugs after induction of neuropathic pain. Induction of neuropathic pain assessment was carried out to detect and quantify neuropathic pain.

Groups

The animals were divided randomly in 3 groups of six animals each. Drug/ distilled water administration was done via oral route in each group.

Group 1: No drug group– Distilled water

Group 2:Control - Cisplatin (2 mg/kg) intraperitoneal injection twice weekly (Monday and Thursday) + Distilled water

Group 3:Test - Cisplatin (2 mg/kg) intraperitoneal injection twice weekly + *Nigella sativa* (500 mg/kg/day) pre-treatment

Ethical considerations

- All experiments were performed
- After approval from IAEC (Institutional Animal Ethics Committee) of Era's Lucknow Medical College and Hospital and
- As per the guidelines of Animal Care by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Pain Assessment

In group 2 &3 pain assessment was done at week 0, 3 and 5 by hotplate and tail flick tests. Histopathological analysis of sciatic nerve of rats of all 3 groups was done to compare changes in sciatic nerve fiber.

1. Eddy's hotplate -

Eddy's hotplate instrument consists of an electrically heated surface made up of aluminium whose temperature is maintained by the thermostat 'Knob' at 55^{o} to 56^{o} C. After maintaining the temperature 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-up 20-30 seconds to avoid any further injury.

2. Tail flick method (Analgesiometer)

Animal was placed into restrainer and leaving the tail exposed outside the restrainer. Tail was cleaned with help of cotton soaked in ethanol & left for drying. Meanwhile rat was also familiarized with restrainer. Then, kept on the "tail flick analgesiometer". Kept the tail on the place made for tail above *nichrome* hot wire of analgesiometer. The time oftail flick is measured and recorded. The cut-off time was set-up 20-30 seconds to avoid any further injury.

Histopathological analysis

Group 1 (No drug control) rats, group 2 (Control) rats, group 3 (Test) ratswere given i.p. injection sodium pentobarbitone (40 mg/kg) for anaesthesia and euthanized to harvest sciatic nerve for its histopathological examination *(Yuan et al.. 2014*). Histopathological examination was done to compare changes in sciatic nerve histology between groups *(Bansode VJ 2014).*

RESULTS

PAIN ASSESSMENT AT 0, 3 & 5 WEEKS – INDUCTION OF NEUROPATHIC PAIN

In group 1 & 2 cisplatin (2 mg/kg) i.p. injection was given in rats twice weekly from week 0 to week 5 to induce neuropathic pain. Total number of rats were 12 (6 rats in each group). We assessed induction of neuropathy ay week 0, 3 & 5 by hotplate and tail flick tests. Induction of neuropathy was observed in all groups receiving cisplatin injection in dose (cumulative) and time dependent manner. On exploring data at 0-5 weeks we found significant decrease in reaction time indicating induction of neuropathic pain. Figure 1 & 2 display decrease in reaction time on stem and leaf plot showing quantitative data in a graphical format. On hotplate test at week 0 reaction time ranged from 4.0 - 5.2 (4.60±0.37) second which decreased to 2.7 - 4.0 (3.30±0.20) second at week 3 and 2.2 - 3.2 (2.68±0.20) second on week 5. On tail flick test at week 0 reaction time ranged from 4.1 - 5.7 (4.65±0.47) second which was decreased to 2.6 – 3.2 (2.81±0.16) second and 2.0 – 2.6 (2.25±0.15) second on week 5. Table 1 shows response to cisplatin induced neuropathy was assessed on week 3 and week 5 by thermal stimuli on hotplate & tail flick apparatus which showed significant difference among week 0, week 3 & week 5 ($P \leq 0.000$ & 0.000 respectively).

Table 1: Response to thermal stimuli on Hotplate & Tail flick apparatus in cisplatin induced neuropathic pain in rats. (n=12)

	0 Week		3 Weeks		5 Weeks		ANOVA	
	Mean	SD	Mean	SD	Mean	SD	F	P
Hotplate	4.60	0.37	3.30	0.20	2.68	0.20	339.390	0.000
Tail flick	4.65	0.47	2.81	0.16	2.25	0.15	727.447	0.000

* $P < 0.05$ in comparison to Control (Independent T-test)

INDUCTION OF NEUROPATHY AND ITS AMELIORATION

Control vs*Nigella sativa* Pre-treatment group - Induction of Neuropathy - Reaction time on Hotplate in seconds (0-5 weeks)

Table 2 & figure 3 show that during induction of neuropathy at week 0 control group and *Nigella sativa* pre-treatment group were similar on hotplate apparatus (P = 0.856). At 3 and 5 weeks there was significant difference between control group and *Nigella sativa* pre-treatment group (P = 0.001 & 0.001 respectively). All rats in cisplatin induced neuropathic pain group (n=12) showed fall in reaction time on hotplate apparatus form week 0 to week 5.

Table 2: Control vs*Nigella sativa* Pre-treatment group - Induction of Neuropathy - Reaction time on Hotplate in seconds (0-5 weeks)

	0 Week		3 Weeks		5 Weeks		
Groups (n=6)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**% change**
Control (DW)	4.60	0.38	3.30	0.20	2.68	0.20	-46.15%
***N. sativa* (500 mg/kg/day) Pre-treatment**	4.63	0.21	3.83*	0.16	3.25*	0.22	-25.00%
t	-0.187		-5.060		-4.661		-4.917
P	0.856		0.001		0.001		0.001

* P < 0.05 in comparison to Control (Independent T-test)

Table 3 & figure 4 show that during induction of neuropathy at week 0 control group and *Nigella sativa* pre-treatment group were similar on tail flick apparatus (P = 0.544). At 3 and 5 weeks there was significant difference between control group and *Nigella sativa* pre-treatment group (P ≤ 0.002 & 0.000 respectively). *Nigella sativa* pre-treatment group showed significantly lesser percentage of decrease in reaction time compared to control.). All rats in cisplatin induced neuropathic pain group (n=12) showed fall in reaction time on tail flick apparatus form week 0 to week 5.

Table 3: Control vs*Nigella sativa* Pre-treatment group - Induction of Neuropathy - Reaction time on Tail flick in seconds (0-5 weeks)

	0 Week		3 Weeks		5 Weeks		
Groups (n=6)	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**% change**
Control (DW)	4.65	0.47	2.82	0.16	2.25	0.15	-55.32%
***N. sativa* (500 mg/kg/day) Pre-treatment**	4.50	0.34	3.53*	0.32	2.85*	0.25	-37.50%
T	-0.631		-0.4900		-6.103		-4.949
P	0.544		0.002		0.000		0.002

* P < 0.05 in comparison to Control (Independent T-test)

DISCUSSION

Effect of various drugs on neuropathic pain was studied in cisplatin induced neuropathy model. *Nigella sativa* extract was given for prevention as well as treatment of neuropathy & compared with pregabalin, tramadol & their combination in different groups. Assessment was done using hotplate, tail flick apparatus & histopathology. The ethanolic extract obtained by maceration was 10% w/w, dark brown in colour& with a characteristic smell which was in conformity to earlier reports (*Kushwah et al.. 2013*). Administration of cisplatin led to significant decrease in reaction time on hotplate & tail flick apparatus at 3 & 5 weeks in all rats. Histopathological findings of sciatic nerve also showed signs of neurodegenaration. These findings confirm development of neuropathy after 5 weeks of cisplatin treatment *(Mansour et al.. 2013)*. Our findings are in conformity to dose of *Hogan et al.. (2004), Joseph et al.. (2009), Mansour et al.. (2013), Bansode et al.. (2014)* who reported hyperalgesia in response to thermal (*Mansour et al.. 2013*), mechanical stimuli (*Hogan et al.. 2004*) & neurodegenerative changes (*Bansode et al.. 2014*) in similar rat models. The mechanism of action of cisplatin induced neuropathy can be predicted by previous studies. *Gill et al.. (1998)* showed that cisplatin caused primary sensory neurons in the dorsal root ganglion die by apoptosis. *Malik et al.. (2008)* demonstrated that reactive oxygen species cause damage to mitochondria. Cisplatin-induced neuropathy is characterized by damage to large mylinated nerve fibers and cell bodies of dorsal root ganglia. High affinity DNA adducts are formed between cisplatin and either genomic or mitochondrial DNA. Pre-treatment with *Nigella sativa*ethanolic extract showed a protective effect in cisplatin induced neuropathy as evidenced by a significant difference in reaction time on hotplate, tail flick apparatus & minimal neurodegenerative changes in sciatic nerve at 3 & 5 weeks in comparison to control group. To the best of our

knowledge this activity has not been reported earlier & this is the first report of amelioration of cisplatin induced neuropathy by oral administration of *Nigella sativa*. *Al-Shabanah et al.. (1998); Nagi et al.. (2000); Badary et al.. (2000)* demonstrated *N. sativa*'s active constituent, thymoquinone, was found to exhibit protective effect in rats through its antioxidant action in nephrotoxicity, cardiotoxicity and benzopyrene induced cancer in mice. In our study we can also predict similar antioxidant mechanism involved in prevention of induction of neuropathy in *Nigella sativa* pre-treatment group. Histopathological changes in sciatic nerve of rats were examined and neuroprotective effect of *Nigella sativa* were observed as compared to cisplatin control group. *Javanbakht et al.. (2013)* has also shown same effects using *Nigella sativa* who evaluated the possible protective effects of *Nigella sativa* on the neuronal injury in the sciatic nerve of rats. They found that treatment of *Nigella sativa*markedly reduced degenerating neurons after trauma and the distorted nerve cells were mainly absent in the *Nigella sativa*treated rats. They concluded that *Nigella sativa* therapy causes morphologic improvement on neurodegeneration in sciatic nerve after trauma in rats. *Bansode et al.. (2014)* screened synthetic compound Ethyl Pyruvate (EP) for its potential use in chemotherapeutic drugs induced neuropathic pain. They demonstrated that rats subjected to cisplatin showed morphological alterations like necrosis of neurons, cellular infiltration and edema along with nerve fiber derangement. Treatment with Ethyl Pyruvate 100 mg/kg significantly attenuated these reactive changes. They concluded that Ethyl Pyruvate 100 mg/kg was effective in attenuation of thermal, mechanical hyperalgesia and cold allodynia in vincristine and cisplatin induced neuropathy. It was also found that the histopathological changes and oxidative stress took place after neuropathy induction was significantly reversed by Ethyl Pyruvate 100 mg/kg. The observed neuroprotective effect may be due to the antioxidant property of Ethyl Pyruvate. *Nigella sativa* also showed neuroprotective effect which can be because of its antioxidant property which was reported in earlier studies (*Ebru et al.. 2008; Ismail et al.. 2010*).

CONCLUSION

These results agree with ameliorative effects on neuropathy due to anti-cancer drug using*Nigella sativa* in cisplatin induced neuropathy in rats. Further studies are required to advocate clinical use of *Nigella sativa* in anti-cancer induced neuropathy and related conditions.

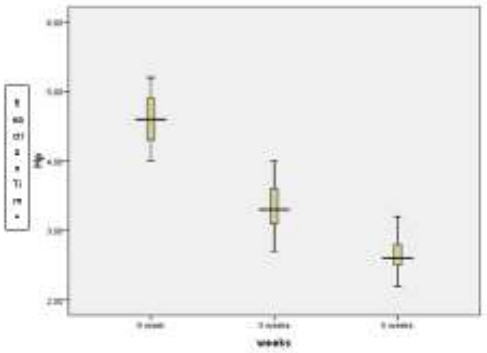


Figure 1

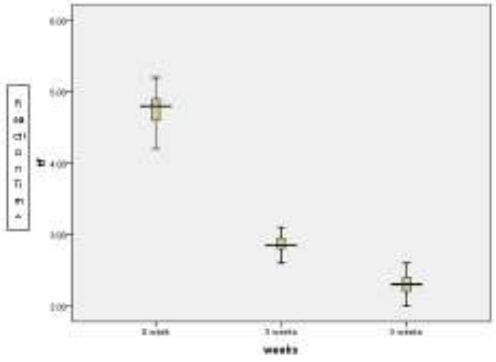


Figure 2

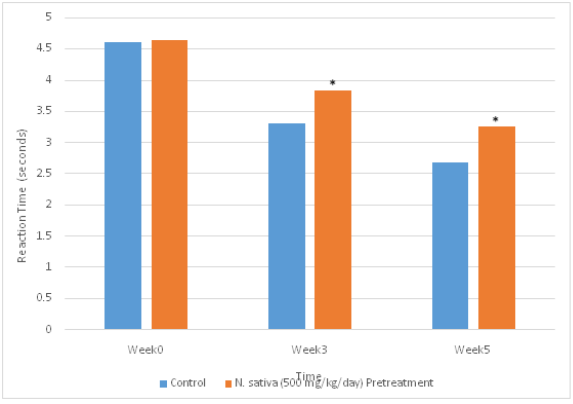


Figure 3

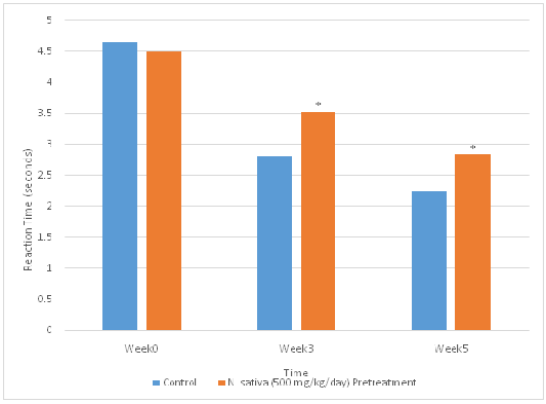


Figure 4

Histopathology of sciatic nerve

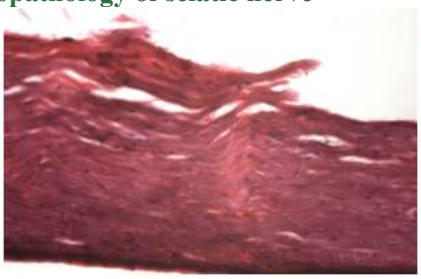


Figure 5(a)

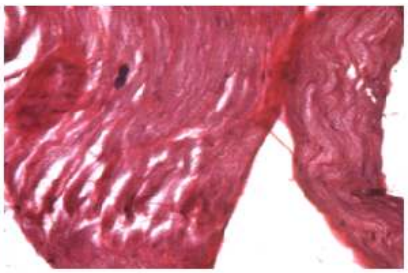


Figure 5(b)

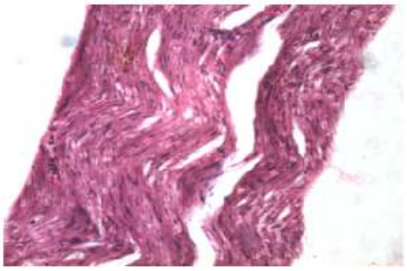


Figure 5(c)

Legend

Figure 1: Response to thermal stimuli on Hotplate apparatus in cisplatin induced neuropathic pain in rats. Stem-and-Leaf Plot for weeks 0 – 5 (n=12)

Figure 2: Response to thermal stimuli on Tail flick apparatus in cisplatin induced neuropathic pain in rats. Stem-and-Leaf Plot for weeks 0 – 5 (n=12)

Figure 3: Induction of Neuropathy - Reaction time on Hotplate in seconds (0-5 weeks). Control vs *Nigella sativa* Pre-treatment group - Induction of Neuropathy - Reaction time on Tail flick in seconds (0-5 weeks)

Figure 4: Induction of Neuropathy - Reaction time on Tail flick in seconds (0-5 weeks)

Figure 5(a): No drug group rat showing a normal sciatic: H & E (100x)

Figure 5(b): Control group rat showing cisplatin induced degeneration of nerve fiber and focal areas of infiltration by mononuclear inflammatory infiltrate: H & E (100x)

Figure 5(c): Test - Nigella sativa pre-treatment + cisplatin group rat showing resolution of cisplatin induced neuropathy and decreased degenerative features as compared to cisplatin control group: H & E (100x)

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