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ORIGINAL RESEARCH

# Motor co-ordinative roles of *Nigella sativa* oil in mice models of phenol-induced essential tremor

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**Abstract**

**Background:** Essential tremor, regarded as the world's most common movement disorder, is a neuronal disorder characterized by uncontrollable shaking (tremor) of different parts of the body. *Nigella sativa* is a medicinal herb with pharmacologically proven therapeutic potentials in various ailments including neurological disorders.
**Objective:** To evaluate the therapeutic roles of *Nigella sativa* on cerebellar phenotypes in phenol-induced mice models of essential tremor.
**Methods:** Tremor response, body weight, temperature, motor coordination (using the parallel bars and static rods tests), relative brain weights, cerebellar glutamate and Glutathione peroxidase (GPX) and histoarchitecture were assayed in 75 adult male BALB/cm ice weighing 21-30g. The animals, equally divided into five groups were respectively administered feed and water only (CTRL); 100mg/kg phenol and 1ml/kg *Nigella sativa* oil concurrently (PNSC); 100mg/kg phenol (P); 1ml/kg *Nigella sativa* oil followed by 100mg/kg phenol (NSP); and 1ml/kg *Nigella sativa* oil (NS).
**Results:** The PNSC, NSP and NS mice displayed significant weight reduction. Histoachitectural defects, stagnancy in weight, high Glutathione peroxidase (GPX) and high glutamate levels and poor motor coordination were exhibited by the P group. The CTRL and NS animals demonstrated good motor coordination while the PNSC and NSP groups showed better coordination than the untreated P group. The CTRL group showed no histoarchitectural defects while the NS and PNSC animals showed histoarchitectural regeneration.
**Conclusion:** This research affirmed the weight-reducing, neuroprotective, neuroregenerative and motor coordinating effects of *Nigella sativa* in the modelled tremor condition.

***Keywords: Albino, Cerebellum, Essential tremor, Mice, Nigella sativa, Phenol.***

## Introduction

Essential tremor is a neuronal disorder characterized by uncontrollable shaking (tremor) of different parts and sides of the body. [1] The phrase essential tremor was first used by Buressi in 1875 to describe patients who had only action tremor and no other neurologic manifestations. It is the most common movement disorder in the world with a prevalence of about 0.9% across all ages. [2] The prevalence of essential tremor increases with age. [3] Essential tremor presents with kinetic, postural and intention tremor. The main cause of essential tremor is unknown but models suggesting the aetiology of essential tremor have been proposed. One

of these models includes the gamma-aminobutyric acid (GABA) hypothesis, which suggests that essential tremor, occurs as a result of GABAergic dysfunction in the cerebellar dentate nucleus and brainstem leading to tremulous activity within the cerebellothalamocortical circuit.[4]Other hypotheses include the olivary model and the degenerative cerebellum model. Essential tremor may be suggested by a patient's failure to complete some designated tasks such as Archimedes spiral. Essential tremor, although considered benign, subjects the patient to social embarrassment arising from the inability to perform daily tasks. Several measures have been put in place to manage essential tremor; these measures include medical therapy, behavioural therapies, physical therapy, and surgical therapy. [5]

Evidence showing the relationship between the cerebellum and essential tremor has been proven by recent advances in medical imaging techniques. However, the extent of neural degeneration of the cerebellum which contributes to essential tremor is unknown.[6]The dysfunction of the cerebellum often presents with a wide range of symptoms depending on the part of the cerebellum that is affected; some of these symptoms include dysdiadochokinesia, ataxia, nystagmus, intention tremor, scanning speech, hypotonia, and loss of balance and abnormal gait. [7] The cerebellum, which develops from the metencephalon, a subdivision of the hindbrain of the developing human brain, is located at the posterior aspect of the brain just inferior to the occipital and temporal lobes, and within the posterior cranial fossa. [7] The cerebellum is divided into an outer cortex made up of grey matter and an inner layer made up of white matter.

Phenolic compounds have been shown in animal models to induce tremor or convulsions, denature proteins and cause muscular atrophy; a phenotype known to be exhibited in essential tremor cases. Phenol, also known as carbolic acid, is a naturally-existing toxic colourless to white solid. It is found naturally in foods, human and animal wastes as well as decomposing organic material. Phenol is manufactured artificially, by extraction from water and other natural sources, using conventional and advanced methods such as ozonation, adsorption, extraction, photocatalytic degradation, biological, electro-Fenton, adsorption and ion exchange, and membrane-based separation.[8]In clinical terms, phenolic compounds have been useful to the dentists as sedatives and antiseptics for topical application to the dentine and pulp.

*Nigella sativa* (N. sativa) is an annual flowering plant of Latin etymology, endowed with lots of pharmacologic properties. [9] *Nigella sativa* contains many active components, such as thymoquinone, alkaloids (nigellicines and nigelledine), saponins (alpha-hederin), flavonoids, proteins, fatty acids and many other constituents, which have positive effects on neurologically impaired patients. [10] The active components of *Nigella sativa* have been pharmacologically proven to have some ameliorative effects on a wide variety of neurological disorders, diseases as well as psychiatric dysfunctions, hence, its use in the treatment of these conditions. Some of these effects include anti-convulsion, anti-inflammation, antihypertensive, immune stimulation, anti-parkinsonism, and antioxidant. [11-13]

As essential tremor remains a prevalent movement disorder across the world, with age and gender-adjusted prevalence estimated at 3 to 4 per 1000, and with an annual incidence of 23.7 per 100,000, identification of more effective alternative therapies with minimal side effects remains a relevant neurological research goal. *Nigella sativa* has been utilised in history and confirmed with modern science, for its medicinal roles in diverse neurological and non-neurological human disorders. However, with a paucity of specific

information on the role of this plant in the treatment of tremors, this study aimed at investigating possible therapeutic potentials of *Nigella sativa* oil on the cerebellar functions of phenol-induced mice models of essential tremor. In an attempt to identify alternative drugs for human disorders, animal models that recapitulate the symptoms partly or holistically (though hardly possible) are important. Amidst human-mice dissimilarities and the complex and heterogeneous nature of neurological disorders such as essential tremor added to the limited information about its aetiology and underlying neural mechanisms, this study was conceived to explore other therapeutic possibilities. Therefore, this research aimed to determine the effect of *Nigellia sativa* on cerebellar functions in mice models using histological, neurochemical, neurobehavioural and morphological mechanisms.

## Methods

*Acquisition of research materials*

The animal experimentations were conducted at the Neurophytotherapy Research Laboratory in the Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu, with seventy-five (75) male adult BALB/c mice weighing 21-30g. Caging and behavioural apparatus were also obtained from the same laboratory. The crystalline phenol and *Nigella sativa* oil utilised were manufactured by Sigma® (USA) and Hemani® (Pakistan) respectively. Ethical permission for the study, numbered OOU-AREC/18/VII/23-017, was obtained from the Anatomical Research Ethics Committee of Olabisi Onabanjo University.

*Experimental design and dosing*

The animals were acclimated for one week. The animals were grouped as described in Table with each group consisting of 15 animals.

**Table I: The grouping of experimental animals and the respective adopted regimens**

| *Groups* | *Description* | *Regimen* |
|---|---|---|
| CTRL | The control group | Received normal saline only |
| P | Phenol group | Received phenol and normal saline |
| PNSC | Phenol + *Nigella sativa* group | Received phenol and *Nigella sativa* concurrently |
| NSP | *Nigella sativa* + phenol group | Pre-treated with *Nigella sativa* and then induced with phenol |
| NS | *Nigella sativa* group | Received *Nigella sativa* and normal saline |

*Administration*

Table II describes the administration process; route of administration, preparation of substances and duration of administration and dosage for each group of animals.

*Scoring of tremor response*

Tremor's response in the animals following the administration of phenol was scored based on the severity of the tremor and variety of tremor. The scoring was as thus;

None- 0

Mild- 1

Moderate 1 -2

Severe-3-4

*Sacrifice*

The animals were sacrificed 48hours after the last administration of therapy, by cervical dislocation to prevent the presence of chemicals which can affect neurochemical contents of the brain in the tissues.

*Histological assay*

Following excision, the brains were immersed in 10% formalin, after which the standard

histological protocols were carried out to illustrate the neuronal architecture within the cerebellum.

*Neurochemical assay*

The cerebelli of the animals were excised, weighed, and then homogenized in phosphate buffer (pH – 7.0). The homogenates were then put in EDTA sample bottles, and were assayed for glutamate and Glutathione peroxidase levels using spectrophotometry.

*Statistical analysis*

The data acquired were expressed in means [with a standard error of the mean (SEM)] and were analysed with One-way Analysis of Variance (ANOVA) using GraphPad Prism (version 5.3) software. Statistical significance was set at $P < 0.05$.

**Table II: The mode of administration, preparation of administered substances and dosage for each animal group**

| *Groups* | *Substances administered* | *Route of administration* | *Dosage (ml/kg)* | *Duration (days)* |
|---|---|---|---|---|
| CTRL | Normal saline | Orally and subcutaneously | 1ml/kg | 16 |
| PNSC | Liquid Phenol | Subcutaneously | 10 ml/kg | 16 |
| | *Nigella sativa* | Orally | 1 ml/kg | |
| P | Liquid phenol | Subcutaneously | 10 ml/kg | 16 |
| NSP | Normal saline | Orally | 10 ml/kg | 16 |
| | *Nigella sativa* | Orally | 1 ml/kg | |
| NS | Phenol | Subcutaneously | 10 ml/kg | 16 |
| | *Nigella sativa* | Orally | 1 ml/kg | |
| | Normal saline | Subcutaneously | 10ml/kg | 16 |

## Results

*Body Weight changes*

The administration of phenol had no effect on the body weight in the P group. In the groups with pre-treatment and concurrent administration of N. sativa, weight reduction was evident as the animals had reduced body weight percentages ranging from - 8% to -2%. The control group increased in body weight throughout the study.

As further shown in Figure 1, the administration of phenol resulted in a slight increase in body weight in the P group although, not very evident. However, pre-treatment and concurrent administration of *Nigella sativa* oil resulted in body weight reduction as shown in the PNSC and NSP groups. The body weight of the control group increased by about 10%. There was a significant difference between the pattern of body weights in the CTRL/PNSC and CTRL/NSP groups.

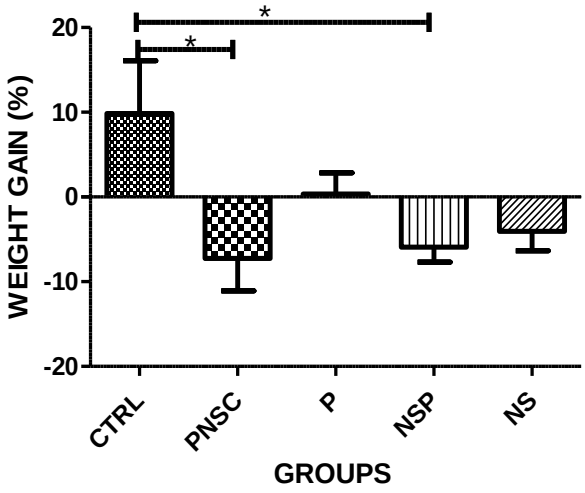


**Figure1: Percentage change in weight across all groups.**

*Rectal temperature changes*

As shown in Figure2, the P group upon first eight days of exposure to phenol, showed increased body temperature (from 37.5 to 37.8˚C)followed by a drop in temperature and then an increase in the last four days(from 36.1 to 37.5˚C). The group with concurrent treatment following the first eight days of administration also showed increased body temperature (from 36.5 to 37.1ºC) but thereafter, showed a reduction in body temperature. This group still showed a slight temperature increase (maintaining an average of 36.7°C) in the last four days of the study. The pre-treated group showed similar fluctuations in body temperature upon first administration but later displayed increased temperature from the 12th day until the 16th day of the study.

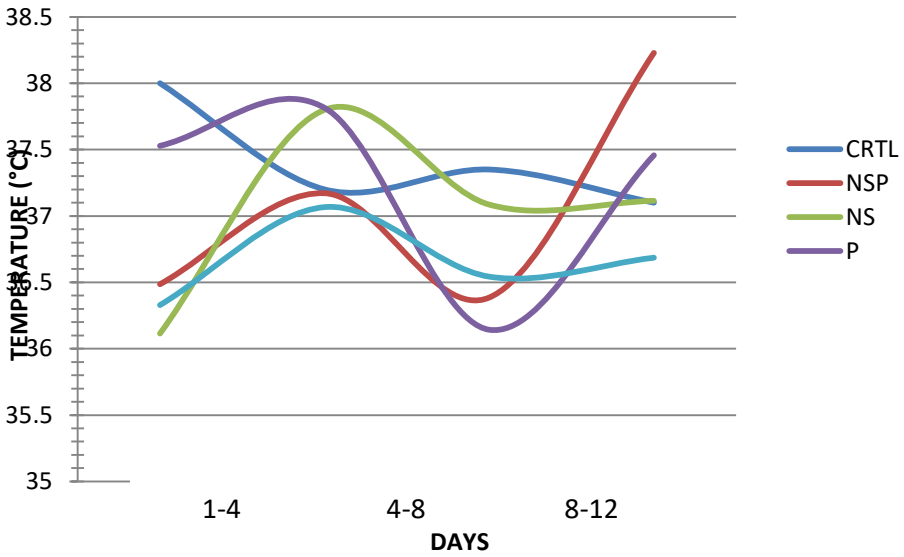


**Figure2: Average rectal temperature of animals across all the groups over the duration of the study**

*Relative brain weight*

As shown in Figure3, the phenol treated group had a high relative brain weight. The pre- and concurrently treated mice, however, had a higher level of relative brain weight value in contrast to the P group(0.2% higher).There existed a slightly strong level of significance between the relative brain weight values of the CTRL and PNSC groups as well as the CTRL and NSP groups with a difference of 0.3%.

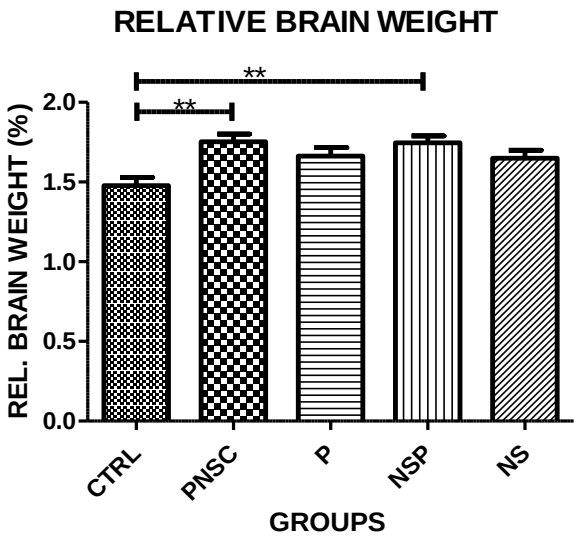


**Figure3: The relative brain weight across all groups**

*Daily feed and water intake*

Figure 4 shows that the administration of phenol was associated with an increase in daily feed consumption as seen in the NSP and P groups with feed intake levels of 65g and 125g respectively higher than that of the PNSC and CTRL groups. Concurrent administration of *Nigella sativa* oil reduced feed consumption as shown in the PNSC group which had a feed intake level close to that of the CTRL group. There exists a slightly strong level of significant difference between the P and PNSC groups. However, in Figure 4B, the untreated (P group) and pre-treated (NSP group) groups respectively drank 7ml and 5ml lower than the PNSC group as well as 12ml and 10ml lower than the control group. These differences were not significant.

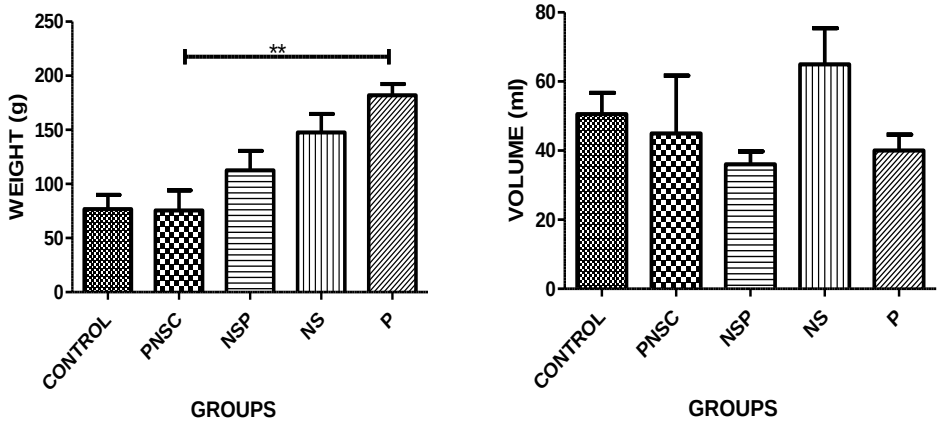


**Figure 4: Pattern of feed and water (in terms of weight and volume respectively) consumption across all groups.**

*Tremor response*

As shown in Figure 5, the mice treated with phenol only (P) demonstrated a constantly high level of tremor response throughout administration at every minute of administration. This response is most evident on the 10th and 11th days of the study. However, in the NSP and PNSC groups, which were treated with *Nigella sativa* oil preventively and concurrently respectively, the tremor score was reduced on these days.

*Neurobehavioral assay*

*Static rods assay*

Figure 6 shows that the phenol treated group took more time to turn on the 28mm and 9mm rods while the *Nigella sativa* pre-treated and concurrently treated groups turned faster.

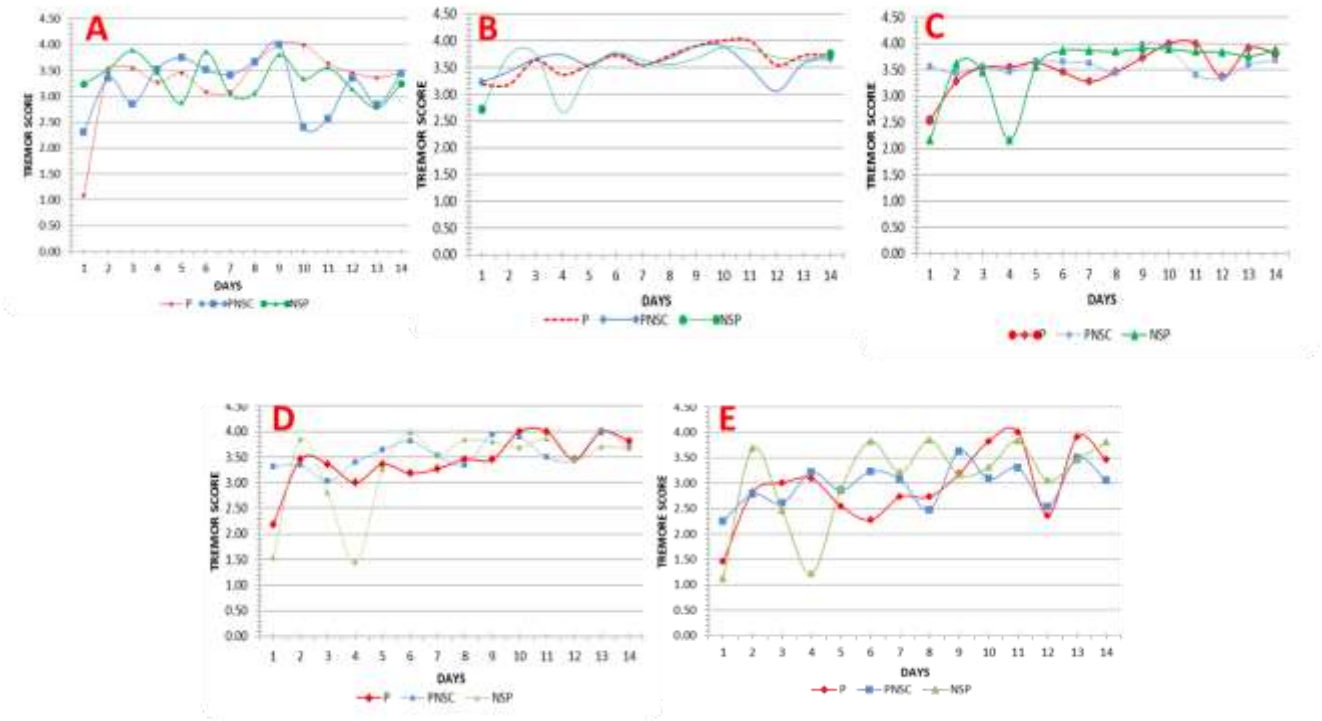


**Figure 5: The average daily tremor response in the phenol-administered mice (P, PNSC and NSP groups) after 2 minutes (A), 4 minutes (B), 6 minutes (C), 8 minutes (D) and 10 minutes (E) of phenol administration.**

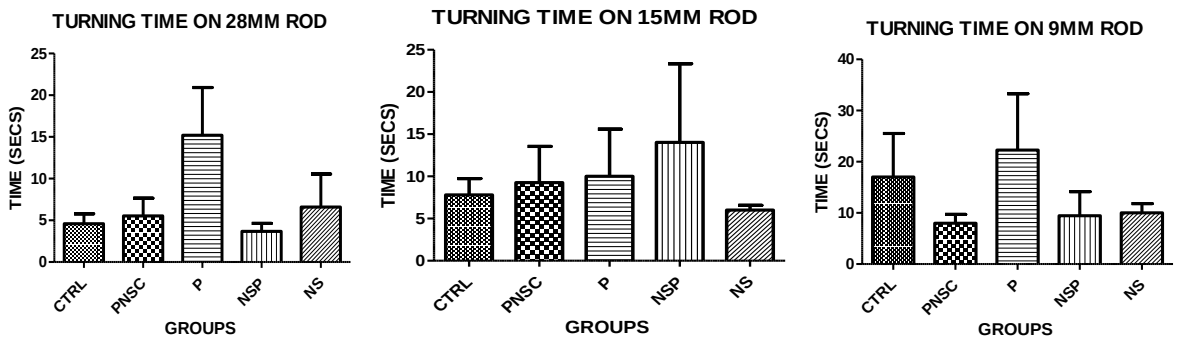


**Figure 6: The average turning time on the 28mm, 15mm and 9mm rods across all groups.**

Figure 7 shows that the phenol treated group had a high value of transit time on the 28mm and 9mm rods. Pre-treated and concurrently-treated groups displayed reduced transit time on the 28mm and 9mm rods. However, on the 15mm rod, the treated group as well as the *Nigella sativa* oil concurrently-treated group showed lesser turning times on the 28mm and 9mm rods. On the 15mm rod, pre-administration increased the turning time while concurrent treatment reduced the turning time. P group showed a reduced transit time while the pre-treated group showed even reduced transit time. The concurrently-treated group displayed increased transit time. However, these differences were not statistically significant.

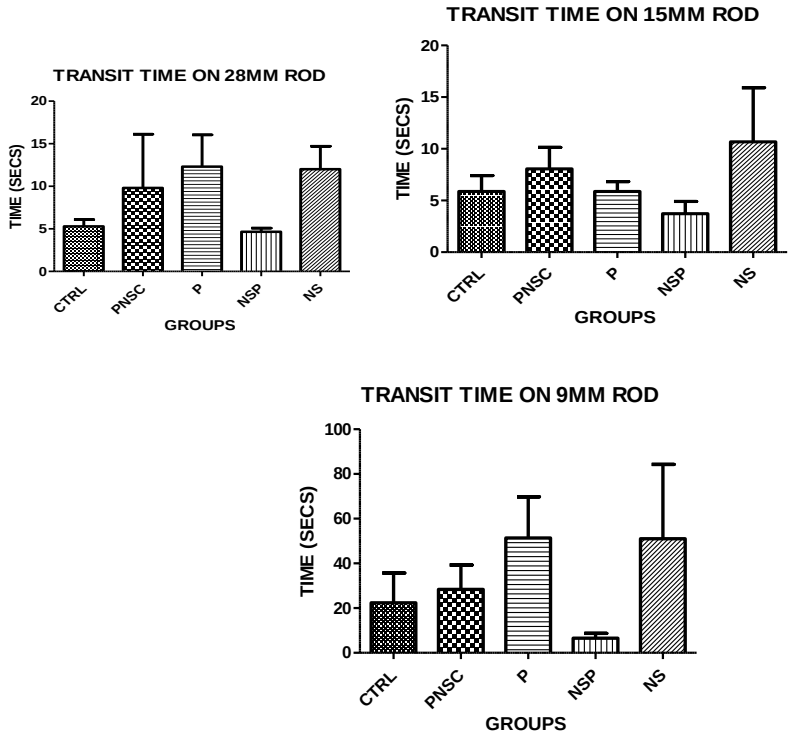


**Figure 7: Average turning and transit time exhibited by animals across all groups on the 28mm, 15mm and 9mm rod.**

*Parallel bars assay*

As shown in Figure 8, the phenol-treated group displayed reduced turning time but an increased transit time. However, the NSP and PNSC groups had increased turning time but displayed a reduced transit time. The NS and CTRL groups displayed high turning times but had reduced transit time. These differences were not statistically significant.

*Histological slides*

Plate 1 shows the molecular layer (ML), granular layer (GL) and white matter (WM) of the cerebellum of the mice across all groups. Haemorrhage (H) was noticed in the ML and region between subsequent ML (s) in the NS group. This haemorrhage (H) can also be seen in the molecular layer of the control group. However, it was not present in the three other groups. There was a loss of Purkinje cells (PC) in the ganglionic layer (GNL) of the P group as well as that of the PNSC group, though not as much as that of the P group. Loss of PC can also be noticed in the NSP group. This PC loss was absent in the NS and CTRL groups.

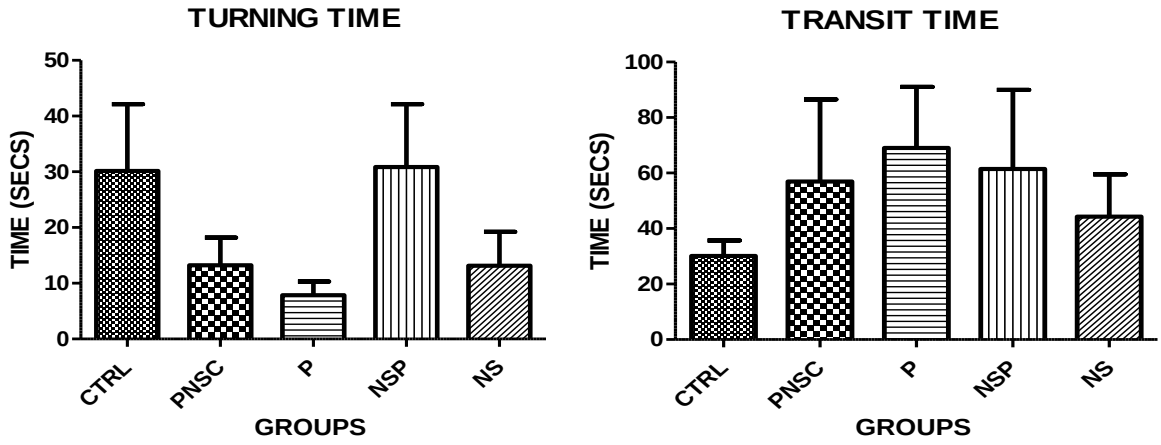


**Figure8: Turning and transit time exhibited by animals across all groups on the parallel bars**

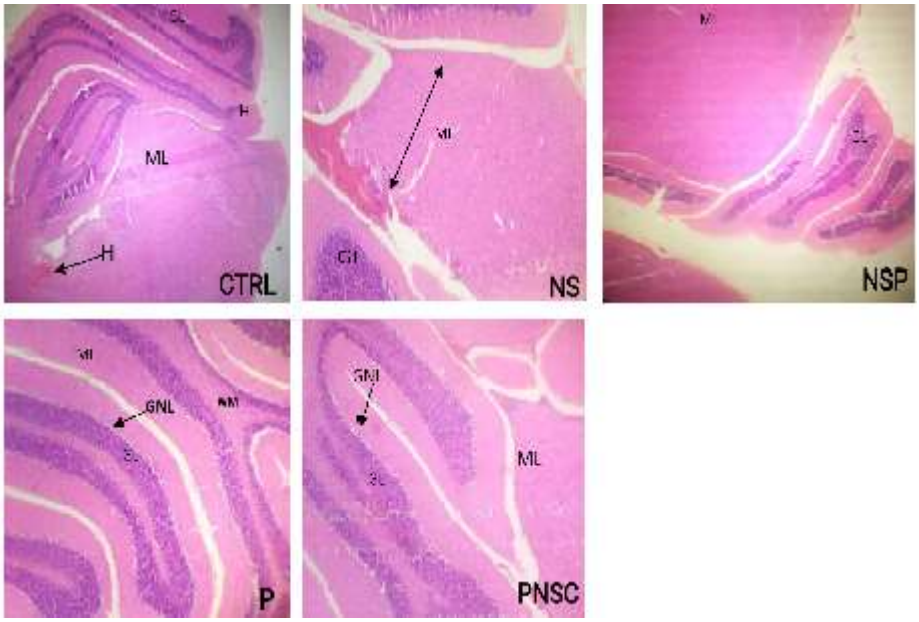


**Plate 1: 40x Micrographs of the cerebellum across all groups**

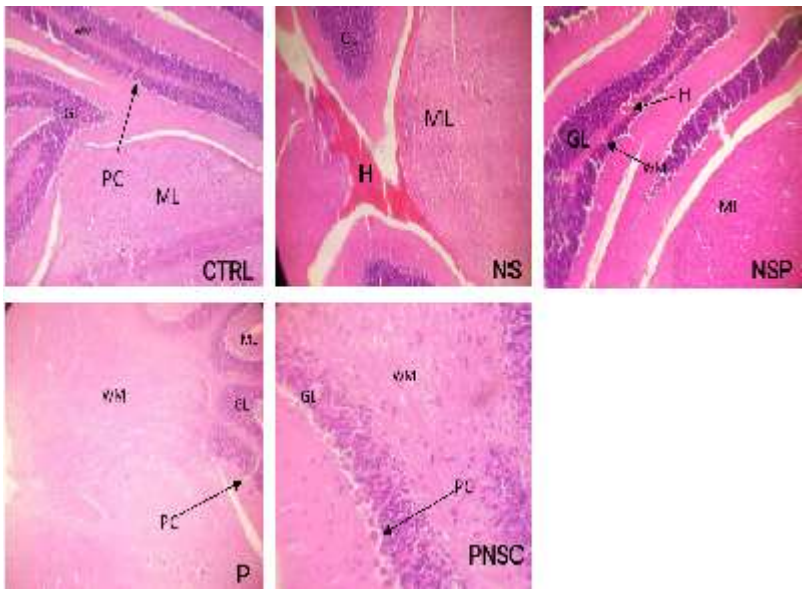


**Plate 2: 100x Micrographs of the cerebellum across all groups**

Plate 2 shows the features of the cerebellar layers across all groups. Haemorrhage (H) can be seen in the molecular layer of the cerebellum of the NS group. Similarly, haemorrhage (H) can be seen in the white matter (WM) of the NSP group. Reduced Purkinje (PC) cell was observed in the NSP group while the NS group has intact Purkinje cells (PC). The PNSC group also had reduced Purkinje cells (PC) but not as much as that of the P group.

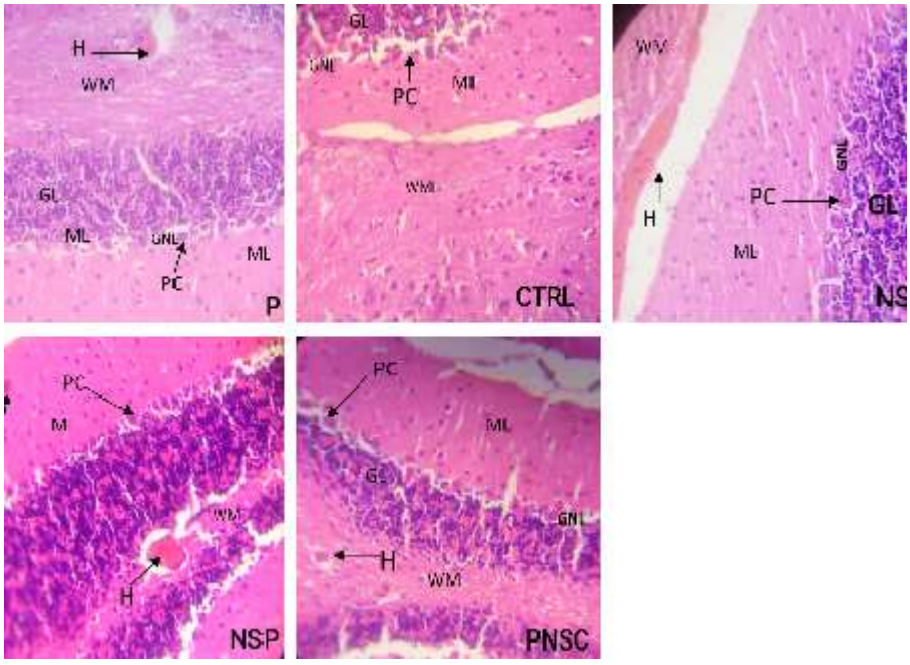


**Plate 3: 400x micrographs of the cerebellum across all groups**

Plate 3 shows the layers of the cerebellum across all groups. There is reduced Purkinje cells (PC) in the PNSC and P groups with the P group exhibiting the highest rate of reduction.

This reduction in Purkinje cells can also be seen in the NSP group though not as much as that of the P group. Haemorrhage (H) was seen in the white matter (WM) of the P, PNSC, NS and NSP groups in varying degrees. The NS group showed the highest degree, followed by the NSP group and the least was observed in the PNSC group. Little droplets of haemorrhages were also seen in the molecular layer in the NSP group.

*Neurochemical assay*

In Figure 9, there was a reduced level of glutamate in the phenol-treated group (0.06µg/g lower than the PNSC group). The glutamate level was high in the group with concurrent treatment with *Nigella sativa* oil(0.1µg/g higher than the control group) while it was more reduced in the *Nigella sativa* oil pre-treated group with a result just slightly lower than the P group. The control and NS groups displayed the lowest level of glutamate levels.

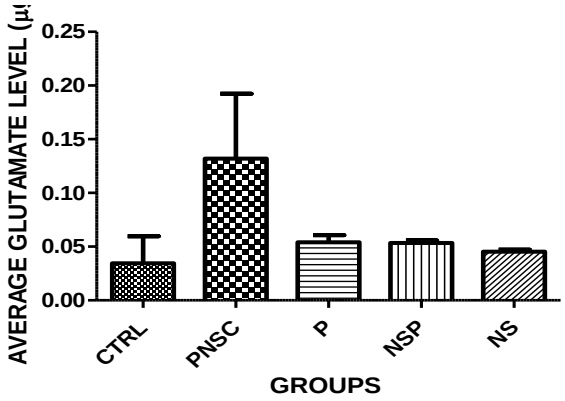


**Figure 9: Mean brain glutamate level across groups**

Figure 10 shows that the phenol-treated group exhibited increased dopamine levels (about 0.04 higher than the CTRL group). Pre-treated and concurrently *Nigella sativa* oil-treated group, however, had reduced dopamine levels respectively of about 0.021µg/g and 0.014µg/g lesser than the untreated P group. There was weak significance in the difference between dopamine levels in the CTRL and NS groups while the difference between the CTRL and P groups was slightly significantly strong.

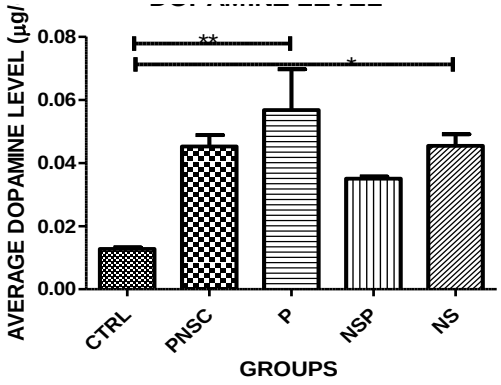


**Figure 10: The mean brain dopamine level across groups**

From Figure 11, the phenol-treated group had an increased level of GPX while the concurrently *Nigella sativa* oil-treated group showed even a more increased GPX level. The pre-treated group (NSP), however, had a reduced GPX level (0.035µg/g), which was 0.05µg/g lower than the value recorded in the P group. The CTRL group demonstrated the lowest level of GPX. There was a great level of significant difference between the CTRL, PNSC, P, NSP and NS groups while it was *vice-versa* between the PNSC/P, P/NSP, NSP/NS, and P/NS groups. Also, there was an average level of significant difference between the PNSC/NS groups while there was a relatively lower level of significant difference between the P/NSP groups.

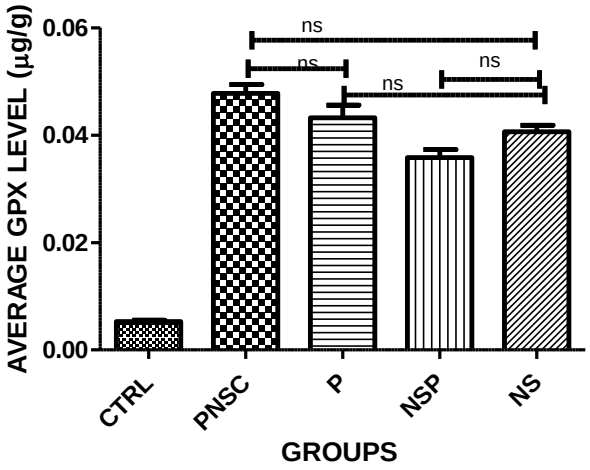


**Figure 11: The mean brain Glutathione peroxidase (GPX) level across groups**
**ns – Not significance**

## Discussion

From physical observations, following the administration of phenol, the P group of mice displayed several kinds of tremor, ranging from cranial and action tremor to limb and gait ataxia as well as reduced movement and subtle eye motion abnormalities. This is in ratification of earlier reports that essential tremor is characterized by 8 – 12Hz action tremor of the arms; other forms of cranial tremor, limb and gait ataxia as well as subtle eye motion abnormalities. [12] The amplitude of these tremulous symptoms, however, began to recede 10 to 15 minutes after treatment. Co-administration of *Nigella sativa* oil did not stop or reduce the rate of tremor in the PNSC group. However, it did in the pre-treated group of animals.

The administration of phenol had a weight stagnating effect on the animals. Pre-treatment and concurrent-treatment with *Nigella sativa* caused a reduction in the bodyweight of the animals. A reversal was subsequently demonstrated when pre-treatment and concurrent treatment with *Nigella sativa* oil led to an increased brain to body weight ratio in contrast to the group that received phenol. This observation suggests that the weight-reducing effects of *Nigella sativa* have no debilitating effect on neuronal integrity.

The pattern of feed intake showed that the phenol-treated group had a better appetite, but this was not translational as the animals showed no reasonable increase in body weight. This observation supports earlier

reports on the mode of action of phenol; there is a suggestion that phenol acts by denaturing protein, thus causing muscular atrophy. On the other hand, the pre-treated and concurrently-treated group of mice had reduced feed intake which was translational as they showed reduced body weights. This reduced feed intake might be as a result of increased water intake in the PNSC animals.
The administration of phenol increased the body temperature of the animals. This might be a result of tremorgenic responses and restlessness coupled with increased dopamine level which culminated into stress as observed physically following tremor induction as shown in the results of neurochemical assays. Increased body temperature in the animals corroborates earlier reports that increased body temperature is a feature of stress. [14]Concurrent administration of *Nigella sativa* oil increased daily water intake in the animals while pre-treatment with the same oil caused a reduction in water intake. However, the phenol administered group recorded an average level of water intake, but the reason for this observation is yet unknown.

From static rods assessment, the administration of phenol resulted in reductions in motor coordination in the P group of mice. This supports Deacon's explanation that animals with good motor coordination will spend lesser time turning on the static rod apparatus; however, animals with lesser motor coordination will spend more time turning and transiting the static rods apparatus. [15] Pre-treatment and concurrent treatment with *Nigella sativa* oil ameliorated this feature as demonstrated in the PNSC and NSP groups.

The parallel bars apparatus is a standard neurobehavioural assessment apparatus used to assay motor coordination in research animals. The parallel bar apparatus was used in this study to assay motor coordination in the mice. The mice with a higher value of transit and turning time are considered less coordinated. [15] The administration of phenol did not affect the turning ability of the animals as demonstrated by the P group of mice which displayed a good ability to turn on the apparatus. However, it had motor in-coordination effects on transit as the P group displayed increased transit time. Pre-treatment and concurrent treatment with *Nigella sativa* oil led to reduced transit time, which is a sign of good motor coordination.[15] While some of these differences were not statistically significant, it shows a high tendency worthy of confirmation with further studies.

Cerebellar dysfunctions are associated with traumatic injuries to the cerebellum. These injuries present with a wide variety of features such as ataxia, abnormal gait, nystagmus, inability to carry out a planned movement, and motor learning. However, the manifestations depend on part of the cerebellum that is affected. Cortical layers of the cerebellum which include the molecular, Purkinje and granular layers might get implicated in these traumatic injuries.

From the histological assessment of the brain, the administration of phenol induced deterioration of the Purkinje cell layer of the P group. However, this was ameliorated in the *Nigella sativa* pre-treated and concurrently treated groups. Glutamate is a neuroexcitatory neurotransmitter. It is the most abundant of all neurotransmitters in the brain.[16] Over stimulation of this neurotransmitter results in excitotoxicity. From neurochemical assessment, the administration of phenol had no significant effect on the glutamate level in the mice as shown in the P group. The concurrent administration of *Nigella sativa* oil increased glutamate levels while pre-treatment lowered glutamate levels.

Dopamine is a neuromodulator that modulates the level of excitation as well as the level of inhibition, thus regulating certain

behaviours such as motivation as well as focus. However, constant stimulation of dopamine can lead to the depletion of dopamine levels over time.[16, 17] In the present study, neurochemical assessment presented phenol as an up-regulator of dopamine as displayed by the P group of mice. The concurrent and pre-treatment with *Nigella sativa* oil, however, lowered the rate of dopamine production, thereby confirming the anxiolytic effect of *Nigella sativa* oil. This might have been the source of good motor coordination demonstrated by the pre-treated and concurrently-treated group of animals as their levels of anxiety have been regulated.

Glutathione peroxidase (GPX) is an antioxidant enzyme with the ability to scavenge free radicals. This scavenging action of GPX also helps to prevent lipid peroxidation and thus, maintain intercellular homeostasis as well as redox balance. [18] The high GPX level in the mice induced with tremor signifies the oxidative effects of phenol. This is however ameliorated with pre-administration of *Nigella sativa* oil as demonstrated in the NSP group of mice.

Despite the medicinal potentials of *Nigella sativa* in the treatment of essential tremor, as suggested in the present study, the use of dendritic, Nissl and immunohistochemical markers for further characterization of the cerebellar neuroarchitecture is required. This shall herald the translational phase of the study towards human therapeutic use of *Nigella sativa* in the management of essential tremor.

## Conclusion

This study demonstrated the weight-reducing, neuroprotective, motor coordinating and anxiolytic potentials of *Nigella sativa* oil in the modelled tremor condition. This was established in the neurochemical, neurobehavioural and histological evidence presented in the groups of mice pre-treated and concurrently-treated with *Nigella sativa* oil, in contrast to the observations made in the phenol-treated-only (essential tremor induced) counterparts. It is recommended that the findings from this study be further validated using other motor coordination assays like the Rotarod, as well as with immunohistochemical assays for further neuroarchictectural appreciation of the potentials of *Nigella sativa* oil in the cerebellum of animal essential tremor models. Clinical trials may, thereafter, be conducted to translate these findings into human benefits.

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