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Short Communication

Effect of Aqueous Seed Extract of *Nigella sativa* on Neurobehaviour in Lead Induced Neurotoxicity

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Abstract

The manipulation of lead for many uses like the manufacturing of protective paints for iron and steel, explosives, rodenticide and batteries has led to lead contamination of air, dust and soil. It is considered as one of the most important environmental pollutants of both urban and semi urban areas. *Nigella sativa* is known to have protective effects on some ailments such as hypertension, diabetics, tumor and some neurological problems. Twenty-five adult Long Evans rat of weight range of 150g-200g were used in this study, the animals were randomly divided into five groups of 5 animals per group. Group I (control) was administered distilled water, group II were administered 1000mg/kg of aqueous seed extract of *Nigella sativa* for 14 days, group III were administered 60mg/kg of lead acetate for 21 days, group IV were administered 60mg/kg of lead acetate for the period of 21 days and then aqueous seed extract of *Nigella sativa* at 1000mg/kg using orogastric tube for the period of 14 days and group V were administered 60mg/kg of lead acetate for the period of 21 days and then aqueous seed extract of *Nigella sativa* at 500mg/kg of animals for the period of 14 days. Neurobehavioural testing using beam walking resulted in significant differences the group's performances on the beam ($p<0.05$). It was observed that the extract of *Nigella sativa* decreased the time spent crossing the beam and also decreased the time spent on the platform. These results suggest that *Nigella sativa* administration alleviates the effects of lead induces neurotoxity in relation to neurobehaviour.

Keywords: lead, *nigella sativa*, neurotoxicity, rat, beam walking

INTRODUCTION

Nigella sativa (*N. sativa*) is an annual plant and widely used as medicinal plant throughout the world. The seeds of the plant have been used traditionally in various disorders and as a spice to ranges of Persian foods. *N. sativa* has therapeutic effects on Tracheal responsiveness (TR) and lung inflammation on induced toxicity by Sulfur mustard. *N. sativa* has been widely used in treatment of various nervous system disorders such as Alzheimer disease, epilepsy, and neurotoxicity (Khazdair, 2015). Most of the therapeutic properties of this plant are due to the presence of some phenolic compounds especially thymoquinone (TQ), which is major bioactive component of the essential oil (Khazdair, 2015). *Nigella sativa* is an annual flowering plant in the family *Ranunculaceae*, native to south and southwest Asia. It grows in countries bordering the Mediterranean Sea, Pakistan, India and Iran (Ali and Bluden, 2003). The seeds of *Nigella sativa* plant have been used to promote health and fight diseases for centuries especially in the Middle East and Southeast Asia. The plant is widely grown in different parts of the world and it is an annual plant cultivated in India and Pakistan. As an oriental spice, *Nigella sativa* has long been used as a natural medicine for the treatment of many acute as well as chronic conditions. This plant has been a great focus of research for centuries and has several traditional uses and consequently has been extensively studied for its chemical constituent and biological activities. The seeds of *N. sativa* are the source of the active ingredient of this plant (Ghaznavi, 1991).

Historical use of black seeds has been mentioned in various religious and ethnic books. The actual importance of *N. sativa* to the Muslims came from Prophetic sayings that black seed is the medicine for every disease except death (Ghaznavi; 1991). Black seeds are identified as the curative black cumin in the holy bible; it is also described as the melanthion of Hippocrates and Dioscordes. In the Greco Arab/ Unani-Tibb system of medicine which originates from Hippocrates, his contemporaries Galen and Ibn- Sina regarded black seed as a valuable remedy in hepatic and digestive disorders. The famous book of medicine by Ibn-Sina "The cannon of medicine (980-1037) revealed historical importance of this Black seeds as the seeds "That stimulates the body's energy and help recovery from fatigue (Ghaznavi, 1991; Chevallier, 1996).

Millions of people in the Mediterranean region and Far East countries have used the oil of *N. sativa* seeds daily as a natural protective and curative remedy. Historically, it has been recorded that *N. sativa* seeds were prescribed by ancient Egyptian and Greek physicians (Hajhashemi *et al*., 2004). The seeds have also been used in mercury poisoning, sores and leprosy (Ahmad *et al*., 2004).

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Chemical composition of *N. sativa* seeds includes oil, protein, carbohydrate, fiber, and saponin. The fixed oil chemical compositions of *N. sativa* are linoleic acid, oleic acid, palmitic acid, arachidic acid, eicosadienoic acid, stearic acid, linoleic acid, and myristic acid (Eltahir *et al*, 2004). The major phenolic compounds of *N. sativa* seeds are p-cymene (37.3%), thymoquinone (TQ) (13.7%), carvacrol (11.77%), carvone (0.9%), and thymol (0.33%) (Khazdair, 2015). .*Nigella sativa* seed contains more than 30% fixed oil and 0.4-0.45% volatile oil. The fixed oil is composed mainly of unsaturated fatty acids. Thymoquineone (TQ) is the major active ingredient of the volatile oil (Worthen *et al*., 1998). Thymoquinone has a strong antioxidant potentials due to its scavenging activity towards free radicals (Kanter *et al*., 2005). There are many reports on its biological activities including antihypertensive, antidiabetic, antibacterial, antitumor and immunomodulator (Kokdil *et al*., 2009).

Lead occurs in a variety of organic and inorganic compounds with a multitude of additional uses in the manufacturing of protective paints for iron and steel, explosives, rodenticide, batteries etc. The manipulation of lead for these uses has caused lead contamination of air, dust and soil. It is considered as one of the most important environmental pollutants of both urban and semi urban areas. Because of its persistence in the environment, exposure to lead has become public health concern (Vaglenov *et al*.,2001).
In humans, lead has been shown to have widespread of effects, targeting the nervous system, cardiovascular system, reproductive system, red blood cells and kidneys (Landrigan and Todd,1994; Goyer,1993). Compared to other organ system, the nervous system appears to be the most sensitive and chief target for lead induced toxicity (Cory-slechta, 1996). Both the central nervous system and peripheral nervous system become affected when exposed to lead. The effects on the peripheral nervous system are more pronounced in adults while the central nervous system is more prominently affected in children (Brent, 2006; Bellinger, 2004). Encephalopathy (a progressive degeneration of certain part of the brain) is a direct consequence of lead exposure and the major symptoms include dullness, irritability, poor attention span, headache, muscular tremor, loss of memory and hallucination. More severe manifestation occurs at a very high exposure and includes delirium, lack of coordination, convulsion, paralysis, coma and ataxia (Flora *et al*., 2006). Xu *et al* (2009) reported that pre and post natal exposure to lead can damage short and long term memory in rats.

MATERIAL AND METHODS

Experimental animals and treatment: Twenty five adult Long Evans rat of weight range of 150g-200g were used in this study. The animals were randomly divided into five groups of 5 animals per group. Group I (control) was administered distilled water, Group II were administered 1000mg/kg of aqueous seed extract of *Nigella sativa* for 14 days, Group III were administered 60mg/kg of lead acetate for 21 days, Group IV were administered 60mg/kg of lead acetate for the period of 21 days and then aqueous seed extract of *Nigella sativa* at 1000mg/kg using orogastric tube for the period of 14 days and group V were administered 60mg/kg of lead acetate for the period of 21 days and then aqueous seed extract of *Nigella sativa* at 500mg/kg of animals for the period of 14 days.

Neurobehavioural test using balance beam test for coordination: 24 hours after the final administration, the animals were subjected to neurobehavioural testing using the beam test. This test is used to assess fine motor coordination. This test essentially examines the ability of the animal to remain upright and to walk on an elevated and relatively narrow beam. The protocol is based on those of Southwell *et al*., (2009) and Carter *et al*., (2001). The time spent crossing the beam and the number of times the animals slipped were recorded for each group.

Statistical Analysis: All data obtained from the neurobehavioral studies were expressed as Mean ± SEM (Standard Error of Mean). One-way analysis of variance (ANOVA) was used to compare the mean differences between groups. Values were considered statistically significant at $p<0.05$. All result were analyzed using Statistical Package for Social Scientist (SPSS version 21), Chicago Illinois U.S.A.

RESULTS

Neurobehavioural tests (time spent crossing beam)
The results of the time spent crossing the beam showed significant difference between the control group (group 1) and groups 3, 4 and 5 at $p<0.05$. From the result of the beam walking analysis, there was no statistical significant change in all the groups both in the time spent on platform and time taken to cross the beam.

Table 1
Time spent crossing beam

Group	Treatment	Latency
1	Control	2.00 ± 0.00
2	NSE(1000mg/kg)	2.75 ± 1.70
3	Lead acetate (60mg/kg)	5.00 ± 2.82*
4	Lead acetate+ 1000mg/kg NSE	3.50 ± 1.91*
5	Lead acetate+ 5000mg/kg NSE	3.25 ± 0.95*

Values are Mean±SEM of 5 animals in each group

Table 2
Time spent on platform

Group	Treatment	Latency
1	Control	10.67 ± 5.50
2	NSE(1000mg/kg)	10.50 ± 2.37
3	Lead acetate (60mg/kg)	18.20 ± 1.68*
4	Lead acetate+ 1000mg/kg NSE	7.75 ± 1.13*
5	Lead acetate+ 5000mg/kg NSE	6.00 ± 3.35*

Values are Mean±SEM of 5 animals in each group

Group 2 (Extract only), showed an increase time taken to cross the beam (2.75 ±1.70 sec) that was not statistically significant when compared to the control group (2.00±0.00 secs). Group 3 (lead only), showed a significant increase in time spent crossing the beam (5.00±2.82) when compared to the control group. Group IV (lead and then extract high dose), showed a significant increase in the time spent crossing the beam (3.50 ±1.91) when compared to control and the extract only group (group 2). The timing was however less than the lead only group (group 3) although this was not statistically significant. Group 5 ((lead and then extract 500mg/kg) also showed an insignificant decrease in the time spent crossing the beam (3.25 ±0.95) when compared to the lead only group (group 3) and group 4 ((lead and then extract 1000mg/kg).

DISCUSSION

In spite of the strict regulatory measures taken by most countries to decrease environmental lead burden, human lead exposure continues to remain an important public health issue particularly in developing countries with a lack of public control. Lead is dispersed throughout the environment, in ambient air, in many foods, in drinking water and in dust. The major environmental sources of metallic lead and its salts are paint, auto exhaust, and contaminated food and water (Shalan *et al*., 2005). This work studied fine motor skills using an elevated beam. Administration of lead brought about an increase in the times spent on the platform and crossing the beam when compared with the control group which is indicative of damage to the cerebral cortex. Administration of *N.sativa* showed reductions in the times spent on the platform and crossing the beam suggesting an improvement in motor skills. These results are similar to the findings of Al-Naggar *et al* (2003) who evaluated the central analgesic effects of methanol and aqueous extract of *N. sativa* using hot-plate test and pressure test and that reaction time in the hot-plate test and pressure tests are significantly increased by both of extracts and Azzubaidi *et al* (2012) who demonstrated that *N. sativa* seeds could significantly preserve the spatial cognitive in rats challenged with chronic cerebral hypoperfusion. Other researchers have reported that *N. sativa* can prevent the damage of spatial memory after scopolamine administration and reduced the acetylcholinesterase (AChE) activity as well as oxidative stress of the brain tissue in rats (Hossini *et al*, 2015). Researches using animal models have shown that lead toxicity could be associated with oxidative stress via the generation of reactive oxygen species and can be mitigated by some antioxidants, materials of animal origin and extracts of plant origin (Adikwu *et al.*, 2013). The present findings agree to the fact that natural compounds that are rich in antioxidants help to reduce oxidative stress thus alleviating the effect of oxidative agents (Ahamed and Siddiqui, 2007; Burger *et al*., 2011). The findings also indicate a dose dependant relationship.

In summary, the aqueous extract of *N sativa* seeds improves the fine motor activity of animals exposed to lead

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