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PROTECTIVE ROLE OF THE *NIGELLA SATIVA* OIL AGAINST ARSENIC-INDUCED NEUROTOXICITY IN MALE RATS

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ABSTRACT

Objective: Arsenic (As) exposure causes oxidative stress and tissue damage in the rat brain. This study was under taken to investigate the role of Nigella sativa oil (NSO) in ameliorate the neurotoxicity induced by arsenic exposure in male rats. Design: Forty male Sprague Dawley rats were divided into four groups each 10 rats, Group I (normal control group): received saline solution orally. Group II (As group): rats received arsenic as sodium arsenite orally (5 mg/kg bw/day) for 30 days. Group III (NSO group): rats administered orally with *Nigella sativa* oil at a dose of (0.5 ml/kg bw/day) for 30 days. Group IV (As +NSO group): rats received both sodium arsenite orally (5 mg/kg bw/day) and NSO oil at a dose of (0.5 ml/kg bw/day) for 30 days. At the end of the experimental period, rats were sacrificed; brain samples were obtained for different biochemical analyses. The biochemical analyses included determination of tumor necrosis factor-α (TNF-α), interleukin-1b (IL-1b), FAS, B-cell lymphoma 2 (Bcl2), Brain derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF). Results: In comparison with normal control group, the As group recorded significant increase in the brain levels of TNF-α, IL-1b, FAS and VEGF levels in brain tissue. On the other hand, significant decrease in brain Bcl2 and BDNF levels were detected in As group as compared to normal control group. In contrast, the treatment of As group with NSO caused an improvement in the most studied biochemical parameters as indicated by decreased brain levels of TNF-α, IL-1b, FAS and VEGF accompanied with significant increase in the levels of Bcl2 and BDNF as compared to As group. Conclusion: This study revealed that Nigella sativa oil significantly ameliorates the neuroinflammation and neurodegenerative disorders

characterizing to neurotoxicity induced by sodium arsenite in male rats due to its anti-inflammatory property and antioxidant capacity.

Keywords: Sodium arsenite, Nigella sativa oil, anti-inflammatory, antioxidant capacity

1. INTRODUCTION

Arsenic (As) is the 20th most abundant element in the earth crust that naturally occurs in many minerals, usually combined with sulfur and metals. It is widely distributed in the environment and is released from the minerals by human activities, wind erosion and groundwater solutions [1]. Today, chronic As exposure in the general population has been reported worldwide [2]. Most human As exposure occurs from consumption of drinking water containing high amounts of inorganic arsenic (iAs). Inorganic arsenic, i.e., arsenite (As [III]) and arsenate (As[V]), is most toxic and also most abundant in water [1]. According to the As limitation of drinking water distributed by the World Health Organization (WHO), it is estimated that more than three million people worldwide are exposed to high amounts of arsenic and more than 30,000 arsenicosis patients have been confirmed in China [3]. Chronic exposure to high concentrations of arsenic in drinking water in humans has been found to be associated with skin lesions [4], peripheral vascular disease [5], hypertension [6], blackfoot disease [7], and high risk of cancers [8]. In addition, impaired cognitive functions and deficiencies in learning and memory have been reported in epidemiological and experimental studies in conjunction with the findings that arsenic compounds can cross the blood–brain barrier and accumulate in brain tissues [1]. Symptoms of arsenic neurotoxicity such as subclinical nerve injuries including peripheral neuropathies, tingling and numbness of the limbs, reduced muscle strength, delirium, encephalopathy, confusion, disorientation, unusual visual sensations, mental sluggishness, blurred vision and loss of hearing and taste have been reported in copper smelter employees [9]. *Nigella sativa* is an herbaceous, flowering plant that is native to southwest Asia, but it is cultivated in different parts of the world including Southern Europe, Northern Africa and Asia Minor. For thousands of years, many cultures have traditionally used *Nigella sativa* as a spice, food additive, preservative, as well as herbal remedy for various diseases and conditions such as asthma, diarrhea, headache, toothache, nasal congestion, and several types of cancer [10]. The pathophysiological and immunopharmacological properties of *Nigella sativa* and its active ingredients such as thymoquinone are evident. Many studies have demonstrated that *Nigella sativa* exerts anti-oxidant and anti-microbial effects *in vitro* and *in vivo* [11]. Hence, the present study was

carried out to investigate Nigella sativa oil (NSO) as potent anti inflammatory and antioxidant agent capable of protecting brain and neurons from arsenite induced neurotoxicity.

2. MATERIALS AND METHODS

2.1. Drugs and chemicals

Sodium arsenite (soluble in dist water), Sodium chloride, Tris–Hcl and sucrose were purchased from Sigma Chemical Co., USA. *Nigella sativa* oil (NSO, 100% pure, endotoxin free, obtained by cold processing, Egyptian Ministry of Health Registration No. 2906/2002, Eng. Mohamed Dorra Co., Alexandria, Egypt).

2.2. Experimental animals

Mature male Sprague Dawley rats (130 ± 10 gm), 12 weeks old were obtained from the animal house of Theodore Bilharis Institute (Cairo, Egypt). They were kept in plastic cages at room temperature (25 ± 2 ◦C) and humidity (55%) under a 12 h dark-light cycle. All animals were accommodated with laboratory conditions for at least two weeks before the experiment and maintained under the same conditions all over the experiment. Diet and water were allowed *ad libitum*.

2.3. Experimental design

Animals were randomly divided into four groups (10 rats for each). Group I (normal control group): received saline solution orally. Group II (As group): rats received arsenic as sodium arsenite orally (5 mg/kg bw/day) for 30 days [12]. Group III (NSO group): rats administered orally with *Nigella sativa* oil at a dose of (0.5 ml/kg bw/day) [13] for 30 days. Group IV (As+NSO group): rats received both sodium arsenite orally (5 mg/kg bw/day) and NSO oil at a dose of (0.5 ml/kg bw/day) for 30 days

2.4. Samples collection

At the end of the experimental period, the animals were sacrificed and the whole brain of each animal was rapidly dissected, thoroughly washed with isotonic saline, dried and then weighed. Brain samples were homogenized immediately to give 10% (w/v) homogenate in ice-cold medium containg 50 mM Tris–Hcl (pH 7.4) and 300 mM sucrose [14]. The homogenate was centrifuged at 3000 rpm for 10 min at 4 ◦C. The supernatant (10%) was separated for biochemical analysis.

2.5. Biochemical analysis

Quantitative estimation of total protein level in the brain homogenate was carried out according to the method of [15]. Brain tumor necrosis factor-α (TNF-α) level was determined according to [16] using ELISA technique using human TNF-α ELISA kit purchased from orgenium Co., Vantaa FINLAND. IL-1 beta (IL-1b) level was estimated using ELISA technique using kit purchased from Uscn life science Inc., USA. FAS level in the brain was assayed by ELISA according to the method of [17] by using ELISA kit purchased from Bioscience Co., Vienna, Austria, Europe. Bcl2 in the brain tissue was estimated by ELISA technique by using ELISA kit purchased from Glory Science Co., Ltd, USA, according to manufacturer's instruction. Brain derived neurotrophic factor (BDNF) was estimated by ELISA technique [18] using kit purchased from RayBiotech Co., USA. Brain vascular endothelial growth factor (VEGF) was estimated by ELISA procedure [19] using a kit purchased from Invitrogen Co., CAMARILLO, and VENTURA, California, USA.

2.5. Statistical anaysis

In the present study, all results were expressed as mean ± S.E. of the mean. Statistical Package for the Social Sciences (SPSS) program, version 14.0 was used to compare significance between each two groups. Difference was considered significant when $P \leq 0.05$.

3. RESULTS

The results in Table 1 showed the effect of NSO treatment on arsenic-induced alterations in brain levels of inflammatory markers TNF-α and IL-1b. In comparison with the normal control group, there was a significant increase in the levels of TNF-α (90.65%) and IL-1b (97.07%) in arsenic group. The oral administration of NSO to normal animals caused a slight increase in the levels of TNF-α (5.29%) and IL-1b (2.66%) as compared to normal control group. On the other hand, the treatment of arsenic group with NSO produced significant reduction in the levels of TNF-α (-55.01%) and IL-1b (-33.85%) as compared to the arsenic group.

The data in Table 2 showed the effect of NSO treatment on apoptic/antiapoptic markers in brain tissue of arsenic-induced neurotoxicity in rats. It was revealed that, daily administration of sodium arsenite to rats resulted in significant elevation in FAS level (172.98%) associated with a reduction of Bcl2 level (-62.24%) as compared to the normal control group. In contrast, the treatment of both As and NSO produced significant reduction in the level of

FAS (-42.47%) and significant elevation in the level of the Bcl2 (89.19%) as compared to the As group.

The results in Table (3) showed the effect of NSO on brain growth factors of arsenic-induced neurotoxicity in rats. In comparison with normal control group, there was a significant decreases in BDNF level (-53.33%) associated with an increment in the VEGF level (26.62%) in the As group. On the other hand, treatment of As group with NSO caused a significant elevations in the brain level of BDNF (66.67%) and reduction in the brain level of VEGF (-49.41%), compared to As group.

Table 1: The effect of Nigella sativa oil treatment on arsenic-induced alterations in brain levels of inflammatory markers: TNF-α and IL-1b.

Groups / Parameters	Con	As	NSO	As+NSO
TNF-α (Pg/mg protein)	209.26±10.56	398.96±9.62[a] C (90.65%)	220.34±6.48[a] C (5.29%)	247.83±9.21[b] C (18.43%) D (-55.01%)
IL-1b (Pg/mg protein)	122.67±5.43	241.75±6.81[a] C (97.07%)	125.93±3.56[a] C (2.66%)	159.92±4.34[b] C (30.37%) D (-33.85%)

- *Values are expressed as mean ± standard error (SE) for 10 animals/ group.*
- *a: Significant change at P <0.05 in comparison with normal control group.*
- *b: Significant change at P < 0.05 in comparison with As group.*
- *C%, percentage of change from normal control group.*
- *D%, percentage of change from As group.*

Table 2: The effect of Nigella sativa oil treatment on FAS and BcL2 levels in brain tissue of arsenic-induced neurotoxicity in rats.

Groups / Parameters	Con	As	NSO	As+NSO
FAS (Pg/mg protein)	65.38±3.45	178.48±7.52[a] C (172.98%)	83.53±2.16[a] C (27.76%)	102.68±5.25[b] C (57.05%) D (-42.47%)
BcL2 (ng/mg protein)	0.98±0.03	0.37±0.05[a] C (-62.24%)	0.81±0.06[a] C (-17.35%)	0.70±0.02[b] C (-28.57%) D (89.19%)

- *Values are expressed as mean ± standard error (SE) for 10 animals/ group.*
- *a: Significant change at P <0.05 in comparison with normal control group.*
- *b: Significant change at P < 0.05 in comparison with As group.*
- *C%, percentage of change from normal control group.*
- *D%, percentage of change from As group.*

Table 3: The effect of Nigella sativa oil treatment on brain growth factors of arsenic-induced neurotoxicity in rats.

Groups / Parameters	Con	As	NSO	As+NSO
BDNF (ng/mg protein)	0.45±0.006	0.21±0.004[a] C (-53.33%)	0.39±0.005[a] C (-13.33)	0.35±0.004[b] C (-22.22%) D (66.67%)
VEGF (Pg/ mg protein)	327.78±10.87	415.05±7.81[a] C (26.62%)	320.32±9.56[a] C (-2.28%)	365.64±6.52[b] C (11.55%) D (-49.41%)

- *Values are expressed as mean ± standard error (SE) for 10 animals/ group.*
- *a: Significant change at P <0.05 in comparison with normal control group.*
- *b: Significant change at P < 0.05 in comparison with As group.*
- *C%, percentage of change from normal control group.*
- *D%, percentage of change from As group.*

4. DISCUSSION

The present study demonstrated an increase in the brain level of proinflammatory molecules, such as TNF-α, IL-1b in As group compared to the normal control group. This may be due to that arsenic exposure produces free radicals that cause damage to lipid, protein, and DNA of the body [20]. Arsenite, like other heavy metals, has been associated with tissue damage. In this line, [21] revealed that arsenite induces oxidative injury in the nigrostriatal dopaminergic system of rats' brain and induces apoptosis in the central nervous system of rat. Also, arsenite-induced oxidative injury in rats' brain was also reported by [22]. The secretion of TNF-α and IL-1b, the major pro-inflammatory cytokines, increased due to the environmental toxicants culminates the acute phase inflammatory responses [23]. These inflammatory mediators are involved in various biological and cellular responses including tumor progression, growth factor, transcription factor and activation of proapoptotic proteins [24]. Besides the inflammatory responses, these inflammatory cytokines are also involved in the generation of free radicals via mitochondrial respiratory chain reaction [24]. Several studies reported that impairment in astrocytes leads to the increased secretion of inflammatory mediators especially TNF-α and IL-1b which are associated with the development of brain inflammation [25]. These studies suggest that oxidative stress plays an important role in pathogenesis of neurodegenerative disorders. Programmed apoptotic cell death (or apoptosis) is a process by which unwanted cells are eliminated from multicellular organisms in a non-harmful way. Several genes have been identified that control the regulation of apoptosis, namely Bcl-2, p53 and c-myc [26]. It is widely recognized that the main entry pathways for

drug discovery in neurotoxicity include (1) antiapoptotic compounds, (2) antioxidant compounds, and (3) substances with a mitochondrial impact [27]. The current study shows a significant increase in the level of APO-1 FAS in brain tissue in the group of arsenic administered rats compared to the normal control group. FAS and FAS ligand have been recently reported to be associated with neuritic degeneration [28]. In accordance with the present results, [29] demonstrated that sodium arsenite decreased cell viability and caused cellular changes typical of apoptosis. Arsenite-induced cerebellar neuron apoptosis was blocked by inhibitors of protein synthesis, RNA synthesis, and caspases activity. The c-Jun amino-terminal kinases (JNKs) are critical regulators of transcription. The p38 MAPKs are activated by inflammatory cytokines and environmental stresses. Interestingly, JNK3 and p38 were activated by arsenite at concentrations that induced apoptosis. Furthermore, [29] demonstrated that inhibition of either JNK or p38 signaling using inhibitors protected cerebellar neurons from arsenite toxicity. The present results revealed that rats treated with NSO showed significant decrease in brain FAS level compared to the As group. This finding implies the neuroprotective action of NSO through its significant antioxidant activity against As-induced oxidative stress. NSO, known as an antioxidant agent, ameliorated oxidative injury in the tissues and functional deteriorations [30]. Moreover, the Bcl2 protein has been demonstrated to be important for regulating apoptosis induced by a variety of stimuli [31]. The present study revealed that, the level of Bcl2 in brain tissue was reduced in As group compared to the normal control group. This result was agreement with [32] who suggest that As is known to generate ROS and free radicals like hydrogen peroxide, hydroxyl radical, nitric oxide, superoxide anion, dimethyl arsenic peroxy radical, and dimethylarsinic radical in living systems that are currently considered to be the major factors for oxidative stress-induced cell damage. Although the downstream pathway for arsenic-induced cell damage is not well defined, evidences suggest that oxidative stress following As exposure. Dwivedi et al., [32] have provided experimental evidences demonstrating the importance of mitochondria in arsenic induced toxicity, as it could be a preferred medium for cellular apoptosis but the mechanism still requires better understanding. Several studies have demonstrated that ROS could lead to neuronal apoptosis in neurodegenerative disorders. Overproduction of ROS can cause deleterious effects, such as an increase in peroxynitrite production or the weakening of the cell antioxidant defenses, including antioxidant enzymes, lipophilic, and hydrophilic scavengers. ROS has been shown to increase the permeability of the mitochondrial membrane and result in mitochondrial failure [32]**.** It has been found that As-induced mitochondrial dysfunction is induced by increased ROS accumulation, the decreased

mitochondrial membrane potential and cytochrome c release. All of these lead to the decreasing of bcl2 level [32,33]. The mitochondrial membrane permeability and the release of cytochrome c are also regulated by Bcl-2 family proteins Bcl-2 stabilizes the mitochondrial membrane permeability, thus preventing mitochondrial cytochrome c release and inhibiting cell death [32,33]. On the other hand, the treatment of As administered group with NSO caused an increase in the level of Bcl2 in brain tissue compared to As administered group. This finding implied that NSO potentially prevents apoptosis by regulating the mitochondrial path way due to beneficial properties of NSO as antioxidant agent to decrease the production of ROS [34]. Since its discovery over 20 years ago, BDNF has been shown to play a key role in neuronal survival, in promoting neuronal regeneration following injury, regulating transmitter systems and attenuating neural-immune responses [35]. The present study recorded a significant decrease in the brain level of BDNF in As group in comparison to the normal control group. This decrease of BDNF was guessed to be a result of the injuries of the nerve and glial cells in the brain due to the production of reactive oxygen species (ROS) and this coincides with [36]. On the other hand, the progression of neurodegenerative disorder can be inhibited by the use of free radical scavengers and anti-oxidants [30]. The present study demonstrated that all rats treated with NSO recorded a significant decrease in brain inflammatory cytokines TNF-α and IL-1b as compared to the As group. *Nigella Sativa* was reported to decrease inflammatory markers in septic rats like IL-1, IL-10, TNF and IL-2 [36]. This effect could be attributed to the antinflammatory, antioxidant protective properties of Nigella Sativa oil [11]**.** In support to the current results, [30,38,39] demonstrated that Thymoquinone (TQ) the main active principle in NSO has been reported to be effective against transient forebrain ischemia-induced inflammation in the rat hippocampus. At present, it is considered that As-induced oxidative stress may lead to inflammation. These findings imply that NSO mitigated As-induced neurotoxicity through its anti-inflammatory action probably via an anti-oxidative mechanism. On the other hand, the brain level of BDNF was increased after the treatment of As groups with NSO. This increase was suggested to be a result of the ability of NSO to eliminate the free radicals which considered to be the first cause for libration of the reactive oxygen species (ROS) that caused apoptosis and injuries in the brain due to sodium arsenite exposure [30]. In addition to the ability of NSO to reduce the inflammation in brain tissue contributed positively to the high increase in the brain level of BDNF [39]. Vascular endothelial growth factor (VEGF) is a critical angiogenic factor known to be required for the normal development of the vasculature as well as for pathologic angiogenesis [40]. As group recorded a significant increase in the brain VEGF level

compared to the normal control group. VEGF is a homodimeric glycoprotein which acts as a highly specific mitogen for vascular endothelial cells, being capable of inducing angiogenesis. In addition, it is a potent inducer of vascular permeability and it acts as a survival factor for the newly-formed blood vessels [41]. However, As group recorded an increase in the brain level of VEGF as compared to the normal control group. This may be due to that sodium arsenite has been found to cause brain damage due to its role in induction of oxidative stress and production of ROS which may offer new insight into understanding the arsenite toxicity associated with vascular instability and subsequent development of vascular disease [42]. On the other hand, the treatment of As group with NSO caused a reduction in the brain level of VEGF. This may be due to the ability of NSO to reduce the inflammation in brain tissue and its antioxidant capacity to remove the ROS librated from sodium arsenite [43].

In conclusion, the present study suggests that Nigella sativa oil could modulate brain inflammatory markers, apoptic, antiapoptic markers and growth factors levels *via* its anti-inflammatory property and antioxidant capacity. So, the present study provides clear evidence that Nigella sativa oil represents a novel approach for protective the brain from arsenic toxicity due to its ability to ameliorate the neurodegeneration characteristic for arsenic neurotoxicity in rats.

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