The Protective Effect of *Nigella sativa* Oil on Neurodisorder and Oxidative Stress Driven by Imidacloprid in Mice Mitochondria

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Abstract: Mitochondria, a main site of cellular energy generation and oxygen consumption, symbolize a likely target for imidacloprid poisoning. Imidacloprid (IMC) is a systemic neonicotinoid insecticide belongs to chloronicotinyl nitroguanidine chemical family and the most frequently detected once in fruits and vegetables. So, the current study was designed to investigate the effect of IMC treatment on mice brain mitochondrial oxidative phosphorylation chain complexes (OXPHC) as well as the protective effect of 4 ml/kg bw *Nigella sativa* oil (Ns oil) against IMC adverse effects. Male albino mice were subjected to oral sublethal dose (2.6 mg / Kg bw) of IMC for four weeks (5 doses/week). Two of mitochondrial bioenergetics biomarkers; nicotinamide adenine dinucleotide dehydrogenase (NADH) and adenosine triphosphatase (ATPase) activities in addition to mitochondrial oxidative stress biomarkers; glutathione S- transferase (GST), superoxide dismutase (SOD) activity, protein carbonyl (PC) and lipid peroxidation (LPO) contents were *in vivo* assessed in mice brain mitochondria post-exposure of IMC. The alterations in the mentioned parameters were confirmed by the changes in mitochondrial OXPHC proteins pattern induced by IMC identified by blue native polyacrylamide gel electrophoresis (BN-PAGE). Results showed a significant decline in brain mitochondrial; NADH, ATPase and SOD activities in IMC treated mice, while the GST activity, LPO and PC levels were significantly increased compared to control. Furthermore, BN-PAGE depicted some alterations in the mitochondrial OXPHC proteins pattern. The previous effects were attenuated by Ns oil supplementation. This study revealed that IMC toxication impaired mitochondrial bioenergetics and induces oxidative stress which may lead to brain mitochondrial dysfunction. Moreover, Ns oil attenuated the IMC adverse effect due to its high content of thymoquinone (TQ) component supporting its wide use as a protective antioxidant food supplement.

Keywords: Neonicotinoid, Black cumin, Brain, Complex I, ATPase, Oxidative stress, BN - PAGE

1. Introduction

IMC is extensively used in agriculture and veterinary medicine **(Ware and Whitacre, 2004)**. Due to its insect's selectivity and human's safety the IMC became the most wildly sold insecticide **(Acin-Perez *et al.,* 2009)** .It acts as neurotoxin by interfering with the transmission of stimuli in the insect nervous system **)Acin-Perez *et al.,* 2009)**.

Brain's bioenergetic demands varying significantly according to the brain activity levels and neuron type **(Moujahid *et al.,* 2014)** .Mitochondrial OXPHOS are responsible for fuelling cell signaling and neuronal activity processes, such as neurotransmitter release, synaptic action potentials and the postsynaptic currents **(Hall *et al.,* 2012)** .The OXPHOS injury affect the central nervous system bioenergetic balance that may chief several neuronal dysfunctions. In fact, the complicated OXPHOS dysfunctions, initiating from both genetic and environmental influences, as pesticides, have been described in lots of disorders and diseases, as schizophrenia (SCZ), and bipolar disorder (BD), Alzheimer and Parkinson diseases **Ben-Shachar (2009)** .Deficiencies in OXPHOS complexes formation and function can lead to several defects, including synapse destruction, axon demolition, ROS formation, apoptosis, and cell death **(Breuer *et al.,* 2013)** .Previous studies have linked individual pesticides to mitochondrial dysfunction as, paraquat, rotenone, pyridaben, fenpyroximate, fenazaquin and tebufenpyrad have been listed to directly inhibit complex I **(Sherer *et al.,* 2007)**.

Although producing energy required to convert adenosine diphosphate into adenosine triphosphate, mitochondria also yields superoxide radical, hydrogen peroxide and hydroxyl radical as by-products of the consumption of molecular oxygen in the electron transport chain (ETC) **(Raha and Robinson, 2000)** and the accumulated O_2 is eliminated by manganese superoxide dismutase (MnSOD), which generates H_2O_2 **(Raha and Robinson, 2000).** Because mitochondria do not contain catalase (CAT), the glutathione system plays crucial role in reduction of H_2O_2 and protects mitochondria against peroxidative stress **(Maher, 2005)**. Also, oxidative damage to lipids or proteins may be seriously lethal and parallel **(Tomlin, 2006 .)**

Nigella sativa is an annual herbal plant from Ranunculaceae family that commonly used for protection and treatment of various ailments in Muslim nations. *N. sativa* oil (Ns oil) exhibits pharmacological activities including the ability to act as a chelating agent **(Ahmad and Beg,(2013** , antioxidant and anti-inflammatory **(Ahmed and Hassanein 2013)**, immunomodulatory and antitumor substances **(Bai *et al.,* 2013)**.

Although the IMC is widely global used, we still need more toxicological research related to its toxicity in mammals nervous system. So, two of mitochondrial bioenergetics, antioxidant and oxidative stress biomarkers will be tested to investigate IMC-induced mitochondrial dysfunction in mice brain.

2. Materials and Methods

2.1. Chemicals

Imidacloprid technical grade (97%) was obtained from Zhejiang Rayfull Chemicals Co., China. *Nigella sativa* oil was obtained from Egyptian Natural Oils Co., Cairo. All other chemicals were purchased from Sigma – Aldrich Co.

2.2. Animals

Male albino mice of 3±0.5 months age and weighing, 30 ± 3 g were obtained from the animal house, Faculty of Agriculture, Alexandria University. Animals were settled in groups each of five mice in a stainless

still cages and kept under laboratory conditions; 65 –75% humidity, 25 ±°C and 12 h light/12h dark. Animals provided with balanced standard rodent diet and water *ad libitum* for two weeks before being experimented. All procedures involving animals were achieved in accordance with the guidelines of the standard procedures established by **OECD, (2008)**

2.3. Experimental design

The animals were divided into four groups, each five animals were orally treated by gavage with 5 doses / week for 4 weeks as follows:
Group C: Corn oil (1 ml/kg bw) used as a control. Group Ns oil: *Nigella sativa* oil (4 ml/kg bw). Group IMC: Imidacloprid 2.6 mg/kg bw equivalent to (1/50 LD_{50}; 130 mg/kg bw cited by **WHO, 2004**). Group IMC+Ns oil: Imidacloprid (2.6 mg/kg bw) plus *Nigella sativa* oil (4 ml/kg bw). The animals were weighed weekly. At the end of the experiment (4 weeks), mice brains were quickly removed and weighed individually and percent of relative organ weight was calculated.

2.4. Isolation of mitochondria

2.4.1. from mice brain

Brain mitochondria were isolated using a modified protocol of **Krause *et al.,* (2005**). A brain tissue was homogenized, in buffer containing (5 mM HEPES - NaOH, 320 mM sucrose, 1 mM EDTA, and 0.5 mM PMSF, pH 7.4) with the ratio (1:5 w/v). The homogenate was centrifuged at 1300 g (3 min, 4 °C). The pellet was extracted twice with using 2 and 1 ml homogenization buffer, respectively, and the combined supernatants were re-centrifuged at 17,000 g (10 min, 4 °C). The pellet was washed once and re-suspended in 0.5 mM PMSF, 320 mM sucrose. Isolated mitochondria were stored at -20 °C.

2.4.2. from chicken heart

Chicken heart mitochondria were prepared according to the method reported by **Smith, (1967)**. The heart was placed in cracked ice during transport from the grosser market to the laboratory and the temperature was maintained at 4 C° through all subsequent steps. The heart was carefully cleaned of fat and connective tissue and cut into 3 to 4 cm cubes then washed three times with saline solution (0.9%NaCl). Each 100 gm of the minced meat was supplemented with 100 ml of solution (A), (10 mM Tris-Cl, 0.25 M sucrose, 0.2 mM EDTA, and 1mM sodium succinate pH 7.8). The mince was re-washed with 100 ml of solution (A) and blended for 45 sec at medium speed. The pH was controlled before and after homogenization and brought to 7.8 with 1 M KOH. The muscle homogenate was centrifuged for 20 min at 1200 xg. The supernatant solution was filtered through a triple layer of cheese cloth then readjusted to pH 7.8 with 1 M KOH. The pellet was re-suspended in solution (A) and re-centrifuged as mentioned before. The collected homogenate was then centrifuged for 15 min at 26000 xg. The resulting mitochondrial pellet was homogenized in solution (A) and frozen at -20 C°.

2.5. Determination of protein

Protein content of the mitochondrial preparations and oxidative phosphorylation complexes was estimated by the method of **Lowry *et al.,* (1951)** and **Bradford (1976)**; respectively using bovine serum albumin as a standard.

2.6. Solubilization of mitochondrial oxidative phosphorylation complexes.

according to **Schägger *et al.,* (1994)**, The mitochondria were precipitated at 17,000 xg for 10 min at 4°C. Sediments (0.2 mg of total protein) were solubilized in 40 µl of solution "A" (1.0 M 6-aminocaproic acid and 50 mM Imidazole pH 7.0) followed by the addition of 5 µl n –Dodecyl β-D-maltoside (DDM) (10%). The samples were extracted for 20 min undercooling then centrifuged at 35000 xg for 30 min at 4 C°. To each 90 µl DDM extract complexes, 5 µl from solution "B" (1.0 M caproic acid and 5.0 % Coomassie G-250) was added.

2.7. Protein pattern of mitochondrial oxidative phosphorylation complexes on blue native polyacrylamide gel electrophoresis (BN-PAGE).

As described by **Schägger and Jagow, (1991)**, equal quantities of protein (90 µg) were loaded per lane and subjected to BN-PAGE using Bio-Rad Mini-Protein cell (4 % stacking gel and linear gradient 5-13% separating gel). Gels were run at 4 C° using anode buffer (50 mM Imidazole, pH 7.0) under conditions of constant electrophoretic current (40V) until the sample was within the stacking gel, then the run was completed at 80 V as well as after 2/3 of the run the cathode buffer "A" (15 mM Imidazole, 50 mM Tricine and 0.02 % Coomassie blue G-250) was changed with cathode buffer "B" (The same buffer as in (A) except the dye concentration was 0.002 %). The protein bands were fixed in; 10 % glacial acetic acid and 50 % methanol for 1hr, stained for 2hr with 0.025 % Coomassie blue G-250 in the fixative solution and destained with 10 % glacial acetic acid.

2.8. Biochemical analysis

2.8.1. Bioenergetics' biomarkers

2.8.1.1. NADH dehydrogenase (Complex I) activity

NADH dehydrogenase activity was measured by the oxidation of NADH as described by **Galante and Hatefi (1978)**. Brain mitochondria (40 µg of protein/ml) were mixed with a mixture containing 40 mM phosphate buffer, pH 7.4, 0.1% sodium cholate and 1.3 mM potassium ferricyanide as well as incubated for 1 min at 30 °C, then 0.14 mM NADH was added. The decrease of absorption was followed spectrophotometrically at 340 nm for 1-3 minutes. Results were expressed as µmol NADH oxidized/min/mg protein. All mitochondrial suspensions were subjected to rotenone (1µM) sensitivity test. The activity was calculated using the molar extinction of NADH ($3.11 \mu M^{-1}\ cm^{-1}$).

2.8.1.2. Total ATPase activity

Basically, this method measures the amount of inorganic phosphate produced from the hydrolytic reaction of ATP by the ATPase. Mitochondrial suspension (30µl of 1mg protein/ml) was added to a medium containing, 20 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$ and 5 mM ATP, then the mixture was incubated at 37°C for 5 min with shaking. The reaction was stopped by the addition of 5% trichloroacetic acid (TCA) and then the inorganic phosphate (Pi) was measured spectrophotometrically at 740 nm according to **Taussky and Shorr**, **(1953**). The concentration of Pi was calculated from a standard curve and the specific activity was expressed as µmole Pi/mg protein.

2.8.2. Oxidative stress biomarkers

2.8.2.1. Superoxide Dismutase (SOD) activity

SOD as an enzymatic antioxidant was measured spectrophotometrically at 25 °C by the method of **Marklund and Marklund (1974**) with some modifications. The assay medium was 1.0 ml containing 50 mM Tris – HCl buffer (pH 8.0) and 0.24 mM pyrogallol. Autoxidation of pyrogallol was monitored at 420 nm for 3 min in the absence and presence of enzyme at three concentrations which produced between 30 to 60 % inhibition of pyrogallol. One unit of the enzyme activity is defined as the amount of enzyme which produced 50 % inhibition of pyrogallol autoxidation under the standard assay conditions. Mitochondrial SOD activities were expressed as Units/mg protein.

2.8.2.2. Glutathione S-Transferase

Glutathione S -Transferase activity was measured by the simplified procedure of **Vessey and Boyer (1984**). Mitochondrial suspension (10µl of 1 mg protein/ ml) was added to a medium containing, 0.1M phosphate buffer pH 6.5, 4mM GSH and 1 mM CDNB. The mixture was incubated for 20 min at room temperature, and then the absorbance was measured at 340 nm. The GST activity was expressed as U/mg protein/ml using the molar extinction of CDNB (0.0096 μM^{-1} /cm).

2.8.2.3. Lipid peroxidation (LPO) level

Malondialdehyde (MDA) has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535 nm. The amount of thiobarbituric acid reactive substance (TBARS) in mitochondrial suspension was measured by the Thiobarbituric acid assay (TBA) as previously described by **Buege and Aust (1978)**. Briefly, 0.5 ml of mitochondrial suspension was react with 2 ml of TBA reagent containing 0.375 % TBA, 15 % TCA and 0.25 N HCL. Samples will be boiled for 15 min, cooled and centrifuged. The yields supernatants was measured at 535 nm. The TBARS concentrations was calculated using the molar extinction $\mathscr{E}$ = 155 $mM^{-1}cm^{-1}$ and results was expressed as nmol MDA/mg protein.

2.8.2.4. Protein carbonyls content

The carbonyl proteins assay provides a convenient technique for detecting and quantifying oxidative modification of proteins. 2, 4- Dinitrophenyl hyrdazine (DNPH) reacts with protein carbonyls to produce hydrazones **(Yan *et al.,* 1995)**. For derivatization of the samples; two 4-mg aliquots were needed for each sample to be assayed. A final concentration of 10% (w/v) TCA was used to extract the samples. The precipitates were treated with 500 µL of 0.2% DNPH or 500 µL of 2 *N*HCl. Samples were incubated at room temperature for 1 h with shaking at 5-min intervals. Then proteins precipitated by 55 µL of 100% TCA, centrifuged and washed the pellets three times with 500 µL of the ethanol: ethyl acetate mixture. The pellet was then dissolved in 0.3 % SDS in 0.2 M Tris-Cl pH 6.5. The carbonyl content was determined by reading the absorbance at the optimum wavelength (λ = 375 nm) of each sample against its appropriate blank. The protein carbonyls content was expressed as nmol/mg protein/ml using the molar extinction (10^{-6} / 22000).

2.9. Statistical analysis

Data were expressed as mean ± standard error (SE). The one-way analysis of variance (ANOVA) was used to analyze data followed by the Student-Newman-Keuls Test. Statistical significance was set at $p < 0.01$. These tests were performed using the computer CoStat program, version 6.400, Copyright © 1998-2008, CoHort Software.

3. Results

3.1. Body and relative organ weights

Change in body and relative organ weights was noted to detect the physiological status of tested animals. No mortality occurred in any group of animals throughout the experiment. IMC treated group showed a significant increase in mice's weight during the experimental period, whereas the IMC intoxication doesn't affect the brain relative weight compared to control (Table 1).

3.2. Biochemical studies

Biochemical replies are considered as primary warning indices of pollution in the environment **(Liu *et al.,* 2011)**. Figures 1-6: summarized the *in vivo* effects of one-fifth the LD_{50} of IMC, 4 ml / kg bw of *Nigella sativa* oil and their combination after four weeks on NADH dehydrogenase, ATPase, SOD, GST activities, and MDA, PC contents in mice brain mitochondria.

3.2.1. Effect of treatments on NADH dehydrogenase activity

IMC treatment decreased significantly ($P < 0.01$) the activity of neuro mitochondrial NADH dehydrogenase by 69.3%, whereas supplementation with Ns oil modulates the IMC adverse effect to 33.7% compared with control (Fig. 1).

3.2.2. Effect of treatments on total ATPase activity

Oral administration of IMC significantly ($P < 0.01$) reduced the mitochondrial ATPase activity by 62%, while the Ns oil and IMC combination significantly attenuated the IMC lesion to be 32.69% compared with control (Fig. 2).

3.2.3. Effect of treatments on SOD activity

Administration of IMC significantly ($P < 0.01$) depleted the brain mitochondrial SOD activity by 36.8 %, while Ns oil supplementation lowered the IMC adverse effect on SOD activity to be 16.8 % less than control values (Fig. 3).

3.2.4. Effect of treatments on GST activity

IMC intoxication induced a significant increase in the mitochondrial GST activity to be 2.4 fold. In the meantime, co - administration of Ns oil significantly ($P < 0.01$) declined imidacloprid toxic effect to be 1.3 fold compared with control values (Fig. 4).

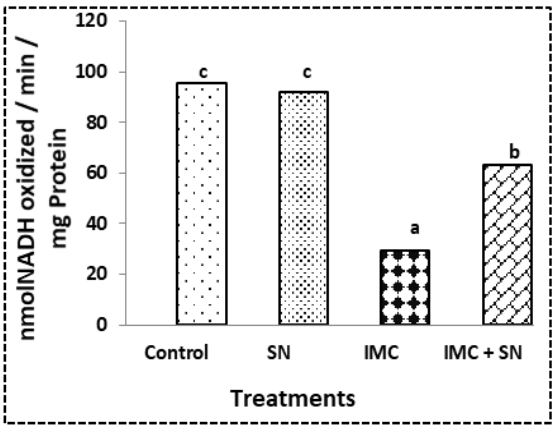

Fig.1. NADH dehydrogenase activity in brain mitochondria of male mice orally treated with imidacloprid (2.6 mg/kg bw), *Nigella sativa* oil (4 ml / kg bw) and their combinations after four weeks. Values represent the mean ± SE of five animals/group. a, b, & c different superscripts indicating statistical significant differences between groups ($p \leq 0.01$).

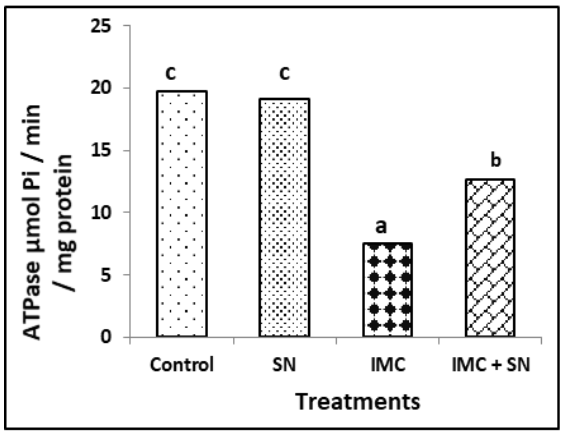

Fig.2. ATPase activity in brain mitochondria of male mice orally treated with imidacloprid ((2.6 mg/kg bw), *Nigella sativa* oil (4 ml / kg bw) and their combinations after four weeks. Values represent the mean ± SE of five animals/group. a, b, & c different superscripts indicating statistical significant differences between groups ($p \leq 0.01$).

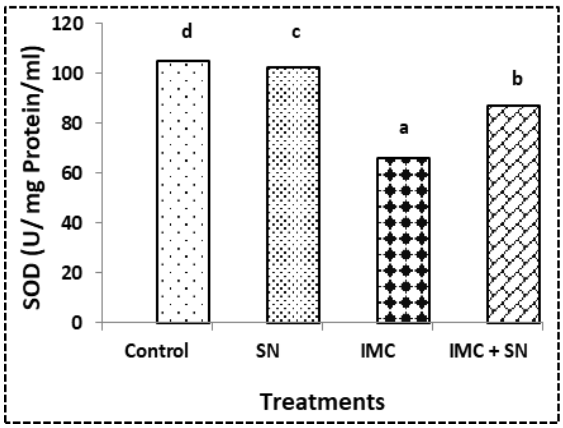

Fig.3. SOD activity in brain mitochondria of male mice orally treated with imidacloprid ((2.6 mg/kg bw), *Nigella sativa* oil (4 ml / kg bw) and their combinations after four weeks. Values represent the mean ± SE of five animals/group. a, b, c & d different superscripts indicating statistical significant differences between groups ($p \leq 0.01$).

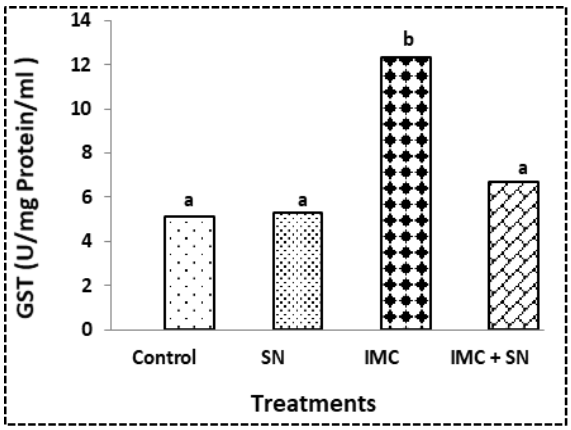

Fig.4. GST activity in brain mitochondria of male mice orally treated with imidacloprid (2.6 mg/kg bw), *Nigella sativa* oil (4 ml / kg bw) and their combinations after four weeks. Values represent the mean ± SE of five animals/group. a, b, & c different superscripts indicating statistical significant differences between groups ($p \leq 0.01$).

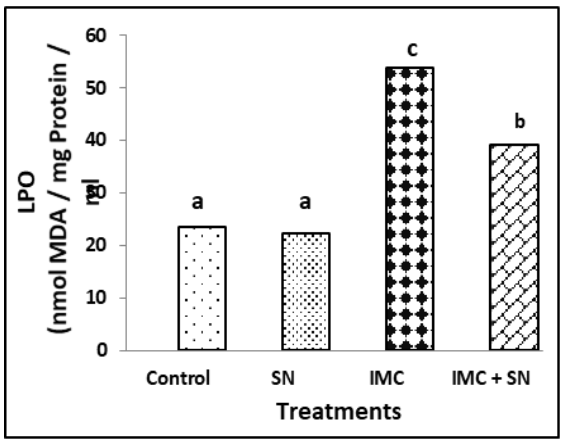

Fig.5. LPO level in brain mitochondria of male mice orally treated with imidacloprid (2.6 mg/kg bw), *Nigella sativa* oil (4 ml / kg bw) and their combinations after four weeks. Values represent the mean ± SE of five animals/group. a, b, & c different superscripts indicating statistical significant differences between groups ($p \leq 0.01$).

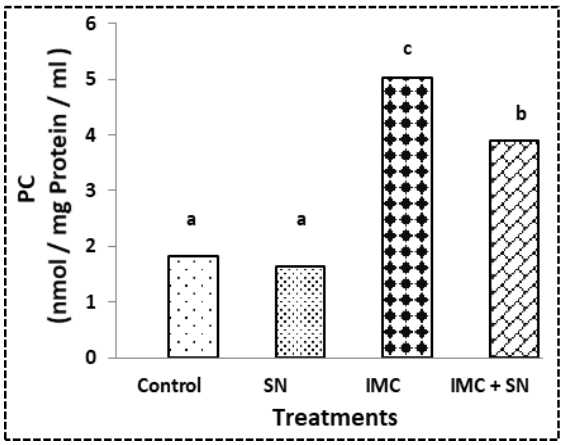

Fig.6. PC level in brain mitochondria of male mice orally treated with imidacloprid (2.6 mg/kg bw), *Nigella sativa* oil (4 ml / kg bw) and their combinations after four weeks. Values represent the mean ± SE of five animals/group. a, b, & c different superscripts indicating statistical significant differences between groups ($p \leq 0.01$).

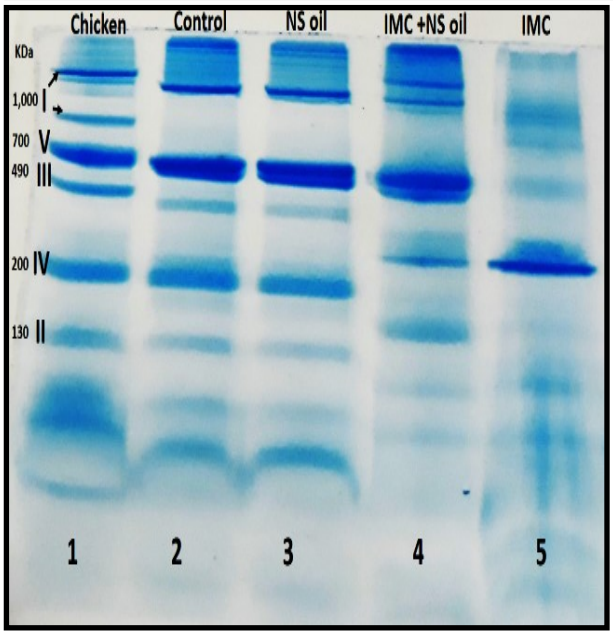

Fig. 7. Blue Native PAGE for resolution of (OXPHOS) membrane protein complexes solubilized from brain mitochondria of male mice orally treated with IMC (2.6 mg/kg bw), Ns oil (4 ml/kg bw) and their combinations for four weeks. Lane 1: preparation from chicken heart mitochondria serve as molecular weight protein marker Lane 2: the control, lane 3: Ns oil (4 ml / kg bw), lane 4: the combination of IMC+Ns oil and lane 5: IMC (2.6 mg/kg bw). The five lanes were loaded with the same amount of protein (90 µg) of each.

3.2.5. Effect of treatments on lipid peroxidation

Oral treatment of IMC was significant ($P < 0.01$) duplicated the brain mitochondrial MDA content by 2.29 fold while Ns oil co-administration with IMC mitigated the IMC oxidative effect to be 1.6 fold compared with control values (Fig. 5).

3.2.6. Effect of treatments on protein carbonyls content (PC)

The brain mitochondria of IMC- treated mice showed a significant enhancement in the protein carbonyl levels by 2.74 fold, whereas Ns oil supplementation with IMC braked the IMC adverse effect to be 2.13 fold respects to control value (Fig. 6).

3.3. The electrophoretic analysis

Fig. 7, inspects the BN - PAGE for resolution of (OXPHOS) membrane protein complexes solubilized from intact mitochondria. Lane 1, 2, and 3 represent solubilized multisubunit protein complexes of chicken heart (lane 1), mice brain lane 2, 3, 4, and 5; respectively. Lane 2 and 3 display five distinct multisubunit protein complexes; I, V, III, IV and II in active and native form. Lane 5, IMC- treated animals, showed deterioration in complex I, V, III and changes in migration distance of complex, IV, III and II as well as both complex II and III have faint and hardly detected bands. All the previous notes reveals that a changing in the multiprotein complexes molecular size have been made as a result of IMC- treatment compared to the control. However, in lane 4, Ns oil supplemented with IMC, is characterized by a distinct bands of membrane protein complexes, I, V, IV, II, but, complex III did not be recognized.

4.Discussion

In mitochondria, reduced energy production and increased oxidative damage are initial pathological events that direct to neurodegeneration **(Beal, 2005)**. Some authors stated that many compounds have the ability to induce alterations in oxidative phosphorylation **(Bridges *et al.,* 2014)**. Proton-translocating NADH: ubiquinone oxidoreductase is represent the first complex of the mitochondrial respiratory chain. Complex I contribute to the

Table 1: Body and relative organ weights of male albino mice orally administrated with IMC (2.6 mg/kg bw), Ns oil (4 ml/kg bw) and their combinations

Animal Group	Body Weight (g)	Brain Weight (g)	Brain Relative Weight (%)	% Change[a]
Control	$34.8^{b} \pm 0.30$	$0.433^{a} \pm 0.010$	1.24^{a}	0.0
Ns oil	$33.8^{c} \pm 0.42$	$0.439^{a} \pm 0.028$	1.29^{a}	-4.0
IMC	$36.2^{a} \pm 0.63$	$0.446^{a} \pm 0.026$	1.23^{a}	0.81
IMC+Ns oil	$33.4^{c} \pm 0.66$	$0.439^{a} \pm 0.028$	1.31^{a}	-5.6

Values are expressed as means (5 mice) ± Standard Error (SE)
Values in the column with different letters are significantly different at ($p \leq 0.01$).
[a] Percentage of increase (-) in treatment weights compared with control

proton-motive force that pushes ATP synthesis. In the present study, the reduction of mitochondrial NADH dehydrogenase by IMC intoxication may hinge on the convoluted, structure of this enzyme complex, which contains of at least 40 different polypeptides strongly embedded in the mitochondrial inner membrane and that may elucidates the great vulnerability of mitochondria to lipophilic molecules **(Don and Hogg 2004)**. **Gassner *et al.,* (1997)** found that submicromolar concentrations of pyrethroid inhibit the respiratory complex I of rat liver mitochondria which is in accordance with the present data. This decrease in the activities of complex I in turn affects the activity of ATPase adversely.

ATPase activity represent a meaningful index of cellular activity and introduce a useful toxicological tool **(Sushma and Rao, 2007)**. At the present data, IMC significantly diminish the brain ATPase activity, which manage to reduced ATP production, and increase the reactive oxygen species (ROS). In line with other studies stated that some pesticides alter the Mg^{2+} ATPase, which may be a causative factor of neuronal / cellular dysfunction **(Rahman *et al.,* 2000 and Mehta *et al.,* 2005)**. **Maioli *et al.*, (2012)** demonstrated that abamectin inhibits the ATPase activity and adenine nucleotide translocator (ANT) when added at micromolar concentration. And sequentially with **Paulo *et al.,* (2018)** who revealed that,

IMC significantly suppresses the ATP synthesis in a concentration-dependent manner.

Respiratory dysfunction develops oxidative stress by increasing escape of electrons and superoxide production **(Marmolino, 2011)**. Once free radicals, such as ROS or RNS beat the cellular antioxidant system and produce cellular damage, ROSs and following oxidative damage lead to disturbance of cellular membrane integrity and mitochondrial dysfunctions, significant pathways guidance to neuronal cell death **(Lee *et al.*, 2011)**.

Mitochondrial SOD and GST are the first line of defense against ROS, which alter superoxide into H_2O_2 then into H_2O and O_2 maintaining ROS balance as they scavenge the excess ROS. The reduction in SOD activity observed in the current study agrees with the conclusions of **Kapoor *et al.*, (2010)**. Similar decline in the SOD activity in rats was noticed with different pesticides; chlorpyrifos, cypermethrin, carbofuran, dimethoate and malathion, **(Khan *et al.*, 2005; Rai and Sharma 2007; Mansour and Mossa, 2009)**. In contrast, **El-Gendy *et al.*, (2010)** cited that, post treatment of 15 mg/ kg IMC significantly raised CAT, SOD, glutathione peroxidase, and GST activities in male mice. The present decrease in SOD activity of IMC intoxicated mice may be due to use of this enzyme in converting O_2- to H_2O_2. **Williams *et al.*, (1998)** showed that heterozygous MnSOD knockout mice ($MnSOD^{-/+}$) mitochondria have a reduction in the respiratory control ratio (RCR) compared to wild type mice ($MnSOD^{+/+}$) and this declined activity was owing to oxidation of the iron-sulfur center of complex I. **Wheeler *et al.*, (2001)** stated that, 50% decrease of mitochondrial SOD activity resulted in a functional decline of oxidative phosphorylation, an accretion in oxidative stress and elevate the rate of apoptosis. This data proposes that SOD plays a vital role for balances of mitochondria redox status.

The carbonylation is an irreversible alteration of proteins, producing changes in structure, function and protein degradation **(Sledzinski *et al.*, 2008)**. At the present data, the magnitude of accumulation of protein carbonyls in the brain mitochondria of IMC exposed mice was more pronounced than control group, and this finding in line with **Sundari *et al.*, (1997)** who suggested that the aggregation of oxidized proteins in the liver may be an early sign of chloropyrifos induced liver injury and that protein oxidation may be distinct of lipid peroxidation. **Dalle Donne *et al.*, (2003),** cited that the relative stability and the relative early formation of carbonylated proteins, may signify a possible occurrence of chronic oxidation. In the present study, based on control values, the percentage of variation in the brain's content of carbonyl groups was higher in comparison to the percentage of variation in the MDA levels, which may point to that protein oxidation is affected more by IMC treatment than by lipid oxidation. Almost all types of ROS and lipid peroxidation products, as MDA, can motivate carbonyl groups formation **(Shacter, 2000).** The present data suggest that, the heightening of ROS group was demonstrated by the increased levels of carbonyl groups in the brain mitochondria of IMC-treated mice.

In human mitochondrial disorders, structural modifications primarily affecting one given complex often direct pleiotropic damaging effects of the other complexes. For example, pathogenic alterations in complex III subunits or associate genes chief to pleiotropic deficiencies of complex I and Complex IV in affected tissues **(Lamantea *et al.*, 2002)**. **D'Aurelio *et al.*, (2006)** mentioned that, mutations in complex IV subunits may produce secondary complex I deficiencies and mutations affecting complex I-specific genes can yield combined complex I and complex III, or complex I and complex IV, deficiencies in patients as cited by **Budde *et al.*, (2000) and Saada *et al.*, (2012)**.

The study of mitochondrial OXPHC using BN-PAGE ،reveals conformational changes in mitochondrial multisubunit protein complexes pattern; dissociation, depletion and indistinct of the IMC toxicated protein complexes, point to the low level of enzymes activity and may also, to the pesticide lipophilic property. Phospholipids are vital for supercomplex formation in addition to its existence as an integral part of each complex. **Schwall *et al.*, (2012)** demonstrated that the phospholipid environment is essential for optimal stability, structure and enzymatic activity of supercomplex, as well as decrease of ROS production. The disfiguration of the lipid bilayer produced by peroxidation and the alteration of the tightly bound phospholipids destabilizes the multisubunit protein complexes resulting in a very low levels of activity and dissociation of subunits **(Gomez and Robinson, 1999)**, and this supported the present findings about the deleterious effect of IMC on mice brain mitochondria.

Supplementation of the Ns oil with IMC showed noticeable improvement as manifested by biochemical results and electrophoretic analysis, Ns oil attenuates the IMC adverse effects, due to antioxidant and cytoprotective property. **Gali-Muhtasib *et al.*, (2006)** mentioned that Ns oil containing thymoquinone (TQ) are known to have strong antioxidant activities, TQ has been shown to inhibit non-enzymatic peroxidation in ox brain phospholipid liposomes, and also it has excessively high superoxide anion radical-scavenging capacities as effective as SOD. Furthermore, it was cited that Ns oil can activate the antioxidant defense system in rats which neutralize the biochemical parameters and lipid peroxidation in dimethoate, **(Attia and Nasr 2009)** and acetamiprid induced toxicities, **(Mosbah *et al.*, 2018)** due to its antioxidant and antiapoptotic properties, **(Erboga *et al.*, 2016)**.

Conclusion

The present findings suggest that IMC intoxication causes an imbalance of the bioenergetics, antioxidant enzyme activities, and increases MDA level as well as protein carbonyl groups, which weaken the ability of brain mitochondria to manage the IMC oxidative stress. Furthermore, Ns oil supplementation can attenuate the IMC adverse effect. So, the sufficient dietary intake of Ns oil may provide significant protection against the IMC harmful impact.

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التأثير الوقائى لزيت حبة البركة *Nigella sativa* على الإضطراب العصبي والإجهاد التأكسدى الناتج عن الإميداكلوبريد في ميتوكوندريا الفئران

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الملخص العربى:

الميتوكوندريا هى مصدر الطاقة واستهلاك الأوكسجين فى الخلية وتعتبرهدفا محتملا للتسمم بالإيميداكلوبريد (IMC) ، وهو مبيد حشرات جهازى ينتمي إلى عائلة كيميائية من الكلورونيكوتينيل نيتروجوانيدين وهو من المبيدات ذات المتبقيات الأكثر اكتشافًا في الفواكه والخضروات. لذا اجريت الدراسة الحالية بهدف تقدير تأثيرات التعرض لمبيد IMC على سلسلة نقل الإلكترونات فى ميتوكوندريا مخ الفئران. وتم معاملة ذكور الفئران البيضاء بجرعات تحت مميتة عن طريق الفم (٢,٦ جم / كجم من وزن الجسم وتمثل، ١/٥٠ LD_{50}) لمدة أربعة أسابيع (٥ جرعات / الأسبوع)، بالإضافة إلى التأثير الوقائي لـ ٤ مل / كجم من وزن الجسم زيت حبة البركة (Ns oil) ضد الآثار الضارة لـ IMC. تم تقييم اثنين من مؤشرات الطاقة الحيوية فى الميتوكوندريا، الناد ديهيدروجينيز (NADH)، الأدينوزين ثلاثى الفوسفاتيز (ATPase) ومؤشرات الاكسدة الحيوية فى الميتوكوندريا وهى، انزيم السوبر اوكسيد ديسميوتيز(SOD)،انزيم الجلوتاثيون إس ترانسفراز (GST) ومستوى اكسدة الدهون (LPO) و مجاميع الكربونيل للبروتين (PC) في مخ الفئران. وتم تأكيد تأثير IMC على المؤشرات الحيوية السابقة من خلال تحليل معقدات بروتينات الفسفرة التأكسدية فى الميتوكوندريا (OXPHC) باستخدام تقنية الهجرة الكهربائية (BN- PAGE) للكشف عن التغير الحادث فى تتابع وحدات معقدات بروتينات سلسلة نقل الطاقة والتنفس فى ميتوكوندريا المخ كنتيجة للتأثير السلبى لـ IMC . أيضا، تم دراسة الدور الوقائي لزيت حبة البركة ضد الآثار السلبية لـ IMC. وأظهرت النتائج: انخفاض نشاط كلا من انزيم NADH, ATPase and SOD بينما زاد نشاط انزيم GST، مستوى كل من PC and LPO و بشكل ملحوظ، مقارنة بالمجموعة الضابطة فى ميتوكوندريا مخ الفئران المعاملة بـالـ IMC. وتمكن زيت حبة البركة بالجرعة المستخدمة من تقليل الضرر الناتج عن المبيد على المؤشرات الحيوية السابقة فى الحيوانات المعاملة بالإميداكلوبريد. أظهرت الهجرة الكهربائية باستخدام الـ BN- PAGE تغييرفي نمط وتتابع معقدات بروتينات ميتوكوندريا مخ الفئران المعاملة بالإميداكلوبريد. وقد أوضحت الدراسة: أن التسمم بالإميداكلوبريد أضعف الطاقة الحيوية للميتوكوندريا وحفز الإجهاد التأكسدي الذي قد يؤدي إلى اختلال وظيفي في ميتوكوندريا المخ. علاوة على ذلك ، فإن زيت حبة البركة بمحتوياته العالية من مكون ثيموكينون (TQ) مكن من تخفيض التأثير السلبي بالإميداكلوبريد مما يدعم استخدامة كمكمل غذائى مضاد للأكسدة.