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Nigella sativa oil alleviates doxorubicin-induced cardiomyopathy and neurobehavioral changes in mice: *In vivo* and *in-silico* study

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ABSTRACT

Objective: To investigate the effect of *Nigella sativa* oil on cardiomyopathy and neurobehavioral changes induced by doxorubicin in mice.

Methods: Swiss strain of albino female mice were divided into 6 groups of 5 animals in each: Group Ⅰ (control group), group Ⅱ (doxorubicin, 10 mg/kg, *i.v.*), group Ⅲ, Ⅳ, and Ⅴ (*Nigella sativa* oil; 1.5, 3, and 6 mL/kg, respectively), group Ⅵ (*Nigella sativa* oil *per se*; 6 mL/kg, *p.o.*). The duration of treatment was 15 d (10 days' pre-treatment and 5 days' post-treatment) and doxorubicin was administered on day 11th of the treatment schedule. Following *Nigella sativa* oil treatment, neurobehavioral tests, cardiac hypertrophy tests, and biochemical tests in serum and tissues were performed. Neurological tests included assessment of anxiety-like behavior in the elevated plus maze, spontaneous alternation behavior in the cross maze, and depression-like behavior in modified forced swim tests. Biochemical tests included serum lactate dehydrogenase and creatinine kinase-MB, malondialdehyde and reduced glutathione in tissues. Lastly, molecular docking was used to estimate the affinity of the phytoconstituents of *Nigella sativa* oil with histone deacetylases.

Results: *Nigella sativa* oil treatment significantly ($P<0.001$) restored doxorubicin-induced neurobehavioral changes, decreased lactate dehydrogenase and creatinine kinase-MB in the plasma, malondialdehyde contents in tissues, and increased reduced glutathione level. Besides, no significant alteration was observed in *Nigella sativa* oil *per se* group as compared to the control. Molecular docking showed that *Nigella sativa* oil components had appreciable binding affinitiy with the protein cavities of HDAC1 and HDAC6.

Conclusions: The result shows that *Nigella sativa* oil exerts anxiolytic, antidepressant, and memory-enhancing effects in addition to cardioprotective effect against doxorubicin-induced cardiomyopathy in mice. The modulatory effect of *Nigella sativa* oil on oxidative stress could contribute to the cardioprotective effect and associated neurobehavioral changes in mice.

KEYWORDS: *Nigella sativa* oil; Doxorubicin; Cardiomyopathy; Neurobehavioral changes; Lactate dehydrogenase; Creatinine kinase-MB; Malondialdehyde; Reduced glutathione; Mice; HDAC docking

Significance

The current experimental study provides additional evidence for the traditional uses of *Nigella sativa* oil in cardioprotection and the improvement of associated neurobehavioral changes. The mechanisms of action of dual beneficial effects of *Nigella sativa* oil may possibly be through its potential interaction with histone deacetylases and the antioxidant effects.

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1. Introduction

Anxiety, depression, and impaired memory are common neuropsychiatric illnesses in patients with heart failure (HF) and/or myocardial infarction (MI), negatively affecting the quality of life and cardiovascular therapeutic outcomes[1]. Treatment strategies mainly focus on the management of cardiovascular conditions, however, associated anxiety, depression, and memory impairments are relatively forsaken by clinicians and researchers, which may hamper the recovery and become a risk factor for further incidence of cardiovascular diseases. Anxiety, depression, and impaired memory are frequent comorbid conditions in HF patients. A total of 72% of HF patients show elevated symptoms of anxiety[2] whereas the prevalence of depression is reported as 60%[3].

Despite the tremendous advancement in the treatment of HF and/or MI, frequency of hospitalization is relatively high and mortality rate is as high as 50% during the 5th year of initial diagnosis[4]. Surprisingly, the incidence of subsequent cardiac events and premature death has been reported as 6 fold higher in patients with existing anxiety, depression, and impaired memory following acute cardiovascular events[5]. Dysregulation of the hypothalamic-pituitary axis, excessive activity of the sympathetic nervous system, suppression of parasympathetic tone in anxiety and depression patients leading to reduced heart rate variability contribute to further cardiac disorders and vascular dementia[6]. In addition, alteration of serotonergic tone and increased thrombogenesis in anxiety and depression patients have been found to hamper the therapeutic outcome in patients with cardiac diseases. Animals with anxiety, depression, and impaired cognitive function have shown reduced mobility, exploration to the environment, escape-related activity in the elevated plus maze and modified forced swim test[7]. In animals with cardiomyopathy/cardiotoxicity, these neurobehavioral changes may be used to mimic the negative health impact and poor quality of life in patients with cardiac diseases.

Doxorubicin (Adriamycin, DOX), an anthracycline anticancer antibiotic and an extremely effective chemotherapeutic agent, has been used for more than three decades for the treatment of a variety of solid tumors including hematological, breast, Kaposi's sarcoma, and non-Hodgkin's lymphoma. The cumulative cardiotoxicity is the dose-limiting side effect of DOX, which limits its optimal therapeutic potential. Administration of DOX produces a variety of changes including blood pressure, cardiac arrhythmia, congestive HF, and cardiomyopathy through multifactorial mechanisms especially oxidative stress *via* generation of reactive oxygen species (ROS) and lipid peroxidation in cardiac tissue. There are a plethora of experimental and clinical reports on the neurobehavioral effects of DOX treatment[8,9], which surprisingly has poor penetration through the blood-brain barrier. However, DOX-induced peripheral increase in oxidative stress results in increased circulating tumor necrosis factor-alpha, which in turn aggravates brain oxidative stress and neuroinflammation following its permeability to the blood-brain barrier through increased production of proinflammatory cytokines[10]. The resulting neuronal inflammation and exacerbation of oxidative stress in the brain can promote apoptosis of neuron cells and produce neurobehavioral changes[11]. Further, male mice exhibited anxiety-like behavior in the elevated plus maze and Vogel's conflict tests following DOX treatment[12]. DOX is considered a first-line anticancer drug, however, DOX-induced cardiomyopathy is clinically well known. Cardiovascular pathologies are known to cause neurological, cognitive, and psychopathic disorders. Therefore, DOX-induced cardiomyopathy and associated neurobehavioral changes in rodents serve as a model for investigation of possible intervention. Moreover, there is a gender disparity in neuropsychiatric disorders among cardiac patients wherein higher incidence has been reported in females compared to males[13]. To the best of our knowledge, there is a dearth of animal data for such observations as reported in clinical settings. Therefore, it is interesting to mimic such findings in animal models to enable the further investigation of interventions in such clinical conditions.

It is apparent from the evidence-based studies that the effective management of psychosocial determinants would result in improved therapeutic outcomes and reduce the risk of further cardiovascular disorders[14]. Since antiquity, several herbs and their preparation have been regarded as valuable remedies for human maladies. Over the past two decades, complementary medicines have gained tremendous growth and worldwide popularity as health care components and alternative medicine for several disorders[15]. *Nigella sativa* (*N. sativa*) L., family Ranunculaceae is a small yearly blooming dicot plant, native to the Middle East, Central Asia, and North Africa. The seeds of *N. sativa* have been used traditionally as a spice and also as a natural remedy for various diseases[16]. It is noteworthy that most of the biological activities of *N. sativa* have been associated with its thymoquinone content. Nonetheless, in addition to thymoquinone, the seed also contains other bioactive components including fixed oil, volatile oil, and alkaloids that also contribute to the diverse pharmacological activities of *N. sativa*[17]. The antioxidant effect of *N. sativa* and its active constituents has been widely explored for mechanisms of its diverse bioactivities. Interestingly, several lines of evidence suggest that the fixed oil extract has more antioxidant effect than the thymoquinone which is the most active component of volatile oil obtained from *N. sativa*[18]. Furthermore, in a rodent model, *N. sativa* oil (NSO) exhibited anxiolytic activity by increasing brain serotonin levels following treatment with NSO for 28 d[19]. Although, several clinical and preclinical studies demonstrated the cardioprotective effect of NSO[20,21]. However, there is no preclinical data available that supported the effect of sexual differences in cardiotoxicity and

associated neurobehavioral changes. Therefore, the present study aims to study the effect of NSO on DOX-induced cardiomyopathy and anxiety, depression and memory impairment in female mice.

2. Materials and methods

2.1. Animals

Female albino mice of Swiss strain (25-30 g) were procured from the animal house facility of Qassim University, Saudi Arabia. The animals were housed in polypropylene cages with 5 animals each under natural light-dark cycle (7:00 AM-7:00 PM) and maintained at (25 ± 2)℃. The animals were allowed free access to pellet feed supplied by local animal feed vendors for rats and mice and water was provided *ad libitum*.

2.2. Drugs and chemicals

DOX was procured from the Oncology center of King Fahad Specialist Hospital, Buraydah, Saudi Arabia. NSO was purchased from the local pharmacy shop in Buraydah. Serum lactate dehydrogenase (LDH) and creatinine kinase-MB (CK-MB) levels were estimated by using a commercially available diagnostic kit from Alsafwa diagnostic center in Unaizah, Al-Qassim, Saudi Arabia. All others reagents and chemicals used in this study for biochemical estimation of oxidative stress parameters were of analytical grade.

2.3. Experimental protocol

Female albino mice of Swiss strain were divided into 6 groups of 5 animals each. DOX was administered intravenously while NSO was administered orally. Animals in group Ⅰ were treated with distilled water (10 mL/kg). Animals in group Ⅱ (pathogenic control) were treated with DOX (10 mg/kg, *i.v.*). The animals of groups Ⅲ, Ⅳ, and Ⅴ were treated with graded doses of NSO (1.5, 3 and 6 mL/kg *p.o*. respectively)[22]. Group Ⅵ (*per se*) was treated with NSO (6 mL/kg, *p.o*.) only. The animals received their respective treatment for 10 d (pre-treatment) and DOX was administered on day 11th and then continued for 5 d (post-treatment). Learning and memory were assessed on day 14th using cross maze, anxiolytic and antidepressant action on day 15th using elevated plus maze and modified forced swim test after 30 minutes of NSO administration. Blood sample was collected for biochemical estimation in serum (LDH and CK-MB) and then the hearts were dissected by sacrificing the animal under ether anesthesia for estimation of malonedialdehyde (MDA) and reduced glutathione (GSH) levels in cardiac tissues.

2.4. Assessment of spontaneous alternation behavior in the cross maze

The alternating behavior of rodents to different arms of a cross maze is the principle of this test. The reduction in alternation behavior is an indication of amnesia and restoration of alternating behavior indicates the nootropic effect. Thus, the utility of elevated plus maze has been proposed for assessing cognition and spontaneous alternation behavior in rodents, and it is estimated by calculating the percentage alternation on elevated plus maze. In brief, the animals were allowed to explore the plus maze with all arms marked as A, B, C, and D by individually placing animals on the central square. The sequence of arm entries and numbers were recorded over an observation period of 6 minutes. One alternation is considered as entry into all the four arms from amongst overlapping quintuple (five consecutive entries) set of entries. The percentage alternations were calculated by using the formula:

Percentage alternation = (Actual alternations)/(Possible alternation) ×100

The total number of actual alternations is calculated from the overlapping quintuple set whereas possible alternation is the total number of entries minus 4.

2.5. Assessment of anxiety-like behavior in the elevated plus maze

The test was performed according to the method described by Anwar *et al*.[12]. Briefly, the mice were individually placed on the central platform (5 cm × 5 cm) of elevated plus maze with two perpendicularly closed arms (25 cm × 5 cm × 16 cm) and open arms (25 cm × 5 cm × 0.5 cm), respectively and elevated at 50 cm from the ground. The number of open arm entries and time spent in open arms were recorded for a 5-minute observation period. The reduction in open entries and more confinement to closed arms was associated anxiety-like behavior of mice.

2.6. Assessment of depression-like behavior in modified forced swim test

The depressive behavior was assessed by recording the total duration of immobility, swimming as well as climbing by individually placing animals on a cylinder (40 cm diameter and 15 cm height) filled up to 30 cm of height with fresh water maintained at (22 ± 2)℃. The test was performed on day 15th after the pre-test session on day 14th of treatment schedule. The animals were subjected to a forced swim test 24 h after the pre-test and the duration of immobility, swimming, and climbing was recorded for the last 5 minutes of the 6-minute session.

2.7. Assessment of cardiac hypertrophy

The hearts of treated animals from all groups were excised out and they were weighed individually. The ratio of heart weight (HW, mg) to body weight (BW, g) (HW/BW) was calculated to assess the degree of cardiac hypertrophy.

2.8. Biochemical estimations in serum

On day 15th of treatment, blood sample was collected after behavioral studies and serum was separated for biochemical estimations. Serum LDH activity and CK-MB were estimated by using commercially available diagnostic kits.

2.9. Biochemical estimations in tissue

The animals were sacrificed under ether anesthesia and hearts were immediately excised out, cleaned in ice-cold normal saline, and weighed. A 10% homogenate of heart tissues was prepared in 10 mM Tris-HCl buffer (pH 7.4). The clear supernatants of tissue homogenate were used for estimation of MDA and GSH.

2.10. Molecular docking studies

Molecular docking was used to estimate the affinity of the phytoconstituents of NSO with histone deacetylases (HDAC). The 3D structures of HDAC1 and HDAC6 proteins with PDB IDs of 4BKX and 5EF7 were obtained from the RCSB PDB database (https://www.rcsb.org/). The 2D structures of NSO chemical components were gathered from the literature, drawn in ChemDraw, and converted to PDB format using the PyMol application (version 2.4). M.G.L. Tools (version 1.5.7) software was used to add hydrogens, combine polar hydrogens, and compute charges of the ligand and protein receptors. The M.G.L. Tools were used to identify the docking site in each protein in addition to assessing the torsion root of the ligands. All of the proteins and ligands were then converted to the pdbqt format. AutoDock Vina 1.1.2 was used to perform molecular docking, with an exhaustiveness value of 100 and the rest of the parameters left at their defaults. The conformations of the bound ligands inside the target proteins were viewed using PyMol 2.5.1, LigPlot+ 2.4.2, and Biovia Discovery Studio Visualizer 2021 software after the docking experiment was completed as previously discussed[23].

2.11. Statistical analysis

All the data expressed as mean ± SEM were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer for multiple comparisons. The statistical analyses were performed using Graphpad Prism 3.0 (San Diego, CA, USA). *P* values <0.05 were considered statistically significant.

2.12. Ethical statement

The animal experiment was performed as per the ethical guidelines of Qassim University, and the protocol was approved by the Committee of Research Ethics, Deanship of Scientific Research, Qassim University, Saudi Arabia (No. 21-04-04, dated 24th Nov. 2021).

3. Results

3.1. Effect of NSO on cardiac hypertrophy

The measurement of HW/BW ratio is generally used as an index for measurement of cardiac hypertrophy. As shown in Table 1, DOX (10 mg/kg, *i.v.*) treatment produced an increase ($P<0.05$) in the HW/BW ratio as compared with the control group. NSO produced a dose-dependent reversal of DOX-induced hypertrophy with the significant reversal of HW/BW ratio, though the reduction by lower dose (1.5 mL/kg, *p.o.*) of NSO was insignificant as compared to the pathogenic group (group Ⅱ). NSO *per se* also produced a reduction in HW/BW ratio as compared to the pathogenic group.

Table 1. Effect of *Nigella sativa* oil treatment on HW/BW ratio, serum LDH and CK-MB, as well as tissue MDA and GSH levels in mice (n=5).

Items	Distilled water (10 mL/kg)	DOX (10 mg/kg)				*Nigella sativa* oil *per se* (6 mL/kg)
		0 mL/kg *Nigella sativa* oil	1.5 mL/kg *Nigella sativa* oil	3 mL/kg *Nigella sativa* oil	6 mL/kg *Nigella sativa* oil	
HW/BW ratio (mg/g)	4.22 ± 0.30	5.9 ± 0.39$^{\#}$	4.77 ± 0.06	4.44 ± 0.27*	4.20 ± 0.39*	4.00 ± 0.39**
LDH (IU/L)	3 374.8 ± 42.39	4 851.2 ± 42.42$^{\#\#\#}$	4 032.0 ± 43.96$^{\#\#,***}$	3 663.6 ± 23.51***	3 624.2 ± 167.4***	3 664.4 ± 156.98***
CK-MB (U/L)	286.8 ± 21.31	637.2 ± 101.85$^{\#\#\#}$	310.8 ± 12.75***	305.4 ± 13.72***	304.8 ± 30.64***	395.4 ± 11.99*
MDA (nM of MDA/mg of protein)	0.34 ± 0.06	0.72 ± 0.02$^{\#\#\#}$	0.47 ± 0.03**	0.42 ± 0.03**	0.40 ± 0.02***	0.40 ± 0.08***
GSH (μM of GSH/mg of protein)	1.49 ± 0.10	0.75 ± 0.11$^{\#\#\#}$	1.26 ± 0.13*	1.37 ± 0.04**	1.44 ± 0.10***	1.42 ± 0.08***

All values are expressed as mean ± SEM. DOX: doxorubicin, HW/BW ratio: heart weight-body weight ratio; LDH: lactate dehydrogenase; CK-MB: creatinine kinase-MB; MDA: malonaldehyde; GSH: reduced glutathione. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ *vs.* Group Ⅰ (Normal). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs.* Group Ⅱ (DOX only), considered significant by ANOVA followed by Tukey-Kramer multiple comparison test.

3.2. Effect of NSO on serum LDH and CK-MB level

Administration of DOX (group Ⅱ) resulted in significant elevation (P<0.001) of mean serum LDH and CK-MB levels as compared to the control animals (group Ⅰ). NSO treatment at 1.5, 3, and 6 mL/kg significantly decreased the elevated serum LDH and CK-MB level (P<0.001) (Table 1). NSO produced a dose-dependent reversal effect and the effect of the higher dose (6 mL/kg, *p.o.*) appeared to be more significant (P<0.001). *Per se* treatment with NSO (6 mL/kg, *p.o.*) failed to produce any apparent change in the serum LDH and CK-MB as compared to the control animals.

3.3. Effect of NSO on cardiac MDA level

Acute administration of DOX (10 mg/kg, *i.v.*) significantly increased (P<0.001) lipid peroxidation in cardiac tissues as compared to the control animals (Table 1). However, NSO treatment significantly (P<0.01) reduced tissue MDA levels as compared to the DOX treated animals. The reduction of tissue MDA was more significant (P<0.001) with the higher dose of NSO. *Per se* treatment with NSO (6 mL/kg, *p.o.*) did not produce any apparent changes in tissue MDA level as compared to the control group.

3.4. Effect of NSO on cardiac GSH level

Table 1 summarizes the effect of NSO on cardiac GSH level. DOX treatment significantly decreased (P<0.001) GSH level in cardiac tissues as compared to the control group. NSO treatment restored the DOX-induced reduction in GSH level in a dose-dependent manner in comparison to the pathogenic group. NSO *per se* (6 mL/kg, *p.o.*) did not produce any change in GSH levels in cardiac tissues compared to the control group.

3.5. Effect of NSO on behavior of mice in the elevated plus maze

As shown in Figure 1 (A & B), DOX-induced cardiotoxic mice exhibited anxiety-like behaviors in elevated plus maze test manifested by a decrease (P<0.05) in open arm entries and time spent in open arms compared to the control. NSO treatment (10 days' pre-treatment and 5 days' post-treatment) exhibited dose-dependent anxiolytic action by an increase in the number of open arm entries (P<0.001) and time spent in open arms (P<0.01) compared to cardiotoxic animals (group Ⅱ). Besides, NSO *per se* (6 mL/kg, *p.o.*) also significantly increased open arm entries (P<0.001) and time spent in open arms (P<0.01) in elevated plus maze.

3.6. Effect of NSO on spontaneous alternation behavior of mice in the cross maze

DOX administration resulted in a reduction (P<0.05) of percentage alternation behavior compared to the control animals, whereas, NSO treatment improved the DOX-induced reduction in percentage alternation as compared to the pathogenic control. The higher dose (6 mL/kg, *p.o.*) of NSO produced a more significant increase in percentage alternation behavior in cardiotoxic mice (P<0.001). NSO *per se* (6 mL/kg, *p.o.*) treatment also increased percentage alternation behavior as compared to the DOX treated animals (Figure 2).

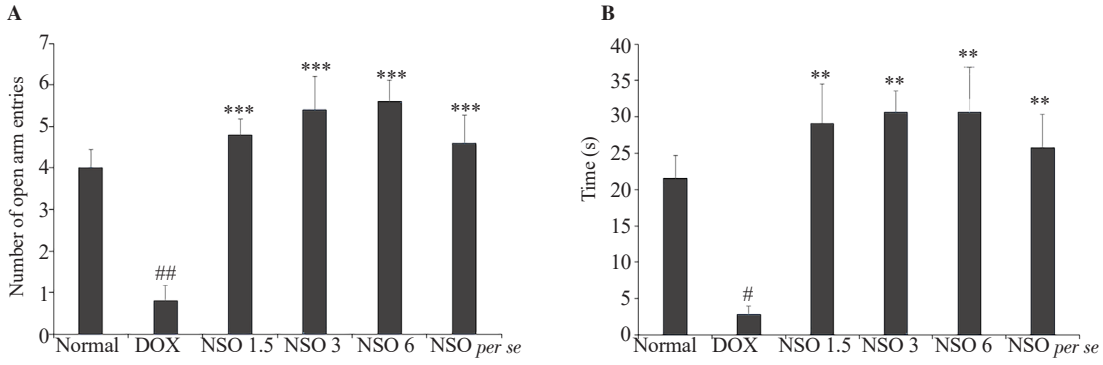

Figure 1. Effects of *Nigella sativa* oil (NSO) treatment on behavior of mice in the elevated plus maze test. All values are expressed as mean ± SEM. DOX was administered intravenously (10 mg/kg, *i.v.*) while NSO was administered by oral route (*p.o.*). The treatment duration was 15 days (10 days' pre-treatment and 5 days' post-treatment with NSO). DOX was administered on day 11th of NSO treatment. $^{\#}P$<0.05, $^{\#\#}P$<0.01 *vs.* Group Ⅰ (Normal). $^{**}P$<0.01, $^{***}P$<0.001 *vs.* Group Ⅱ (DOX only), considered significant by ANOVA followed by Tukey-Kramer multiple comparison test.

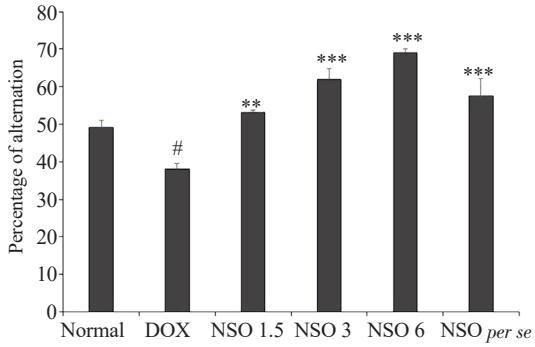

Figure 2. Effect of NSO treatment on spontaneous alternation behavior of mice in the cross maze test. All values are expressed as mean ± SEM. DOX was administered intravenously (10 mg/kg, *i.v.*) while NSO was administered by oral route (*p.o.*). The treatment duration was 15 days (10 days' pre-treatment and 5 days' post-treatment with NSO). DOX was administered on day 11th of NSO treatment. $^{\#}P<0.05$ *vs.* Group Ⅰ (Normal). $^{**}P<0.01$, $^{***}P<0.001$ *vs.* Group Ⅱ (DOX only), considered significant by ANOVA followed by Tukey-Kramer multiple comparison test.

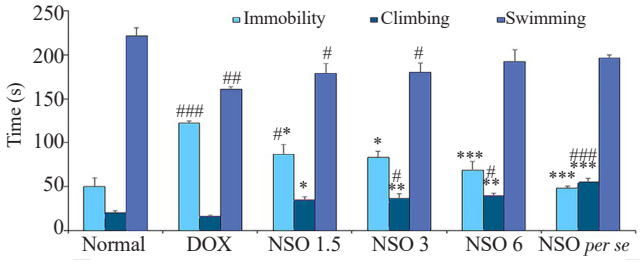

Figure 3. Effects of NSO treatment on the behavior of mice in the modified forced swim test. All values are expressed as mean ± SEM. DOX was administered intravenously (10 mg/kg, *i.v.*) while NSO was administered by oral route (*p.o.*). The treatment duration was 15 days (10 days' pre-treatment and 5 days' post-treatment with NSO). DOX was administered on day 11th of NSO treatment. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ *vs.* Group Ⅰ (Normal). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *vs.* Group Ⅱ (DOX only), considered significant by ANOVA followed by Tukey-Kramer multiple comparison test.

3.7. Effect of NSO in modified forced swim test

Figure 3 illustrates the behavior of mice in modified forced swim test. DOX-induced cardiotoxic mice exhibited depressive behavior in modified forced swim test evidenced by an increase in immobility time and decreases in swimming time and climbing time as compared to the control. Although cardiotoxic mice exhibited a reduction in climbing time, it was insignificant. Treatment with NSO (10 days' pre-treatment and 5 days' post-treatment following DOX administration) produced dose-dependent antidepressant action evidenced by a reduction in immobility time and increases in swimming time and climbing time as compared to the pathogenic group ($P<0.05$).

3.8. Molecular interaction of NSO constituents with HDACs

The molecular docking approach was utilized to assess the possible binding of NSO components to the protein cavities of HDAC1 and HDAC6, which showed binding energies ranging from –5.2 to –6.9 and –5.4 to –7.4 kcal/mol, respectively (Figure 4). In general, HDAC6 was shown to have a higher affinity for docked molecules than the HDAC1 target. Among all docked compounds, nigellidine exhibited maximum affinity with HDAC1 and HDAC6 targets with binding energy of –6.7 kcal/mol and –7.4 kcal/mol, respectively. However, nigellimine-*N*-oxide was noted as least active among the docked molecules exhibiting –5.2 kcal/mol and –5.4 kcal/mol binding energy with HDAC1 and HDAC6, respectively. Molecular interaction of the docked compounds involves both hydrophilic and hydrophobic types. Figures 5&6 represent minimum energy conformations of potential compounds such as nigellidine and thymoquinone with HDAC1 and HDAC6 binding sites. The binding site of HDAC6 is composed of Ser531, His574, Gly582, Phe583, His614, Phe643, Asp705, Leu712, Gly743, Tyr745, Thr777, Thr780, Val781, Asn784. Most of the docked compounds exploited these amino acid residues for their interaction with HDAC6 (Figure 5A-5D). However, residues such as His140, His141, Asp174, Asp176, His178, Asp181, Asp264, Gly301, Tyr303 encircle the binding area of HDAC1 providing a platform for contact with NSO constituents (Figure 6A-6D).

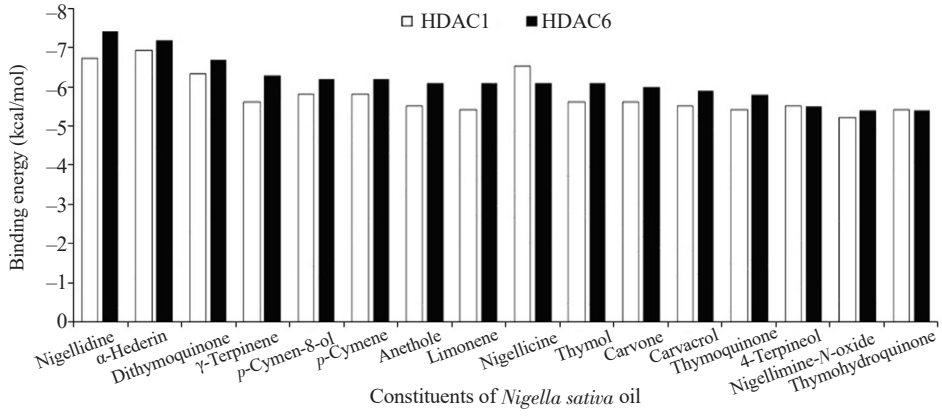

Figure 4. Bar plot showing AutoDock Vina predicted binding energy of *Nigella sativa* constituents against HDAC1 and HDAC6.

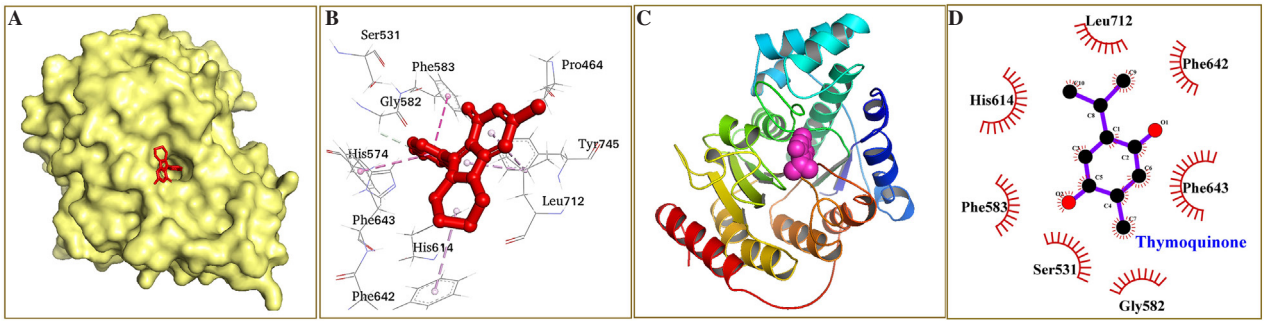

Figure 5. Minimum energy conformation of the docked nigellidine (shown in red ball and stick style) in the binding pocket of HDAC6 protein. Non-bond interactions are presented as dashed lines (A & B). Docking conformation of thymoquinone in the HDAC6 protein (shown as ribbon) (C & D).

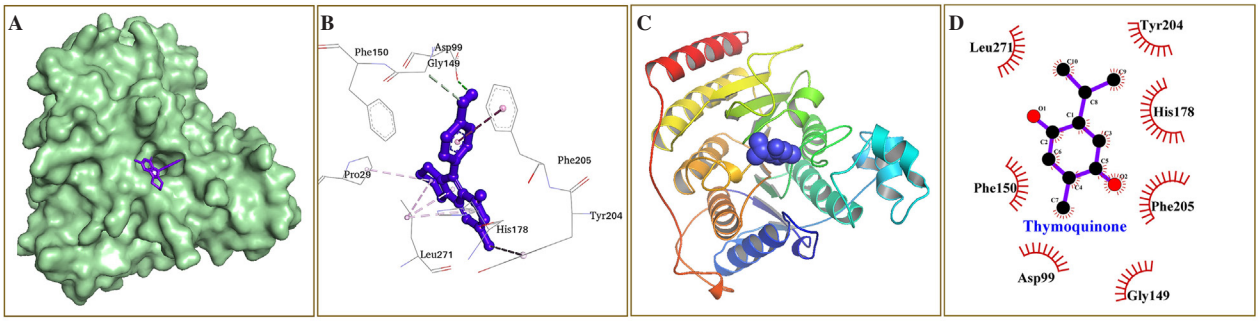

Figure 6. Docked nigellidine (shown as blue ball and stick style) in the binding pocket of HDAC1 protein. Non-bond interactions are presented as dashed lines (A & B). Docking conformation of thymoquinone in the HDAC1 protein (shown as ribbon) (C & D).

4. Discussion

It is well known that cardiomyopathy and/or MI are often associated with impairment of the quality of life following such cardiac events. The rodent model of HF may be used to replicate such psychosocial issues including anxiety, depression, and memory impairments which are frequently observed in clinical settings. In addition to cardioprotective therapy, the coexisting psychological problems also require effective management as they may become a major risk factor of future cardiovascular incidence. The pathogenesis of impaired quality of life in cardiac patients is multifactorial and most of the post-infarct treatment targets mainly at improving longevity whereas minimal or no attention is paid to the associated psychological issue that hampered the quality of life, especially anxiety, depression, and memory. Thus, it would certainly be beneficial to have an agent that has cardioprotective effects as well as improves quality of life through anxiolytic, antidepressant, and nootropic potential.

In the present study, we used DOX-induced cardiomyopathy as a model of HF in female mice, while many earlier studies reported anxiety-like behavior in male mice[12]. Since the prevalence of anxiety and depression in cardiac patients exhibited gender disparity and more prevalent in females than males, the current study aims to mimic such clinical findings in female mice.

DOX (10 mg/kg, *i.v.*) produced cardiotoxicity in female mice evidenced by a significant increase in HW/BW ratio which is considered an important marker of cardiac hypertrophy[24]. In the present study, NSO treatment significantly reversed the DOX-induced increase in HW/BW ratio though lower dose (NSO, 1.5 mL/kg) being statistically insignificant.

Myocardial tissue contains a large amount of LDH in the cytosol which is released by damaged myocardium into the extracellular fluids and thus an increase in the serum level of LDH serves as a marker of myocardial injury. DOX treatment significantly increased the serum LDH level which is in agreement with previous findings[25] and treatment with NSO (1.5, 3 & 6 mL/kg, *p.o.*) produced a significant reduction in serum LDH level in a dose-dependent manner. Although LDH is a nonspecific enzyme, the changes in serum LDH correlate with CK-MB. Therefore, it is plausible that the changes in serum LDH mainly contribute to cardiomyocyte damage, which is consistent with previous reports showing increased serum LDH after DOX treatment[26]. Our results corroborated with the earlier findings showing reduced serum LDH level after thymoquinone treatment, an active constituent of NSO in rats with cardiotoxicity induced by cyclophosphamide[27]. CK-MB is primarily present in cardiac muscles and served as a serum

marker for myocardial damage. The results of the present study showed that DOX administration resulted in a significant increase in serum CK-MB level compared to the control group. However, NSO treatment (10-day pretreatment and 5-day posttreatment at 1.5, 3, and 6 mL/kg doses orally) significantly reduced DOX-induced increase in serum CK-MB level. However, NSO *per se* treatment does not produce significant changes in serum enzyme levels which demonstrates that NSO could maintain the integrity of myocardial membrane, thus restricting the DOX-induced myocardial damage and release of these cardiac enzymes. The current findings corroborated with previous reports wherein NSO and its active constituent, thymoquinone inhibit the lipid peroxidation in membrane and generation of leukocytes.

The generation of ROS is undoubtedly an important mechanism of oxidative stress leading to DOX-induced cardiotoxicity[28]. Further, the increase in ROS results in increased lipid peroxidation leading to an increase in the MDA level in cardiac tissues as reported in the previous study[29], which is also observed in our finding. The increased MDA level in heart tissues following DOX (10 mg/kg, *i.v.*) administration was significantly prevented by NSO treatment (10 days of pretreatment followed by 5 days of posttreatment). This could be due to the antioxidant effect of NSO which is attributed to the neutralising effect of thymoquinone on ROS including superoxide free radicals, singlet oxygen, and nitric oxide[30]. Moreover, GSH plays a vital role in cellular defense against oxidative stress by scavenging highly reactive free radicals such as ROS[31]. The depletion of GSH may lead to impaired cellular defense and thus tissue damage due to oxidative stress. We found significant depletion of GSH level in heart tissue following administration of DOX compared to the normal group, which is similar to previous observation[32]. However, treatment with NSO (1.5, 3, 6 mL/kg) significantly restored the DOX-induced depletion of GSH level in cardiac tissue, which is similar to a previous finding wherein fixed and essential oil of *N. sativa* modulates the redox enzymes system including GSH in oxidative stress induced by potassium bromate[33]. The cardioprotective effect of NOS as observed in this study also corroborated the previous findings wherein NSO supplementation (80 mg/kg/dose in 3 divided doses) showed a cardioprotective effect in children with acute lymphoblastic leukemia receiving DOX treatment[20]. The maintenance of antioxidant condition in normal tissue that enhances an effect on endogenous antioxidant system by augmentation of endogenous antioxidant mechanism of NSO could possibly play a vital role in cardioprotective action.

In this study, the administration of acute dose (10 mg/kg *i.v.*) of DOX produced neurobehavioral alterations evidenced as anxious, depressive, and amnesic behavior of mice. The activation of sympathetic nervous system and hypothalamo-pituitary-adrenal axis in conjunction with anxiety are involved in the pathogenesis of HF[6]. Thus, the persisting anxiety following cardiomyopathy and/or MI leads to an increase in myocardial contractility, heart rate, and myocardial O_2 demand and thus precipitates further cardiac events. Therefore, effective management of anxiety becomes equally important in addition to cardioprotection. Our results showed that DOX-induced anxiety-like behavior in female mice is shown by a significant reduction in the number of open arm entries and time spent in open arms in addition to freezing, reduced mobility, frequent urination, and defecation. This is in agreement with our previous findings wherein mice exhibited anxious behavior in the elevated plus maze and Vogel's conflict test which was well reversed by psychoactive drugs alprazolam and escitalopram[12,34]. The anxiolytic effect of NSO could be due to its antioxidant and GABA secretory action as reported previously[35].

Depression is a multifaceted disorder and is considered one of the risk factors of cardiovascular diseases. The results of the present study demonstrate that there was an increase in depressive behavior following cardiomyopathy induced by DOX in mice as evidenced by a significant increase in immobility time in the modified forced swim test. However, the mechanism of such an association between cardiomyopathy and depression is not fully deciphered but reduced brain-derived neurotrophic factor (BDNF) at least could partly contribute to the pathogenesis of depression[36]. However, NSO treatment produced a dose-dependent reversal of DOX-induced increase in immobility time and also decreases in climbing and swimming behavior of mice in the modified forced swim test. Our finding corroborated the previous study that reported the antidepressant effect of polar extract of *N. sativa* seed in tail suspension test and forced swim test[37]. The antioxidant and anti-inflammatory effects of NSO might contribute to the antidepressant action. Moreover, increased BDNF signaling has been reported to produce rapid antidepressant action[38]. Interestingly, thymoquinone, the principal active constituent of NSO, has been reported to increase in BDNF level in experimentally induced neurotoxicity in rats[39].

Cognitive damage is another complication of HF that has a significant impact on the quality of life. In addition to anxiety and depression impairment of memory following cardiac events is another troublesome corner that also requires special attention. Rodents have a normal tendency to alternate and the determination of spontaneous alternation behavior is considered as a reliable measure for short-term memory[40]. In the present study, cardiotoxic mice showed impaired memory evidenced by a significant decrease in percentage alteration compared to the normal group. In agreement with the previous study that reported the nootropic effects of hydroalcoholic extract of *N. sativa* against lipopolysaccharide-induced memory impairment in rats[41], our

results demonstrated the nootropic effect of NSO treatment in mice following DOX administration. The reported anticholinesterase effect of *N. sativa* L.[42] increases the cholinergic activity in addition to antioxidant and neuroprotective effects which possibly contribute to the nootropic action in the present model.

The results of the study revealed that NSO exhibited significant cardioprotection and restored the neurobehavioral changes in cardiotoxic mice through modulating the DOX-induced oxidative stress. In addition to mitochondrial oxidative stress, accumulating evidence suggests the modification in histone deacetylase activity by DOX treatment owing to apoptosis and hypertrophy of cardiomyocytes[43]. DOX treatment changes the amounts of microRNAs and upregulated several HDACs including HDAC1 and HDAC6. Further, DOX-induced cardiotoxic rats showed upregulation of HDAC6 which in turn resulted in deacetylation of α-tubulin and autophagic flux mechanism[44]. Emotional behaviors are linked to dorsal and median raphe nuclei in mice lacking HDAC6, which can localize to these nuclei preferentially. Interestingly, HDAC6-deficient mice are more active and less anxious in behavioral studies. An antidepressant-like effect on mice was also duplicated by administering an HDAC6 inhibitor. According to these findings, reversible acetylation of serotonin by HDAC6 may help maintain appropriate neural function and give a novel therapeutic target for depression[45]. Therefore, a docking study was conducted against HDACs to get insight into the interaction processes of possible phytoconstituents of *N. sativa*. Current study employs molecular docking technique to describe molecular interactions of these compounds with the HDACs. The findings of this study may lead to uncovering *N. sativa*'s ameliorative mechanisms in cardiomyopathies and associated neurobehavioral changes. It may also help to optimize lead compounds for such maladies.

The present study used animal models presenting cardiomyopathy/cardiotoxicity and neurobehavioral changes to demonstrate the negative health impact and poor quality of life in heart patients. Our study shows that rodents with DOX-induced cardiomyopathy and associated neurobehavioral changes could serve as an important model to investigate possible interventions against comorbid afflictions of the brain such as anxiety, depression, and others. Our finding revealed that NSO considerably ameliorated anxious and depressive behaviors including memory impairments in mice having cardiomyopathy in a dose-dependent manner. These ameliorative activities of the NSO were observed besides its protective effect against cardiomyopathy. In cardiac patients, females have a higher incidence of neuropsychiatric disorders compared to males[13], and there is a dearth of animal data for such observations as reported in clinical settings. Our finding in animal models might enable the researcher to further investigate interventions in such clinical conditions. Furthermore, the molecular docking studies of active constituents of NSO with HDAC1 and HDAC6 could potentially lead us to design further experimental studies. However, given the limitation of the present study which used a small number of animals in a limited number of experimental models and the short duration of experimental design, further investigation is warranted to ascertain the effects on some other models of HF in rodents and neurobehavioral changes.

Conflict of interest statement

The authors declare no conflict of interest.

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Authors' contributions

MJA conceptualized and designed the work. MJA and DM were involved in experiments and data collection. FA carried out software assisted molecular docking. MJA, SKA, and FI were involved in validation of methodology. MJA and FA conducted data curation and interpretation. MJA, and FA wrote the manuscript. MJA, SKA, FA, DM, FI and KSA finally revised and approved the final version to be published.

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