

Vol. 17 No. 2 (2018)

Published: 2018-12-31

Original Articles

Anthropometric study of the frontal sinus on plain radiographs in Delta State University Teaching Hospital

DOI: https://doi.org/10.4103/jeca.jeca_45_16

T.M. Enaowho, O.G. Okoro (Author)
49-52



A simple and sensitive immunoassay method for the detection of low-molecular-weight proteins and neuropeptides

DOI: https://doi.org/10.4103/jeca.jeca_36_17

A. Ajayi, B. Withyachumnarnkul (Author)
53-55



Risk of foot complications in diabetes mellitus; how much do the diabetes mellitus patients in Enugu know?

DOI: https://doi.org/10.4103/jeca.jeca_23_1

W.O. Okenwa, A.J. Edeh (Author)
56-59



Subchronic dichlorvos-induced Cardiotoxicity in Wistar rats: Mitigative efficacy of Nigella sativa oil

DOI: https://doi.org/10.4103/jeca.jeca_18_17

A. Imam, M.O. Busari, M.Y. Adana, M.I. Ajibola, A. Ibrahim, F.A. Sulaimon, M.S. Ajao (Author)
60-65



Presence of GFSKLYFamide-like neuropeptide in the nervous tissue of Holothuria scabra: Immunohistochemical evidence

DOI: https://doi.org/10.4103/jeca.jeca_3_18

A. Ajayi, B. Withyachumnarnkul (Author)
66-69



Topographic anatomy of the neurovascular bundle at the tarsal tunnel and its applied significance

DOI: https://doi.org/10.4103/jeca.jeca_5_18

M.N. Nabil, N.A. Homosani, D. Biram (Author)
70-75



Case Reports

Bilateral “quadriceps gastrocnemius” and termination of small saphenous vein outside the popliteal fossa in a cadaver

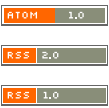
DOI: https://doi.org/10.4103/jeca.jeca_14_18

D.H. Woldeyes, A.M. Moges (Author)
76-78





Latest publications



Make a Submission

Browse

Categories

Keywords

ABOUT JECA

[About the Journal](#)
[Aims & Scope](#)
[Editor Profile](#)
[Editorial Board](#)

RESOURCES

[Editorial Policy & Malpractice Statement](#)
[Guide for Authors](#)
[Guidelines for Reviewers](#)
[Open Access Policy](#)

ACCOUNT

[Editor](#)
[Authors](#)
[Reviewers](#)
[Subscribers](#)

ISSUES

[Current](#)
[Archives](#)

Access this article online

Quick Response Code:

Website :
www.jecajournal.comDOI:
10.4103/jeca.jeca_18_17

Subchronic dichlorvos-induced Cardiotoxicity in Wistar rats: Mitigative efficacy of *Nigella sativa* oil

Aminu Imam, Maryam Oluwatobi Busari, Misturat Yetunde Adana, Musa Iyiola Ajibola¹, Abdulmumin Ibrahim, Fatimoh Ajoke Sulaimon, Moyosore Salihu Ajao

Abstract:

BACKGROUND: Accidental poisoning from indiscriminate use of organophosphates have become endemic in recent decades, most especially in developing nations, coupled with the limitations of the availability of satisfactory antidotes.

AIM OF THE STUDY: Thus, we investigated the cardioprotective efficacy of *Nigella sativa* oil (NSO) following dichlorvos (DDVP)-induced cardiotoxicity in Wistar rats.

MATERIALS AND METHODS: A total of 24 Wistar rats were randomly divided into four groups ($n = 6$); the control was administered sunflower oil (1 ml/kg), DDVP (8.8 mg/kg) to the experimental Group I, whereas DDVP + NSO (8.8 mg/kg + 1 ml/kg) and NSO (1 ml/kg) was administered orally to the experimental Groups II and III, respectively. The animals were euthanized; blood was transcardially collected from the right atrium, centrifuged, and plasma extracted to analyze levels of total cholesterol (TC), triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol (LDL-C). While cardiac muscle tissue was collected from the left heart, processed and stained for general architecture (hematoxylin and eosin) and elastic morphology (orcein).

RESULTS: DDVP significantly ($P \leq 0.05$) increased the plasma levels of TC, LDL, atherogenic and atherosclerotic indices (TC/HDL-C and LDL-C/HDL-C ratios), but this was prevented by co-administration with NSO. Histological investigations showed that DDVP resulted in the pathological appearance of cardiac tissues, such as the lack of striations, myocardial hemorrhage, and necrosis-like features.

CONCLUSION: It can be concluded that NSO was able to attenuate DDVP-induced cardiotoxicity.

Keywords:

Cardiotoxicity, dichlorvos, *Nigella sativa* oil, organophosphate, poisoning

Introduction

Organophosphate (OP) pesticides are widely employed in the control of household and agricultural pests. However, their indiscriminate use has led to great environmental pollution, contaminating air, soil, water, and farm produce leading to unsuspected health burdens (Davies *et al.*, 2016; Farrukh *et al.* 2016; Rashmikka *et al.*, 2016; Weidong *et al.*, 2016).

The toxicity of most of these OPs, which are insecticidally active to animals, is based on their property to inhibit acetylcholinesterase, resulting in the accumulation of acetylcholine in the presynaptic space. This has been associated with several deleterious effects including muscle incoordination, tremors, myosis, chest discomfort, decreased heart irregularities, loss of reflexes, muscular paralysis, autonomic overstimulation, and cardiorespiratory failure among other potentially lethal syndrome (Roth *et al.*,

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

How to cite this article: Imam A, Busari MO, Adana MY, Ajibola MI, Ibrahim A, Sulaimon FA, *et al.* Subchronic dichlorvos-induced cardiotoxicity in Wistar rats: Mitigative efficacy of *Nigella sativa* oil. J Exp Clin Anat 2018;17(2):60-65.

Department of Anatomy,
Faculty of Basic Medical
Sciences, University
of Ilorin, Ilorin, Nigeria,
¹Institute of Neuroscience,
National Yang-Ming
University, Taipei, Taiwan

Address for correspondence:

Dr. Aminu Imam,
Department of Anatomy,
Faculty of Basic Medical
Sciences, University
of Ilorin, Ilorin, Nigeria.
E-mail: imam.a@unilorin.
edu.ng

1993; Mostafalou and Abdollahi, 2013; Rosman *et al.*, 2014; Arthur *et al.*, 2017).

Documented evidence have shown that OP poisonings are mostly accompanied by cardiovascular complications, with abnormalities on electrocardiography (ECG), conduction and ventricular arrhythmias (Karki *et al.*, 2004; Anand *et al.*, 2009; Mostafalou and Abdollahi, 2013).

Other complications of OPs exposure include mutagenicity (Bhinder and Chaudhry, 2013), neurotoxicity (Galal *et al.*, 2014; Shrot *et al.*, 2015), carcinogenicity (Greim *et al.*, 2015), hepatotoxicity (Ogutcu *et al.*, 2008), and nephrotoxicity (Hou *et al.*, 2014).

Of all OPs, Dichlorvos is one of the most used in the developing countries (Deka and Mahanta, 2015), and has been reported to be the cause of severe poisoning and death associated with most OPs products used in these nations (Musa *et al.*, 2010; Brown *et al.*, 2015). It has been implicated to induce a more rapid onset of poisoning symptoms when compared with other OPs, although with rapid recovery (Erdman, 2004).

DDVP-induced toxicity has been linked to a number of mechanisms including cholinesterase and non-cholinesterase pathways, however, treatment with the available antidotes remains a challenge. Thus, finding novel alternative or supplementary regimen with potential efficacy against DDVP and OP poisoning is vital. Thus, research into an alternative regimen in the management of DDVP induced cardiotoxicity is crucial, with great focus on the supplementary regimen.

Nigella sativa oil (NSO) is a high-value traditionally used medical regimen in the management of various diseases. Its efficacy has been extensively studied and reported to be subserved with neuro-protective (Yaman and Balikci, 2010), antioxidant (Kanter *et al.*, 2008), anti-inflammatory (Noor *et al.*, 2015), anti-ischemic (Hobbenaghi *et al.*, 2014), anti-seizure (Farzaneh *et al.*, 2015), memory and recall (Imam *et al.*, 2016) among other activities. Thus, the aim of this study was to investigate the attenuating efficacy of NSO in dichlorvos poisoning-induced cardiotoxicity in rats.

Materials and Methods

Chemicals and drugs

Dichlorvos (PESTANAL®), the analytical standard was purchased from Sigma (Sigma-Aldrich) (St. Louis, MO, USA), while an analytical grade of Sunflower oil (by Africa Sun oil Refineries) was purchased from a local pharmaceutical and supplement shop. The NSO (concentration; 100% black seed; HUSNA black seed oil, Fazhab Agency, Karachi, Pakistan) was

purchased from a trade-medical store in Ilorin, Kwara state, Nigeria.

Animals

Twenty-four adult male Wistar rats with an average weight of 200 ± 20 g were used in this study. The animals were housed under standard laboratory conditions in the animal holding of the Faculty of Basic Medical Sciences, University of Ilorin, Nigeria. They were allowed free access to water and food *ad libitum*.

After the last treatment day, the rats were overdosed with appropriate doses of sodium pentobarbital. Once respiration had ceased and the animals were nonresponsive to vigorous tactile stimuli, blood was collected transcardially for biochemical estimation, and then whole-body intracardial perfusion was performed, initially, with a cold rinse of 0.9% saline solution, followed by 10% buffered formalin. Following fixation, the hearts were carefully removed from the thorax, and postfixed overnight in 10% buffered formalin, for further histopathological processing.

Treatments

The rats were randomly distributed into four groups ($n = 6$) as follows:

- Control: Received subfornical organ (1 ml/kg oral)
- Experimental 1: Received DDVP (8.8 mg/kg/day oral) (Sharma and Singh, 2012)
- Experimental 2: Received DDVP (8.8 mg/kg/day orally) + NSO (1 ml/kg oral) 30 min later
- Experimental 3: Received NSO (1 ml/kg orally) (Atef and Wafa'a, 2010; Nahed and Bassant, 2011).

All procedures were scheduled and carried out during the early light phase between 07:00 and 09:00 h, and treatments were given for 21 consecutive days.

Plasma lipid profile

Blood samples were centrifuged at 3000 rpm for 15 min, and plasma was collected. Plasma levels of high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and total cholesterol (TC) were determined spectrophotometrically using commercially available diagnostic kits according to the standard procedures (Randox Laboratories Limited, United Kingdom). The atherogenic indices (AI) were determined using the Friedewald equation (Friedewald, 1972):

$$AI = (TC - HDL-C)/HDL-C$$

also calculated are: Atherosclerotic indices (TC/HDL-C and LDL-C/HDL-C ratios).

Histology

The heart tissues were subsequently embedded in paraffin, sectioned into 8 μ m sections using a rotary

microtome (MK 1110). These sections were stained with hematoxylin and eosin for general cardiac architecture and Orcein staining for elastic fibers morphology following standard routine laboratory procedures. Six sections were prepared from each heart tissue and evaluated for the degree of injury. Images of the general architecture were captured under $\times 40$ objective lens using the Zeiss AxiostarPlus Light microscope.

Statistical analysis

Data recorded in this study were reported as the mean $\pm$ standard error of the mean. The plasma levels of HDL-C, LDL-C, triglycerides, and TC were analyzed using one-way analysis of variance and for the *post hoc* analyses, we used the Bonferroni test. A value of $P \leq 0.05$ was considered statistically significant in all cases, using the software package Graphpad Prism software (version 5.0, La Jolla, CA) for analysis.

Results

Plasma lipid profile following DDVP exposure and *Nigella sativa* oil treatment

The changes in the level of plasma lipids in the control and experimental rats are shown in Table 1. Significant ($P \leq 0.05$) increase in the levels of TC, TG, and LDL-C were observed between control and DDVP-treated rats. While significant ($P \leq 0.05$) decrease in HDL-C level was observed in DDVP-treated when compared with the control. These variations were markedly improved following the posttreatment with NSO and in the NSO only treated rats.

Atherogenic and atherosclerotic indices following DDVP exposure and *Nigella sativa* oil treatment

Following DDVP exposure, the atherogenic and atherosclerotic indices (TC/HDL-C and LDL-C/HDL-C ratios) were markedly enhanced. However, posttreatment with NSO was able to mitigate the outburst induced by DDVP in the NSO co-treated rats [Figure 1].

Table 1: Lipid profile (total cholesterol, triglyceride, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol) in plasma of control (sunflower oil), Dichlorvos exposed, combined Dichlorvos + *Nigella sativa* oil treated and *Nigella sativa* oil treated rats respectively. Analysis of variance + Bonferroni, * $P \leq 0.05$

Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Sunflower oil	29.64 $\pm$ 4.33	188.57 $\pm$ 12.02	23.39 $\pm$ 5.88	0.68 $\pm$ 0.19
DDVP	31.74 $\pm$ 6.89	224.92 $\pm$ 25.89	10.59 $\pm$ 0.38	1.51 $\pm$ 0.33
DDVP + NSO	30.64 $\pm$ 6.04	220.82 $\pm$ 13.89	20.18 $\pm$ 2.78	1.23 $\pm$ 0.43
NSO	23.12 $\pm$ 2.78	145.78 $\pm$ 14.89	34.89 $\pm$ 4.47	0.54 $\pm$ 0.17

+ *Nigella sativa* oil treated and *Nigella sativa* oil treated rats respectively. Analysis of variance+Bonferroni, * $P \leq 0.05$

Cardiac histoarchitecture following DDVP exposure and *Nigella sativa* oil treatment

Histological assessment showed a normal muscle and elastic fiber morphology in the heart tissue in control rats [Figures 2 and 3]. Exposure to DDVP-induced structural changes in this tissue, characterized by cytoplasmic vacuolization of cardiac muscle cells [Figures 2 and 3]. The latter was significantly decreased when NSO was administered to the DDVP combined treated rats when compared with those exposed to DDVP without treatment [Figures 2 and 3]. In rats treated with NSO alone, heart histoarchitecture was normal [Figures 2 and 3].

Discussion

In the recent decades, we have witnessed a drastic increase in the use of pesticides worldwide; due to growing effort to increase food production and control vector-borne diseases, and this have consequentially become harmful to the environment and to the human health (Hou and Wu, 2010). Biochemical changes associated with myocardial necrosis and toxic myocarditis have been associated with OPs poisoning, accounting for the death of exposed patients even after apparent clinical recovery (Anand *et al.*, 2009; Vijayakumar *et al.*, 2011; Wahab *et al.*, 2016).

Unfortunately, very little is known about the cardiovascular complications from OPs poisoning due to limited studies, whereas cardiotoxic effects have been documented in other insecticides (Papaeftimiou and Theophilidis, 2001; Won *et al.*, 2012; Mariem *et al.*, 2016).

The present study showed that DDVP (8.8 mg/kg) exhibits cardiac toxicity in subchronic exposure (21 days) by inducing morphological damages as well as elevated lipid profiles, atherogenic, and atherosclerotic indices. The results implicated DDVP in the deterioration of

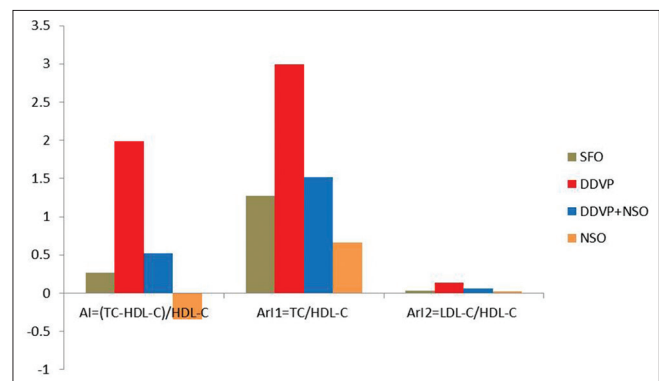


Figure 1: Atherogenic index ((TC-HDL-C)/HDL-C) and atherosclerotic indices (TC/HDL-C and LDL-C/HDL-C) of control (SFO), DDVP exposed, combined DDVP + NSO treated and NSO treated rats, respectively. SFO - Subformal organ, NSO - *Nigella sativa* oil, HDL-C - High-density lipoprotein-cholesterol, TC - Total cholesterol, LDL-C - Low-density lipoprotein-cholesterol, DDVP - Dichlorvos

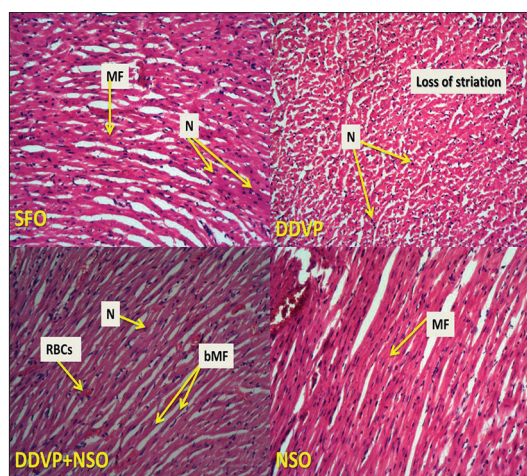


Figure 2: Photomicrograph of the cardiac tissue section following treatment with SFO, DDVP (H and E, ×100). DDVP + NSO and NSO. MF - Muscle fibers, N - Nuclei, bMF - Branching muscle fibers, RBCs - Red blood cells, SFO - Subfornal organ, NSO - *Nigella sativa* oil, DDVP - Dichlorvos

plasma lipid profiles where it markedly increased the plasma concentrations of levels of TC, TG, and LDL-C and depleted HDL-C levels.

The damaging effects of DDVP on cardiovascular functions was confirmed with the outturn of the AI as well as the atherosclerotic indices (LDLC/HDL-C and TC/HDL-C ratios) which are the important central indicators of cardiovascular complications (Meriem *et al.*, 2016). These alterations in lipid profile, atherogenic, and atherosclerotic indices are indicators that DDVP might impair lipid metabolism and cause cardiotoxicity, a characteristic reported of some other OPs (Cetin *et al.*, 2007; Hariri *et al.*, 2010).

There were also some damages in the myocardial architecture, marked with vacuolations and disrupted general morphology of the tissue and these strengthen the extent of alterations in the underlined biochemical profiles. These observed cytoarchitectural changes are not unusual of an OP, as several OPs have been implicated in damaging of cardiovascular functions (electrical and mechanical) and structural architectures (Allon *et al.*, 2005; Yavuz *et al.*, 2005; Ogutcu *et al.*, 2006; Calore *et al.*, 2007; Zamzila *et al.*, 2011).

NSO administration 30 minutes after exposure to DDVP in this study was able to rescue and prevent further perturbation of plasma lipid profiles, atherogenic and atherosclerotic indices induced by DDVP. The prophylactic efficacy of NSO in DDVP-induced cardiotoxicity as observed in this study could be attributed to its antioxidant efficacy (Kanter *et al.*, 2008), and previously reported protection against OPs induced damages to functional and biochemical activities in various body organs (Atef and Wafa'a, 2010; Mohamadin

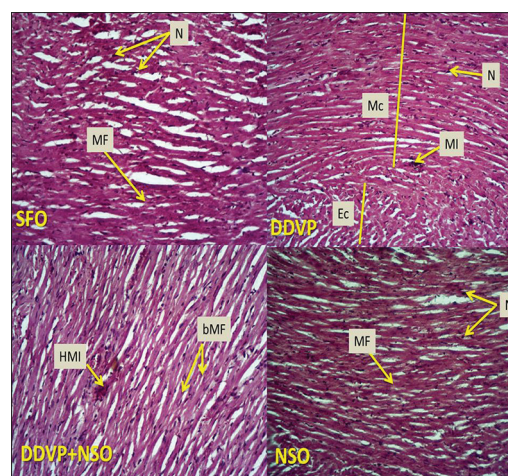


Figure 3: Photomicrograph of the cardiac tissue section following treatment with SFO, DDVP (Orcein, ×100). DDVP + NSO and NSO. Ec - Endocardium, Mc - Myocardium, MI - Myocardial infarct, HMI - Healing myocardial infarct, MF - Muscle fibers, bMF - Branching muscle fibers, N - Nucleus, NSO - *Nigella sativa* oil, SFO - Subfornal organ, DDVP - Dichlorvos

et al., 2010; Nahed and Bassant, 2011; Hashem, 2012; Halil *et al.*, 2015).

Various research works have also reported the therapeutic effect of *N. sativa* on lipid profile disturbance, atherogenesis, endothelial dysfunction, cardiac mass and contractility abnormality, platelet aggregation, heart rate, blood pressure disorder, and cardiotoxicity (Dehkordi and Kamkha, 2008; Shabana *et al.*, 2013; Zahra *et al.*, 2016).

Conclusion

Based on the results of this study, it can be inferred that NSO has potential therapeutic efficacy against subchronic DDVP impaired lipid profile, atherogenic index, atherosclerotic indices and myocardia architecture in Wistar rats.

Acknowledgment

The authors would like to acknowledge the technical staff of the Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Ilorin for helping with technical modalities during implementation of this research.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Allon N., Rabinovitz I., Manistersky E., Weissman B.A., Grauer E. (2005). Acute and long-lasting cardiac changes following a single whole-body exposure to sarin vapor in rats.

- Toxicol Sci 87:385-90.
2. Anand S., Singh S., NaharSaikia U., Bhalla A., Paul S.Y., Singh D. (2009). Cardiac abnormalities in acute organophosphate poisoning. Clin Toxicol (Philadelphia, PA) 47:230-5.
3. Arthur S., Ran M., Sigal E., Noah LC., Yossi R., Shai S., et al. (2017). QT prolongation as an isolated long-term cardiac manifestation of dichlorvos organophosphate poisoning in rats. Cardiovasc Toxicol 18:24-32. [Doi: 10.1007/s12012-017-9409-z].
4. Atef M.A., Wafa'a A.A. (2010). Preventive effects of black seed (*Nigella sativa*) extract on Sprague Dawley rats exposed to Diazinon. Aus J Bas App Sci 4 (5):957-68.
5. Bhinder, P., & Chaudhry, A. (2013). Evaluation of toxic potential of acephate and chlorpyrifos by dominant lethal test on *Culex quinquefasciatus*. Journal of environmental biology, 34(3), 573.
6. Brown H., Oruambo F., Kenanagha B. (2015). Poor anted effects of copper and manganese on ratsexposed to acute dose of dichlorvos. Ejpmr 2 (1):290-303.
7. Calore E., Perez N., Herman M. (2007). Morphometric studies of cardiac miocytes of rats chronically treated with an organophosphate. Ecotoxicol Environ Saf 66:447-50.
8. Cetin N., Cetin E., Eraslan G., Bilgili A. (2007). Chlorpyrifos induces cardiac dysfunction in rabbits. Res Vet Sci 82:405-8.
9. Davies M.S., Boniface M., Gibson S. (2016). Determination of dichlorvos residue levels in vegetables sold in Lusaka, Zambia. Pan Afr Med J 23:113. [Doi: 10.11604/pamj. 2016.23.113.8211].
10. Dehkordi F.R., Kamkhah A.F. (2008). Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. Fundamen Clinic Pharmacol 22:447-52.
11. Deka S., Mahanta R. (2015). Dichlorvos toxicity on fish – A review. Eur J Biol Res 5 (3):78-85.
12. Erdman A.R. (2004). Insecticides. In: Dart R.C., Caravati E.M., McGuigan M.A., editors. Medical Toxicology. 3rd ed. Lippincott Williams and Wilkins, Philadelphia, p. 1475-96.
13. Farrukh J., Quazi S.H., Sangram S. (2016). Interrelation of glycemic status and neuropsychiatric disturbances in farmers with organophosphorus pesticide toxicity. Open Biochem J 10:27-34.
14. Farzaneh V., Mahmoud H., Zahra H., Mohammad A.E., Hamid R.S., Masoumeh S., et al. (2015). The effects of *Nigella sativa* hydro alcoholic extract on memory and brain tissues oxidative damage after repeated seizures in rats. Iran J Pharm Res 14 (2):547-57.
15. Friedewald W.T. (1972). Estimation of concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 18:499-502.
16. Galal M.K., Khalaf A.A.A., Ogaly H.A., Ibrahim M.A. (2014). Vitamin E attenuates neurotoxicity induced by deltamethrin in rats. BMC Complement Altern Med 14:458-65.
17. Greim H., Saltmiras D., Mostert D., Strupp C. (2015). Evaluation of carcinogenic potential of the herbicide glyphosate, drawing on tumor incidence data from fourteen chronic/carcinogenicity rodent studies. Crit Rev Toxicol 45:185-208.
18. Halil B., Nigar Y., Esin S.C., Yasar T., Hatice T., Hamdi S., et al. (2015). The Effects of thymoquinone on nitric oxide and superoxide dismutase levels in a rat model of diazinon-induced brain damage. Ethno Med 9 (2):191-5.
19. Hariri A., Moallem S., Mahmoudi M., Memar B., Hosseinzadeh H. (2010). Sub-acute effects of diazinon on biochemical indices and specific biomarkers in rats: Protective effects of crocin and safranal. Food Chem Toxicol 48:2803-8.
20. Hashem H.E. (2012). Light and electron microscopic study of the possible protective effect of *Nigella sativa* on metalaxyl induced hepatotoxicity in adult albino rats. J Cell Sci Ther 3:118. [Doi: 10.4172/2157-7013.1000118].
21. Hobbenaghi R., Javanbakht J., Sadeghzadeh Sh., Kheradmand D., Abdi F.S., Jaber M.H. et al. (2014). Neuroprotective effects of *Nigella sativa* extract on cell death in hippocampal neurons following experimental global cerebral ischemia reperfusion injury in rats. J Neuro Sci 337:74-9. [doi: http://dx.doi.org/10.1016/j.jns. 2013.11.019].
22. Hou B., Wu L. (2010). Safety impact and farmer awareness of pesticide residues. Food Agric Immunol 21:191-200.
23. Hou Y., Zeng Y., Li S., Qi L., Xu W., Wang H., et al. (2014). Effect of quercetin against dichlorvos induced nephrotoxicity in rats. Exp Toxicol Pathol 66:211-8.
24. Imam A., Ajao M.S., Ajibola M.I., Amin A., Abdulmajeed A.I., Lawal A.Z., et al. (2016). Black seed oil reversed scopolamine-induced Alzheimer and cortico-hippocampal neural alterations in male Wistar rats. Bull Fac Pharm Cairo University. 54:49-57. [Doi: 10.1016/j.bfopcu. 2015.12.005].
25. Kanter M., Coskun O., Kalayci M., Cagavi F. (2008). Neuroprotective effects of *Nigella sativa* on experimental spinal cord injury in rats. Hum Exp Toxicol 25 (3):127-33.
26. Karki P., Ansari J.A., Bhandary S., Koirala S. (2004). Cardiac and electrocardiographical manifestations of acute organophosphate poisoning. Singa Med J 45:385-9.
27. Mariem C., Meriem T., Safa H., Ons B., Kamel J., Tahia B., et al. (2016). Improvement of heart redox states contributes to the beneficial effects of selenium against penconazole-induced cardiotoxicity in adult rats. Biol Trace Elem Res 169:26170. [DOI: 10.1007/s1201101504260].
28. Mohamadin A.M., Sheikh B., Abdel-Aal A.A., Elberry A.A., Al-Abbasie F.A. (2010). Protective effects of *Nigella sativa* oil on propoxur-induced toxicity and oxidative stress in rat brain regions. Pest Biochem Phys 98:128-34.
29. Mostafalou S., Abdollahi M. (2013). Pesticides and human chronic diseases: Evidences, mechanisms, and perspectives. Toxicol Appl Pharmacol 268 (2):157-77.
30. Musa U., Hati S.S., Mustapha A., Magaji G. (2010). Dichlorvos concentrations in locally formulated pesticide (Ota-piapia) utilized in Northeastern Nigeria. Sci Res Essay 5:49-54.
31. Nahed S.K., Bassant A.E. (2011). Prophylactic effect of green tea and *Nigella sativa* extracts against fenitrothion-induced toxicity in rat parotid gland. Arch Oral Biol 56 (11):1339-46.
32. Noor N.A., Fahmy H.M., Mohammed F.F., Elsayed A.A., Radwan N.M. (2015). *Nigella sativa* ameliorates inflammation and demyelination in the experimental autoimmune encephalomyelitis-induced Wistar rats. Int J Clin Exp Pathol 8 (6):6269-86.
33. Ogutcu A., Suludere Z., Kalender Y. (2008). Dichlorvos-induced hepatotoxicity in rats and the protective effects of Vitamins C and E. Environ Toxicol Pharmacol 26:355-61.
34. Ogutcu A., Uzunhisarcikli M., Kalender S., Durak D., Bayrakdar F., Kalender Y. (2006). The effects of organophosphate insecticide Diazinon on malondialdehyde levels and myocardial cells in rat heart tissue and protective role of Vitamin E. Pesticide Biochem Physiol 86:93-8.
35. Papaefthimiou C., Theophilidis G. (2001). The cardiotoxic action of the pyrethroid insecticide deltamethrin, the azole fungicide prochloraz, and their synergy on the semi-isolated heart of the bee *Apis mellifera* macedonica. Pestic Biochem Phys 69:77-91.
36. Rashmika S., Manju B.G., Bhat L.R., Noel N., Swaminathan S., Uma M.K., et al. (2016). Simultaneous detection of monocrotophos and dichlorvos in orange samples using acetylcholinesterase-zinc oxide modified platinum electrode with linear regression calibration. Sensors Actuators B Chem 230:306-13.
37. Rosman Y., Eisenkraft A., Milk N., Shiyovich A., Ophir N., Shrot S., et al. (2014). Lessons learned from the Syrian sarin attack: Evaluation of a clinical syndrome through social media. Ann Inter Medic 160:644-8.
38. Roth A., Zellinger I., Arad M., Atsmon J. (1993). Organophosphates and the heart. Chest 103:576-82. [doi: 10.1378/chest. 103.2.576].
39. Shabana A., El-Menyar A., Asim M., Al-Azzeh H., Al Thani H. (2013). Cardiovascular benefits of black cumin (*Nigella sativa*). Cardiovascular Toxicol 13:9-21.

40. Sharma P., Singh R. (2012). Dichlorvos and Lindane induced oxidative stress in rat brain: Protective effects of ginger. *Pharm Res* 4 (1):27-32. [doi: 10.4103/0974-8490.91031].
41. Shrot S., Tauber M., Shiyovich A., Milk N., Rosman Y., Eisenkraft A., et al. (2015). Early brain magnetic resonance imaging can predict short and long-term outcomes after organophosphate poisoning in a rat model. *Neurotoxicology* 48:206-16.
42. Vijayakumar S., Fareedullah M., Ashok K.E., Mohan R.K. (2011). A prospective study on electrocardiographic findings of patients with organophosphorus poisoning. *Cardiovascular Toxicol* 11:113-7.
43. Wahab A., Hod R., Ismail N.H., Omar N. (2016). The effect of pesticide exposure on cardiovascular system: A systematic review. *Int J Commun Med Public Health* 3 (1):1-10.
44. Weidong T., Feng R.M.M., Qi C., Suping C., Xuebo S., Jianbo G.M.M., et al. (2016). Independent prognostic factors for acute organophosphorus pesticide poisoning. *Resp Care* 61:967-70. [DOI: 10.4187/respcare. 04514].
45. Won K.J., Lin H.Y., Jung S., Cho S.M., Shin H.C., Bae Y.M., et al. (2012). Antifungal miconazole induces cardiotoxicity via inhibition of APE/Ref-1-related pathway in rat neonatal cardiomyocytes. *J Toxicol Sci* 126:298-305.
46. Yaman I., Balıkcı E. (2010). Protective effects of *Nigella sativa* against gentamicin-induced nephrotoxicity in rats. *Exp Toxicol Pathol* 62:183-90. [doi: 10.1016/j.etp. 2009.03.006].
47. Yavuz T., Delibas N., Yildirim B., Altuntas I., Candir O., Cora A., et al. (2005). Vascular wall damage in rats induced by organophosphorus insecticide methidathion. *Toxicol Lett* 155:59-64.
48. Zahra G., Shahrzad H., Mohammad H.B. (2016). Preclinical and clinical effects of *Nigella sativa* and its constituent, thymoquinone: A review. *J Ethnopharmacol* 190:372-86. [doi: 10.1016/j.jep. 2016.06.061].
49. Zamzila A.N., Aminu I., Niza S., Razman M.R., Hadi M.A. (2011). Chronic Organophosphate Pesticide Exposure and Coronary Artery Disease: Finding a Bridge. IIUM Research, Invention and Innovation Exhibition (IRIIE).