ORIGINAL | INDEPENDENT JOURNAL OF ALLIED HEALTH SCIENCES

IJAHS-0206

CHLORPYRIFOS – INDUCED DISTURBANCES IN HEMATOLOGICAL PARAMETERS: A MODEL STUDY FOCUSING PROTECTIVE EFFECT OF NIGELLA SATIVA SEED EXTRACT

Raheela Adil, Department of Physiology, Al-Tibri Medical College, Isra University Karachi
Sadia Sundus, Department of Anatomy, College of Medicine and Dentistry, Hamdard University Karachi
Qadir Bux Memon, Department of Anatomy, Al-Tibri Medical College, Isra University Karachi
Mohammad Ahmed Azmi, Department of Physiology, Al-Tibri Medical College, Isra University Karachi

Date of Received: 11/07/2018 **Date of Acceptance:** 22/01/2019

ABSTRACT

Objective: To assess the protective role of *Nigella sativa* in chlorpyrifos- induced changes in some of the hematological parameters of Wistar albino rats. **Study Design:** An experimental Study. **Place and Duration of Study:** Conducted at the department of Physiology Al-Tibri Medical College, Isra University Karachi Campus from January 2015 to December 2015. **Methodology:** Total Sixty fours rats were taken for this study. The animals were grouped and sub-grouped on the basis of 3 weeks and 6 weeks of treatment protocol. Control group A1 and A2 were given distilled water only. Group B1 and B2 were given chlorpyrifos 4.2mg/kg. bw; 1/20 LD_{50} orally. Animals of Group C1, C2, and D1, D2 were the combination groups and were given 250mg/kg. bw low dose and 500mg/kg. bw high dose of *Nigella sativa* orally to these chlorpyrifos – induced rats respectively. After treatment blood samples 5cc were collected from each animal through cardiac puncture for hematological studies. **Results:** The results revealed that the hematological parameters like RBC count, Hb, Hct, MCV, MCH and MCHC all were significantly reduced in chlorpyrifos- induced group of animals with p-value <0.001 but when compared the hematological data with low dose 250mg/kg. bw and high dose 500mg/kg. bw of *Nigella sativa* all the hematological parameters were significantly increased in *Nigella sativa* treated groups. More over when compared the data between chlorpyrifos induced group and low or high dose of *Nigella sativa* treated group all the parameters were significantly increased. However when the results were compared between low and high dose of *Nigella sativa*, all the parameters were increased non-significantly in high dose of *Nigella sativa* treated groups. **Conclusion:** It is therefore concluded that *Nigella sativa* has the beneficial role of possessing antioxidant property and thus provide beneficial influences in ameliorating the disturbances in hematological parameters caused by chlorpyrifos – induced toxicity.

Keywords: Chlorpyrifos, *Nigella sativa*, Oxidative damage, Hematological parameters, Albino rats.

Correspondence Address
Mohammad Ahmed Azmi
Professor of Physiology
Al- Tibri Medical College & Hospital old Thana, Gadap town Malir, P.O. Box. 7540 Karachi.
azmiahmed@hotmail.com

Article Citation: Adil R, Sundus S, Memon B Q, Azmi A M. Chlorpyrifos – induced disturbances in Hematological parameters: A model study focusing protective effect of nigella sativa seed extract. **IJAHS,** Jul-Sep 2019;03(129-137):01-09.

INTRODUCTION

As pesticides are basically poisonous causing various health problems in the population even then they are widely used chemicals in the world today without knowing their poisonous nature.[1] Though some pesticides can be beneficial in eliminating the pests but on the other hand can cause serious disturbances in the environment.[2] It has also been reported in studies that in some countries pesticides are commonly used for the control of health related issues.[3] Organophosphorus pesticides which represent one of the major classes of pesticides growing importance in pest control because of their rapid action and less likely accretion in the environment.[4,5] Frequent exposure of this class of pesticide causes a major public health issue in the general population in connection to death, health care and safe measures from toxicity and poisoning.[6]

Among all the organophosphorus pesticides, chlorpyrifos is an important class of this group of pesticide which in chemically and structurally represented as [0,0 – diethyl 0- 3, 5, 6 – trichloro – 2- pyridinol) phosphorothionate] is a broad spectrum chlorinated organophoshorus group of pesticides commonly used in domestic agriculture sectors particularly for pest control throughout the world.[7,8] As far as the mechanism of action of organophosphorus pesticide is concerned it binds with cholinesterase enzyme and inhibits the activity of the enzyme through irreversible phosphorylation thus causing in the elevation of the level of acetylcholine and therefore stimulate the muscarinic and nicotinic receptors.[9] Like other organophosphorus, chlorpyrifos is an important and well known acetylcholinesterase (AChE) inhibitor that causes the accumulation of acetylcholine resulting in the marked stimulation of postsynaptic receptors and related sign and symptoms of toxicity and poisoning.[10,11,8] Recent studies reported that other mechanisms are also involved in the inhibition of AChE due to the toxicity of chlorpyrifos such as oxidative stress in this connection.[12-14] In considering the adverse effects and toxicity caused by chlorpyrifos exposure, phytochemically –based medicine and extracts are gaining importance to overcome the health problems and various diseases.[15-20] Among the most prominent medicinal – based plant having therapeutic effect is the *Nigella sativa* which is commonly called as black cumin and Kalunji belongs to the family *Ranunculaceae.* It is an amazing herb possessing characteristic medicinal properties frequently used in traditional medicine.[21]

As far as the active ingredients of the seed extract of *Nigella sativa* is concerned it mainly depends on quinone constituents consisting of thymoquinone, dithymoquinone, thymohydroquinone and thymol.[22] Among all, thymoquinone (TQ) is the most abundant and active component of the seed oil.[23] Recent studies reported that thymoquinone have shown improvement in the oxidative status through modulation of oxidative enzymes and immunity.[24] In a study it has been reported that *Nigella sativa* and its active constituent thymoquinone has a therapeutic role in the different components of metabolic syndrome including risk factors related to cardiovascular problems like hypertension, dyslipidemia and hyperglycemia.[25] In another study it has also been cited that most of the active ingredients of *Nigella sativa* particularly thymoquinone present in the seeds extract has beneficial effect for treating many illness like asthma, hypertension, diabetes, bronchitis, cough, fever, dizziness and gastrointestinal disturbances.[26] Protective effect of *Nigella Sativa* particularly , thymoquinone as main constituents were also studied against diazinon and cardiotoxicity and reproductive toxicity acetamipril induced in male albino rats.[27, 28] Therefore, present study aimed to assess the phytotherapeutic role of *Nigella sativa* seed extract on some of the hematological parameters of rats treated with toxic dose of chlorpyrifos.

MATERIALS AND METHODS 5 *Animals*

The present study was carried out on locally breed albino rats of Wistar strain having weight 150180g were purchased from the animal house of Al-Tibri Medical College, Isra University Karachi Campus. Rats were kept in specially designed plastic cages in a quiet room with free access of water and cubes of standard rat diet one week before starting the experimental protocol so that the animals could adopt the laboratory environment.

Chemicals

All the chemical such as chlorpyrifos 40EC, ethanol, *Nigella sativa* seeds, distilled and de-ionized water were purchased from local marked for experimental work.

Extraction procedure

Nigella sativa seed extract was prepared according to the method with slight

modifications.[29] Different steps involved in the extraction procedure include dryness procedure of *Nigella sativa* seeds, checking level of dryness of seeds, sifting of *Nigella sativa* seed powder, soxhlation process and storage of extract for further use.

The seeds of *Nigella sativa* were purchased from local market and were cleaned with distilled water and air dried. The seeds were then grinded for fine powder using electrical grinder and stored at 5°c until further use. The fine powder was then placed in 95% ethanol solution for extraction process. The extraction process will be done by using soxhlet apparatus. The mixture obtained was kept in a glass jar without cover lid at room temperature overnight for the evaporation of ethanol. The resultant material after evaporation will be dissolved in distilled water and the extracted material obtained will be finally used for experimental work.

***Preparation* of *Working Solution* 6*Chlorpyrifos*:** 4.2mg/kg bw; $1/20^{th}$ LD_{50} was prepared accordingly.[28]

***Nigella sativa seed extract*:** 250mg/kg. bw low dose and 500mg/kg. bw high dose were also prepared accordingly.[29]

Experimental protocol

Sixty four (64) rats were assigned for control and test groups as A, B, C, D and each will be divided into two sub-groups on the basis of 3 weeks and 6 weeks of treatment.

Group A: Control group

A1: treated with distilled water for 3 weeks

A2: treated with distilled water for 6 weeks

Group B: Chlorpyrifos – treated group

B1: treated with chlorpyrifos 4.2mg/kg. bw for 3 weeks

B2: treated with chlorpyrifos 4.2mg/kg. bw for 6 weeks

Group C: Low dose *Nigella sativa* treated group (combination group)

C1: chlorpyrifos + low dose 250mg/kg. bw of *Nigella sativa* for 3 weeks

C2: chlorpyrifos + high dose 250mg/kg. bw of *Nigella sativa* for 3 weeks

Group D: High dose *Nigella sativa* treated group (combination group)

D1: chlorpyrifos + high dose 500mg/kg. bw of *Nigella sativa* for 3 weeks

D2: chlorpyrifos + high dose 500mg/kg. bw of *Nigella sativa* for 6 weeks

The calculated dose of chlorpyrifos was given orally through feeding tube to all group of animals for 3 weeks and 6 weeks. In the combination group *Nigella sativa* seed extract was given two hours later to chlorpyrifos – treated rats daily for 3 weeks and 6 weeks respectively.

Sacrification and Collection of blood samples

7Each animal was anesthetized after the end of 3 weeks and 6 weeks of treatment (32 animals were sacrificed separately). After sacrification blood sample 5cc through cardiac puncture was collected in heparinized tube for the investigation of different blood parameters like red cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentrations using the specified method.[32]

STATISTICAL ANALYSIS

The results are presented in the form of mean standard deviation. The comparison between the mean of control groups and treated groups was done using ANOVA SPSS version 21 to find out the significant differences among the groups. In case of significant result Turkey–multiple comparison post HOC test was applied to check the pair- wise comparison at 5% level of significance (95% confidence interval. CI).

RESULTS

8In the present study the effects of low and high dose of *Nigella sativa* seed extract were observed when administered orally for three and six weeks of assessment. Data regarding hematological parameters are presented in the form of mean SD. The comparison between the control and treated group of animal i.e., chlorpyrifos – treated

and low or high dose of *Nigella sativa* seed extract – treated groups.

1. RBC Count (10^6/mm^3)
Three Weeks Assessment
Mean SD of group
A1: 7.8 1.17114
B1: 4.9265 0.62
C1: 7.2375 1.52
D1: 7.375 1.82
Six Weeks Assessment
Mean SD of group
A2: 7.8 1.204
B2: 4.76 0.96.
C2: 7.3125 1.78
D2: 7.425 2.56

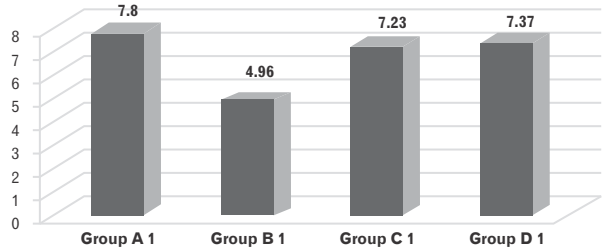

Figure 1. Bar chart showing mean number of RBC (10^6/mm^3)

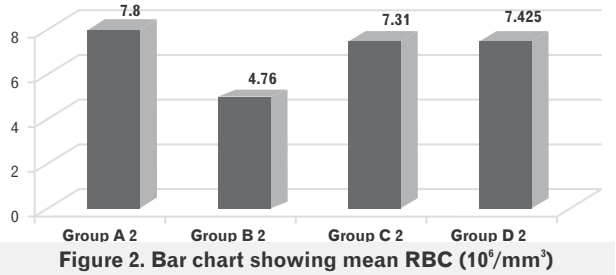

Figure 2. Bar chart showing mean RBC (10^6/mm^3)

2. Hemoglobin (gm/dl)
Three Weeks Assessment
Mean SD of group
A1: 17.1735 1.4808
B1: 13.6625 0.83
C1: 16.0125 1.68
D1: 16.7875 1.98
Six Weeks Assessment
Mean SD of groups
A2: 17.1375 1.4971
B2: 13.44 0.76
C2: 16.2625 1.90
D2: 16.8125 2.39

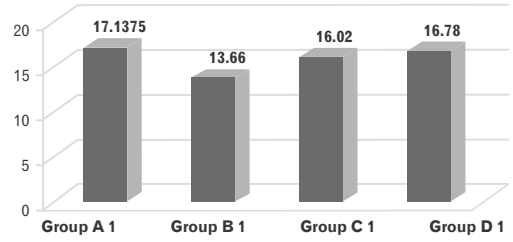

Figure 3. Bar chart showing mean Hb (gm/dl)

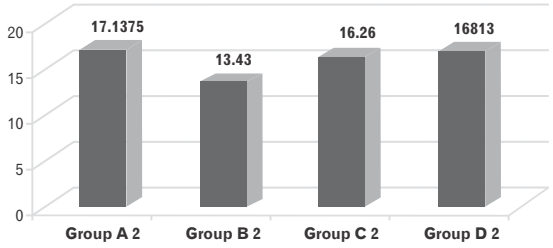

Figure 4. Bar chart showing mean Hb (gm/dl)

3. Hematocrit (%)
Three Weeks Assessment
Mean SD of group
A1: 41.5 5.391
B1: 28.5 3.94
C1: 38.75 6.33
D1: 39.125 7.09

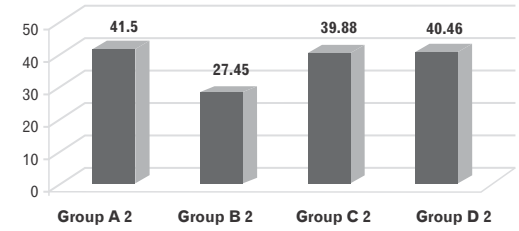

Figure 5. Bar chart showing mean Hct (%)

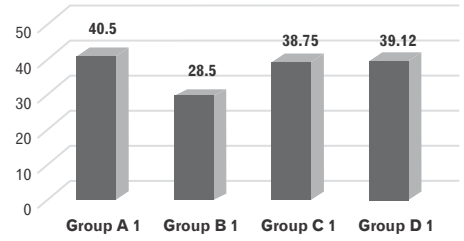

Figure 6. Bar chart showing mean Hct (%)

Six Weeks Assessment
Mean SD of groups
A2: 41.5 5.849
C2:39.8875 4.94
D2: 40.4625 6.04

4. Mean Corpuscular Volume (f1)
Three Weeks Assessment
Mean SD of group
A1: 60.9 12.522
B1: 31.487 10.06
C1: 58.775 11.59
D1: 59.6 13.65
Six Weeks Assessment
Mean SD of groups
A2: 60.9 12.472
B2: 31.176 10.98
C2: 59.575 11.45
D2: 60.1625 13.65

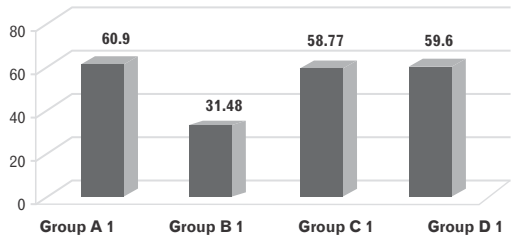

Figure 7. Bar chart showing mean MCV (f1)

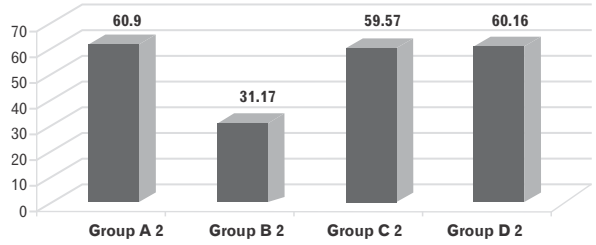

Figure 8. Bar chart showing mean MCV (f1)

5. Mean Corpuscular Hemoglobin (pg)
Three Weeks Assessment
Mean SD of group
A1: 17.975 2.1138
B1: 12.7625 1.31
C1: 16.95 2.98
D1: 17.2 3.41

Six Weeks Assessment
Mean SD of groups
A2: 17.975 2.149
B2: 12.7625 0.59
C2: 17.025 1.95
D2: 17.575 3.09

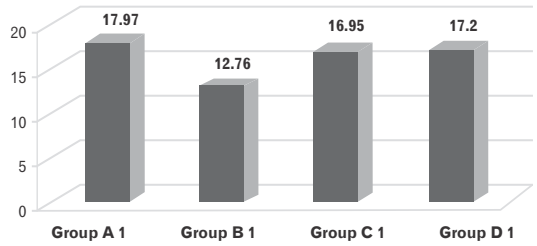

Figure 9. Bar chart showing mean MCH (pg)

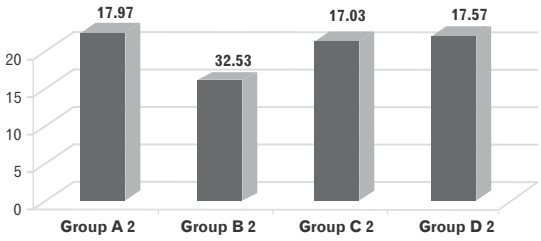

Figure 10. Bar chart showing mean MCH (pg)

6. Mean Corpuscular Hemoglobin Concentration (gm/dl)
Three Weeks
Mean SD of group
A1: 28.7625 2.227
B1: 24.4 1.52
C1: 27.7 2.98
D1: 28.6 3.92

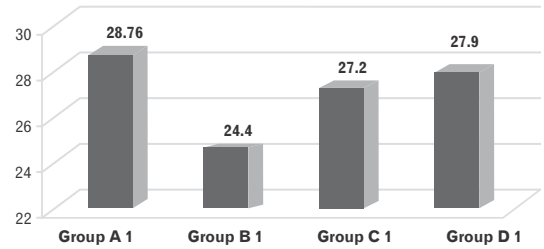

Figure 11. Bar chart showing mean MCHC (gm/dl)

Assessment Six Weeks Assessment
Mean SD of group

A2: 28.7625 2.1718
B2: 24.4 0.90
C2: 27.98 1.87
D2: 28.23 3.09

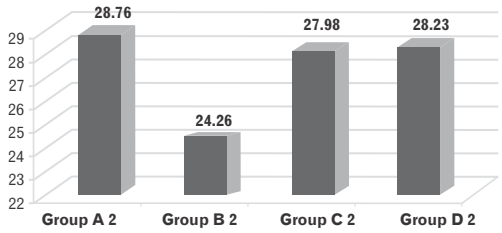

Figure 12. Bar chart showing mean MCHC(gm/dl)

DISCUSSION

12The present study is concerned with the toxic effects of chlorpyrifos which is the most extensively used organophosphorus pesticide in the agricultural sector with its prominent adverse health effects. The present study also assessed that the exposure of chlorpyrifos showed marked disturbances in some of the hematological parameters of albino rats. It has also been reported in many studies that hematological assessment tools are used for the diagnosis of different types of illness and health issues that are caused by the frequent use of some of the industrial compounds and chemicals.[31,32] Chlorpyrifos which is one of the important class of organophosphorus pesticide when given orally in a dose of 4.2mg/kg.bw; 1/20th of LD_{50} to the albino rats, adverse effects have been noticed particularly in the disturbances of hematological parameters.[33-37] The toxic effects of chlorpyrifos was also noticed by some of the researchers who reported that administration of chlorpyrifos disturbs the various body's functional system including the hemopoietic system.[38-40] These findings are in agreement with the results of our study.

However recent data of the present study revealed that exposure of chlorpyrifos lowers the levels of red cell count, hemoglobin and hematocrit in the chlorpyrifos treated groups of animals. This might be happened due to the repeated or frequent administration of chlorpyrifos in the animal causing reduction in the red cell count thus resulting in anemia which may occur either by the impaired production of red cells or increased destruction of red cells. The same findings were also noticed by some researchers in many studies where it has been reported that continuous exposure of chlorpyrifos causes destruction of red cells.[11] Significant reduction of red cell count, hemoglobin and hematocrit was also noted during three and six weeks of treatment in rats due to the exposure of diazinon which is also an important class of organophosphorus pesticide.[41] This is almost in line with the present study where animals were treated with chlorpyrifos instead of diazinon. This inconsistent finding might be due to the nature of chemical compound.[13]

The results of some studies showed that when the animals were treated with diazinon or aluminum, reduction in hematological parameters were noted but when *Nigella sativa* was given to these animals for 3 weeks and six weeks extensive protective effects were seen.[42-43] The result of these studies confirm the findings of the present study that when *Nigella sativa* was given in a dose of 250mg/kg. bw and 500mg/kg. bw orally protective effects were also seen but not markedly. A recent report however explained that thymoquinone is considered to be the most active ingredient of *Nigella sativa* because of having phytotherapeutic role.[44,26,45]

CONCLUSION 14The present study concludes that *Nigella sativa* seed extract exerts antioxidant effect against chlorpyrifos - induced hemopoietic changes but treatment with *Nigella sativa* seed oil minimizes the changes in hematological parameters partly due to having antioxidant property and partly by increasing the immune system due to having thymoquinone which is the most pharmacologically active ingredient of the extract or oil.

REFERENCES

1. Imran H, Dilshaad KA. **Adverse health effects of pesticide exposure in agricultural and industrial workers of developing countries. Impacts of pesticide exposure**. InTech Open, Croatia 2011; pp : 155-178.

2. Azmi MA, Naqvi SNH. **Pesticide pollution, resistance and health hazards. Pesticides: The impacts of pesticide exposure**. InTech 2011; 1-24.

3. Konradren F, Vander – Hoek W, Cole DC, Hutchinson G, Daisley H, Sing S et al. **Reducing acute poisoning in developing countries : Options for restricting the availability of pesticides.** Toxicology 2003; 192 : 249 – 261.

4. Aardema H, Meertens JHJM, Ligtenberg JJM, Peter-Polman OM, Tulleken JE, Zijlstra, JG. **Organophosphorus poisoning: cases and developments**. Netherland. J.Med. 2008; 66 (4): 149-153.

5. Leibson T, Lifshutz M. **Organophosphate and carbamate poisoning : review of the current literature and summary of clinical and laboratory experience in Southern Israel**. Israel. Med. Ass. J. 2008; 10 : 767-770.

6. Jaga K, Dharmani C. **Sources of exposure to and public health implications of organophosphate pesticides**. Am. J. Pub. Health. 2003; 14 (3) : 171-185.

7. Mitra NK, Siong NH, Nadarajah VD. **Evaluation of neurotoxicity of repeated dermal application of chlorpyrifos on hippocampus of adult mice**. Ann. Agric. Env. Med. 2008; 61 : 4-9.

8. Mehta A, Verma RS, Srivastava N. **Chlorpyrifos induced alteration in the level of hydrogen peroxide, nitrate and nitrite in rat brain and liver**. Pesticide. Biochem. Phys. 2009; 94 : 55-59.

9. Abou – Donia MB. **Organophosphorus ester-induced chronic neurotoxicity. Arch**. Envir Health. 2004; 58 (8): 484-497.

10. Zheng Q, Oliver K, Won YK, Pope CN (2000). **Comparative cholinergic neurotoxicity of chlorpyrifos exposure in preweaning and adult rats**. Toxicol. Sci., 55 : 145-150.

11. Al-Badrany YMA, Mohammad FK. **Effect of acute and repeated or exposure on open field activity in chicks**. Tox. 2007; Lettr., 174: 110-116.

12. Gultekin F, Patat S, Akca M, Akdogan M. **Melatonin can suppress the cytotoxic effect of chlorpyrifos on human Hep G2 cell lines. Hum**. Exp. Toxicol. 2006; 35: 47-55.

13. Ambali SF, Akanbi D, Igbokwe N, Shittu M, Kawu M, Ayo J. **Evaluation of sub-chronic chlorpyrifos in mice and the protective effect of vitamin C.** J. Toxicol. Sci. 2007' 32 (2): 111-120.

14. Durak DK, Uzun FG, Uzunhisarcikli M, Kalender S, Kalender y, Ogutcu A. **Malathion – induced oxidative stress in human erythrocyte and the protective effect of vitamin C and E *in vitro*.** Env. Tox. 2008; 24: 234-242.

15. Salem ML, Hossain MS. **Protective effect of black seed oil from Nigella sativa against murine cytomegalo virus infection.** Int. J. Immunopharmacol. 2000; 22 (9) : 729-740.

16. Chattopadhyay D, Arunachalam G, Mandal AB, Sur TK, Mandal SC, Bhattacharya SK. **Antimicrobial and anti-inflammatory activity of folklore: Mallotus peltatus leaf extract**. J. Ethnopharmacol. 2002; 82 (2-3): 229-237.

17. Dattner AM. **From medical herbalism to phytotherapy in dermatology: back to the future.** Dermatol Ther. 2003; 16 (2) 106-113-

18. Huffman MA. **Animal self medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants.** Proc. Nutr. Soc.

2003; 62 (2) : 371-381.

19. Miller KL, Liebowitz RS, Newby LK. **Complementary and alternative medicine in cardiovascular disease : a review of biologically – based approaches**. Am. Heart. J. 2004; 147 (3) : 401-411.

20. Aherne AS, Joseph P, Kerry JK, O'Brien NM. **Effect of plant extracts on antioxidant status and oxidant – induced stress in Caco-2 cells**. Br. J. Nutr. 20087; 97 (2): 321-328.

21. Salem ML. **Immunomodulatory and therapentic properties of *Nigella sativa* L. seed. Int. Immunopharmacol.** 2005; 5 (13-14) : 1749- 1770.

22. Venkatachallam SK, Pattekhan H, Divakar S, Kadimi US. **Chemical composition of *Nigella sativa* L. seed extracts obtained by supercritical carbon dioxide**. J. Food Sci. Technol. 2010; 47 (6): 598-605.

23. Nehar S, Kauser H, Rani P, Alam I. **Effects of *Nigella sative* seed extract on insulin resistant non-insulin dependent diabetic guinae pigs.** Am. J. Ethnomedicine. 2015; 2 (1) : 58-67.

24. Lev E, and Amar Z. **Ethnopharmacological survey of traditional drugs sold in Israel at the end of 20th century**. J. Ethnopharmacol. 200; 72 : 191-205.

25. Razavi BM, Hosseinzadeh. **A review of the effects of *Nigella sativa* L and its constituent, thymoquinone in metabolic syndrome**. J. Endocrinol. Invest. 2014, 33 (11) : 1031-1040.

26. Amin B, Hosseingadeh H. **Black cumin Nigella sativa and its active constituent, thymoquinone: An overview on the analgesic and anti-inflammatory effects**. Planta. Med. 2016, 82 (01/02) : 08-16.

27. Hadjzadeh MAR, Khoei A, Hadjzadeh Z, Parizady M. **Ethanolic extract of Nigella sativa L seeds on ethylene glycol-induced kidney calculi in rats**. Urol. J. 207, 4 (2): 86-90.

28. Ambali SF, Orieji C, Abubakar WO, Shittu M, Kawu MU. **Ameliorative effect of Vitamin C on alterations in thyroid hormones concentrations induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats**. Thyroid Research. 2011; 10: 1-6.

29. Nehar S, Kauser H, Rani P, Alam I. **Effects of sativa seed extract on insulin resistant non-insulin dependent diabetic guinae pigs.** Am. J. Ethnomedicine. 2015; 2 (1) : 58-67.

30. Dacie JV, Lewis SM. **Practical Hematology**, 7th Edn. Churchill Livingstone, London. 1991; 659-661.

31. Alahyary P, Poor MI, Azarbaijani FF, Nejati V. **The potential toxicity of diazinon on physiological factors in male rats**. Pak. J. Biol. Sci. 2008; 11 (1): 127-130

32. Afshar S, Heidari R, Farshid AA, Ilkhanipour M. **Effect of oral administration of fenitrothion on biochemical and hematological parameters in rats**. Pak. J. Biol. Sci. 2008; 11 (13): 1742-1745.

33. Yousef MI, El-Demerdash FM, Kamel KI, Al- Salhen KS. **Changes in some hematological and biochemical indices of rabbits induced by isoflavones and cypermethrin.** Toxicology. 2003; 189 : 223-23.

34. Suleiman FA, Dayo OA, Mufta S, Abdul Ganiyu G, Olushola OO, Joseph OA. **Chlorpyrifos – induced clinical, hematological and biochemical changes in Swiss albino mice – mitigating effect of co-administration vitamin C and E. Life.** Sci. J. 2010; 7 (3) : 37-44.

35. Heikal TM, Mossa ATH, Marei G1, Rasoul MAA. **Cyromazine and chlorpyrifos induced renal toxicity in rats: The ameliorating effects of green tea extract.** J. Environ. Anal. 2012l 2 (5) :2-7.

36. Newairy AA, Abdou HM. **Effect of propolis consumption on hepatotoxicity and brain damage in male rats exposed to chlorpyrifos.** Afr. J. Biotechn.

2013l; 12 (33) 5232-5243.

37. Noaishi MA, Abdallah AA, Afify MM. **Oral and dermal exposure of chlorpyrifos and cypermethrin mixture induced cytogenetic, histopathological damage and oxidative stress in rats.** J. Am. Sci.2013; 9 (3) : 56-65.

38. Steenland K, Robert BD, Ronald JH, Chrislip DW, Thomas MR. **Neurological functions among termiticid applicators exposed to chlorpyrifos.** Environ. Health Perspect.2000; 108 :293-300.

39. Ambali SF, Abubakar At, Shittu M, Yaqub LS, Anafi SB, Abdullahi A. **Chlorpyrifos– induced alteration of hematological parameters in Wistar rats**: Ameliorative effect of zinc. Res. J. Environ. Toxicol. 2010-al 4 (2) 55-66.

40. Olorunshola KV, Achie LN, Akpomiemei ML. **Ascorbic acid ameliorates toxic effects of chlorpyrifos on testicular functions of albino rats**. Brit. J. Pharmacol. Toxicol.2011; 2 (2): 262-269.

41. Al-Attar AM, Al-Taisan WA. **Preventive effect of Black seed *(Nigella sativa)* extract on Sprague Dawley rats exposed to Diazinon**. Aust. J. Basic. Appl. Sci.2010; 4 (5): 957-968.

42. Mohammad MA, Awad SM. **Effect of *Nigella sativa* oil on some hematological values in aluminum – treated rats**. Aust. J. Basic. Appl. Sci. 2008; 2 (4) : 1157 - 1164.

43. Asgary S, Najafi S, Ghannadi A, Dashti G and Helalat A. **Efficiency of black cumin seeds on hematological factors in normal and hypercholestroemic rabbits.** ARYA Atheroscler. 2012; 7 (4): 146-150.

44. Kaatabi H, Bamosa AO, Badar A, Al-Elq A, Abou – Hafiza B, Lebda F, Al-Khadra A Al-Almaie S. **Nigella sativa improves glycemic control and ameliorates oxidative stress in patients with type-2 diabetes mellitus : Placebo controlled participant blinded clinical trial.** Plos One. 2015; Doi : 10. 1371 : 1-15.

45. Ibrahim TAA. **Beneficial effects of diet supplementation with *Nigella sativa* (Black seed) and Sesame seeds in alloxan – diabetic rats.** Int. J. Curr. Microbiol. Appl. Sci.2016; 5 (1): 411-423.

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author's Full Name	Contribution to the paper	Author's Signature
1	Mohammad Ahmed Azmi	Study conception, Drafting and grammatical mistake chek-up of manuscript	
2	Raheela Adil	Critical revision and data analysis	
3	Sadia Sundus	Plagiarism check-up, analysis and interpretation of data	
4	Qadir Bux Memon	Acquisition of data and cross-checking of references