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Chlorpyrifos Induced Alterations in the Status of White Cells Profile: Protective Role of *Nigella sativa* Seed Extract

Azmi MA[1]*, Adil R[1] and Qamar N[2]

[1]Department of Physiology, Isra University Karachi Campus, Pakistan

[2]Department of Pathology, Isra University Karachi Campus, Pakistan

Research Article

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***Corresponding Author:** Mohammad Ahmed Azmi, Professor of Physiology Director, Postgraduate Studies, Al-Tibri Medical College & Hospital, Old Thana, Gadap Town, Malir, P.O. Box. 7540, Karachi, Pakistan, E-mail: azmiahmed@hotmail.com

Abstract

Objective: The aim of this study is to evaluate the protective role of *Nigella sativa* seed extract in chlorpyrifos-induced changes in the status of white cells profiles of Wistar albino rats.

Methodology: An experimental study. Total sixty four healthy adult rats of either gender were taken for this experimental study. The animals were divided into A, B, C, D groups and each was further divided into two sub-groups on the basis of 3 weeks and 6 weeks of treatment protocol. Control group A1 and A2 were given only distilled water. Group B1 and B2 were given chlorpyrifos 4.2 mg/kg.bw; $^1/_{20}$ of LD_{50} orally. Animals of group C1, C2 and D1, D2 were the combination groups and were treated with low dose 250mg/kg.bw and high dose 500mg/kg.bw of *Nigella sativa* seed extract to these chlorpyrifos – induced group of rats respectively. After treatment 5CC blood sample were taken out from each group of animals through cardiac puncture for the evaluation of white cell profile. The parameters under evaluation were total leucocytes count and differential leucocytes counts such as neutrophils, eosinophils, basophils, lymphocytes and monocytes respectively. Data was analyzed using SPSS version 21.

Results: The findings revealed that significant reduction in white cells profile were noted in chlorpyrifos-induced animals with p-value <0.001. Significant improvement was also seen in the animals of group C and D when treated with low and high dose of *Nigella sativa* orally.

Conclusion: Based on the results of this study it is concluded that *Nigella sativa* seed oil has a potent phytotherapeutic role due to having antioxidant characteristics thus providing beneficial preventive role in ameliorating the alterations in the white cell profile status due to chlorpyrifos-induced toxicity.

Keywords: Chlorpyrifos; *Nigella sativa* seed extract; White cell profiles; Oxidative damage; Albino rats

Introduction

All pesticides are potentially toxic and hazardous to human beings. Pesticides on one side are beneficial in agriculture for crop protection if used according to the standard protocol but if used indiscriminately may cause considerable health problems to the general population and can affect the environment and to the surrounding as well [1-3]. One of the most important group of organ phosphorus which is widely used in agriculture sector is

the chlorpyrifos which is considered to be potential for pest control because of having quick action and produced minimum hazardous effects in the environment [4,5]. Chlorpyrifos is a broad spectrum chlorinated group of pesticide structurally and chemically resembling as [0,0 diethyl 0-3,5,6-trichloro -2 pyridinol) phosphorothionate] commonly used in residential and agricultural sectors [6-8]. Several studies have shown that chlorpyrifos which is a well known acetyl cholinesterase inhibitor causes the accumulation of acetylcholine thus resulting in the stimulation of postsynaptic receptors with related sign and symptoms of toxicity and poisoning [9,10]. It has also been reported in many studies that some other mechanism beside this also involved in the inhibition of acetyl cholinesterase (AChE) because of the toxicity caused by the oxidative stress [11-13].

Considering the toxic nature of chlorpyrifos, some phytochemically related products and extracts have been used therapeutically to minimize the adverse effects of pesticides [14-16]. Among the most prominent medicinal plant used therapeutically to treat various illness is the *Nigella sativa* commonly called as black seed or black cumin. It is an amazing herb belonging to the family *Renunculaceae* used as a traditional phytochemical drug in folk medicine to treat many diseases and ailments [17-19]. It has also been reported in some studies that the seed oil of *Nigella sativa* contains some active ingredients named as thymoquinone, dihydrothymoquinone, thymo-hydroquinone and thymol. Among them thymoquinone is the most active ingredient causing oxidative stress through the involvement of oxidative enzymes and immunity [20]. Another study reported that thymoquinone which is the most active component also has a phytotherapeutic involvement in health related problems such as hypertension, hyperglycemia and dyslipidemia. Similarly recent studies also reported that this active ingredient thymoquinone also has a prominent role in the treatment of various ailments such as asthma, diabetes, bronchitis, nephritis and hematological related disturbances [21,22]. Keeping the adverse effects and toxicity caused by chlorpyrifos exposure, present study in this regard was carried out to investigative the preventive role of *Nigella sativa* seed extract in minimizing the disturbances in the status of white cells profile caused by chlorpyrifos toxicity.

Methodology

Study Design

An experimental study that was conducted to determine the effect of chlorphyrifos on the status of white cell profile as well as in ameliorating the effect of *Nigella sativa* seed extract.

Study Setting

This experimental study was performed at Al-Tibri Medical College, Isra University Karachi Campus, Pakistan.

Duration of Study

It was two years study period extending from January 2013 to January 2015.

Experimental

Chemicals

Different materials such as chlorpyrifos 40EC, *Nigella sativa* seed extract distilled water and deionized water were used. Herbal product *Nigella sativa* seed were collected from local market of Karachi city Sindh Pakistan. The seeds were identified by a Botanist, Faculty of Science University of Karachi, Sindh – Pakistan.

Preparation of working solution

Chlorpyrifos: 4.2mg/kg bw; $1/20^{th}$ LD_{50} was prepared accordingly [23].

Nigella sativa seed extract

250mg/kg. bw low dose and 500mg/kg. bw high dose were prepared accordingly [24].

Experimental Animals

The study was carried out on albino rats of either gender having weight 150~180 grams. They were obtained from the animal house of Al-Tibri Medical College Isra University Karachi Campus. The animals were kept in a quiet room under standard laboratory condition of 12hrs dark-light cycle at 23^{o}C to 24^{o}C. They were fed on standard rat pellets and water was provided ad libitum.

Groping of animals

Group A: **Control group**
A1: Rats given distilled water only for 3 weeks
A2: Rats given distilled water only for 6 weeks
Group B: **Chlorpyrifos – treated group**
B1: Rats given chlorpyrifos at a dose of 4.2mg/kg. bw 3 weeks
B2: Rats given chlorpyrifos at a dose of 4.2mg/kg. bw 6 weeks

Group C: **Low dose *Nigella sativa* treated group (combination group)**
C1: Rats given chlorpyrifos + low dose 250mg/kg. bw of *Nigella sativa* for 3 weeks
C2: Rats given chlorpyrifos + high dose 250mg/kg. bw of *Nigella sativa* for 6 weeks
Group D: **High dose *Nigella sativa* treated group (combination group)**
D1: Rats given chlorpyrifos + high dose 500mg/kg. bw of Nigella sativa for 3 weeks
D2: Rats given chlorpyrifos + high dose 500mg/kg. bw of Nigella sativa for 6 weeks

Chlorpyrifos (4.2mg/kg. bw) was given orally through feeding tube to all groups of rats 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.

Extraction procedure

The procedure for extraction is based on different steps that include dryness, checking level of dryness, sifting and lastly storage of extract for experimental work.

The extract was prepared accordingly with slight modification in the procedure [25]. After collection the seeds were immediately washed with distilled water. All the seeds were kept in an open air for complete dryness prior to grinding. After checking the dryness level the seeds were grinded into fine powder which was then placed in 95% ethanol solution for extraction which was carried out by using soxhlet apparatus. The mixture thus obtained was kept in a glass jar without cover at room temperature overnight for complete evaporation of ethanol. The resultant material obtained after evaporation dissolved in distilled water and the final extracted material was then used for experimental work.

Animal Scarification

Each animal was anesthetized by giving deep ether anesthesia in a glass jar after the end of 3 weeks and 6 weeks of treatment and animal was sacrificed separately.

Collection of Blood Sample

Before the collection of blood samples, thoracic cage of each animal was cut and retracted. Tissues were cleared and heart was exposed. Blood sample 5CC was collected via cardiac puncture from each rat with the help of syringe in heparinized tubes and was sent immediately to the laboratory for the analysis of white cell profile.

Statistical Analysis

The data will be collected and reported as mean ± standard deviation. One way ANOVA SPSS version 21 was used to find out the significant result Turkey-Multiple Comparison post HOC test was applied to check the pair wise comparison at 5% level of significance (95% confidence interval C1).

Results

The results of low and high dose of *Nigella sativa* seed (NSS) extract were studied in the present era. The data obtained after three and six week of assessment of white blood cells profile are presented in the form of mean ± standard deviation. The results were then compared between the control and treated groups of animals i.e., chlorpyrifos-induced group and low or high dose of *Nigella sativa* seed (NSS) extract treated groups.

Effect of NSS on Total Leucocyte count (TLC) ($10^3/mm^3$)

The effects of oral administration of low and high dose of NSS extract on total leucocyte count in chlorpyrifos-induced group of animals during 3 weeks and 6 weeks of assessment are presented in (figure 1a, 1b). Compared with control group significant reduction in TLC was noted in the chlorpyrifos - induced group of animals during both 3 week and six week of assessment ($p<0.00$ at C1 of 95%). In case of NSS extract when low dose 250mg/kg.bw were given, also significant reduction on TLC was seen in 3 weeks and non-significant reduction was noted in 6 weeks of treatment. In addition a non-significant reduction in TLC count was noted in chlorpyrifos with high dose 500mg/kg. bw during both 3 weeks and 6 weeks of treatment (Table 1) (Figures 1a & 1b).

Three Week Assessment	Six Week Assessment
Mean ± SD of group	Mean ± SD of group
A1 9.7 ± 1.5077	A2 9.7 ± 1.5354
B1 5.952 ± 1.5077	B2 5.725 ± 1.5354
C1 8.725 ± 1.5077	C2 8.825 ± 1.5354
D1 8.9125 ± 1.5077	D2 9.1375 ± 1.5354

Table 1: Effect of NSS on TLC ($10^3/mm^3$) during 3 weeks and 6 weeks of treatment.

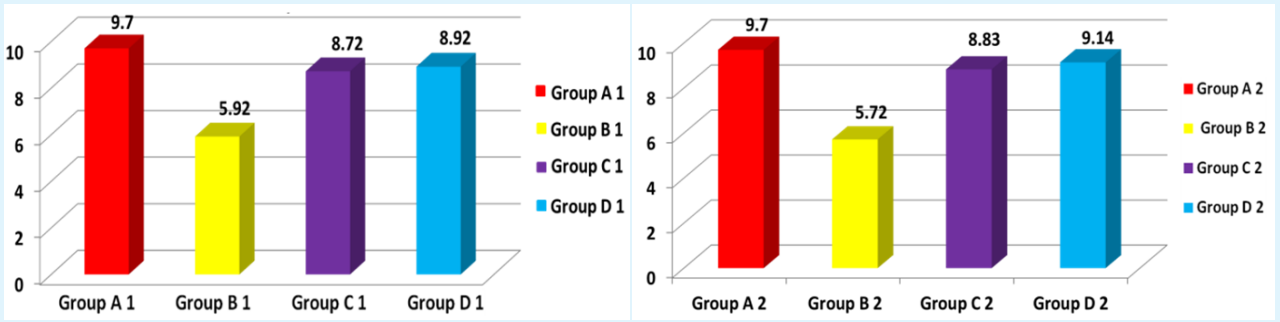

Figure 1a: Bar chart showing mean number of TLC $10^3/mm^3$. Figure 1b: Bar chart showing mean number of TLC $10^3/mm^3$.

Effect of NSS Extract on Neutrophil (%) Count

Effect of NSS extract on neutrophil count was also noted in chlorpyrifos – induced group of animals. When compared with control group significant reduction with ρ-value 0.00 at C1 95% was seen in the group of rats treated with chlorpyrifos during 3 weeks and 6 weeks of treatment (Table 2) (Figure 2a, 2b). When animal were treated with low dose 250mg/kg.bw of NSS extract, significant reduction and non-significant reduction was noted during 3 weeks and 6 weeks of treatment respectively. On the other hand non-significant decrease in neutrophil count was also noticed with high dose 500mg/kg. bw during 3 week and 6 weeks of assessment.

Three Week Assessment	Six Week Assessment
Mean ± SD of group	Mean ± SD of group
A1 19.1125 ± 1.3299	A2 19.1125 ± 1.662
B1 15.887 ± 1.3299	B2 15.1817 ± 1.662
C1 17.612 ± 1.3299	C2 18.5 ± 1.662
D1 17.937 ± 1.3299	D2 18.925 ± 1.662

Table 2: Effect of NSS extract on Neutrophil (%) Count during 3 weeks and 6 weeks of treatment.

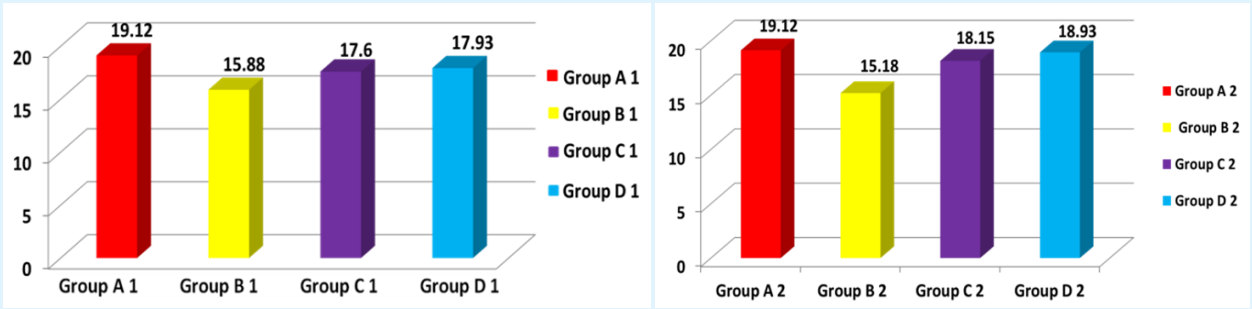

Figure 2a: Bar chart showing mean number of Neutrophils (%). Figure 2b: Bar chart showing mean number of Neutrophils (%).

Effect of NSS extract on Eosinophil (%) Count

As presented in (figure 3a, 3b) eosinophil count was significantly reduced in the chlorpyrifos treated group compared to control group during 3 weeks and 6 weeks treatment with ρ-value 0.00 at C1 95%. Oral administration of NSS at the dose of 250mg/kg.bw significant reduction was seen during 3 weeks and 6 weeks of treatment but with high dose 500mg/kg.bw of NSS extract non-significant reduction was seen in eosinophil count (Table 3) (Figure 3a, 3b).

Three Week Assessment	Six Week Assessment
Mean ± SD of group	Mean ± SD of group
A1 0.375 ± 0.1653	A2 0.375 ± 0.206
B1 0.0075 ± 0.1653	B2 0.00125 ± 0.206
C1 0.2 ± 0.1653	C2 0.3 ± 0.206
D1 0.2125 ± 0.1653	D2 0.3125 ± 0.206

Table 3: Effect of NSS extract on Eosinophil (%) Count during 3 weeks and 6 weeks treatment with ρ-value 0.

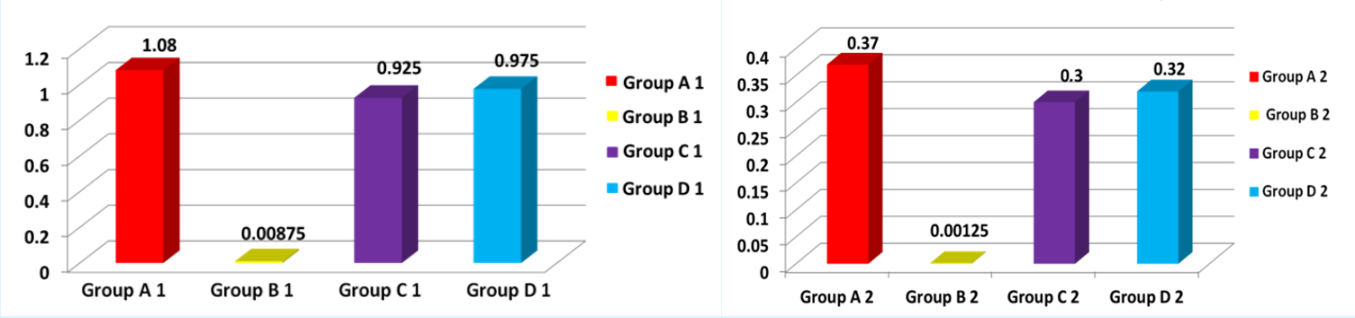

Figure 3a: Bar chart showing mean number of Eosinophil (%). Figure 3b: Bar chart showing mean number of Eosinophil (%).

Effect of NSS extract on Basophil (%) Count

As shown in (figures 41, 4b)significant of basophil count significant was observed in chlorpyrifos treated groups with ρ-value 0.00 at C1 95% as compared to control group. In comparison between control group and chlorpyrifos-treated group with low dose 250mg/kg.bw, non-significant reduction was seen in both 3weeks and 6 weeks of treatment. The same finding was also noticed when treated with high dose 500mg/kg.bw during assessment (Table 4) (Figures 4a, 4b).

Three Week Assessment	Six Week Assessment
Mean ± SD of group	Mean ± SD of group
A1 0.3125 ± 0.1537	A2 0.3125 ± 0.1822
B1 0.00875 ± 0.1537	B2 0.00125 ± 0.1822
C1 0.221 ± 0.1537	C2 0.2825 ± 0.1822
D1 0.2375 ± 0.1537	D2 0.3 ± 0.1822

Table 4: Effect of NSS extract on Basophil (%) Count during 3 weeks and 6 weeks treatment.

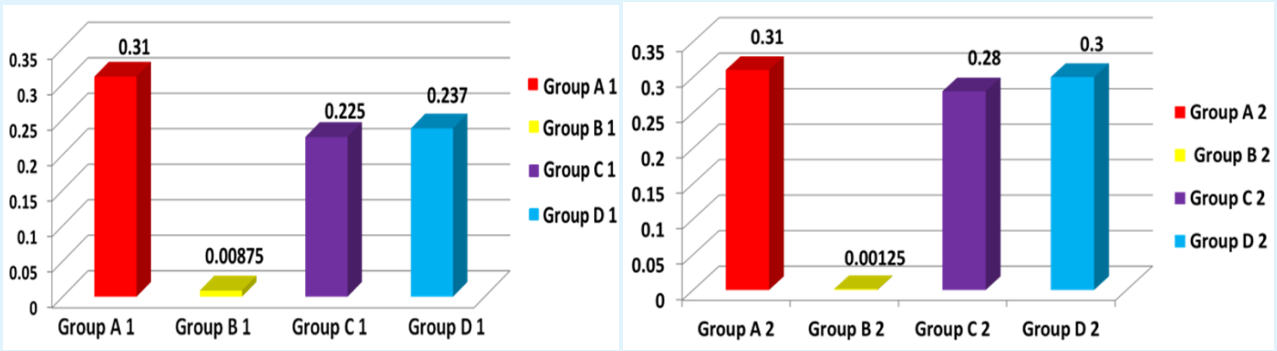

Figure 4a: Bar chart showing mean number of Basophils (%). Figure 4b: Bar chart showing mean number of Basophils (%).

Effect of NSS Extract on Monocyte (%) Count

Effect of NSS extract on monocyte count was also seen in chlorpyrifos-induced group. As compared with control show in (figures 5a, 5b), significant reduction was noted in chlorpyrifos-treated group (ρ-value 0.00 at C1 of 95%) during 3 week and 6 week of treatment. In comparison with control group and treated group at low dose 250mg/kg.bw, non-significant reduction of monocyte count was seen. Similarly with dose 500mg/kg.bw of NSS extract non-significant reduction in monocytic count was also noticed during the treatment protocol (Table 5) (Figure 5a, 5b).

Three Week Assessment	Six Week Assessment
Mean ± SD of group	Mean ± SD of group
A1 1.0875 ± 0.6298	A2 1.0875 ± 0.621
B1 0.00875 ± 0.6298	B2 0.00875 ± 0.621
C1 0.925 ± 0.6298	C2 1.05 ± 0.621

Table 5: Effect of NSS extract on Monocyte (%) Count during 3 week and 6 week of treatment.

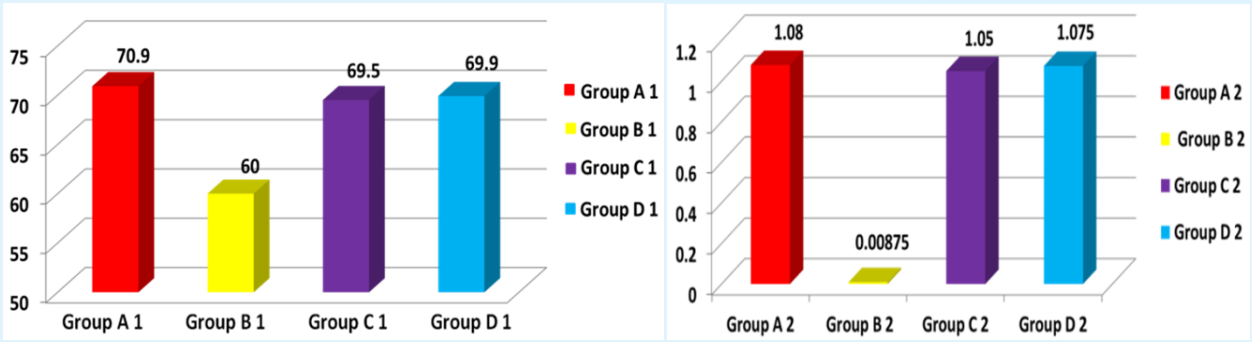

Figure 5a: Bar chart showing mean number of Monocyte (%). Figure 5b: Bar chart showing mean number of Monocyte (%).

Effect of NSS extract on Lymphocyte (%) Count

In comparison with control group as shown in (figures 6a and 6b) significant reduction with ρ–value 0.00 at C1 95% in lymphocytic count was seen during 3 week and 6 week of treatment. When chlorpyrifos induced group was treated with low dose 250mg/kg. bw of NSS and high dose 500mg/kg.bw of NSS, non-significant reduction in lymphocytic count was noted during both 3 weeks and 6 weeks of treatment procedure (Table 6) (Figure 6a, 6b).

Three Week Assessment	Six Week Assessment
Mean ± SD of group	Mean ± SD of group
A1 70.9125 ± 4.722	A2 70.9125 ± 4.722
B1 60.075 ± 4.722	B2 60.075 ± 4.722
C1 69.5 ± 4.722	C2 69.5 ± 4.722
D1 69.925 ± 4.722	D2 69.925 ± 4.722

Table 6: Effect of NSS extract on Lymphocyte (%) Count during 3 week and 6 week of treatment.

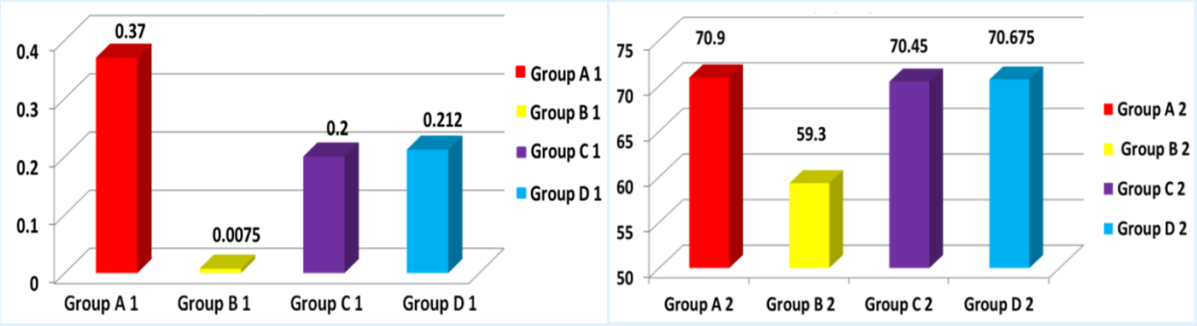

Figure 6a: Bar chart showing mean number of Lymphocyte (%). Figure 6b: Bar chart showing mean number of Lymphocyte (%).

Discussion

Nigella sativa is an amazing herb mainly found in the Indian sub-continents. Its seed extract in particular has been used traditionally as a phytochemical drug in folk medicine for treating many diseases [25]. In our study, the NSS extract has been used to assess its preventive role against the disturbances in white cell profile caused by chlorpyrifos which is an important class of organ phosphorus group of pesticide. The increased levels of some of the white cell parameters were detected in treated group which confirm that chlorpyrofos has successfully disturbed the profile of white cell parameters. After confirmation of the developmental changes in white cell profile, then potential phytotherapeutic effect of NSS extract in chlorpyrifos-induced changes in white cells were evaluated. The present study investigated some of the parameters of leucocytes like total leucocyte count and differential leucocyte count. The overall results showed that NSS extract successfully causes significant and non-significant reduction in almost all the parameters of white cells during 3 weeks and 6 weeks of treatment. The same findings were also noted in a study where significant

reduction in neutrophil count was seen when animals were exposed to chlorpyrifos. In another study the total leucocyte count, lymphocyte and monocyte counts were significantly increased when the animals were treated with another organophosphorus pesticide like diazinon [26]. These inconsistent changes might be an important element that disturbed the first line of defense mechanism which may be due to the rupture of some of the neutrophils during the immune process. Thus, neutrophil count was consistently decreased during treatment with different doses of NSS extract. The result of the present study also agreed with the findings of other studies as reported in the literature [27,28].

Currently it is best to know that the primary mechanism involved in the organophosphate compound is the inhibition of acetyl cholinesterase (AChE) through phosphorylation. This mechanism is common for all the insecticide of this group of compound irrespective to the differences in their chemical structure or due to the effectiveness as inhibitor of acetyl cholinesterase [29,30]. This inhibition increases the level of endogenous acetylcholine in the living subjects thus resulting in its binding to muscarinic and nicotinic receptors in both peripheral and central nervous system. Chlorpyrifos which is used in the present study act in the same way as other organophosphate that leads to the accumulation of acetylcholine and therefore results in the stimulation of postsynaptic receptors that ultimately causes the signs of toxicity [31].

Some previous studies also reported that when animals were treated with diazinon and aluminum causes reduction in some of the hematological parameters but when NSS extract were given orally in diazinon and aluminum-treated rats during 3 weeks and 6 weeks of treatment marked protective effects were seen in hematological parameters. The result of these studies are almost same to the present study when *Nigella sativa* seed extract was given orally in a dose of 250mg/kg.bw and 500mg/kg.bw also giving ameliorating effects but not seen markedly which may be due to the nature of chemical structure or the ability of effectiveness of the pesticide used in this study to induce the rats in causing the disturbances in hemopoietic system.

The exact mechanism by which *Nigella sativa* seed extract produces its protective role against chlorpyrifos – induced toxicity is still unknown but in some studies it has been clearly reported that NSS extract that contains some quinone-like active ingredients such as thymoquinone, dithymoquinone, thymohydroquinone and thymol have prominent free radical scavenging characteristics [32,33]. However, some recent reports explained that thymoquinone is considered to be the most pharmacologically active component of the black seed oil or extract having phytotherapeutic role in folk medicine because of possessing characteristic antioxidant properties [34-35].

Conclusion

Based on the findings of this study it is concluded that oxidative stress plays a vital role in chlorpyrifos-mediated injury but the treatment with *Nigella sativa* seed extract ameliorated chlorpyrifos-induced alterations in hematological parameters partly due to its antioxidant ability or partly by increasing the immune system by possessing the most active phytotherapeutic agent thymoquinone in the seed extract of *Nigella sativa*.

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