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Alteration of Hematological and Immunological Parameters in Rabbits Treated with Parathion

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ABSTRACT

Background: Organophosphorus compounds (OP's) increase endogenous acetylcholine levels by inhibiting acetylcholinesterase. Their suppression of the immune responses might be due to direct action of acetylcholine on the immune system. **Objective:** To investigate the effect of organophosphorus pesticide, parathion (0.2 mg/kg/day) for 14 consecutive days on the hematological parameters and the immune response of rabbits after different time intervals (1 hour, 24 hours, 1 week, 2 weeks and 4 weeks after the end of treatment). **Materials and Methods:** Blood samples were analyzed for hemoglobin content (Hb), packed cell volume (PCV), erythrocytes and leukocytes. The cellular immunity was assessed by lymphocyte proliferation response to mitogens; phytohemagglutinin (PHA) and lipopolysaccharide (LPS) and humoral immunity was measured by plaque-forming cell (PFC) generation and hemagglutination titer (HA). Also, nonspecific immunity was assayed by phagocytic activity. **Results:** They showed that parathion caused a significant increase of total leukocytes and monocytes, while blood erythrocytes, Hb and PCV were insignificantly reduced. Parathion caused a pronounced suppressive effect on the cellular immunity (lymphocyte proliferation response to PHA and LPS) and humoral immunity (PFC and HA). Also, a significant reduction in nonspecific immunity was observed. The suppressive effect of parathion on immune response was time dependent. **Conclusion**: The results of the present study suggested that the determinations of hematological and immunological parameters are useful tools for evaluating the toxic effects of parathion on animals.

Key words: Erythrocyte, humoral immunity, leukocyte, parathion, rabbit

INTRODUCTION

Due to a continuously growing human population, modern agriculture has relied heavily on pesticides to produce high crop yields, prevent diseases and control pests. OP's are extensively used to replace the persistent organochlorine due to the fast degradation rate and hence less persistence in any environmental

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compartment.[1,2] OP's; are known as potential inhibitors of cholinesterase (ChE) activity that lead to acetylcholine (ACh) accumulation in the synaptic cleft, which causes nerve exhaustion and consequently a failure of the nervous system.[3] OP compounds not only inhibit ChE activity, but also interfere with the immune system of organisms.[4,5] These insecticides are reactive and labile that can directly damage cell membranes, protein and DNA.[6,7] They can also reduce vertebrate ability to make either humoral immune or cytotic T lymphocyte responses.[8] OP compounds were first reported as immunotoxicants in the early 1970s by Ercegovich.[9] Traditional methods for toxicological assessment have implied that the immune system is a frequent target of toxic insult following acute or sub-chronic exposure to environmental chemicals.[10] The toxic action of OP compounds on the immune system has been investigated by several authors.[11,12] Also, Casale et al.[13] and Kim et al.[14] reported suppression of primary humoral immune responses to a T-cell dependent antigen in rodents treated orally with cholinergic doses of parathion (16 mg/kg).

Hematological parameters in general are commonly used in disease diagnosis in domestic animal health practice.[15] They usually reflect the physiological responsiveness of the animal to its external and internal environments and this serves as a veritable tool for monitoring animal health.[16,17]

Therefore, the present investigation was designed to evaluate the alterations of the hematological and immunological parameters in rabbits treated with repeated sub-lethal dose; 0.2 mg/kg/day of parathion for 14 consecutive days.

MATERIALS AND METHODS

Animals

Male New Zealand white rabbits (six months old, 3-4 kg), were purchased from Abbis Farm, Faculty of Agriculture; Alexandria University. Animals were housed one to a cage in 22-26 ºC

temperature, 40-70 % humidity and controlled environment with a 12 hour light / dark cycle. Food and water were given *ad libitum*. All maintenance and care were in accordance with the animals welfare guidelines established at the university.

Chemicals

Technical material (99.6%) parathion was obtained from EPA, Research Triangle Park, N.C.

Animal treatments

The animals were divided into two groups (5 animals per each). The first group was treated orally with 0.2 mg/kg/ day parathion for 14 consecutive days. The second group was treated with corn oil and used as control. Animals were examined throughout the experimental period. All animals were immunized with 0.2 ml antigen (5 x 10^8 SRBC in PBS buffer) 4 days before the end of treatment.

The blood samples were collected from the ear vein of each rabbit at different time intervals (1 hour, 24 hours, 1 week, 2 weeks and 4 weeks) after the end of treatment into three tubes. The first tube containing ethylene diamine tetra acetate (EDTA) was used for the hematological studies, while a heparinized tube was used for lymphocytes preparation. Another aliquot of the blood was taken in plain tube for serum preparation.

Hematological studies

Blood samples were analyzed for hemoglobin content, packed cell volume (PCV), erythrocytes and leukocytes (total count and differential) according to Dacie and Lewis.[18]

Immunological studies

Lymphocytes were prepared according to Boyum.[19] under sterile conditions. Heparinized blood was diluted with PBS (1:1). The diluted blood (4 ml) was carefully poured onto the ficoll solution (5 ml) and centrifuged at 1800 rpm for 20 minutes. The ring of lymphocytes was harvested and washed three times with Hanks' balanced salt solution. The final pellet was suspended in RPMI-1640 medium containing 10 % fetal calf serum. The

viability of the cells was measured using 0.5 % trypan blue dye exclusion technique according to Kawabata and White.[20]

Cellular immunity

Lymphocytes proliferation to mitogens were measured in a 3-days microculture assay using T-cell mitogen, phytohemagglutinin (PHA, 5 µg / ml, Sigma USA) and B cell mitogen, lipopolysaccharide (LPS, 40 µg / ml, Sigma USA) as described by Anderson et al.[21] All steps were done under complete sterile conditions. Cell suspensions with the mitogens were incubated for 72 hours at 37 ºC and 5 % CO_2. Twenty four hours prior to the end of the incubation, 1 µ Ci of (^{3}H)-thymidine (sp.act. 5 Ci / mmol Amersham) was added. Cells were harvested onto glass-fiber filter paper and measured in a scintillation counter. Proliferation response was expressed as Stimulation Index (SI) that is; mitogen-stimulated thymidine incorporation divided by thymidine incorporation in non stimulated cultures.

Humoral immunity

Plaque forming cells (PFC)

Lymphocyte suspensions (2 x 10^6 cells / ml) were added to tubes containing SRBCs (12 %) and guinea pig complement. The mixtures were mixed and transferred to the double slide chambers. The slides were incubated at 37 ºC for 3 hours and plaques were enumerated.[22] The PFC response was expressed as the number of plaque forming cells per 10^6 viable lymphocytes.

Hemagglutination titer (HA)

Two fold dilutions (25 µl) of sera were made in the microtiter V-shaped plates. To each well, 25 µl of 20 % v/v SRBCs was added. The plates were incubated at 37 ºC for 1 hour and then observed for hemagglutination. The highest dilution giving hemagglutination was taken as the antibody titer.[23]

Non specific immunity

Phagocytic activity was measured using the fluorescence microscope.[24] The acridine orange positive cells were counted and expressed as

percentage.

Statistical analysis

Student's t-test was used to estimate statistically significant differences between the mean values of treated and control animals ($P < 0.05$). The data were expressed as mean ± standard error (mean ± S.E.).

RESULTS

The presented results indicated that oral administration of parathion to rabbits in a dose of 0.2 mg/kg/day for 14 days did not show any signs and symptoms of overt toxicity, neurotoxicity or mortality.

Hematological study

Table 1 presents the hematological parameters of male rabbits treated with 0.2 mg/kg/day for 14 days of parathion after different time intervals. The data showed no-significant decrease in Hb content, PCV percentage and erythrocyte counts among various experimental time intervals. The study revealed a significant increase in the leukocyte count; 11.98, 12.89 and 12.99 x 10^3 cells / ul after 1, 2 and 4 weeks, respectively in parathion treated groups compared to the count obtained in the control group (9.55 x 10^3 cells/ul). There were non-significant changes in the lymphocytes and neutrophils percentage at the different times, while a significant increase ($P < 0.05$) in the monocyte count was found in the treated animals compared to the control group. Consequently leukocyte and monocyte counts exhibited a gradual increase with time after parathion exposure.

Table 1. Hematological parameters of male rabbits treated with 0.2 mg/kg/day of parathion after different time intervals (1 hour, 24 hours, 1 week, 2 weeks and 4 weeks after the end of treatment)

Time after treatment	PCV % Mean ± SE	Hemoglobin g/dl Mean ± SE	Erythrocytes count 10^6 cells / µl Mean ± SE	Leukocytes (Mean ± SE)			
				Total count 10^3 cells / µl	Lymphocytes %	Neutrophils %	Monocytes %
Control	39.50±0.57	14.70±0.59	6.22±0.32	9.55±0.57	70.44±0.79	23.98±0.90	3.467±0.05
1 hour	38.87±0.49	14.59±1.24	6.08±0.61	9.98±0.59	66.94±1.18	24.67±1.18	5.67±0.14 *
24 hours	37.59±0.45	14.36±0.90	5.89±0.64	10.05±0.71	70.11±2.06	25.36±1.74	4.26±0.14 *
1 week	40.12±0.90	15.12±0.84	6.42±0.51	11.98±1.16 *	68.87±1.48	23.46±1.24	4.33±0.14 *
2 weeks	36.50±1.24	14.23±0.96	5.56±0.61	12.89±1.08 *	67.00±0.88	22.67±0.96	6.23±0.23 *
4 weeks	35.98±0.89	14.07±1.18	5.49±0.70	12.99±0.90 *	67.35±1.48	24.36±1.19	5.87±0.33 *

* Significantly different from control ($P < 0.05$).

Immunological study

Cellular immunity

The mitogenic responses of lymphocytes to PHA and LPS in treated rabbits after different time intervals are illustrated in Figure1. The data showed gradual significant reduction in the SI for T and B lymphocytes after 1 week and reached its maximum depression after 4 weeks (73.9 and 70.2 % for T and B lymphocytes proliferation, respectively) when compared to the control group.

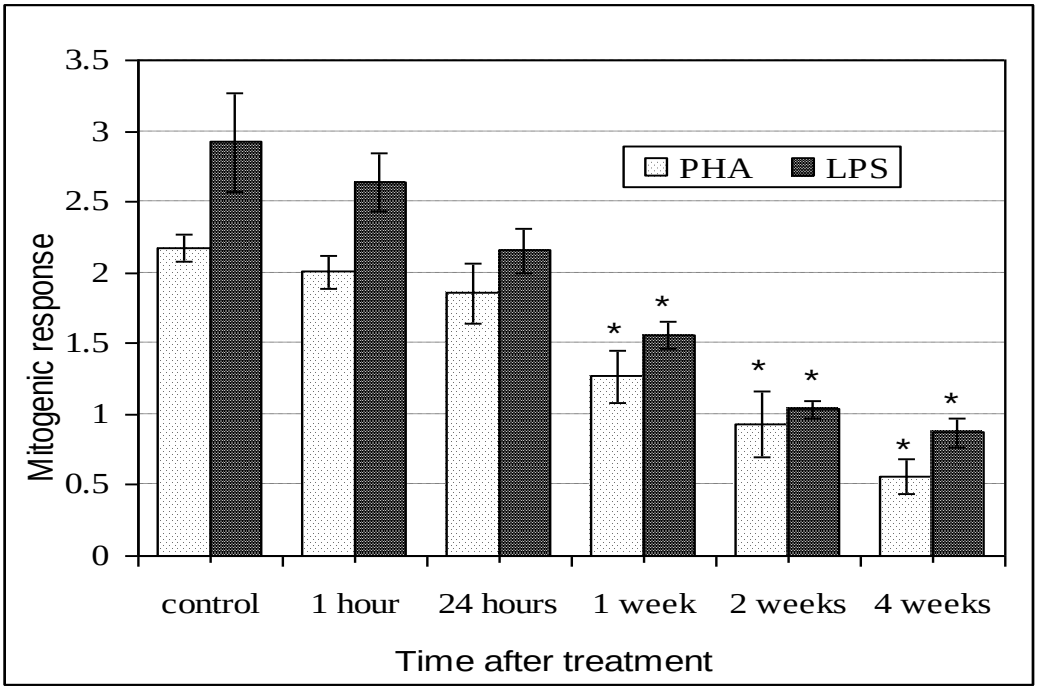

Figure 1. Mitogenic response of lymphocytes to phytohemagglutinin (PHA) and lipopolysaccharide (LPS) in male rabbits treated with 0.2 mg/kg/day of parathion after different time intervals (1 hour, 24 hours, 1 week, 2 weeks and 4 weeks after the end of treatment)

Humoral immunity

A significant ($P < 0.05$) decrease in the number of PFC/10^6 lymphocytes and the antibody titers were observed after 1, 2 and 4 weeks of parathion treatment when compared to the control group, while after 1 and 24 hours non-significant decrease was found (Table 2). The data also, showed a gradual decrease in a time dependant fashion of both PFC and antibody titers in the treated rabbits. The reduction of humoral immunity reached 59.19 % and 83.05 % in PFC and HA after four weeks of the last treatment.

Non specific immunity

Parathion treated rabbits showed a significant ($P < 0.05$) reduction in phagocytic activity compared to the respective control group (Table 2). The percentage positive phagocyte cells decreased by time and reached its maximum after four weeks of treatment (21.35%) compared to the control group (44.13%).

Table 2. Humoral and non specific immunity of male rabbits treated with 0.2 mg/kg/day of parathion after different time intervals [a]

Time after treatment	Humoral immunity		Non specific immunity % Positive phagocytes Mean ± SE
	PFC / 10^6 lymphocytes Mean ± SE	HA ($\log_2$ titer) Mean ± SE	
Control	79.321 ± 0.76	213.330 ± 25.13	44.13 ± 0.71
1 hour	78.643 ± 1.35	200.561 ± 7.13	41.321 ± 0.76
24 hours	75.326 ± 0.85	184.321 ± 14.04	39.862 ± 1.03
1 week	60.108 ± 1.76*	106.625 ± 10.53*	31.567 ± 1.34*
2 weeks	38.524 ± 1.23*	64.326 ± 7.12*	25.643 ± 0.37*
4 weeks	32.364 ± 1.73*	36.156 ± 4.24*	21.346 ± 0.59*

[a] At 1 hour, 24 hours, 1 week, 2 weeks and 4 weeks after the end of treatment
* Significantly different from control ($P < 0.05$).

DISCUSSION

Using agrochemicals to control pests has become a necessity and an accepted worldwide practice to improve crop production. Pesticides exposure poses a serious risk to all domestic animals and non target species in the environment and the public health.[25] The widespread use of OP insecticides in agriculture, veterinary and public health applications has acted as a stimulus for the study of their toxicity to the non target organisms. Such toxicity studies will provide data for the enforcement of regulatory rules aiming to protect autochthonous species and to periodically monitor traces of pesticides in the environmental components. With this in mind, our group has concentrated efforts to evaluate the effects of the agrochemicals on hematology, immunology, biochemistry, hormonal balance and oxidative stress.[26-28] The impact of residual agrochemicals on indigenous species has become a matter of concern to researchers and environmentalists. The measurements of hemoglobin, PCV, erythrocyte and leukocyte counts with its differential disclose the possible relations of blood forming tissue to parathion treatment. In the present study Hb content, PCV percentage and erythrocyte count were not significantly decreased, while, leukocyte count significantly increased in the rabbits treated with 0.2 mg/kg/day parathion. Similar effects have also been reported in rabbits by Yousef et al.[16] and Capcarova et al.[29] Reduction in hemoglobin content can be related to the decreased size of red blood cells, the impaired biosynthesis of heme in bone marrow or the increased rate of destruction / reduction in the formation rate of total erythrocyte count (TEC).[30] One of the molecular mechanisms of the toxicity of some pesticides seems to be lipid peroxidation; as a consequence these compounds can disturb the biochemical and physiological functions of the erythrocyte.[31] The susceptibility of red blood cells to oxidative damage is due to the presence of polyunsaturated fatty acid, heme iron and

oxygen, which may produce oxidative changes in erythrocytes.[32] The hematological alterations in parathion exposed rabbits might be due to physiological dysfunction of hemopoietic stem, which is considered to be the most sensitive indicator towards environmental pollutants.[33] The increase in leukocyte count may indicate activation of the animal's defense mechanisms and immune system [16] or due to inflammation caused by pesticide general toxicity.[34]

The immune system is important for defense against a variety of insults. It is a highly evolved system and is distributed throughout the body. The complex nature of the immune system with its multiple humoral and cellular components makes it an easy target for many drugs and chemicals.[35] Inhibition of ChE causes accumulation of ACh in synapses, resulting in different malfunctions of the nervous system.[36] The immunosuppression may result from direct action of ACh upon the immune system or it may be secondary to the toxic chemical effect associated with cholinergic poisoning.[37] The present results clearly showed that the proliferative response of lymphocytes to mitogens PHA and LPS significantly decreased in parathion treated rabbits and reached maximal depression after 4 weeks. Our data suggested that parathion can interfere with DNA synthesis and inhibit the PHA and LPS induced lymphocyte transformation *in vitro* which is correlated with depressed cell mediated immunity. Any agent that alters the delicate regulatory balance of the immune system may affect the functions of several cell types or alter their proliferation or differentiation. Decreased lymphocyte proliferation is thought to represent the impaired host immune competence and to be inactive of an immunotoxic effect of the chemical being tested.[38]

The parathion exposure could have directly inhibited antibody synthesis or caused chronic stress situation which was responsible for the reduced titers. Regarding the gradual decrease in

antibody titer and PFC in rabbits treated with parathion, it may be due to the decrease in the total number of circulating B-cells. In addition, it may have a selective direct suppression effects on the functional capacity of B-cell indicating direct toxic influence on those lymphocytes especially after prolonged exposure or it may be due to increased degeneration of B-lymphocytes in special sensitive areas as germinal cortex, lymph nodes and follicles of spleen.[39] Parathion may be metabolically converted to a reactive electrophilic derivative and once it was formed, it may bind to critical sites on DNA and / or other molecular targets that are important in the PFC response.

Phagocyte cells are considered as an important component of defense mechanisms as they act against any foreign invasion not only to kill and remove them from the body but also these cells act as antigen presenting cells and participate actively in the specific immunity.[40] A significant reduction ($P < 0.05$) in the phagocytic index was observed in cells from rabbits exposed to parathion for 14 days compared to cells from control animals. Previously, we reported that both carbaryl and cypermethrin caused a significant reduction in the phagocytic index.[26] In addition, the phagocytic capacities of macrophages were significantly reduced in carbaryl and dimethoate treated cells.[40,41] The reduction in the number of active phagocyte cells in parathion treated animals may also lead to decreased natural resistance or innate immunity to infections. Our results are in agreement with those of Aly and El-Gendy.[11] Riahi et al.[42] who reported similar marked depression of cellular immunity in animals treated with a variety of pesticides.

CONCLUSION

The present investigation showed that 14-day exposure of male rabbits to sub-lethal dose of parathion (0.2 mg/kg/day) has caused a non significant reduction in blood erythrocytes, Hb content and PCV,, while a significant increase of total leukocytes and

monocytes was found. Also, parathion caused a pronounced suppressive effect on the cellular, humoral and non specific immunity. The effect of parathion on the tested parameters was time dependent. Finally, our results showed that the selected hematological and immunological parameters were used as useful biomarkers for detecting the effects of pesticides on the non target organisms.

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