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Effects of Sublethal Doses of Lampcon® and Saturn® Pesticides on Some Clinicopathological and Immunological Parameters in Rats

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ABSTRACT

This study aimed to evaluate the clinicopathological and immunological profiles of two pesticides widely used in Egypt, Lampcon® and Saturn® in rats. Rats divided into three groups of 10 animals in each. The first group received no treatment and served as control, the second group received Lampcon at a dose of 168 ppm in food, and the third group received Saturn at a dose of 4230 ppm in food. Pesticides (treatments) were administrated daily via food for 60 consecutive days. Blood samples were collected for evaluating the selected hematological, biochemical and immunological parameters. The results of hematological variables implicated no significant effect on red blood cell parameters, but there was a significant leukopenia, lymphopenia and neutrophilia in both treated groups. The blood biochemical parameters showed a significant increase in serum levels of urea and enzymatic activities of ALT, AST and ALP. The findings of immunological studies clarified that Lampcon and Saturn induced oxidative stress in leukocytes and had immunosuppressive effects. The main histopathological alterations induced by Lampcon and Saturn included lymphoid depletion in lymphoid organs. Conclusion: we can conclude that the exposure of rats to Lampcon and Saturn pesticides pose many hematological, biochemical, and histopathological alterations in addition to immunotoxic effects. It is thus advisable that Lampcon and Saturn should be used with caution and at the recommended levels indicated by the producing company to ensure minimal exposure to these pesticides during domestic, veterinary, agricultural or industrial use.

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1. INTRODUCTION

Pesticides are toxic chemicals that are deliberately introduced in the environment and therefore exposure of people and animals to them is unavoidable (SERA, 2010). Pesticides are widely used throughout the world in agriculture to protect crops and their residues affect the environment adversely. The uses of such biologically active compounds possess potential problems of toxicity among those who manufacture, formulate or use these compounds. The exposure usually occurs either through the skin (dermal) or as a result of inhalation or even from its residues in food "especially field crops. In farm animals, the most common route of pesticide exposure is through the ingestion of contaminated food and/or water (IPCS INCHEM, 1990). The toxicity of pesticides to mammalian animals has received much attention in recent years because animals exposed to these insecticides exhibited changes in their physiological activities beside other pathological features (Saxena and Saxena, 2010). Due to the pervasive use of pesticides, we are all exposed to them on a daily basis whether we work with them directly or not (Sharma, 2005; Brundage and Barnett, 2010). The pyrethroid represents a relatively new group of synthetic insecticides, although members of the group have been commercialized since mid-1950s (Saka *et al*., 2011). They are derived from pyrethrins, natural substances obtained from the flowers of Pyrethrum species. Their popularity has been increasing substantially in recent years, and new members are constantly being developed and commercialized. Synthetic pyrethroid insecticides are now used as substitutes for pest control (Sangha *et al*., 2011). Lampcon (lambda-cyhalothrin) is a pyrethroid insecticide, highly active against a wide range of insects. It is widely used in developing countries and has public and animal health applications in which it effectively controls a broad spectrum of insects including cockroaches, flies, mosquitoes, ticks and mites (SERA, 2010). Saturn (thiobencarb) is a systemic broad-spectrum carbamate herbicide used to control many broadleaf

weeds and grasses (Fan and Alexeeff, 2000). Although extensive research work has been performed on various aspects of synthetic pesticides, including metabolism, toxicological characteristics, ecotoxicology and detection of residues, little attention has been paid to their hematological, biochemical and immunological effects. Therefore, the present investigation aims to study the effects of sub lethal doses of Lampcon and Saturn as new generations of pesticides on some hematological, biochemical and immunological variables with particular emphasis on the immunotoxicity of these two widely used pesticides in albino rats after 60 days continuous feedings.

2. MATERIALS and METHODS

2.1. Animals: A total of sixty two (62) male Sprague Dawley rats weighing (90-120 g) obtained from the National Institute of Ophthalmology, Giza, Egypt were used, 32 in determination of the acute oral LD50 and 30 in the experimental protocol. The animals were housed under hygienic conditions in plastic cages, 5 animals per cage and were provided with balanced ration and water adlibitum. All animals were kept under observation for two weeks before the experiment starts for acclimatization.

2.2. The Pesticides: Lampcon® (*lambda-cyhalothrin*), a synthetic pyrethroid insecticide (procured from Healthy life Pharma. Co., India) and Saturn® (*thiobencarb*), a thiocarbamate herbicide (procured from Kumiai Chemical Industry Co., Ltd. Japan).

2.3. Determination of the acute oral LD50: Thirty two male rats were divided into two groups of sixteen in each. For each treatment, the rats were divided into four equal groups (4 rats each). The animals were dosed orally with either the formulated Lampcon or Saturn using a stomach tube at dose levels with a geometric progression. Mortalities were recorded within 24 hours of pesticide ingestion and the symptoms of intoxication were recorded. The LD50 value was then calculated (Weil, 1952).

2.4. The experimental protocol: Thirty male rats were divided into 3 groups each of 10 animals. Doses were selected on the basis of LD50. The first group received no treatment and served as control. The second group received 1/10th oral LD50 of Lampcon (11.2 mg/kg body weight equivalent to168 ppm in food). The third group received 1/10th oral LD50 of Saturn (282 mg/kg body weight equivalent to 4230 ppm in food). The treatments were administrated daily via food for 60 consecutive days. All rats were immunized by intraperitoneal (I/P) injection of 0.5 ml of a 20% sheep red blood cells (SRBC) suspension at the 40th day of exposure. A second (booster) dose was given 15 days after the first immunization. Animals were then sacrificed 5 days after injection of the booster dose (Monis and Valentich, 1993).

2.5. Samples: Blood samples were collected from venous canthus of the eye of each rat. The blood sample was divided into three parts. The first part (1 ml) was collected on disodium ethylene diamine tetracetic acid (EDTA) for hemogram. The second part (1 ml) was collected on heparin (20 IU/ml) for evaluating the phagocytic activity of neutrophils. The third part (2 ml) was placed in a plain centrifuge tubes for separation of serum. Serum samples were divided into aliquots in Eppendorf tubes and the tubes were stored at -20°C until assayed for the rest biochemical and immunological parameters. Liver, spleen, thymus, lymph nodes and payer's patches were collected for histopathological examination.

2.6. Analytical methods:

2.6.1. Hemogram: The evaluated hematological parameters included estimation of red blood cell count (RBCs), hemoglobin concentration (Hb), hematocrit (Hct), total (TLC) and differential leukocytic counts. These parameters were done using the routine hematological procedures according to Feldman *et al*. (2000).

2.6.2. Serum biochemical parameters: Serum samples were investigated for the concentrations of total protein (TP), albumin (Alb), glucose, calcium (Ca), inorganic phosphorus (iP), blood urea nitrogen (BUN), creatinine, cholesterol, triglycerides, high density lipoprotein (HDL) and serum enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and creatine kinase (CK). parameters were determined spectrophotometrically using commercial available kits supplied by Biomed diagnostics (Germany) and following the manufacturer's instructions. Total globulin was determined by subtracting albumin from serum total protein and then albumin-globulin ratio (A/G) was estimated.

2.6.3. Immunological Parameters:

2.6.3.1. Leukocyte oxidant-antioxidant status: Leukocytes were collected after lysis of RBCs using ammonium chloride solution as described by Ercal *et al*. (2000) and Griffin (2012). Oxidative stress in leukocytes was then evaluated by measuring malondialdehyde (MDA), reduced glutathione (GSH) and enzymatic activities of glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT). These parameters were determined spectrophotometrically using commercial kits of Biodiagnostics (Egypt).

2.6.3.2. Phagocytic activity: The phagocytic activity of polymorphonuclear cells towards *Candida albicans* was performed according to the method described by Wilkinson (1981).

2.6.3.3. Cell-mediated immune response: Assessment of cell-mediated immune response was performed by evaluating the delayed-type hypersensitivity reaction to intradermal injection of 0.5 ml/animal of a 20% SRBC suspension one day before the end of experiment (59^{th} day). The diameter and thickness of skin reactions 24 and 48 hours after the inoculation were then measured (Dean *et al*., 1989).

2.6.3.4. Electrophoretic pattern of serum proteins: Serum protein electrophoretic fractionation profile was carried out by Polyacrylamide Gel Electrophoresis for Proteins "SDS-PAGE" (Laemmli, 1970).

2.6.3.5. Determination of antibody titer and immunoglobulin levels: The hemolysing antibody titer was measure according to Seinen *et al.* (1977). Titers represent the reciprocal of the highest dilution giving total hemolysis. Immunoglobulin M (IgM) and immunoglobulin G (IgG) concentrations in serum were determined using single radial immunodiffusion methods derived from the works of Fahey and McKelvey (1965) and Mancini *et al*. (1965) for the quantitative determination of individual protein groups in biological fluids.

2.6.4. Histopathological studies: Liver, spleen, thymus, lymph nodes and payer's patches were fixed in 10% neutral formalin and prepared for histopathological examination according to Bancroft *et al*. (1996).

2.7. Statistical analysis: The data were presented as mean± standard error (SE) and were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by a multiple comparison Duncan test (Gad, 2001). Differences at $p \leq 0.05$ were considered significant.

3. RESULTS

3.1. The Median Lethal Dose (LD50): Table 1 reveals the mortality data in rats received graded oral doses of Lampcon and saturn insecticides during the first 24 hours after administration. Symptoms of intoxication were observed few minutes after administration including: piloerection, ataxia, choreoathetosis, salivation, lacrimation, reduced body temperature, labored breathing and decreased motor activity.

Table (1): Mortality data in rats received single oral doses of Lampcon or Saturn.

Group	No. of rats	Dose (mg/kg)		No. of dead rats	
		Lampcon	Saturn	Lampcon	Saturn
1	4	40	1000	0	0
2	4	80	2000	1	1
3	4	160	4000	3	3
4	4	320	8000	4	4

The oral LD_{50} of the commercial formulation Lampcon in rats was considered to equal 112.2 mg of the active ingredient (lambda-cyhalothrin) per kilogram of body weight while the oral LD_{50} of the commercial formulation Saturn was considered to equal 2818.38 mg of the active ingredient (thiobencarb) per kilogram of body weight.

3.2. Effect of Lampcon and Saturn on hemogram: Data presented in table 2 showed that, there is no significant alterations were observed in RBC, Hct and Hb values in rats treated with Lampcon or Saturn. With respect to leukocytic parameters, intoxicated animals showed a significant decrease of total leukocytes, lymphocytes count and higher neutrophil count.

Table (2): Effect of Lampcon and Saturn on hematological parameters:

Parameter	Control	Lampcon	Saturn
RBCs ($x10^6$/µl)	6.32±0.99	8.40±0.35	5.90±1.01
Hct (%)	41.16±0.45	41.20±0.35	40.68±0.55
Hb (gm/dl)	13.72±0.52	14.40±0.91	13.56±0.59
WBCs ($x10^3$/µl)	6.23 ± 0.031^{a}	6.04 ± 0.043^{b}	5.92 ± 0.031^{c}
Lymphocytes (%)	79.5 ± 1.29^{a}	70.8 ± 2.18^{b}	64.9 ± 0.97^{c}
Neutrophils (%)	15.9 ± 0.78^{c}	24.3 ± 1.18^{b}	29.9 ± 1.17^{a}
Monocytes (%)	2.9 ± 0.50	2.9 ± 0.60	3.3 ± 0.47
Eosinophils (%)	1.4 ± 0.27	1.6 ± 0.31	1.8 ± 0.33
Basophils (%)	0.3 ± 0.15	0.4 ± 0.16	0.1 ± 0.1

Values are presented as mean ± SE. Means in the same row followed by different letter superscripts are significantly different at ($P < 0.05$).

3.3. Effect of Lampcon and Saturn on blood biochemical parameters: Data shown in table 3 demonstrated a significant (P< 0.05) increase in serum concentrations of blood urea and serum enzymatic activities of ALT, AST and ALP in the treated groups compared to the control. Other biochemical variables did not show significant alterations.

3.4. Effect of Lampcon and Saturn pesticides on immunological parameters:

3.4.1. Leukocyte oxidant-antioxidant status: The effects of Lampcon and Saturn on leukocytic oxidant-antioxidant status as indicated in table 4 implicated a significant (P<0.05) elevation in the levels of malondialdehyde and a significant (P<0.05) decrease in leukocytic reduced glutathione values. Treatment of rats with Lampcon and Saturn also resulted in significant (P<0.05) decrease in the activities of leukocytic glutathione reductase, glutathione peroxidase and catalase enzymes (Table 4).

3.4.2. Phagocytic activity: The results of phagocytic activity in table 5 demonstrate that Lampcon did not affect the phagocytic activity of neutrophils in subchronically intoxicated rats while Saturn significantly (P<0.05) reduced it.

3.4.3. Cell-mediated immunity: Cellular immune response was estimated by evaluating delayed-type hypersensitivity response following I/P injection of 0.5 ml of a 20% SRBC suspension one day before the end of treatments exposure. The results shown in table 6 indicated a significant (P<0.05) decrease in the diameter of skin reaction in both Lampcon and Saturn treated groups after 24 and 48 hours. The thickness of skin reaction was significantly (P<0.05) reduced in both test groups at 24 hours while only the Saturn treated rats showed a significant difference from control values in this parameter at 48 hours.

Table (3): Effect of Lampcon and Saturn on blood biochemical parameters:

Parameter	Control	Lampcon	Saturn
Glucose (mg/dl)	46.84±2.33	51.35±2.24	53.30±1.40
Triglyceride (mg/dl)	52.63±9.13	49.85±11.46	56.39±4.18
Cholesterol (mg/dl)	60.91±6.85	59.63±5.67	63.96±4.61
HDL (mg/dl)	22.86±3.93	24.70±1.94	22.35±2.01
Ca (mg/dl)	12.87±1.74	13.27±0.49	13.76±1.10
iP (mg/dl)	7.38±0.14	6.61±0.68	6.59±0.27
Urea (mg/dl)	43.35±6.65 [c]	52.26±4.44 [b]	60.64±3.20 [a]
Creatinine (mg/dl)	1.15±0.07	1.12±0.02	1.29±0.02
ALT (U/l)	31.33±0.88 [c]	52.00±2.51 [b]	59.33±0.33 [a]
AST (U/l)	19.33±1.20 [b]	39.33±1.66 [a]	33.66±0.88 [b]
ALP (U/l)	12.31±0.88 [b]	21.53±0.31 [a]	19.08±0.14 [a]
LDH (U/l)	50.12±3.35	52.21±1.83	48.28±3.88
CK (U/l)	30.28±2.44	31.17±2.41	35.52±2.41

Values are presented as mean ± SE. Means in the same row followed by different letter superscripts are significantly different at (P< 0.05)

Table (4): Effect of Lampcon and Saturn on leukocyte oxidant-antioxidant status:

Parameter	Control	Lampcon	Saturn
Malondialdehyde (nmol/g)	2.56 ± 0.044 [b]	7.26 ± 0.24[a]	8.03 ± 0.136[a]
Reduced glutathione (mg/g)	8.03 ± 0.20 [a]	6.59 ± 0.28[b]	5.25 ± 0.20[b]
Glutathione reductase (U/mg)	0.143 ± 0.005 [a]	0.127 ± 0.004[b]	0.130 ± 0.005 [b]
Glutathione peroxidase (U/mg)	0.102 ± 0.006 [a]	0.065 ± 0.005[b]	0.041 ± 0.004[b]
Catalase (U/mg)	1.77 ± 0.061 [a]	1.20 ± 0.039[b]	1.18 ± 0.048[b]

Values are presented as mean ± SE. Means in the same row followed by different letter superscripts are significantly different at (P< 0.05).

Table (5): Effect of Lampcon and Saturn on phagocytic activity of neutrophils:

	Control	Lampcon	Saturn
Percentage of Phagocytosis	82.0 ± 1.32 [a]	79.1 ± 1.28 [a]	69.9 ± 1.52[b]

Values are presented as mean ± SE. Means in the same row followed by different letter superscripts are significantly different at (P< 0.05).

3.4.4. Electrophoretic fractionation pattern of serum proteins: Results of serum protein electrophoresis fractionation profile presented in table 8 revealed that treatment of male rats with Lampcon and Saturn induced a significant ($P<0.05$) decrease in serum concentrations of total protein and albumin while the mean values of serum globulin levels did not show significant ($P<0.05$) differences from control values. The A/G ratio significantly ($P<0.05$) decreased only in the Saturn treated rats. Compared to the control, there was a significant ($P<0.05$) reduction in the mean values of α1 and γ globulins while, serum levels of β globulins were significantly ($P<0.05$) elevated. Significant changes in serum concentrations of α2 globulins were not observed (Table 7).

3.4.5. Hemolysin antibody titer and serum immunoglobulin levels: The results in table 8 shows that, sera from animals treated with Lampcon and Saturn demonstrated significantly lower values of antibody titer, IgM and IgG levels in comparison with the control group.

3.5. Histopathological changes: Oral administration of 1/10*th* oral LD_{50} of Lampcon and Saturn induced many histopathological changes in various organs of treated rats. In Lampcon group, the liver showed Kupffer cell hyperplasia, various degrees of hydropic degeneration, formation of newly formed bile ducts, sporadic cell necrosis and congestion of central vein (Fig. 1, A & B). In Saturn treated group, the liver showed also Kupffer cell hyperplasia, congestion of sinusoids and central vein, and sporadic and single cell necrosis (Fig. 1, C & D). The spleen in both test groups showed periarterial lymphoid sheath exhaustion and depletion with necrosis of lymphocytes (Fig. 2, A & B). Thymus from Lampcon treated rats demonstrated necrosis in cortical lymphocytes and depletion in medulla while in Saturn group there was congestion and single cell necrosis in thymus gland (Fig. 2, C & D). The ileum and mesenteric lymph nodes showed lymphoid depletion in both treated groups (Fig. 3).

Table (6): Effect of Lampcon and Saturn on delayed type hypersensitivity reaction

	24hs			48hs		
	Control	**Lampcon**	**Saturn**	**Control**	**Lampcon**	**Saturn**
Diameter of skin reaction (mm)	11.15 ± 0.20^{a}	9.84 ± 0.16^{b}	9.29 ± 0.27^{b}	8.73 ± 0.18^{a}	8.16 ± 0.075^{b}	8.01 ± 0.17^{c}
Thickness of skin reaction (mm)	1.91 ± 0.01^{a}	1.84 ± 0.02^{b}	1.83 ± 0.01^{b}	1.58 ± 0.02^{a}	1.53 ± 0.02^{ab}	1.50 ± 0.01^{b}

Values are presented as mean ± SE. Means in the same row followed by different letter superscripts are significantly different at ($P< 0.05$).

Table (7): Effect of Lampcon and Saturn on serum protein electrophoresis pattern

Parameter	**Control**	**Lampcon**	**Saturn**
Total protein (g/dl)	6.94 ± 0.03^{a}	6.61 ± 0.02^{b}	6.53 ± 0.03^{b}
Albumin (g/dl)	3.37 ± 0.03^{a}	3.09 ± 0.02^{b}	3.02 ± 0.02^{b}
Globulin (g/dl)	3.60 ± 0.03	3.52 ± 0.02	3.51 ± 0.03
A /G ratio	0.94 ± 0.01^{a}	0.91 ± 0.00^{a}	0.86 ± 0.01^{b}
α_1 globulin (g/dl)	1.23 ± 0.02^{a}	1.15 ± 0.02^{b}	1.05 ± 0.02^{c}
α_2 globulin (g/dl)	0.55 ± 0.02	0.52 ± 0.02	0.51 ± 0.02
β globulin (g/dl)	1.36 ± 0.03^{b}	1.44 ± 0.02^{a}	1.57 ± 0.03^{a}
γ globulin (g/dl)	0.46 ± 0.02^{a}	0.40 ± 0.01^{b}	0.38 ± 0.01^{b}

Values are presented as mean ± SE. Means in the same row followed by different letter superscripts are significantly different at ($P< 0.05$).

Table (8): Effect of Lampcon and Saturn on antibody titer and IgM and IgG levels

	Control	**Lampcon**	**Saturn**
Antibody titer (log2)	7.65 ± 0.26^{a}	6.75 ± 0.35^{b}	6.15 ± 0.27^{b}
IgM (mg/dl)	38.55 ± 1.65^{a}	28.52 ± 1.74^{b}	28.23 ± 1.54^{b}
IgG (mg/dl)	352.4 ± 4.85^{a}	331.3 ± 4.44^{b}	313.9 ± 2.95^{c}

Values are presented as mean ± SE.Means in the same row followed by different letter superscripts are significantly different at ($P< 0.05$).

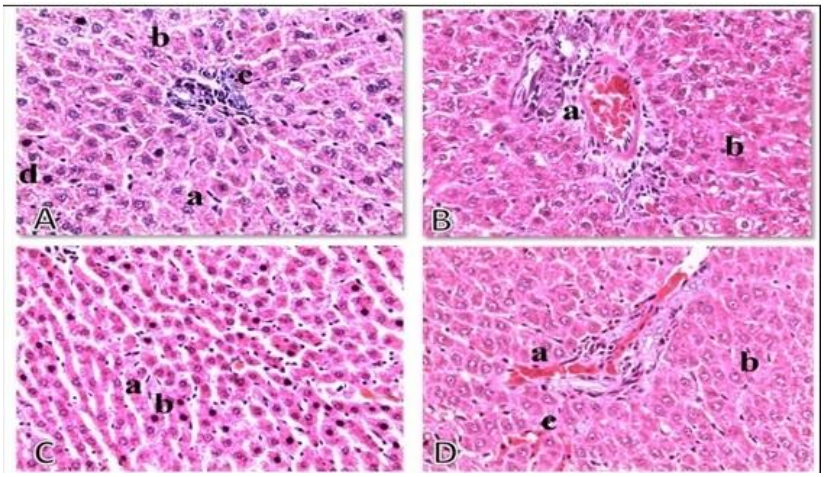

Figure 1: A. Liver of Lampcon-treated rats showing Kupffer cell hyperplasia (a), mild degree of hydropic degeneration (b), formation of newly formed bile ducts (c) and sporadic cell necrosis (d) (H&E, X 40). **B**. Liver of Lampcon-treated animals showing congested central vein (a) and hydropic degeneration (b) (H&E, X40).**C**. Liver from Saturn-treated rats showing Kupffer cell hyperplasia (a) and sporadic cell necrosis (b) (H&E, X40).**D**. Liver from Saturn-treated rats showing congestion in central vein (a), Kupffer cell hyperplasia (b) and congestion of sinusoids (c) (H&E, X40).

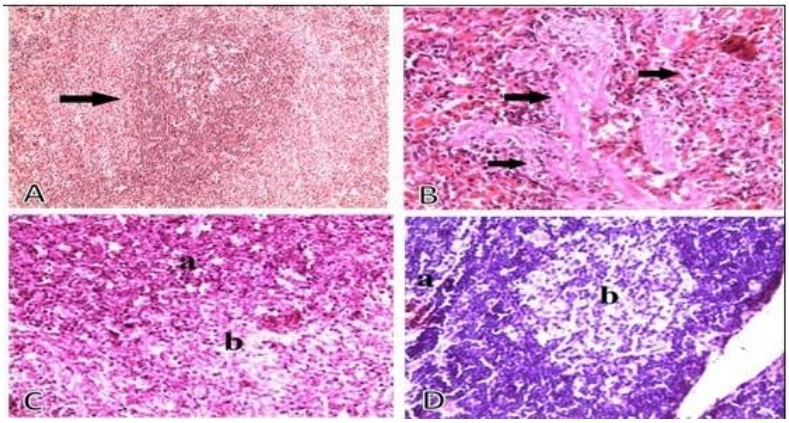

Figure 2: A. Spleen from Lampcon-treated rats showing lymphoid exhaustion (arrow). H&E, X20. **B.** Spleen from Saturn-treated rats showing lymphoid depletion (arrows). H&E, X40. **C**. Thymus from Lampcon-treated rats showing necrosis in cortical lymphocytes (a) and depletion in medulla (b). H&E, X40. **D.** Thymus from Saturn-treated rats showing congestion (a) and single cell necrosis (b). H&E, X40.

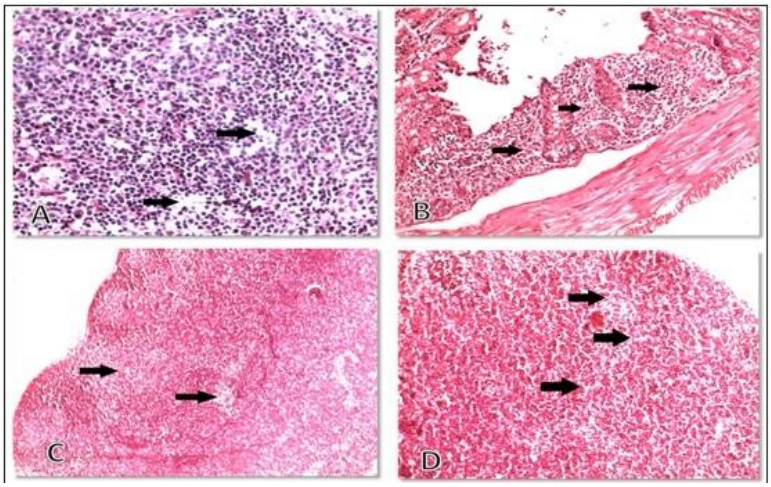

Figure 3: A. Ileum from Lampcon-treated rats showing lymphoid depletion (arrows). H&E X40. B. Ileum of Saturn-treated rats showing lymphoid depletion (arrows). H&E, X40.C. Mesenteric lymph node from Lampcon-treated rats showing lymphoid depletion (arrows). H&E, X10.D. Mesenteric lymph node from Saturn-treated rats showing lymphoid depletion (arrows). H&E, X40.

4. DISCUSSION

Pesticides are chemical substances that are widely used throughout the world in agriculture to protect crops. When used properly, they have been found to pose very little risk to human and animal health and to the environment. But exposure to very large amounts of these chemicals may experience adverse health effects among domestic animals and human beings as well. There is a common perception of users about the safety of these substances because accumulation of a compound in excess in the blood is usually a pointer to a clinicopathological condition (Saka *et al.,* 2011; Sangha *et al.,* 2011). In the present study, two pesticides, Lampcon a synthetic pyrethroid insecticide and Saturn, a thiocarbamate herbicide were tested for their hematological, biochemical and immunological effects. The oral LD_{50} in rats was calculated as 112.2 mg lambda-cyhalothrin per kg body weight for Lampcon and 2818 mg thiobencarb per kg body weight for Saturn. These values were higher than those reported in previous studies (US EPA, 1988; Royal Society of Chemistry, 1991; Tomlin, 1994; US EPA RED, 1997; CPHG, 2000). This could be attributed to use the insecticide formulations in our study, while other investigators used the active ingredients of the compounds. However, the difference might be related to the presence of the dosing vehicles in the insecticide formulation used in this work which may affect the acute oral toxicity of pesticides. (ATSDR, 2003).

Evaluation of blood cells and serum variables has higher predictive value in assessing status of the body because it tells us changes occurring internally particularly those occurring with toxicity or diseases. Blood can be tested for a wide range of cells and substances whose presence in excess or deficit can provide us with better understanding of the pathologic process and thus effective management tool. With respect to the hematological parameters, the data of this study showed that Lampcon and Saturn had no significant effects on red cell parameters. This is consistent with previous studies which reported that feeding permethrin to mice revealed no significant effect on hematological values (Ishmael and Litchfield, 1988). Others also reported no significant changes in red blood cell count, hemoglobin concentration and hematocrit values in animals treated with Cypermethrin (Matsushima *et al.,* 2003; Sayim *et al.,* 2005). However, anemia was reported in mice treated with fenvalerate (EPA, 1991) and carbamate (Zaahkouk *et al.*, 2000). The disparity between studies could be due to the dose, duration of exposure and sex (Luty *et al.*, 2001).

Consistent with the previous reports, Lampcon and Saturn caused significant leukopenia which may be explained as shown in this work by lymphopenia. Possible reasons for the reduction in the total leukocytic and lymphocytic counts were documented to be the cytotoxic and genotoxic effects of lambda-cyhalothrin and thiobencarb and/or their effect on leukocytic membrane (Ladics *et al.,* 1994; Krishnappa *et al.,* 1999; Grosman and Diel, 2005; Naravaneni and Jamil, 2005). In rabbits, administration of lambda-cyhalothrin caused significant decrease in white blood cell and lymphocytic counts, while neutrophils, monocytes and eosinophils were increased (Basir *et al.,* 2011). In broiler chicks, leukopenia was recorded after Cypermethrin (Sharaf *et al.,* 2009) and fenvalerate (Garg *et al.,* 2004) administration. Saturn was found to have a mutagenic effect on bone marrow cells of mice as was evidenced by increased number of micronucleated cells (US EPA RED, 1997). In addition, the toxic effects of Saturn on blood leukocytes were found to be the result of inhibited lymphocyte proliferation (Medjdoub *et al.*, 2011).

The number of circulating lymphocytes in peripheral blood is an index of the functional ability of lymphoid organs thus; reduction in the number of lymphocytes might be due to a direct necrotic effect of the treatments on lymphoid tissue leading to lymphocyte depletion (Garg *et al.,* 2004). This suggestion was further supported in this study by the results of histopathology which demonstrated lymphoid depletion in spleen, thymus and lymph nodes (Fig. 2, 3). The significant neutrophilia seen in the test groups could relate to stress response to the pesticides treatment and endogenous release of corticosteroids (Duncan *et al.,* 1994).

The current findings of serum biochemical parameters implicated a significant increase in serum concentrations of blood urea and serum enzymatic activities of ALT, AST and ALP in the treated groups. Although the concentrations of blood urea were significantly increased, renal injury was not expected to exist as serum creatinine concentrations did not show significant changes excluding the possibility of presence of renal dysfunction. The increased blood urea concentration in rats treated with Lampcon and Saturn could be explained by the accelerated catabolism of body protein and could result as a response to stress of treatments or due to more efficient conversion of ammonia to urea because of increased synthesis of enzyme involved in urea production (Saxena and Saxena, 2010). Elevation of serum activities of ALT, AST and ALP in rats treated with Lampcon and Saturn indicates hepatic injury which is comparable with the results

of previous studies (Zaahkouk *et al.*, 2000; Manna *et al.*, 2003; Garg *et al.*, 2004; Khan *et al.*, 2008). The authors in these reports attributed the hepatic injury to the oxidative damage by free radicals. Additionally, the influence of some male or female hormones on the leakage of these enzymes and thus the increased enzymatic activities were suggested to be a reasonable cause in other studies (El-Tawil and Abdel-Rahman, 1997).

Considering the past demonstrations on the actions of some pesticides on immune response, it was apparent that these compounds may be immunosuppressive leading to a decreased effectiveness of the defense system that is they reduce immune cell number and function. Lampcon and Saturn were evaluated for their effects on some immunological parameters including innate, cell mediated and humoral immune responses. The results in this respect indicated that both Lampcon and Saturn induced oxidative stress in leukocytes via increased levels of malondialdehyde, a lipid peroxidation marker and decreased level of reduced glutathione in these cells. The activities of the antioxidant enzymes, glutathione reductase, glutathione peroxidase and catalase were also inhibited by the two treatments. These findings are identical to those previously reported which confirmed the oxidative stress induced by synthetic pyrethroids and carbamates on leukocytes and other immune cells. Rats received lambda-cyhalothrin orally suffered from oxidative stress with protein oxidation, DNA damage, decreased antioxidants enzyme activities and a significant increase of malondialdehyde levels in erythrocytes, brain, liver and kidney (Fetoui *et al.*, 2008; Abdallah *et al.*, 2012).

Further, malondialdehyde levels were found to be significantly increased in the erythrocytes (El-Demerdash, 2007) and testis (Yousef, 2010) of lambda-cyhalothrin treated rabbits. Carbamate insecticides were also found to be oxidative stressors on neutrophils, lymphocytes and macrophages (Banerjee *et al.*, 1999; Calviello *et al.*, 2006; Pruett *et al.*, 2009; Ahmad *et al.*, 2010; Saquib *et al.*, 2010; Medjdoub *et al.*, 2011). Consistent with the report of Righi *et al.* (2009), the present investigation revealed that administration of Lampcon did not affect the phagocytic activity of blood neutrophils in subchronically intoxicated rats. The author in that study found that cyhalothrin treatment decreased the percentage and intensity of phagocytosis performed by macrophages, but did not alter these parameters in neutrophils. On the other hand, administration of Saturn for 8 weeks to rats suppressed the phagocytic activity. Similarly, the phagocytic capacities of macrophages were significantly reduced in carbaryl treated rats (Pipy *et al.*, 1983) and chicken (Singh *et al.*, 2007). The inhibited phagocytic activity produced by Saturn might be attributed to reduction of the monocyte respiratory burst response (Jaisson *et al.*, 2007).

The findings of the current study in respect to cell-mediated immunity revealed that Lampcon and Saturn inhibited the cell-mediated immunity as shown by the significant reduction in in delayed type hypersensitivity reaction to SRBCs. Similarly, Kuz'minskiĭ and Popko (1992) reported that rats subchronically intoxicated with the synthetic pyrethroid sumi-alpha developed reduction of cell-mediated immunity. Delayed hypersensitivity reaction to tuberculin was reduced to 77% of control values in chicken fed with carbaryl (Singh *et al.*, 2007). These findings are suggestive of impairment of T effector cells which are responsible for elaboration of lymphokines involved in the delayed hypersensitivity reaction via enhancement of accumulation of mononuclear cell infiltrates, mononuclear cell interaction and increased vascular permeability that occur in the vicinity of stimulus (Danneberg, 1991).

The results of humoral immune response presented in this work revealed that Lampcon and Saturn significantly inhibited the hemolysin antibody titer and reduced serum gamma globulins, IgM and IgG levels which may reflect impaired B lymphocyte function with the resultant decreased antibody production (Prater *et al.*, 2003). Feeding of rats emulsifiable concentrate formulation of lambda-cyhalothrin for 90 days was found to result in significant decreases in total immunoglobin concentration following treatment with Brucella abortus antigen (Krishnappa *et al.*,1999). Similar to these findings, Chauhan and Agrawal (1999) recorded reduction in Brucella-specific antibody titers and total IgG and IgM titers in alphamethrin fed calves. Suppression of the humoral immune response by carbamate insecticides was also previously reported. Carbaryl inhalation in rats reduced serum levels of SRBC-specific IgM (Ladics *et al.*, 1994). In another study, rats exposed to propoxur exhibited decrease in antibody titer and plaque forming cell assay (Suke *et al.*, 2006). Chicken exposed to carbendazim showed reduced B-lymphocyte proliferation and decreased serum gamma-globulins, IgG, IgM and IgA levels (Singhal *et al.*, 2003). Some possible causes for these effects were suggested as the genotoxicity of these compounds on lymphocytes (Grosman and Diel, 2005; Naravaneni and Jamil, 2005), inhibition of production of cytokines essential for antibody

production (Diel *et al.,* 1998) or inhibited B lymphocyte proliferation (Singhal *et al.,* 2003). The latter assumption correlates with the recorded histopathological findings in both Lampcon and Saturn-treated rats which demonstrated depletion and degeneration of lymphoid organs with the concurrent lymphocytes necrosis.

The significant decrease in the mean values of serum concentrations of total protein, albumin and α1 globulins could be attributed to liver impairment (Sood, 2006) while, the significant elevation in serum levels of β globulins may implicate some degree of systemic acute phase response to stress of pesticides treatments because important proteins of the acute-phase response are in β globulins (Duncan *et al.,* 1994). The results of histopathological findings revealed the presence of hepatotoxic and immunotoxic effects in both test groups. The liver of both groups showed Kupffer cell hyperplasia, various degrees of hydropic degeneration, congestion of sinusoids and central vein and sporadic cell necrosis, while the main changes observed in lymphoid organs (spleen, thymus and lymph nodes) included lymphoid depletion, congestion and cell necrosis (Garg *et al.*, 2004; Hassan *et al.*, 2004; Petrovova *et al.*, 2010; Petrovova *et al.*, 2011; Dutta and Das, 2011).

In conclusion, the results from the present study have shown that subchronic exposure to Lampcon and Saturn pesticides can be hazardous because they pose many hematological, biochemical, and histopathological alterations. These pesticides also appeared to have immunotoxic effects inhibiting all types of immunity including innate, cell mediated and humoral immune responses. Thus in the light of these observations, it is advisable that Lampcon and Saturn should be used with caution and at the recommended levels indicated by the producing company besides adequate measures be taken to ensure minimal exposure to these pesticides during domestic, veterinary, agricultural or industrial use.

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