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Effects of Subchronic Intoxication with Propoxur on Serum Biochemical Parameters in Pigeon (*Columba livia domestica*).

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ABSTRACT

The pesticides tend to become concentrated as they move up the food chain. They accumulate in organism body (target and non-target organisms) which contained them. The pesticides don't kill the individual birds often, but do affect their bodies so that they lay eggs with very thin shells often these thin-shelled eggs break or the birds are unable to reproduce. Pesticides also affect bird's physiological functions. Natural conservation committee and the Royal Society for bird protection suggested that indirect effect of pesticides was a major cause of decline birds species. The purpose of this study is to investigate the effects of subchronic oral dosage of propoxur (1/10 LD50) on serum biochemical parameters. The bird employed in the present study is the rock pigeon (*Columba livia domestica*), weighing between 320–380g. Birds were classified into four groups, each consists of 5 animals as follows: 1- Control group, this group, non-treated pigeons, were not subjected to oral administration of the insecticide. 2- Three doses group, pigeons in this group treated with a repeated oral dose (1/10 LD_{50}) of propoxur for three consecutive doses. 3- Six doses group, pigeons in this group treated with a repeated oral dose (1/10 LD_{50}) of propoxur for six consecutive doses. 4 – Nine doses group, Pigeons in this group treated with a repeated oral dose (1/10 LD_{50}) of propoxur for nine consecutive doses. (Two-days interval between each two consecutive doses in treated groups) and birds were sacrificed after 24 hours after the last dose. Results of this study, showed significant increase in liver enzymes (ALT and AST), blood glucose, blood urea, serum uric acid, cholesterol, and triglycerides in intoxicated groups with propoxur as compared to control pigeons group.

Keywords: Propoxur, Pigeon, Liver, Kidneys

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INTRODUCTION

Pesticides are compounds or mixtures of substances intended for preventing, destroying, repelling or mitigating any pests, including not only insects, but also other animals, unwanted plants, fungi or microorganism [1]. Carbamates are relatively simple molecules that are characterized by being carbamic acid esters. These molecules have been used as pesticides in agriculture, for human drug therapies (e.g., for the treatment of Alzheimer's disease, myasthenia gravis, glaucoma, and as a prophylactic for organophosphate compound poisoning), and in veterinary medicine as antiparasitic drugs [2].

Propoxur (2-isopropoxyphenyl N-methyl carbamate) is a widely used broad spectrum insecticide. In addition to the control of cockroaches, mosquitoes, bugs, fleas, ants, millipedes, this insecticide is also used against pests in food stores, open areas, and households. Propoxur exhibits a toxic effect characterized by the inhibition of the enzyme cholinesterase [3]. Although mildly toxic to humans and domestic animals (class II), propoxur is highly toxic to birds, fish and honeybees [4]. Several cases of suicidal and occupational poisoning have also been reported [5]. Propoxur have a common mechanism of action toward insect pests and unintended toxicity to non target organisms, including humans, that is, acetylcholinesterase (AChE) inhibition by carbamylating the serine hydroxyl group in the active site of the enzyme in the nervous system, leading to the persistent action of the neurotransmitter, acetylcholine, on cholinergic postsynaptic receptors [6].

It is possible that carbamates may be involved in oxidative stress through the generation of free radicals and changes in antioxidant enzymes. Lipid peroxidation is known to be one of the molecular mechanisms of carbamate-induced toxicity [7]. The study of Waly *et al.* [8] concluded that exposure of animals to diazinon or propoxur are capable of including marked hazardous alterations. The generation of excessive levels of free radicals is one of the basic underlying mechanisms of these changes. Changes observed in oxidative stress markers as a reduction in catalase and SOD activities and GSH concentration support this. Propoxur might also influence general physiological and pathological condition, nutritional status, hormonal function and hepatic metabolism, which may affect immune system [9].

In mammals, some internal organs are usually affected and clinical assessment of extent of toxicity would be necessary by measuring the activity of certain enzymes, which catalyze sensitive biochemical reactions in such tissues. The target of clinical investigation is an evaluation and comparative analyses of tissues, organs and systems that are involved in metabolism, excretion and other processes. Such systems include nervous, hepatic, urinary, hematological and cardiac systems. Obstruction of normal function and changes in the normal level or activities of certain measurable biochemical parameters forms the basis for predicting the course of a clinical investigation. Liver function tests help in the detection, diagnosis and evaluation of liver disease; they also help in monitoring therapy and assessments of prognosis. The major enzymes of clinical significance in liver function are the aminotransferases or transaminases (AST and ALT) and the phosphatases: total, acid and alkaline [10, 11].

The study of Gahelnabi *et al.* [12] concluded that propoxur toxicity caused significant decrease in hemoglobin, hematocrit, and red blood cells count. Biochemically, there were significant inhibition of cholinesterase and a rise in AST and LDH indicative of tissue damage. Increase in creatinine and urea were suggestive of renal damage. Histopathologically, there were degeneration and necrosis of hepatocytes and of cells of the renal convoluted tubules, congestion of the heart, lungs, kidneys, and intestine.

Despite the increasing use of propoxur in Egypt, there is no complete information on the toxic effects of this insecticide in birds. Pigeons are usually fed on the seeds that may be contaminated by the insecticide (propoxur), meanwhile their meat is greatly required as food for people. Therefore, the purpose of this study is to investigate the effect of subchronic oral dosage of propoxur (1/10 LD50) on liver and kidney function tests, also its effects on serum lipids, proteins and glucose.

MATERIALS AND METHODS

Experimental Animal

The bird employed in the present study is the rock pigeon (*Columba livia domestica*) which belongs to order columbiformes, weighing between 320 – 380g. Experimental birds purchased from local market of

Benha city, Egypt. They were apparently healthy, active and free from any abnormalities. Birds were kept for one week under normal conditions of feeding with free access to water before experiments in order to assure their acclimatization.

Insecticide

The carbamate insecticide used in the present work was propoxur. The chemical names are :2-isopropoxyphenyl–N–methyl-carbamate and 2-(1-methylethoxy) phenylmethyl carbamate). The common names are propoxur and PHC . Propoxur has also been called IMPC and IPMC. Ttrade names have included Baygon, Balttanex, Invisi-Gard, Propogon, Sendra, Sendran, Suncide, Tendex, Tugon, Fliegenkugel, Unden and Undene [13].

The required dose of propoxur was mixed with 1gm of wheat dough, formed as pellets, dried, and was given to pigeons by obligatory oral feeding.

Methods

Determination of LD_{50} of propoxur for pigeon (*Columba livia domestica*)

Five groups of pigeons (7 birds each) were treated with a single oral doses of propoxur 30, 36, 42, 48, and 52 mg / kg body weight, respectively . The pigeons died were watched by the end of 24 hrs., and the mortality percentage was determined according to the method of Litchfield and Wilcoxon [14]. This experiment was repeated twice and the average of mortality was taken. The calculated median lethal concentration (LD_{50}) of propoxur for the rock pigeon, *Columba livia domestica ,* at a period of 24 hrs was 38.83 mg/kg body weight .

Experimental Groups: -

Birds were classified into four groups each consists of 5 animals as follow:

Control group

This group, non-treated pigeons, were not subjected to oral administration of the insecticide. Pigeons in this group were given 1gm dried pellets of wheat dough, by obligatory oral feeding (two-days interval between each two consecutive doses).

Three doses group

Pigeons in this group treated with a repeated oral dose (1/10 LD50) of propoxur for three consecutive doses. (two-days interval between each two consecutive doses) and birds were sacrificed after24 hours after the last dose.

Six doses group

Pigeons in this group treated with a repeated oral dose (1/10 LD50) of propoxur for six consecutive doses. (two-days interval between each two consecutive doses) and birds were sacrificed after 24 hours after the last dose.

Nine doses group

Pigeons in this group treated with a repeated oral dose (1/10 LD50) of propoxur for nine consecutive doses. (two-days interval between each two consecutive doses) and birds were sacrificed after 24 hours after the last dose.

Determination of serum Biochemical parameters

Blood sampling

Blood samples were collected by making a puncture in the wing vein, large enough to ensure a free flow, making sure not to take the first drops which contain haemolysed blood, in non-heparinized tubes, and left overnight at 4°C to obtain a full separation of clott. Serum was obtained by centrifugation of the tubes at 5000 r.p.m. for 10 minutes then stored in deep freeze (at -200 C) for kidney function tests.

Determination of liver function tests

Serum aspartate amino-transferase (AST) and alanine amino-transferase (ALT) activities were estimated by UV-method according to Bergmeyer and Horder [15] Serum total protein was determined colorimatrically based on Biuret method [16] by using Bicon Kit (Mittel GmbH and Co. Produktions KG-Hecke 8D-34516 Vöhl-Marienhagen-Germany). Bicon Kit was used for the colorimetric determination of serum albumin according to bromcresol green method (BCG) [17, 18]. Serum globulin can be calculated by subtracting the amount of serum albumin from the serum total protein.

Determination of kidney function tests

Urea was determined by urease Berthelot reaction according to Patton and Crouch [19]. Uric acid was determined by Uricase-PAP method (enzymatic colorimeteric test) using Diamond Kit-Egypt according to Barham and Trinder [20] and Fossati *et al.* [21]. Creatinine was measured using kinetic JAFFE method according to Henery [22].

Determination of Serum lipids and Glucose

The level of glucose was determined by using Bicon Kit as described by Trinder [23] based on GOD-PAP method (enzymatic colorimetric test). Total lipid was estimated colorimetrically by sulfophophovanillic mixture using Diamond Kit-Egypt according to Zollner and Kirsch [24]. Estimation of triglyceride was done by GPO-PAP methods according to Young and Pestaner [25] and Fossatic and Principe [26]. Serum cholesterol was determined as CHOD-PAP method (enzymatic colorimetric test) according to Richmond [27].

Statistical Analysis

Data are expressed as mean ± SE. The level of statistical significance was taken at $P < 0.05$, using one way analysis of variance (ANOVA) test followed by Dunnett test to detect the significance of differences between each group and control. All analysis and graphics were performed by using graphPad Prism software version 5.

RESULTS

Serum AST activity was increased significantly in three doses, and six doses groups, Meanwhile, non-significant change was noticed in serum AST activity after nine doses of propoxur intoxication as compared to the control pigeons. Serum ALT activity in all intoxicated groups showed significant increases as compared to control (Table 1). Significant elevations of serum total protein were noticed for all experimental groups after administration of a repeated oral dose (1/10 LD_{50}) of propoxur. The data shown in table (1) indicated significant increase in serum albumin in all treated groups with a repeated oral dose (1/10 LD_{50}) as compared to that of control pigeons. Statistical analysis for globulin results indicated significant increases in serum globulins concentration in all intoxicated pigeon groups (table 1).

Table (2) illustrates the effects of repeated oral doses of proxour (1/10 LD_{50} each) on kidney function tests. Serum urea concentration was increased significantly in pigeons treated with a repeated dose (1/10 LD_{50}) of propoxur; after 3 doses, 6 doses 9 doses as compared to control pigeons group. Significant increase in serum uric acid concentration .in pigeons treated with repeated doses (1/10 LD_{50}) of propoxur; after 6 and 9 doses compared with control pigeons group as illustrated in table (2). There are no any significant changes were observed in serum creatinine concentration in all intoxicated groups.

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Table 1: Effect of subchronic oral dosage (1/10 LD_{50}) of propoxur on liver function tests of pigeon.

Parameter Mean ± SE	Number of oral doses			
	Control	Three doses	Six doses	Nine doses
sAST (U/l)	59.40 ± 3.25	166.70 ± 4.78 **	145.66 ± 9.02 **	58.0 ± 5.62
sALT (U/l)	46.0 ± 1.41	134.84 ± 0.72 **	137.77 ± 3 **	205.20 ± 21.21 **
Total protein (g/dl)	2.84 ± 0.16	5.24 ± 0.07 **	5.49 ± 0.09 **	6.31 ± 0.22 **
Albumin (g/dl)	1.30 ± 0.06	2.40 ± 0.03 **	2.53 ± 0.05 **	2.45 ± 0.06 **
Globulins (g/dl)	1.54 ± 0.16	2.85 ± 0.07 **	2.97 ± 0.05 **	3.86 ± 0.20 **
A / G (ratio)	0.88 ± 0.09	0.84 ± 0.02	0.85 ± 0.02	0.64 ± 0.04 *

(*) significant difference compared to control group (P < 0.05).
(**) highly significant difference compared to control group (P < 0.01).

Table 2: Effect of subchronic oral dosage (1/10 LD_{50}) of propoxur on kidney function tests of pigeon.

Parameter Mean ± SE	Number of oral doses			
	Control	Three doses	Six doses	Nine doses
Urea (mg/dl)	6.72 ± 0.23	39.04 ± 0.77 **	41.1 ± 0.99 **	38.01 ± 1.13 **
uric acid (mg/dl)	5.68 ± 0.10	6.05 ± 0.32	7.50 ± 0.31 *	6.84 ± 0.27 *
Creatinine (mg/dl)	1.63 ± 0.02	1.63 ± 0.02	1.66 ± 0.02	1.66 ± 0.01

(*) significant difference compared to control group (P < 0.05).
(**) highly significant difference compared to control group (P < 0.01).

Effects of a repeated oral dose (1/10 LD_{50}) of propoxur on serum glucose are presented in table (3). Results showed significant increase in serum glucose concentration after 3 doses and 6 doses of treatment and Non-significant changes were observed after 9 doses. Treatment of pigeons with a repeated oral dose (1/10 LD_{50}) of propoxur caused significant increase in serum triglycerides concentration after 6 doses and non-significant changes after 3 and 9 doses as compared with control pigeons. The data presented in table (3) showed significant increase in serum cholesterol concentration after 9 doses and non-significant changes after 3 and 9 doses of treatment as compared to control pigeons.

Tables 3: Effect of subchronic oral dosage (1/10 LD_{50}) of propoxur on serum glucose and lipids of pigeon.

Parameter Mean ± SE	Number of oral doses			
	Control	Three doses	Six doses	Nine doses
Glucose (mg/dl)	295.80 ± 7.14	399.88 ± 9.87 **	386.78 ± 20.15 **	330.74 ± 13.63
Total lipids (mg/dl)	1185 ± 47.20	1106.2 ± 106.76	1010.4 ± 25.64	1210.66 ± 93.92
Triglycerides (mg/dl)	248.72 ± 4.56	218.84 ± 15.31	316.34 ± 18.49 *	220.43 ± 25.59
Cholesterol (mg/dl)	248.69 ± 6.01	225.3 ± 11.34	253.6 ± 12.86	290.53 ± 19.47 *

(*) significant difference compared to control group (P < 0.05).
(**) highly significant difference compared to control group (P < 0.01).

DISCUSSION

The pesticides tend to become concentrated as they move up the food chain. They accumulate in organism body (target and non-target organisms) which content them. The pesticides don't kill the individual birds often but do affect their bodies so that they lay eggs with very thin shells often these thin-shelled eggs break or the birds are unable to reproduce. Pesticides also affect bird's physiological functions. Natural conservation committee and royal Society for birds protection suggested that indirect effect of pesticides was a major cause of decline birds species [28]. Changes in blood constituents are routinely used to determine various states of the body for clinical purposes or physiological studies. Blood constituents can be used to determine stresses due to intoxication with pesticides, and other environmental pollutants, nutritional and pathological factors.

Alanine amino-transferase (ALT) is an enzyme that helps metabolize protein. When the liver is damaged, ALT is increased in liver and released in the bloodstream, so high level of this enzyme is observed. The estimation of this enzyme is a more specific test for detecting liver abnormalities since it is primarily found in the liver, also this enzyme showed elevated levels during hepatocellular necrosis. Aspartate amino-transferase (AST) is another liver enzyme that aids in producing proteins. It catalyzes the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. Aspartate amino-transferase is the mitochondrial enzyme, predominantly found in liver, skeletal muscles and kidneys. Injury to any of these tissues can cause an elevated blood level. It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury as it can also signify abnormalities in heart, muscle, brain or kidney. The ratio of serum AST to ALT can be used to differentiate liver damage from other organ damage [29, 30]. In the present study ALT and AST were elevated by propoxur intoxication which in accordance with the study of Waly *et al,* [8].

The present work showed that total serum protein, albumin and globulins concentrations were increased significantly in all intoxicated groups of the pigeon. The present results are in agreement with those obtained by Yassin and Shanti [31], who found a significant in serum total protein, albumin and globulins concentrations of farm workers exposed to pesticides compared to controls. Also, other researchers were recorded that a higher levels of total proteins in pesticide-exposed agricultural workers compared with non-exposed controls [32, 33]. The present results do confirm impairments in protein metabolism as a result of pesticide exposure. Serum protein concentrations may be altered due to the toxic effects of pesticides through impairment of protein synthesis by hepatocytes and disturbance of kidney function [31, 34, 35].

On the other hand, serum total proteins of some avian species were decreased as a result of treatment with organochlorine insecticides [36] and with organophosphorus insecticides [37, 38, 39]. The observed reduction in serum proteins of pigeons may be due to several pathological processes including renal damage and elimination of protein in the urine [40]. Saleh *et al.* [41] in their work on pigeons poisoned with synthetic pyrethroid cypermethrin, reported that the insecticide had caused a destructive effect on the lysosomal membranes of different tissue cells, followed by a release of proteases and other protein splitting enzymes, thus accounting for the decrease of serum protein level. In contrast, several investigators reported that serum total proteins of some birds were unchanged after the administration of malathion [42], supermethrin [43] and of benfuracarb [44]. Regarding the effect of some insecticides on blood proteins, the results reported by several authors are contradictory. This contradiction may be attributed to several factors such as the type and dose used, sex of the experimental animals, organ under investigation and period of treatment.

Non-protein nitrogenous substances such as uric acid, urea and creatinine are increased only when renal function is below 30% of its original capacity in birds [45]. Plasma urea appears to be the single most useful variable for early detection of pre-renal causes of renal failure [45]. The elevation of serum urea concentration after a repeated oral doses (1/10 LD_{50}) administration of propoxur to pigeons shows an alteration in normal kidney function which might be related to the propoxur– induced renal dysfunction or may be due to heap to cellular disorder. A similar elevations in serum urea was observed with the chlorinated insecticide in rats [46], and with the carbamate insecticide in mice [47], and in rats [48]. In addition, Cerôn *et al*., [49] observed elevation of plasma urea level at 72 hours of exposure of eel (*Anguilla anguilla*) to a sub-lethal diazinon concentration of 0.042 mg/L. This suggests that probably proteins are being used to meet the increases energy demands during pesticides intoxication. Morever, the overall effect of glucocorticoids (secreted after a stressful stimuli) on metabolism will supply glucose to the organism by the trans -formation of proteins in the liver [50]. An accelerated rate of protein catabolism would result in an increase of amino groups released from amino acids. These groups are converted firstly to uric acids, and secondly to urea in the detoxification process that takes place in liver [49]. Jayasree *et al.* [45] recorded an increase in serum urea in day old male broiler chicks fed on deltamethrin (100 mg/kg feed) for 6 weeks, which may be due to the oxidative damage by free radicals. The elevation of serum urea and uric acid in the present study may be due to the decrease in the glomerular filtration rate induced by kidney dysfunction as a result of the action of propoxur. El-Missiry and Othman [51] reported that in-significant changes in blood urea nitrogen was observed after 1 hrs and 7 days of treatment of rats with a subcutaneously injection with 3.3 mg / kg body weight with lannate. The present results showed significant increase in the serum uric acid after 6 and 9 doses of propoxur (1/10 LD_{50}). Similar observations were reported with pyrethroid insecticide in pigeons [41, 48], and with the carbamate insecticides in rats [46].

Creatinine is the anhydrides of creatine (methyl -guanidinoacetic acid) and a constant constituent of normal human urine and is found in serum in a small amount [52, 53]. Jayasree *et al.* [45] recorded an increase in serum creatinine in day old male broiler chicks fed on deltamethrin (100 mg/kg feed) for 6 weeks, which may be due to the oxidative damage by free radicals. In the present study, repeated oral doses of propoxur administration to pigeons showed nonsignificant changes in serum creatinine . Similar observation was reported in rats treated with methomyl except for a slight, but significant, decrease after the 3rd week of methomyl treatment [53].

Result data showed increased levels of cholesterol and triglycerides in pigeons treated with propoxur. These results coincide with those of Waly *et al.* [8], who found that the serum cholesterol and triglycerides were significantly increased in rats treated orally through gavage with diazinon (10 mg/kg per day in corn oil) or propoxur (10 mg/kg per day in corn oil) for four weeks compared with control animals. Cholesterol and triglycerides levels were considered as valuable indicator of drug-induced disruption of lipid metabolism. Increase of cholesterol and triglycerides levels in pigeons suggest increased synthesis and accumulation of cholesterol and triglycerides. Accumulation of pesticides in the liver is reported to disrupt lipid metabolism and increase serum cholesterol and triglycerides [8, 54]. This disruption may be due to decreased lipoprotein lipase activity in adipose tissue and increased the levels of total serum cholesterol and triglycerides in the affected pigeons [8, 55].

The increase in blood glucose can be viewed as part of stress response triggered by Propoxur intoxication. The mechanism may be attributed to the stimulation produce by Propoxur to secrete epinephrine. This is because epinephrine has been reported to induce hyperglycemia due to its dual action on carbohydrate metabolism; it causes increased liver glycogenolysis and reduction in peripheral utilization of glucose [56]. Similar results were reported by Srivatava and Singh [57] were the treated fish elicited hyperglycemia and glycogenolysis in their blood when exposed to 5.20 and 2.608ppm of formothion and Propoxur for 8 days respectively. In another work by Srivatava and Singh [57] on the acute toxicity of Propoxur on carbohydrate metabolism of India catfish, *Heteropneustis fossilis*, showed a significant increase in blood pyruvate levels at 12 and 48hrs. the results of blood glucose in intoxicated pigeon groups also in accordance with the study of Ezemonye *et al.* [56] which indicated that the pesticide, Propoxur had slight effect on the glucose and phospholipids levels of the fish.

Elevation of serum glucose may be induced by decrease in endogenous insulin release due to a damage of pancreatic tissue [58]. One of the serious effects of organophosphate and carbamate intoxication is the development of acute pancreatitis and subsequent intrapancreatic fluid formation [59, 60, 61]. We recognize the possibility that there was the pancreatic inflammation as a result of the hypoxemic effect on the pancreas [60 - 62]. Hyperglycemia in the present study may be induced by a decrease in endogenous insulin release due to a damage of pancreatic tissue caused by propoxur.

CONCLUSION

Intoxication of pigeons with subchronic oral dosage with propoxur (1/10 LD_{50}) in study, leads to significant increase in serum ALT and AST activities, total protein, albumin, globulins, blood glucose, blood urea, serum uric acid, cholesterol, and triglycerides concentrations in intoxicated groups with propoxur as compared to control pigeons group.

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