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Assessment of non-target toxicity of profenofos insecticide on the aquatic bird; the white egret, *Egretta alba*

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ABSTRACT

Although profenofos is the most commonly used organophosphate in the world, and its residual amounts spread into the environment via air, soil, and water, limited information was found on the toxic effect of this insecticide on birds. Previous toxicological studies on profenofos mainly concentrated on rodents and few studies focused merely on broiler birds. Therefore, this study aimed to assess the toxic effects of profenofos on white egret, *Egretta alba*. White egrets are water birds (Ciconiiformes: Ardeidae) widely spread in all habitats, viz. wetland, fresh and salt habitats. They feed mainly on fish, frogs, small mammals, reptiles, crustaceans and insects. The aggregation sites of this bird are fish ponds, rivers, marshes and channels. In the present study, the median lethal dose (LD_{50}) was determined. Twenty birds were divided into two groups (ten/group). The first group formed the untreated control, while the second group was treated with (1/50 of LD_{50}) profenofos for 5 consecutive days. The toxicological evaluation was performed by assessing changes in body weight gain, relative liver, kidney weights, and serum biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine, as well as the histological changes in liver and kidney. Profenofos caused a significant decrease in body weight gain and relative kidney weights. Serum biochemical analysis revealed significant increases in the activities of AST, ALT, urea and creatinine levels in the treated group compared to the control. Histological results of liver tissue recorded several alterations, such as the focal accumulation of mononuclear inflammatory cells, and the expansion of the hepatic sinusoids with nucleated erythrocytes and congested blood vessels. The renal tissue showed marked hemorrhagic areas in cortical and medullary zones, mild focal interstitial nephritis, and pyknotic nuclei in the epithelial lining renal tubules. These results have potential value to increase public awareness about the excessive use of profenofos.

INTRODUCTION

Birds play a vital part in the environment. They are the most successful, diversified, and evolving group, found in large numbers in the tropics. Bird population reduction is attributed to a variety of factors. Pesticides used in agriculture are responsible for 87 percent of the world's vulnerable bird species **(BLI, 2008)**. Avian species population fluctuations serve as main markers of environmental concerns in ecosystems, while

healthy bird populations act as indicators for ecological safety. The decline in bird populations indicates a deteriorating ecology **(US FWS, 2002)**. Due to their vast distribution, richness, and long life span, birds are considered bioindicators for environmental contamination **(Rothschild & Duffy, 2005)**. In addition, since they graze at various trophic levels, they can accumulate toxins over time **(Boncompagni *et al.*, 2003)**.

The white egret is a global species, found on all continents except for the Antarctica. It is found in a variety of habitats, including wetland, freshwater and saltwater habitats (marshes, coasts, estuaries, river floodplains, the margins of lakes, ponds, reservoirs and mangrove thickets) **(Voisin, 1991; Kushlan & Hancock, 2005)**. Egrets feed mainly on fish, frogs, small mammals, and sometimes small reptiles, crustaceans and insects **(Jones, 2002)**.

Profenofos is the most widely used organophosphate in the world. It is used heavily in cotton growing areas, such as eastern Australia, northern Africa, in addition to different areas of America **(Kumar & Chapman, 2002)**. In Egypt, it is used for controlling Lepidopteron pests of cotton and 12 vegetables (e.g. onion, tomato, sweet potato) **(Greish *et al.*, 2011)**. Organophosphate pesticides (Ops) inhibit acetylcholinesterase (AChE) of all vertebrate species in the postsynaptic membrane of cholinergic synapses within central and peripheral nervous systems **(Bishop, 1998).**

Despite having an economical value to control pests, pesticides are considered toxic to aquatic organisms, especially fishes. The irrational use of these chemicals causes toxicity to non-target organisms and the emergence of environmental pollution. Thus, the use of pesticides has triggered worldwide worry **(Venkateswara, 2004)**.

Living organism can be exposed to pesticides directly or indirectly through contaminated food, water and soil **(Edwards *et al.*, 2013)**. These organophosphate pesticides have negative impacts on the welfare of numerous species of mammals and birds **(Iqbal *et al.*, 2012)**. Birds are usually in direct contact with polluted food, grains, fruits, and fishes that may cause several changes in their metabolic organs due to remaining residues of hazardous pesticides **(Memon *et al.*, 2015)**. Birds are very sensitive to anticholinesterase pesticides for the decreased enzymatic levels of anticholinesterase detoxifying **(Parker & Goldstein, 2000)**. Unfortunately, there is no available literature on the toxicity of profenofos on white egret, *Egretta alba*. Consequently, the present study aimed to evaluate the toxic effect of this insecticide on *Egretta alba*. The results of this study would enhance public awareness of the negative impact of the excessive use of this insecticide profenofos on non-target organisms.

MATERIALS AND METHODS

Chemicals (Insecticide)

Profenofos (Selecron 72% EC; Ciba-Geigy, Pharmacological Company, Scientific office Cairo, Egypt) is a pale yellow liquid, with the following molecular formula: [O-(4-bromo-2- chlorophenyl) O-ethyl S-propyl phosphorothioate]. Other chemicals were of analytical grade and were obtained from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt).

Experimental animals

White egret, *Egretta alba* was selected for the present experiment. Birds (296.2 ± 9.07 g) were collected from Mansoureya Canal, (Giza Governorate, Egypt) by animal dealers. The egrets were apparently healthy, active and free from abnormalities. The collected birds were transferred alive to the animal care house in the Faculty of Science, Ain Shams University. They were kept in their own wooden bird cages. One week prior to the beginning of the trial, the birds were acclimated to the laboratory environment. Birds were maintained at about 25°C of room temperature, with suitable aeration and 12 h light/ dark cycle. Unhealthy individuals were excluded from the study. The birds were allowed free access to small fishes and water *ad libitum*. Birds were humanly treated in accordance with the guidelines of animal care of the National Institute of Health (NIH publication No.86-23, revised 1985). The experimental design was approved by Ain Shams University Research Ethics Committee. All available efforts were exerted to decrease bird suffering. Therefore, a limited number of animals were used to produce reliable scientific data.

Determination of LD_{50} of profenofos for white egret, *Egretta alba*

Seven groups of white egrets (6 birds each) were treated with a single oral dose of profenofos (5,10, 20, 30, 40 and 50 µg/ kg/ body weight, respectively). The dead birds were recorded by the end of 24 hours, and the mortality percentage was determined according to the method of **Chinedu *et al.* (2013)**. The calculated median lethal concentration (LD_{50}) of profenofos for *Egretta alba* at a period of 24 hours was 79.60 µg /kg (Table 1). To measure the LD_{50} of profenofos, the following equation was used:

$$LD_{50} = (LD_{100} - \sum (axb)/n)$$

Where, LD_{50} = Median lethal dose

LD_{100} = Minimum dose required to kill 100%

a = Difference in doses

b = Mean of dead

n = Number of group.

Table1. Determination of median lethal dose of profenofos for the white egret, *Egreta alba*

Group	Dose (µg/bird)	Difference in doses (a)	Number of dead individuals	Mean of dead individuals (b)	Difference in doses x mean of dead (axb)
1	5	0	0	0	0
2	10	5	2	1	5
3	15	5	3	2.5	12.5
4	20	5	4	3.5	17.5
5	30	10	5	4.5	45
6	40	10	5	5	50
7	50	10	6	5.5	55

$LD_{50} = (LD_{100} - \sum (axb)/n) = 50-(185/7)= 23.58$ µg/296.2 g bird=79.60 µg/kg bird

Experimental design

Twenty birds (unsexed) were divided randomly into two groups of ten for each. The first untreated group (control) was given distilled water. The second group was treated with (1/50 of LD_{50}) of profenofos (1.59 µg/ kg/body weight) for 5 consecutive days by oral gavage. Fresh preparation was administered and diluted in distilled water just before use. On the basis of body weight, doses were calculated and accordingly administered. Any clinical signs were recorded within 4 hours of administration of profenofos.

Necropsy and sample collection

After finishing the experiment (final dosing), birds were left for 24 hours then five birds were randomly selected from each group and sacrificed by overdose of chloroform and weighed. Bloods were drawn from the wing vein of each bird by sterilized syringe and collected in simple vacutainers. Blood samples were collected, left to clot and centrifuged at 1,800 x g for 10 min. The supernatant serum was frozen at −20°C for biochemical analysis. Liver and kidney tissues were excised, blotted, weighed then processed for histological assessment.

Biochemical analysis

Stored serum samples were analyzed for aspartate aminotransferase (AST) and alanine aminotransferase (ALT). AST and ALT activities were determined following the guidelines of **Bergmeyer *et al.* (1978)** and using commercial kits from Spectrum, Egyptian company for biotechnology in El Obour City industrial area, Cario, Egypt.

Creatinine and urea levels were estimated according to the colorimetric methods of **Henry *et al.* (1974)** and **Patton and Crouch (1977)**, respectively, using commercial kits from Biodiagnostics Co. (Dokki, Egypt).

Evaluation of body weight and relative organ weights

All birds were weighed using an automatic balance at the start of the experiment (initial body weight) and before dissection (last body weight). The percentage of body weight change was calculated according to the following equation:

Body weight change % = {(last body weight - initial body weight / initial body weight) ×100}

Internal organs; liver and kidney were excised, dried from any adherent tissues and blood and were then weighed. Organ relative weights were calculated with respect to the following formula: {(organ weight/body weight) ×100}.

Gross and histological procedure

Gross examination of any alteration was recorded for sacrificed birds. After weighing the tissues, the samples of each bird's liver and kidney were fixed in Bouin's solution, dehydrated with an escalating sequence of ethanols, cleaned in terpineol and embedded in paraffin. Sections were cut at a thickness of 5 µm, deparaffinized in xylol, rehydrated in a series of ethanols and stained with hematoxylin and eosin (H & E). The slides were then dipped in xylene and mounted with cover slip, using a mixture of distyrene, a plasticizer, and xylene forming a synthetic resin mounting media (DPX) **(Soliman *et al*., 2016; Taha & Soliman, 2019)**. The slides were examined under light microscope.

Statistical analysis

The mean and standard error of the mean (SEM) were used to express numerical data. GraphPad PrismTM (version 5.0) was used to evaluate the differences between the normally distributed means using the t-test for independent samples (GraphPad, San Diego, CA). The statistically significant *P* value was less than 0.05.

RESULTS

General toxicity symptoms

During experimental trial, the birds in the control group were active and healthy with normal behavior. Upon exposure to profenofos, all treated birds showed sluggishness, dullness, depression, decreased water and feed intake (reduced appetite) and general body weakness. No mortalities were recorded during the experimental period.

Evaluation of the profenofos effect on body weight gain and relative organ weights

The net body weight gain in all profenofos treated birds showed a significant decrease, compared to the untreated group (Table 2). There was no significant change in relative liver weight, while a significant decrease was recorded in the relative kidney weight of the treated group compared to the control (Table 2).

Table 2. Effect of profenofos on body weight and relative organ weight of the white egret, *Egretta alba*

Groups	Initial body weight (g)	Last body weight (g)	Body weight change (%)	Relative liver weight (%)	Relative kidney weight (%)
Control	295.2 ± 4.63	306.7 ± 4.79	3.9 ± 0.29	2.93 ± 0.37	2.13 ±0.30
Treated	306.09 ± 3.91	265.6 ± 9.5*	-13.2 ± 2.86***	2.15 ± 0.26	0.71 ± 0.04**

Values are expressed as means ± SEM. Mean values were significantly different from that of the control group; Where, P*<0.005, P**<0.01, and P***<0.001. n=5.

Biochemical results

Table (3) shows that, serum AST and ALT activities of the treated group were significantly elevated ($p < 0.001$) compared to the control group, showing signs of hepatotoxicity. Additionally, significant increases ($p < 0.001$) were detected in kidney biomarkers of creatinine and urea levels of the treated group compared to the control (Table 3).

Gross and histopathological results

Liver

Gross morphology of dissected liver of normal birds appeared normal in both size and color with no marks of toxicity. Microscopic examination of the control group showed normal hepatic plates surrounded by hepatic sinusoids (Fig. 1a). On the other hand, dissected liver of the treated group was more pale in color and small-sized compared to control. Hepatic tissue examination of the treated group showed inflammatory cells aggregation in the hepatic parenchyma (Fig. 1b) and focal accumulation of mononuclear inflammatory cells in the portal area (Fig. 1c), congested blood vessels (Fig. 1d), expansion of the hepatic sinusoids obviously stuffed with nucleated erythrocytes (Fig. 1e).

Table 3. Effects of profenofos on serum biochemical analysis of the white egret, *Egretta alba*

Groups	ALT (U/mL)	AST(U/mL)	Creatinine (nmo/L)	Urea (mg/dL)
Control	40.3 ± 1.4	46.26 ±1.70	40.80 ±0.9	2.14 ± 0.12
Treated	56.9 ± 0.52***	63.74 ± 0.8***	53.4 ± 11.14***	5.73± 0.06***

Values are expressed as means ± SEM. Mean values were significantly different from that of the control group. *P****<0.001. *n*=5.

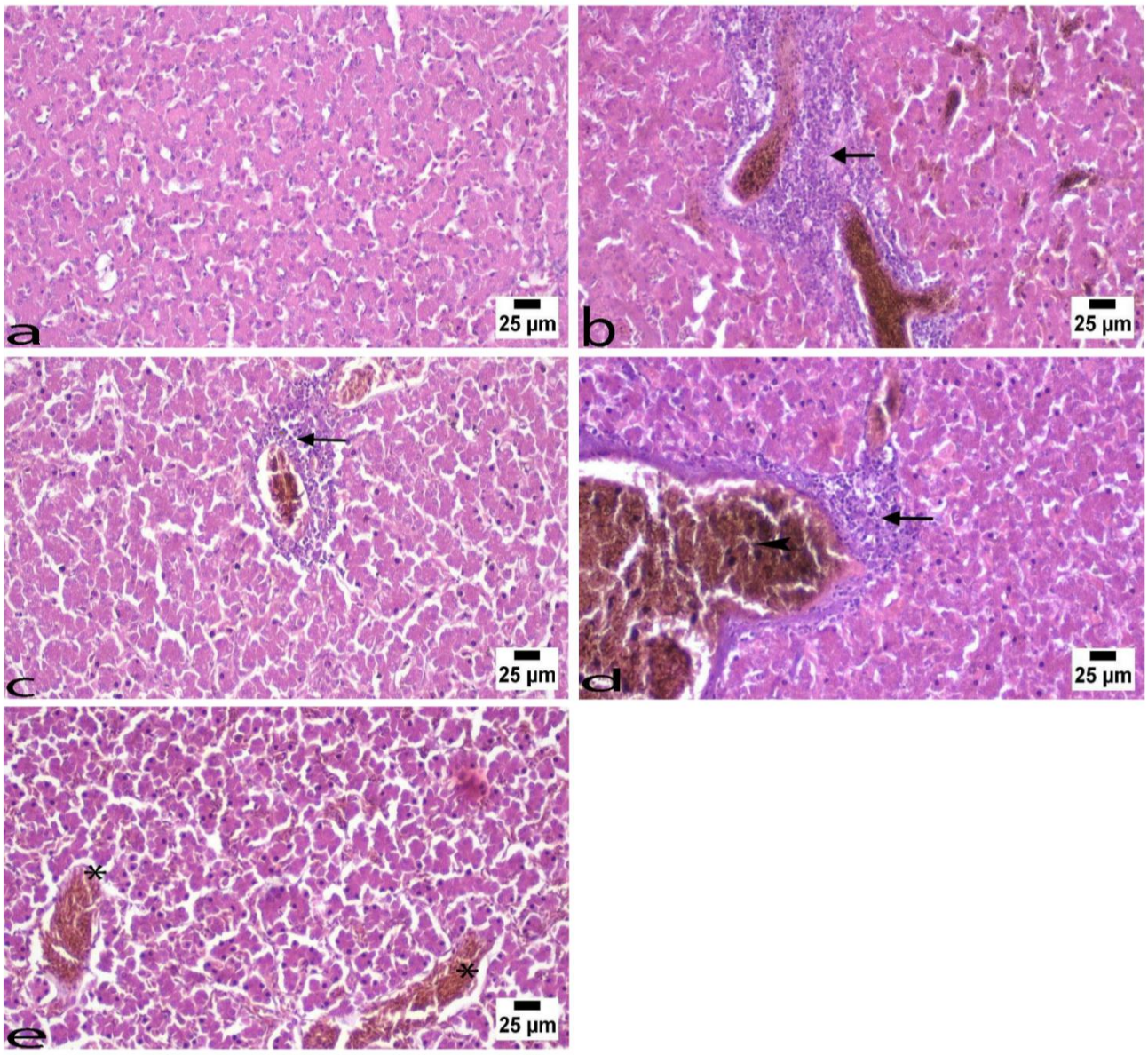

Fig.1. Photomicrographs of liver tissue of white egret, *Egretta alba* stained by H&E showing: **(a)** Normal control group with normal histological structure of liver and normal hepatic plates; **(b-e)** Photomicrographs of liver tissue of treated group with (1/50 of LD_{50}) profenofos stained by H&E showing: **(b)** inflammatory cells aggregation in the hepatic parenchyma (arrow); **(c)** Fewer inflammatory cells aggregation in the portal area (arrow); **(d)** Fewer inflammatory cells infiltration (arrow) and congested blood vessel (arrowhead). **(e)** Marked sinusoidal dilation appeared stuffed with nucleated erythrocytes and congested hepatic sinusoid (asterisks).

Kidney

Gross morphology of dissected kidneys of untreated birds exhibited normal size and color, without marks of toxicity. Histological examination of renal tissue of the untreated group showed normal histological structure of the renal cortex and medullary cones (Fig. 2a, b). On the other hand, dissected kidneys of the treated birds appeared more pale in color and decreased in size, compared to the control. The renal tissue examination of the treated group was characterized by marked hemorrhagic areas, congested blood vessels in cortical and medullary zones (Fig. 2c), severe medullary inflammation with intense inflammatory cells infiltration (Fig. 2d), mild focal interstitial nephritis in the renal cortex (Fig. 2e), and mild necrobiotic changes in the renal cortex with variable number of pyknotic nuclei in the epithelial lining renal tubules in cortical area (Fig. 2f).

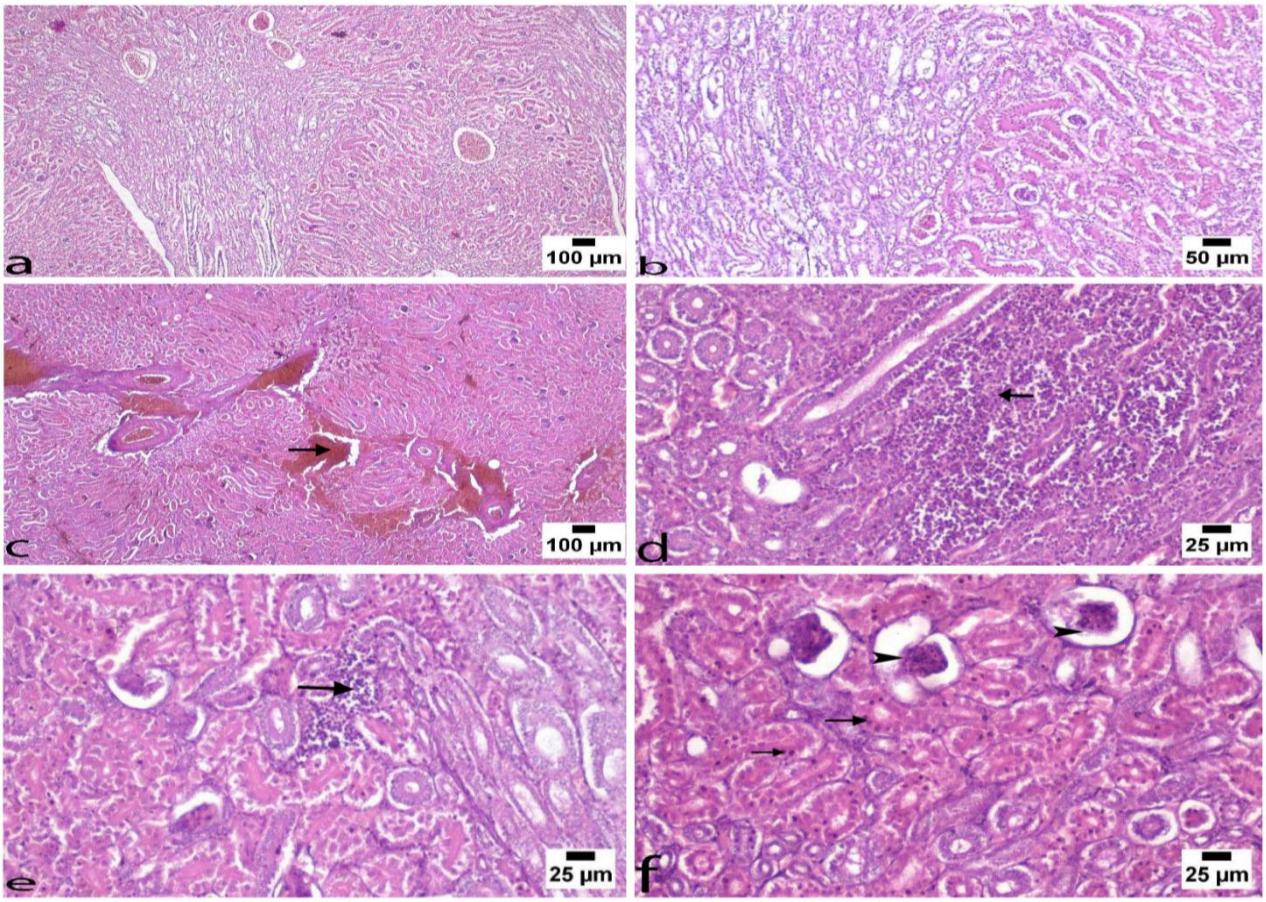

Fig. 2. Photomicrographs of kidney tissues of white egret, *Egretta alba* stained with H&E **(a,b)** Control group showing normal renal parenchyma, with normal cortex and normal medulla; **(c-f)** Photomicrographs of kidney of treated group with (1/50 of LD_{50}) of profenofos stained with H&E showing: **(c)** Excessive hemorrhages and congestion of renal tissue (arrow). (d) Numerous mononuclear inflammatory cells infiltration in the renal medulla (arrow); **(e)** Focal interstitial nephritis (arrow) and **(f)** Mild necrobiotic changes (arrowhead) in the renal cortex, with variable number of pyknotic nuclei in the epithelial lining renal tubules in cortical area (arrow).

DISCUSSION

Profenofos is the most organophosphate widely used around the world for agriculture and domestic purposes. The indiscriminate use of profenofos resulted in the pollution of ecosystem causing hazards to non-target organisms including humans, animals and birds. Continuous use of insecticides even in normal recommended doses may have negative effect on the normal physiological growth processes of non-target organisms. Therefore, this study aimed to clarify the biochemical and histopathological effects of profenofos on white egret, *Egretta alba*.

The present study revealed that LD_{50} of profenofos for white egret, *Egretta alba* is 79.60 µg/kg, which differs from the result obtained in the study of **Kafle *et al*. (2018)**, who determine$1/10^{th}$ LD_{50} of profenofos was 1.6 mg/kg for broiler birds.

The variations in LD_{50} of the current study may be attributed to the variation of species, revealing that *Egretta alba* species is more sensitive to insecticides than boiler birds. This indicates that this species can be used as good bioindicator for environmental pollution due to its high sensitivity for environmental toxicity. The clinical signs appeared during the course of profenofos treatment, including sluggishness, dullness, depression, decreased water and feed intake (reduced appetite), and general body weakness. The appearance of those signs may be due to the accumulation of acetylcholine at the nerve endings, which potentiates inhibition of acetylcholinesterase enzyme, known for its central nervous system effects **(Ghaffar *et al*., 2014)**. Profenofos strongly caused significant decrease in the percentage of the changes recorded in the weights of body and kidney of the treated birds. Remarkably in general toxicity studies, fluctuations in the body and organ weights are strong indicators of potential toxic materials **(Bailey *et al*., 2004)**. **Yano *et al*. (2000)** added the decrease in body weight gain in chlorpyrifos-methyl and chlorpyrifos treated animal groups might be related to toxic stress, oxidative stress and cholinergic stress of pesticides. Moreover, **Mansour and Mossa (2010)** reported that, the decrease in the body weight gain in rats exposed to chlorpyrifos insecticide might be due to the level of stress caused by the Op. Other authors revealed that red-winged blackbirds showed adverse effect on feeding behavior upon exposure to a slight amount of Op (**Nicolaus & Lee, 1999)**. In male starlings, approximately 14% of weight loss was recorded after sub-lethal treatment with the Op **(Grue & Shipley, 1984)**.

Liver is the main organ metabolizing all foreign compounds **(Sarkar *et al*., 2005)**. It is worth noting that, toxic materials entering the body are detoxified in liver **(Baisterri & Shaw, 1987)**. The considerable increase in both ALT and AST serum activities in the current study could be attributed to hepatotoxicity caused by the changes in permeability and leakage of lysosomal enzymes, which induce the release of these enzymes **(Choudhary *et al*., 2003)**. Since ALT is the primary enzyme in the cytoplasm of

hepatocytes, its leakage into the bloodstream and subsequent increase in serum indicates hepatic damage **(Alabdulkarim, 2012)**. The increased AST makes the liver more susceptible to various pathogens and toxicants **(Chamulitrat & Spitzer, 1996)**. Muscle damage, hepatic injury, and toxic hepatitis were all induced by an increase in serum AST and ALT activities **(Farkaset *et al*., 2004)**. These findings are consistent with previous research that linked an increase in ALT and AST serum activities to a negative effect on the liver produced by the pesticide, chlorpyrifos **(Kammon *et al.*, 2010)**.

Results of liver biomarkers confirmed the hepatopathological finding under the toxic effect of profenofos. Treated birds showed several alterations in hepatic tissue, with congested blood vessels and an expansion of the hepatic sinusoids, appearing stuffed with nucleated erythrocytes. In the present study, the portal area of the treated bird's liver showed aggregation of mononuclear inflammatory cell and the presence focal accumulation of mononuclear inflammatory cells. These could be explained as follows: after the oral administration of profenofos, blood supply of the digestive tract reached the liver; therefore, the areas around the portal tract mainly necrosed, and the inflammatory cells were aggregated in differential foci in the liver parenchyma **(El-Bendary *et al*., 2014)**. These histological results agree with those of several authors who addressed the liver of treated animals with $1/10^{th}$ LD_{50} of different Ops and detected blood vessels congestion, degeneration of hepatocytes, with focal infilration of mononuclear cells and periportal fibrosis **(El-Bendary *et al*., 2014)**.

Kidneys represent the major organs that maintain body hemostasis; they are more sensitive to chemicals and drugs causing adverse effect on their functions **(Al-Okbi *et al.*, 2014)**. Elevation of kidney biomarks of creatinine and urea in the treated birds indicates nephrotoxicity. An elevation in blood urea level in the present study could be attributed to the decrease in the rate of glomerular filtration / or total renal blood flow in the treated birds and may reflect an accelerated rate of protein catabolism rather than decreased urinary excretion of urea **(Finco, 1989)**. Moreover, the elevation level of blood urea may be due to faulty excretion, occuring in renal failure **(Hood, 1980)**. Biochemical marker of the elevation in creatinine level in the blood is used as a significant index for toxicity in kidneys **(Finco, 1997)**. **McLauchian (1988)** postulated that, the elevation of serum level of urea and creatinine may be due to the retention of nitrogenous wastes of the impair kidneys' function. Hence, those kidneys fail to excrete these nitrogenous wastes or a failure of these wastes to be delivered to the kidneys could take place as a result of either a decrease in cardiac output resulted in a reduction in renal blood flow or any cause related to circulatory failure. In this context, some authors determined that the increase in serum levels of both urea and creatinine may be a result of kidney damage and renal dysfunction **(Walmsley & White, 1994; Mohseen, 2001)**. Biochemical results of kidney biomarkers confirmed by the pathological findings caused by profenofos. These histopathological findings were as follows: marked hemorrhagic areas, congested blood vessels in cortical and medullary zones, mild focal interstitial nephritis in the renal cortex,

severe medullary inflammation that characterized by intense inflammatory cells infiltration, and pyknotic nuclei in the epithelial lining renal tubules. Lesions in the kidneys indicate nephrotoxicity caused by the tested compound and its metabolites since kidneys are the main way for the elimination of most of the Op compound **(Kammon *et al.*, 2010)**. To the best of my knowledge, this is the first research on the evaluation of the toxic effect of profenofos on the non-target white egret, *Egretta alba*. It was found that profenofos caused adverse effects on non-target white egrets. Consequently, It is recommended to increase the public awareness about the impact of profenfos hazards on non-target organisms.

CONCLUSION

Based on the present findings, profenofos had negative biochemical and histopathological effects on the liver and kidney of non-target white egrets, *Egretta alba*. In the long run, this could cause the extinction of the population of the white egret, *Egretta alba*. To avoid similar mishaps, the public must well understand the proper use of profenofos.

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