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Biochemical and Histological Effects of Cyfluthrinon Liver and Kidney of Quail (*Coturnix couternix*): Potentiating Role of Peg

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Abstract: The aim of this work was to find out the effect of acute single dose level (1/4 LD_{50}) of cyfluthrin onquail and if the amplifying of these effects would occur with the application of polyethylene glycol 600 (PEG-600). The birds were treated with 125mg/kg of cyfluthrin(α-cyano-4-fluoro-3-phenoxy benzyl-3(2,2-dichlorovinyl) either administered individually or in combination with equal volume of PEG and the birds were sacrificed after 96 hours. Control bird received similar quantities of olive oil. Daily body weights were recorded. The histological examination of liver and kidney of cyfluthrin treated bird's revealed disturbances of hepatic lobules, increase of cellular vacuolation and centrilobular necrosis. Kidney tissue showed lymphocytic cells infiltration, marked hemorrhage, hypertrophied glomeruli. Birds groups that treated with the tested insecticide in combination with PEG showed a pronounced increase in hepatic cell degeneration and vacuolation. The kidney tissues showed highly recognized nephritic degeneration, atrophied Bowman's spacesand marked hemorrhage outside the renal corpuscles. Treatment with a single acute dose of baythroid also resulted in a significant increase in glucose, creatinine and total lipids of plasma, significant decrease in plasma total protein and insignificant changes in transaminases activities. The combination with PEG revealed more pronounced biochemical effects. It is noteworthy to point out that the combination with PEG induced more additive toxic effect.

Key words: Cyfluthrin · Peg · Biochemical · Histological · Quail

INTRODUCTION

Liver and kidney are important vital organ in the animal body as they are the site of detoxification and removal of toxic materials that animal is exposed. A foreign body in form of a chemical stress is sufficient enough to give a jolt either mild or severe in their functioning processes. However, some of the synthesized chemicals like pesticides still have received attention as they are highly toxic to different types of animals, including birds [1, 2]. This extent could be magnified for the safe listed chemicals and the joint action of binary combinations. According to Nelson [3], concurrent exposures to a variety of chemical including, industrial agents approximately about two third of 160 of concurrent exposures report are of interactive effects. Synthetic pyrethroids and agricultural adjuvant are such synthesized chemicals, overwhelmingly accepted throughout the globe in farm and industrial practices for different categories. Beta-cyfluthrin of the pyrethroid series is highly toxic to fish [4] and moderately toxic to mammals [5] andwide divergencein toxicityamong bird species [6]. As postulated by many authors, like all synthetic pyrethroids, cyfluthrin is a neurotoxin. It causes hyperexcitation of the nervous system, which leads to convulsions and ultimately death [7]. On the other hand, Polyethylene glycol (PEG) is used in a variety of applications from industrial manufacturing [8] to pesticides formulations [9, 10]. Furthermore, PEG is currently water soluble polymer and widely accepted in therapeutics with market approval for different drugs [11, 12]. Till now pesticide and pharmaceutical adjuvant interaction have not been regulated under the US Federal pesticides because little is known about their bioavailability, environmental fate and physiological effects [13]. Moreover, as reported by many investigators the problem of pesticide interactions with other industrial factors is highly complicated since most of them are

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reactive, unstable and their waste products are characterized by high concentrations [14]. The main objective of this study was to find out whether PEG has a significant effect on the biochemical and histological effect of beta-cyfluthrin at a single acute dose level on the adult quail *Coturnixcoturnix*.

MATERIALS AND METHODS

Rearing of Experimental Animal: The experiment was conducted on acclimated quail weighing 50-70 g, from inbred colony in King Abdulaziz university (Jeddah), maintained at temperature 25+5°C, relative humidity 60±5% and photoperiod of 10 hours per day. All the animals were allowed free access to standard pellet diet *ad libitum*.

LD_{50} Determination: Commercial formulation of beta-cyfluthrin (Bulldock 025SC) was obtained from Bayer Ltd. in the form of emulsion and adequate dilutions were done in water to prepare various concentrations. Freshly prepared solution of beta-cyfluthrin in water in five different concentrations (100, 200, 400, 800 and 1600 mg/kg body weight) either individually or in combination with equal volumes of 5% polyethylene glycol 600 (PEG-600) were given to ten groups, each containing seven individuals of quails orally by gavages tube to determine LD_{50}. The mortality and survival numbers were recorded for each dose after 96-hours. The data were analyzed statistically by log dose/probity regression line method. The statistical calculations were completed by statistical methods given by Fischer and Yates [15]. The calculated LD_{50} of beta-cyfluthrin after 96 hour treatment is 500 mg/kg body weight.

Experimental Design: To assess the potent effects of beta-cyfluthrin, either individually or in combination with PEG-600 three sets each consisting of 10 birdswas considered. The control sets were administered olive oil; Cyfluthrin-treated group was administered with 125 mg/kg (1/4 LD_{50}) and the animals of the 3rd group was administered with the same tested dose level in combination with an equal volume of 5% PEG-600. After 96 hours of oral administration, the animals were scarified and allowed for biochemical and histological investigations. Before sacrificing the animals, the toxicity symptoms body has been recorded.

Biochemical Analysis: At the appropriate time, control and treated birds were sacrificed suddenly. The plasma was collected in heparinized centrifuge tubes, Liver and kidneys were quickly dissected, removed and divided into two equal parts. Each part was divided into two equal parts. One part was weighed carefully, homogenized with 9% cold sucrose solution and kept in deep freezer at -20°C till used. The sera and tissue homogenates were subjected to different biochemical analyses including glucose [16], total protein, [17]; total lipids [18], creatinine [19] and transaminases activity [20] useappropriate BioMerieux Chemicals, Kits.

Histopathological Examinations: At the end of the experimental period, after decapitation selected pieces of liver and kidney were obtained immediately and fixed in 10% formalin-solution. Serial sections were prepared at 5µ then stained with haematoxylin and eosin stain [21].

Statistical Analysis: The data are expressed as mean ± S.E. The data were analyzed by using SPSS (version 11.0) for Windows. Paired samples t-test was used to compare between the data of the control and those of treatments.

RESULTS

Histopathological Aspects

Hepatotoxicity: Histopathological examinations among treated groups showed remarkable pathological changes in liver and kidney cells in response to cyfluthrin toxicity either administered individually or in combination with PEG-600. In liver cells, there was a cloudy swelling, cytoplasm vacuolation and hyperchromatic nucleiand necrobioticchanges (Plate 1 B). Also,sever hyperemia in the central veins and sinusoids, inflammatory infiltrative leucocytes (Plate 2 A) and disorganization of the characteristic pattern of the hepatic cells (Plate 2) were detected as compared to normal architecture in control hepatic tissues . Moreover, focal mononuclear leucocytic inflammatory cells aggregation with hyperemia in the central veins and, granulomatus lesions was seen (Plate 3-A). And (Plate 3-B). Also hypertrophied and hyperchromatic nuclei (Plate 3-C); pyknotic and enlarged nuclei (Plate 3-D) were noticed in PEG-600-combined treatments in association with mononuclear leucocytic inflammatory cells infiltration in portal area and dilatation in the portal vein. Indeed, PEG-combined treatment revealed severe hyperemia, increased mononuclear leucocytic inflammatory cell infiltration and vascular degeration.

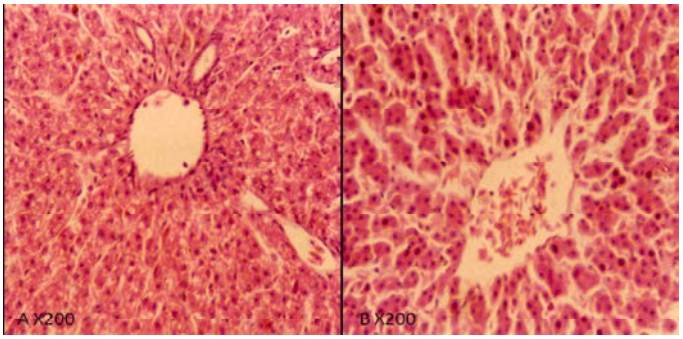

Plate 1: Liver sections of a normal (A,) and cyfluthrin-treated adult quail (B), showing marked disorganization of the hepatic cells, (Heamatoxylin and eosin X200)

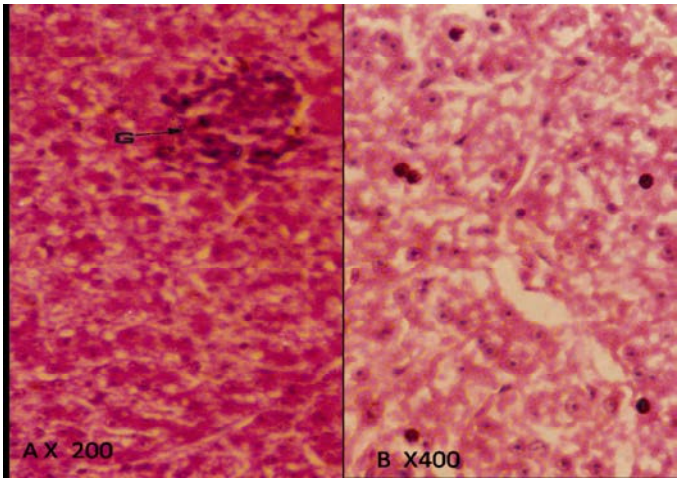

Plate 2: Liver sections of cyfluthrin treated quail, showing, inflammatory infiltrative leucocytes (A,) and disorganization of the characteristic pattern of the hepatic cells (C) , (Heamatoxylin and eosin X400)

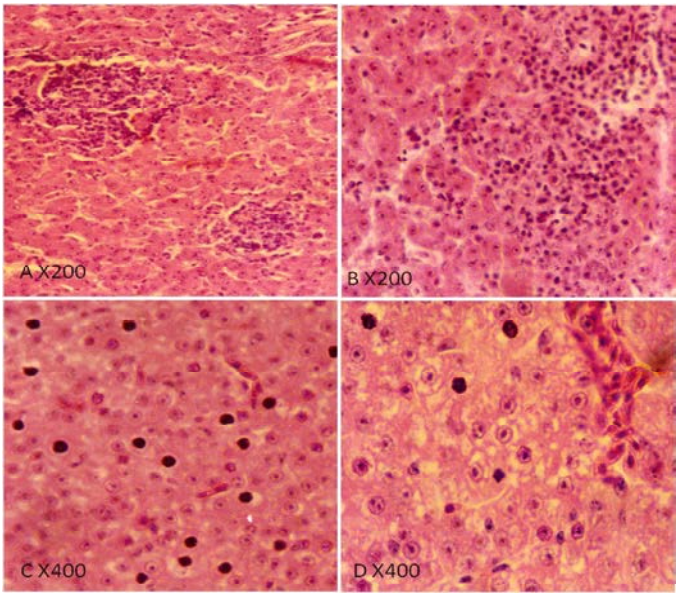

Plate 3: Liver sections of quail treated with /cyfluthrin and PEG, showing granulomatus lesions (A), inflammatory leucocytic infiltration (B) -(Heamatoxylin and eosin X200), hypertrophoid and hyperchromatic nuclei (C); pyknotic and enlarged nuclei (D), (Heamatoxylin and eosin X400)

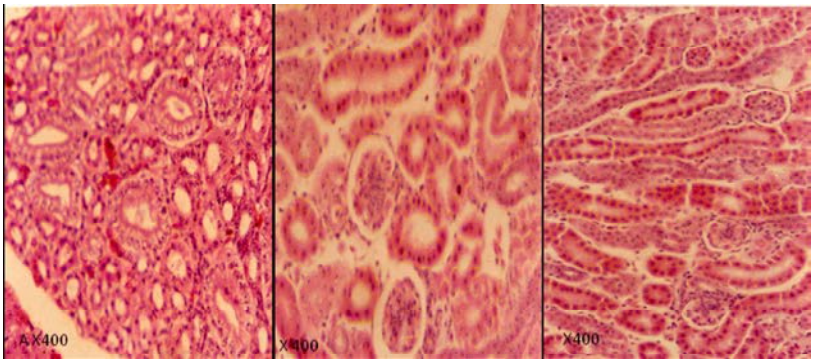

Plate 4: Kidney sections of normal quail showing proximal and distal convoluted tubules, (Heamatoxylin and eosin X400)

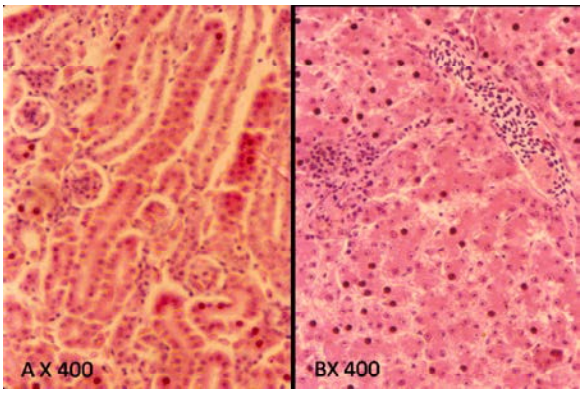

Plate 5: Kidney sections of cyfluthrin treated quail showing shrinkage glomeruli and tubules, destructed glomeruli, diffused stainability of some cells, (A); inflammatory leucocytic infiltration and hypertrophied nuclei (C), (Heamatoxylin and eosin X400)

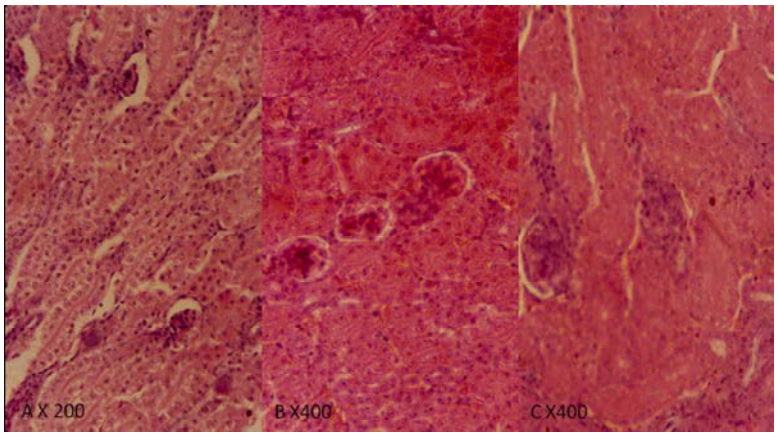

Plate 6: Kidney sections of cyfluthrin and PEG treated quail showing, atrophiedBowman'scapsules (A), degeneration of renal tubules andmarked hemorrhage outside the renal corpuscles (A), necrobiotic changes of epithelial cells of renal tubules (B) , degeneration and necrosis of renal tubules (C), (Heamatoxylin and eosin X400)

Nephropathy: In comparison with the control kidney (Plate 4), pesticide treated group revealed focal mononuclear leucocytic inflammatory cells infiltration in the perivascular area. Sever hyperemic blood vessels and glomeruli were noticed in the cortical portion shrinkage glomeruli, destructed glomeruli, diffused stainability of some cells, (Plate 5-A), marked evidence of hydropic degeneration (Plate 5-B), inflammatory leucocytic infiltration and hypertrophied nuclei () Moreover in the combined treated group,(), the microscopic examination showed marked tubular necrobiotic changes, tubular dilatation, nuclear degeneration, shrinkage glomeruli tuft

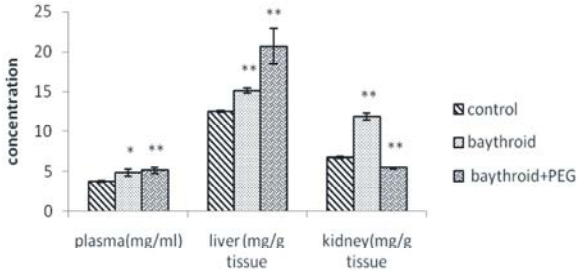

Fig. 1: Effect of cyfluthrin insecticide individually or incombination with PEG on glucose concentrations of different body organs of quail. Each bar represents the mean and vertical lines above denote the S.E.M. of six animals. **p<0.01, *P<0.05 control vs. experimental groups

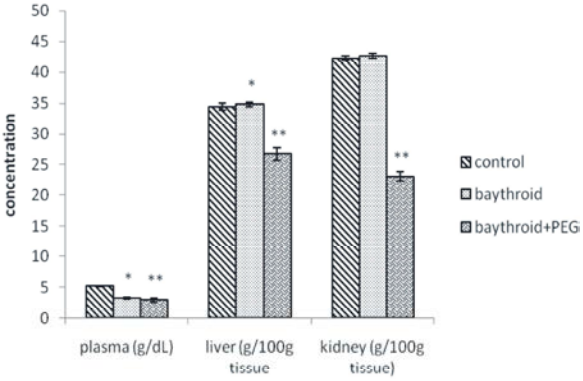

Fig. 2: Effect of cyfluthrin insecticide individually or in combination with PEG on total protein contents in different body organs of quail. Each bar represents the mean and vertical lines above denote the S.E.M. of six animals. **p<0.01, *P<0.05 control vs. experimental groups.

of some glomeruli and focal hyperplasia of the renal epithelial cells. AtrophiedBowman'scapsules degeneration and marked hemorrhage outside the renal corpuscles (Plate 6-A), inflammatory and necrobiotic changes (Plate 6-B) glomerular rupture and release of RBCs (Plate 6-C).

Effect on Biochemical Parameters: The animals treated with pesticide under investigation showed gradual degree of toxic symptoms including, diarrhea and unsteady gait. At the end of experimental duration (96 hours), all the treatments achieved significant decrease in body weights compared to control. The body weight gain in control accounted to 4.57% while ranged between 3.27% to 3.66% and 3.30% to 4.04% following treatment with the acute tested dose of beta-cyfluthrin either administered individually or in combination with PEG-600.

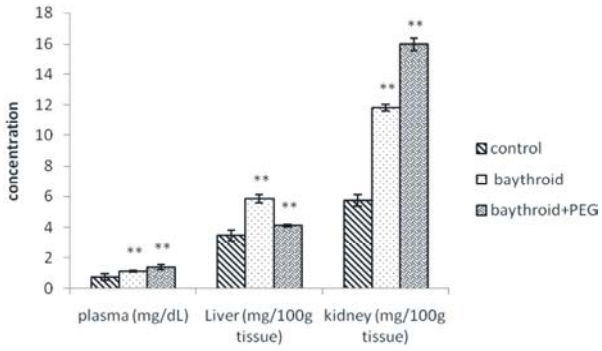

Fig. 3: Effect of cyfluthrin insecticide individually orincombination with PEG on creatinine concentrations in different body organs of quail. Each bar represents the mean and vertical lines above denote the S.E.M. of six animals. **p<0.01, *P<0.05 control vs. experimental groups

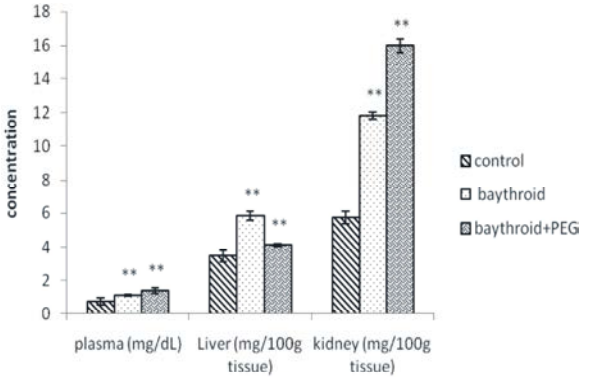

Fig. 4: Effect of cyfluthrin insecticide individually orincombination with PEG on total lipid concentrations in different body organs of quail. Each bar represents the mean and vertical lines above denote the S.E.M. of six animals. **p<0.01, *P<0.05 control vs. experimental groups

The effect of single dose of cyfluthrin on liver, kidney and plasma biochemical's are illustrated in Figs. (1-5). Concurrently, collected data showing that the tested pesticide when administered singly or in combination with PEG-600 attained a general pattern increased effect in glucose, creatinine and total lipids. However, these effects were highly recorded among pesticide plus PEG treated group (Fig. 1). On the other hand, the tested dose levels showed reduced total protein concentration in all the studied organs. However, this reduction was highly recognized in the total protein contents in the kidney tissue, followed by hepatic cells and plasma in a decreasing order (Fig. 2). On the other hand, the present results showed that cyfluthrin caused significant increase

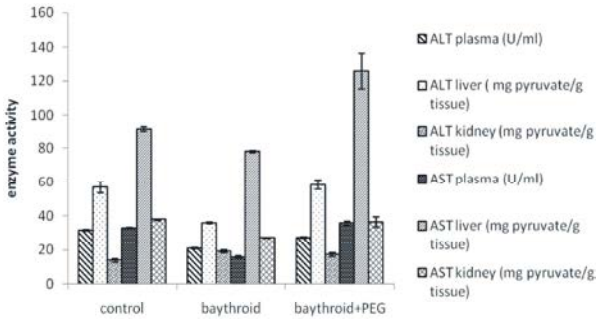

Fig. 5: Effect cyfluthrin insecticide individually orincombination with PEG on transaminases activity in different body organs of quail. Each bar represents the mean and vertical lines above denote the S.E.M. of six animals. **p<0.01, *P<0.05 control vs. experimental groups

in serum and tissue creatinine (Fig. 3). The present study also showed that total lipid content were increased in serum, liver and renal tissues (Fig. 4).

Regarding to the effect of the tested toxicant on the trasnsaminases activity (Fig. 5), the values detected being insignificantly affected. Higher increased effect, however, was obtained in AST activity of liver and renal cells in the animals that administered in combination with PEG-600.

DISCUSSION

The LD_{50} value we obtained in quail (*Couternixcouternix*) approximates the value presented in the report on beta-cyfluthrin of Addy-Orduna *et al*. [6]. At the end of experimental duration (96 hours), all the treatments achieved significant decrease in body weights compared to control. Several investigators have reported body weight decreases in experimental animals exposed to different types of pyrethroid insecticides including rats [22], rabbits, [23], mice [24] and white leghorn [25]. Previous work indicated a highly significant difference in food and water intake [26] of pyrethroid intoxicated animals. This difference, in addition to the observed diarrhea physiological disturbances and cellular and tissue damage may be indicative of body weight loss.

Histopathological examination of liver of quails treated with cyfluthrineither administered individually or in combination with the tested polyethylene glycol, revealed severe inflammation and degenerative changes in hepatocytic characterized by swelling in hepatic cells with increasing granularity of the cytoplasm. However, with PEG, there was a marked increase in the intensity of degenerative changes increased congestion of blood vessels, individual and multiple cell necroses was also observed. Moreover granulomatous lesions, inflammatory leucocytic infiltration hypertrophied and hyperchromatic, pyknotic and enlarged nuclei were evident. The present findings are in consequence with the results of the previous investigations [27, 28] after exposure to different types of pyrethroid insecticides including cyfluthrin. The cytoplasmic vacuolation, cloudy swelling, mononuclear leucocytes inflammatory infiltration and the remarkable abundance of cell vacuolation after intoxication with cyfluthrin either administered individually or in combination with PEG has been subjected to different speculations like; defensive mechanism for collecting the injurious elements [29], breakdown of lipoprotein complexes [30] and mixed micelles actions [31, 32]. Those authors considered hepatocellular damage to be from a reduction in ATP supply. Other reports had elucidated that hepatocellular damage could be correlated with the disrupted enzymes activities [33].

Histological examination of kidney of cyfluthrin - intoxicated birds revealed many alterations such as tubular degeneration, atrophy of glomeruli, leucocytes infiltrations and congestion of renal blood vessels. Such observations were reported by Grewal *et al*. [26] and Tos-Luty *et al*. [27]. Susceptibility of the kidney to toxic injury appears to be related to the complexities of renal anatomy and physiology as reported by Hodgson and Smart [34]. Signs of toxicity and consequent activation of the defensive mechanism could be observed in the form of hyperemia in glomeruli with focal area of necrosis, inflammatory infiltration with perivascular area among all treatments. Severe effects however were attained in the animal group that exposed to mixed treatment. Similar observations had been recorded by Tos-luty *et al.* [27]. Post pyrethroid toxicity; induced oxidative stress, changes in cell morphology and tissue injury [35] and in the cytoskeleton occur during cell injury, oxidative stress is generated by stimulated leucocytes in the areas of inflammation associated with tissue injury. On the other hand, the present results showed that cyfluthrin caused significant increase in serum and tissue creatinine. This indicates diminished ability of the kidneys to filter these waste products from the blood. Similarly, Mongi *et al*. [36] reported an increase in the serum urea and creatinine in rats exposed to deltamethrin and this toxicity are

attributed to its free radical induced oxidative damage. Cellular creatinine levels depend largely on the glomerular infiltration. The change in its concentration together with the histological results indicates a reduction in the glomerular filtration rate as a result of cyfluthrin intoxication. It seems from the aforementioned data that, the combination of the tested insecticide with PEG follows the synergism phenomena.

Theactivity of ALT and AST enzymes in sera and kidney and liver homogenates of treated birds showed fluctuated insignificant changes throughout the tested organs. Higher changes, however, was attained in liver ALT and AST when the tested insecticide was administered in combination with PEG-600. On the other hand, there was a decreased effect in the activity of the studied enzymes when cyfluthrin was administered individually. The decline in the transaminases activities in liver and tissue homogenates as a result of cyfluthrin toxicity may be investigated with the reference of the total protein decreased effect. On the other hand, the elevation in the plasma transaminases activity may reflect the induced hepatic and renal cell injury. These findings are highly supported by Abou El-Magd *et al*. [37]. Concurrently, when the tested pesticide is administered in combination with PEG, transaminases activity was highly affected. Till now little is known about the physiological effect of PEG either administered individually or in combination with the tested pesticide. It is worthy hear to point out that the intensity of the toxicity syndromes were more obvious in animal group treated with the pesticide plus PEG which support Potentiating mixed toxicity which may be related to different metabolic pathway and or to the production of more toxic intermediates. According to Burr and Ray [38], the toxic effect might be varied as a result of differences in pesticides potency and structural modification which potentiate the mode of actions of the tested pesticide.

Concerning, the effect of the tested pesticide on total protein content, the reduced effect might be attributed to disturbances in amino acids level [39], renal dysfunction [40] and disturbance in liver function which in turn affect protein balance. According to El-zayat *et al*. [41]. Free amino acids were significantly reduced in animals that received different types of pesticides. The biochemical and histopathological findings recorded in the present study evidence these contributions. On the other hand, the combined treatment with PEG revealed more pronounced effect on total protein deterioration which may be related to more toxic metabolic effect. Moreover, according to Addy-Orduna *et al*. [6], decreased protein after exposure to beta-cyfluthrin is a consequence of lyses of structural proteins, increased mutagenic activity and excessive cell proliferation.

Regarding to the results obtained for sera and tissue glucose content of beta-cyfluthrin intoxicated bird, the recorded values showed transitory high level of impaired glucose as compared with non-treated group. Our findings are in agreement with Koundinya and Ramamurthi [42]. Elevated blood glucose may be resulted in abnormal glucose oxidation in citric acid cycle [43] also it might be contributed to either a defect in insulin secretion or insulin resistance [44].

The present study also showed that, total lipids contents were increased in serum, liver and renal tissues. Our results are in accordance with the findings of El-Magd *et al.* [37] and Abdel-Rahim *et al.* [45], which recorded different increases in the total lipids in different body organs as a result of intoxication with different types of pyrethroids and brings about an enhanced fat metabolism [46] due to pesticides exposure. Other investigators postulated that, pyrethroid insecticides have the ability to affect NADPH, which is necessary for lipids synthesis [47], also, the present findings support the assumption that PEG, increase cyfluthrin toxicity on the basis of total lipids content. Otherwise, the potent increased effect both in serum, liver and kidney total lipids of cyfluthrin plus PEG may be attributed to accelerated rate of synthesis and release of more fatty acids on the adipose tissue. Moreover, the recorded effect may be attributed to the effect of the tested materials on some hormonal activity affecting lipid mobilization, like catecholamines [48] and insulin hormones [37].

CONCLUSION

The current study support of cyfluthrin toxicity on *Couternixcouternix* on the basis of histological and biochemical changes Also, the present work concluded the achieved potentiating toxicity effect of PEG.

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