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TOXICOLOGICAL IMPACTS OF MALATHION WITH PEG-600 AS FORMULATING ADJUVANT

Sayed M Rawi[1], Mansour A Al-Hazmi [2] ,Seif El-Nasr F 3 and Ayed S Al-Logmani[4]

[1,2]Faculty of Sciences and Arts, King Abdulaziz University, Khulais, Saudi Arabia
[3]Cairo University, Faculty of Science, Egypt
[4]Faculty of Sciences and Arts, Jeddah University, Khulais, Saudi Arabia.

***Author for Correspondence: Sayed M Rawi**
Faculty of Sciences and Arts, King Abdul Aziz University, Khulais, Saudi Arabia.

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ABSTRACT

The effects of the organophosphorus insecticide, malathion and polyethylene glycol-600 as adjuvant, were evaluated on the quail *Coturnix coturnix*. These compounds were tested separately and as a mixture because they can be applied together for different applications and therefore have the potential acts as a binary mixture. The acute mortality (LD_{50}) after 96 hours were estimated to be 583 and 395 mg/kg. for malathion individually and In combination with the studied adjuvant, respectively. At the dose level ¼ LD_{50} malathion alone or in a mixture with PEG was found to induce severe histopathological changes in liver, brain and kidney tissues and has a potent effect on the levels of plasma total protein, albumin, total lipids, and ALP, GOT, GPT and AChE activities. However, exposure to the mixture caused more severe histological changes and significantly induced greater effects in the studied biochemical than either compound alone. These results indicate that agricultural adjuvants and pesticides may cause more damage as a mixture than either product alone. Therefore, future evaluations of pesticide effects should consider the effects of adjuvants as a mixture with pesticides when these products are recommended to be applied together for pest control.

KEYWORDS: Malathion - Polyethylene glycol – Biochemistry – Histopathology.

INTRODUCTION

Malathion is an OP insecticide that is used mostly in agriculture and in public health programs to control infestations of insects (U.S. EPA 2006). In addition to the use of malathion in plant applications, it is a key component of personal hygiene products used for lice control (ATSDR 2003). Currently, malathion is still used in a large scale in agricultural sector and public health programs all over the world. (U.S. EPA 2008). As postulated by many investigators, it is toxic to birds, fish, and other wildlife.

The toxicity of malathion is compounded by its metabolites and contaminants. Malaoxon, the metabolites produced by the oxidation of malathion in mammals, insects, and plants, is the primary source of malathion˝s toxicity and it is 40 times more acutely toxic than malathion (U.S. EPA. 2006,2008).

Additionally, several studies showed that malathion and their metabolites induced various physiological, biochemical, immunological, genetic, and histological changes in experimental animals (Rezg et al. 2007; Saadi et al. 2008; Al-Attar, 2010). Moreover, in laboratory tests with birds, malathion disrupted normal thyroid hormone function and caused genetic damage (De Coster and van Larebeke 2012).

Along with the pesticidal application millions of tons of industrial surfactants are used annually by the textile, paint, cleaning supplies and agricultural industries, and use is increasing (Upham et al. 2014). The agricultural industries rely on adjuvants to emulsify water-insoluble pesticides to optimize the spreading, retention and uptake of active ingredients. These adjuvants can constitute up to 90% of pesticide formulations (Upham et al. 2007). Many of the surfactant types (e.g., polyethylene glycol ethers, alkyl benzene sulfonates) with different molecular weights, different physical properties and are blends of heterogeneous compounds. Since PEGs are considered by industry as „inert ingredients˝ they are widely used In the pharmaceutical and pesticides formulations. They are chemically and biologically active compounds and produce pronounced effects in plants and animals including birds (Bendele et al. 1999; Comber et al. 1993; Edginton et al. 2004;Székács et al. 2014) Moreover, some adjuvants have the potential to

induce behavioral (Rodrigue et al. 2011), biochemical (Wieder and Davis 1983) and histopathological effects (Agrawal et al. 2015). Moreover, reported recently the non-ionic surfactants like polyethylene glycol are partially degraded to more toxic environmentally persistent metabolites (Wang et. 2013; Olkowska et al. 2014), They can be toxic for different types of organisms and disturb their endocrine balance Moreover, they can interact with different interfaces and change natural processes.Moreover, some surfactants are also caused integrated inhibition of mitochondrial FAO (Guo et al. 2013), elevated synthesis of fatty acylcarnitines (Schooneman et al. 2013) significantly reduced hepatic activity of catalase, DNA damage and lipid peroxidation, which are directly related to an imbalance in the redox state (Navarro and Martinez 2014) of different animals including birds. However, no one has examined the effects of PEGs commonly used in pesticide formulations in birds, even though it is known that these chemicals can be more toxic than the active ingredients and due to scarcity of related researches, a knowledge gap has been observed regarding the scope of polyethylene glycol use in pesticides formulations. Hence, this study aims to investigate the underlying effects of PEG-600 use in malathion toxicity of PEG as adjuvant on malathion-induced physiological and histopathological alterations for birds using the quail (*Coturnix coturnix*).

Materials and Methods

Animals

Adult Quails (*Coturnix coturnix*) of both sexes weighing (100-150 g) were obtained from the Experimental Animal Unit of Research Center, Cairo University, Egypt. The birds were reared in plastic cages of 100x100x80 cm3 at the animal house, unit, zoology department, faculty of science, Cairo University under suitable conditions, water and feed were supplied *ad libitum*. Before doing the experiment, the birds were acclimatized for two weeks to the laboratory conditions. They were exposed to a 12 hour day and night cycle and were provided access to food and water 24 hourly. Experiments took place between 10:00 and 15:00 h. The protocol of the study was approved by the Institutional Animal Care and Use Committee (IACUC).

Pesticide and Adjuvant

Malathion: An organophosphate pesticide, has the chemical formula $C_{10}H_{19}O_6PS_2$ (diethyl dimethoxyphosphinothyiol) Thiobutanedioate). It was donated as technical form by Novartis Egypt. The indicated dose level was 145.7 mg/kg dissolved in corn oil.

PEG-600: Polyethylene glycol 600 di-laurate (PEG-600) was available in emulsifier form. It was donated by the Egyptian Company of Starch, Yeast and Detergents in emulsifier form with an average molecular weight of 600. PEG provided for use in this experiment comprised a clear and viscous liquid when kept at room temperature.

Experimental design and treatment

Adjuvant-pesticide dose preparation

Adjuvant-pesticide mixture was prepared by mixing the tested dose level of malathion dissolved in 1 ml of corn oil with PEG-600 glycol (5g %) on the basis of (1:1) volume.

Toxicity testing

After the acclimatization period, quails were divided into four groups comprising 30 animals in each group as follows. -Group 1 quails were untreated, given only corn oil and served as control. -Group 2. Experimental animals were orally given varying doses of malathion dissolved in corn oil for a period of 24 hours. -Group 3. The animals were orally given polyethylene glycol (PEG-600) at a dose level of 50 mg/kg BW and after 3 hours treated with malathion at the same doses given to group 2. -Group 4. Animals were treated with PEG-600 at the same dose given to group 3. After 96 hours of exposure, the number of the dead animals was counted. The probit analysis was used to calculate the lethality percentiles of the studied toxicants by the aid of NCSS 2007 software. After LD_{50} determination, stock solutions were prepared on the basis of 1/4 LD_{50} values of the studied toxicants.

Biochemical analysis

After 2 weeks of acclimatization, 160 animals of nearly a similar weight were selected, separated into 4 groups, each was orally given one of administering the treatment regimen for a period of one week.

–Group 1. Quails were administered 1 ml corn oil and regarded as control.
–Group 2. Experimental animals were orally given malathion at a dose level of 145.7 mg/kg in 1 ml corn oil.
–Group 3. The animals were orally given malathion (145.7mg/kg in 1 ml corn oil)+PEG-600 (1 ml 5% PEG)
–Group 4. Animals were treated with PEG-600 at the same dose given to group 3.

At the end of administering the treatment regimen, at morning time, the animals were sacrificed by sudden decapitation. Blood was collected in heparinized tubes, centrifuged at 2000 rpm for 20 minutes, and blood plasma was then collected and stored at -4°C prior immediate determination of total protein, albumin, uric acid, glucose, glutamic oxaloacetic acid transaminase (GOT), glutamic pyruvic acid transaminase (GPT), alkaline phosphatase (ALP), and acetylcholinesterase (AChE). All of these parameters were measured using reagent kits purchased from Biodiagnostic

Histopathological examination

For light microscopic examination, at the end of the tested period, liver, kidney and brain cerebral tissues from each group were dissected and rapidly fixed with 10% buffered formalin saline. After fixing for 3 to 4 days, the tissues were cleared, and embedded with paraffin. After a routine processing, paraffin sections of each tissue were cut into 5µm thickness and stained with haematoxylin and eosin.

Statistical analysis

The data obtained was analyzed by using SPSS program version 17 and are represented as mean values of standard deviation, i.e. Mean±SE. The statistical results were further calculated by One-way analysis of variance (ANOVA) followed by LSD (Least significant difference) for comparison between groups.

RESULTS

Toxicity testing and general toxic observation

As recorded in the table (1), the toxicity of the tested insecticide was decreased by -32.247% in combination with PEG. The intoxicated animals displayed noticeable behavioral changes as general weakness, salivation, variable sequence of motor symptoms and convulsions. However, the symptoms were obviously recognized with the tested mixture.

Biochemical change

Fig.1, and Table 1 shows the values of plasma AST, ALT, ALP, total protein, total albumin, uric acid and glucose and brain AChE in all the experimental groups. In comparison with the control values, the levels of AST, ALT, ALP, uric acid and glucose were statistically increased, while the levels of total protein, total albumin and AChE were significantly decreased in quails rats exposed to malathion.

Significant elevations in the levels of plasma AST, ALT and uric acid were observed in rats exposed to malathion plus PEG-600, while the levels of total protein, total albumin, glucose and AChE were statistically decreased. Except for glucose content, significant decrease in the levels of all the studied plasma biochemical and elevation in the activities of study enzymes was noted in birds treated with only with PEG-600.

Histopathological effect

Histopathological effects of malathion on the liver of treated birds presented in Figs. 3-5. The histological structure of quails treated with malathion showed varying degrees of changes represented as, damage of liver structure, massive necrosis,. hepatocytes dissociation, marked sinusoidal dilation and multiple focal mononuclear cell aggregations. In PEG-600 treated group, the histopathological changes were represented as marked cellular vacuolation and focal lymphocytic aggregations (Fig.6). In malathion+PEG-600 treated animals (Figs. 7-9), the examined liver sections showed, multiple focal areas of parenchymal cell necrosis associated with mononuclear cell infiltration marked hepatic necrosis associated with hemorrhage. Figures 10-17 shows the histological structures of the kidney in the control group (Figure 10), malathion treated group (Figs 11-13) and malathion plus PEG-600 treated group (Figs.14-17). In comparison with the control, the examined renal sections of the animal group intoxicated with malathion showed pronounced changes represented as, tubular pyknosis of the epithelial lining, focal mononuclear cell aggregation, vacillation, hemorrhage and swelling of epithelial lining Bowman, s capsule and glamour tufts. Additionally, the histopathological changes of birds treated with only with PEG-600 showed tubular pyknosis of the epithelial lining, cellular infiltration, focal mononuclear cell aggregation and vacuolation.In the animal group treated with malathion plus PEG-600, the examined renal sections showed peritubular leukocyte cell infiltration together with karyopyknosis of renal tubular epithelium., vacuolar degeneration, few inflammatory cellular infiltration and marked interstitial cellular infiltration. Moreover, sections of the brain cortex of quails treated with malathion, PEG and malathion plus PEG were also examined (Figs. 18-23). The main changes in the sections from brain cortex of birds treated with malathion revealed neuronal degeneration, pyknosis and necrosis of some purkinge cells (20). In The animal group treated only with PEG-600, the examined sections showed demylination of nerve fibers (gray matter) and pyknosis. (Fig. 21). Moreover, the examined neuronal sections of the brain cerebral cortex of birds treated with malathion plus PEG-600 showed marked congestion and focal minute hemorrhage and pyknosis of some neurons (p) (Fig. 23).

Table 1: Median lethal concentrations (LD50) (96 hours duration) of malathion when administered individually or in combination with PEG-600 to quails *(Coturnix coturnix)*.

Treatment	malathion	malathion +PEG
LD50 (mg/kg)	583	395

Table 2: Plasma AChE, total protein, total albumin, uric acid and glucose in quails treated with malathion, malathion plus PEG-600, and PEG-600.

Parameter	Control	PEG	Malathion	PEG+ Malathion
Total protein (g/dl)	5.03±0.13	2.968±0.04*	3.78±0.09*	2.96±0.04*
Albumin (g/dl)	1.606±0.02	0.720±0.03*	1.233±0.048*	1.19±0.04*
Uric acid (g/dl)	5.297±0.05	3.783±0.12*	7.518±0.074*	10.10±0.07*

Glucose (Mg/dl)	323.7 ±0.82	369.69±0.87*	480.25±1.44*	206.05±1.91*
AChE (µMSH/min./ml)	2.339±0.09	1.832±0.11*	2.035±0.015	1.498±0.143

Values are Means ±SE from six animals in each group
Data were analyzed by student "t" test
*Significant (P<0.05)
Pesticide treated group is compared to control group
Pesticide+adjuvant is compared to PEG-treated group

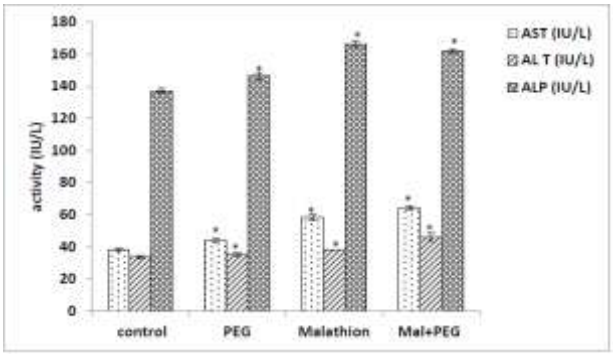
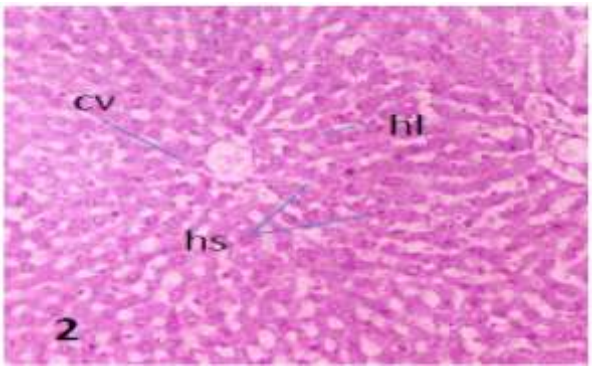
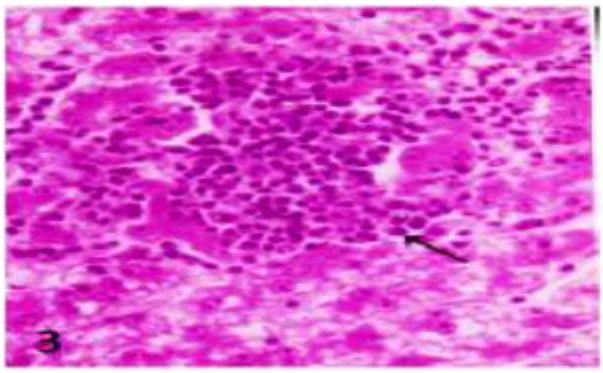
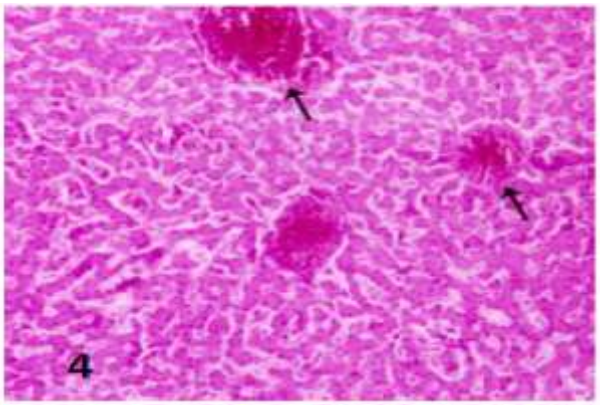
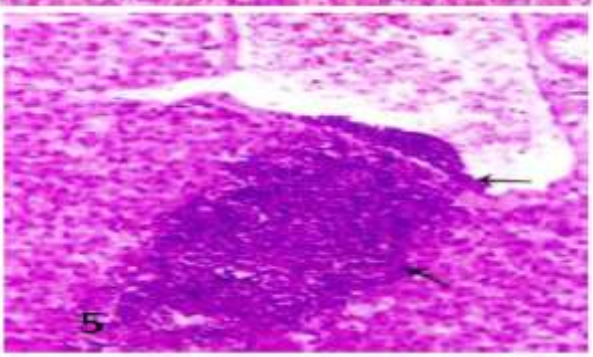


Fig. 1: Transaminases and alkaline phosphate activity in plasma of *Coutrnix coutrnix* after exposure to 1/4 LD_{50} of malathion individually or in combination with PEG.

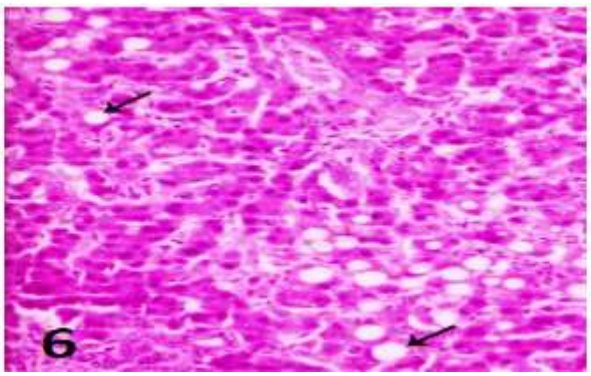


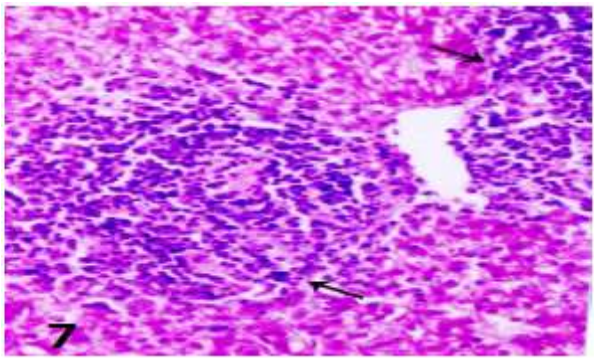


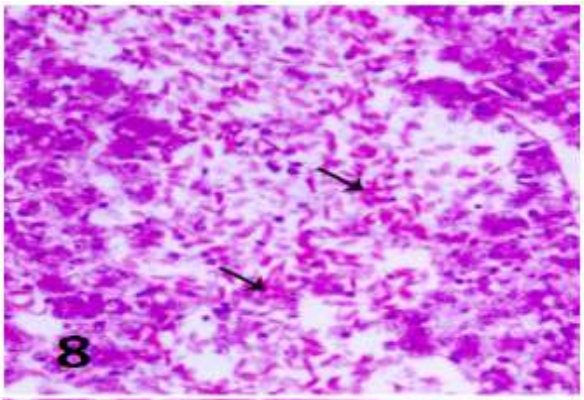


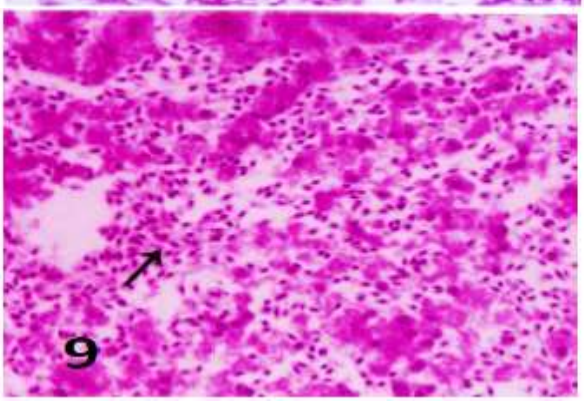


Figs.2-5: Section of liver of, normal adult quail (2) showing normal hepatic lobules (hl), central vein (cv) and hepatic strands (hs) and malathion treated quail (3-5) showing, focal mononuclear cell aggregation (arrow) (3), congestion of central veins and hepatic sinusoids (arrow) (4), and focal lymphatic aggregation and sinusoidal leucoctosis associated with vascular permeation with lymphocytes (arrow) (5). [Figs. X200].

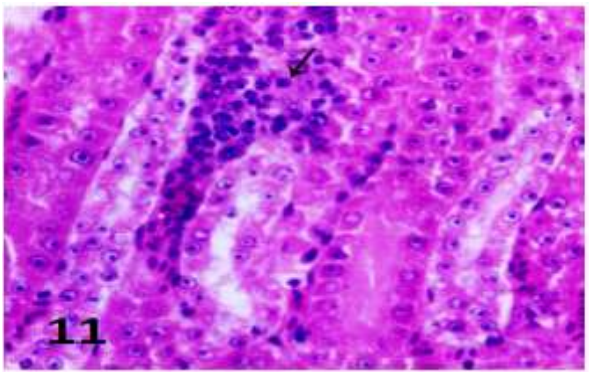


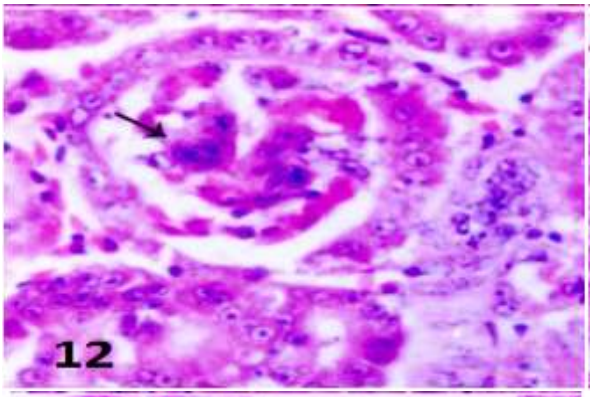


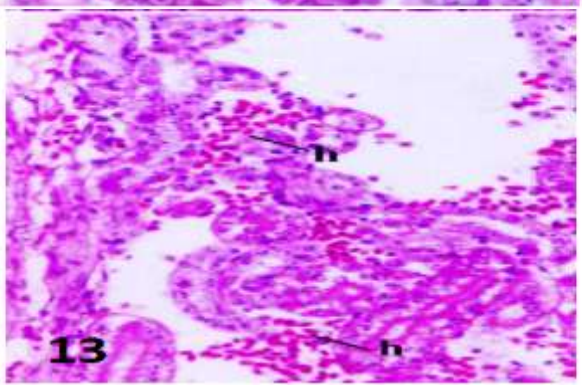




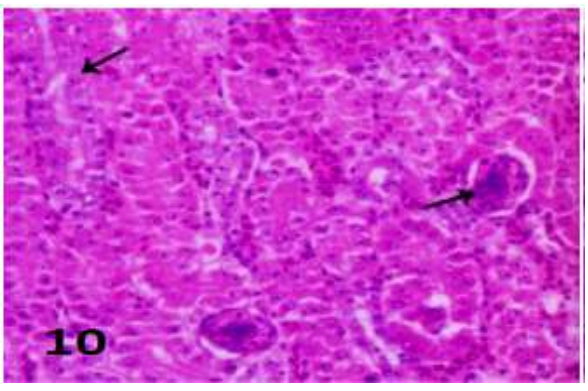
Figs 6-9: Section of liver of, PEG-600 treated animals showing, hepatocellular vacuolation (6), and malathion+PEG treated animals (7-9) showing, multiple focal areas of parenchymal cell necrosis associated with mononuclear cell infiltration (arrow) (7), marked hepatic necrosis associated with hemorrhage (arrows) (8,9). [Figs X200].

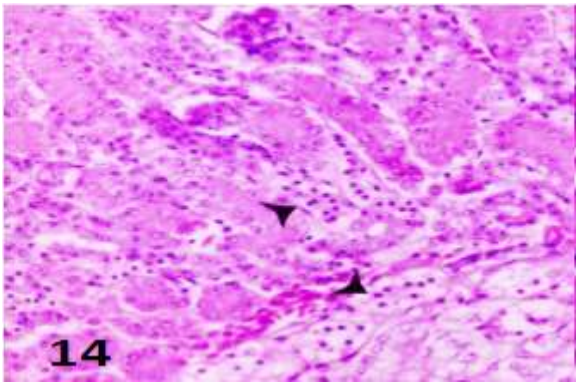


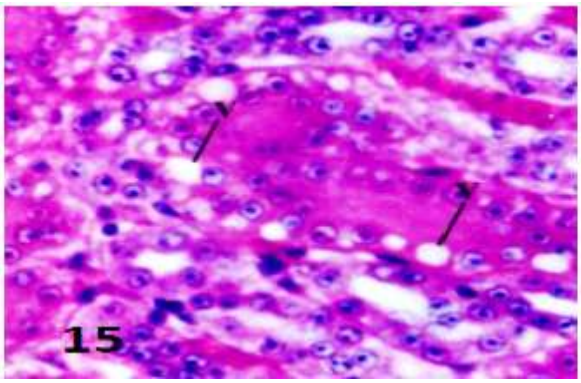


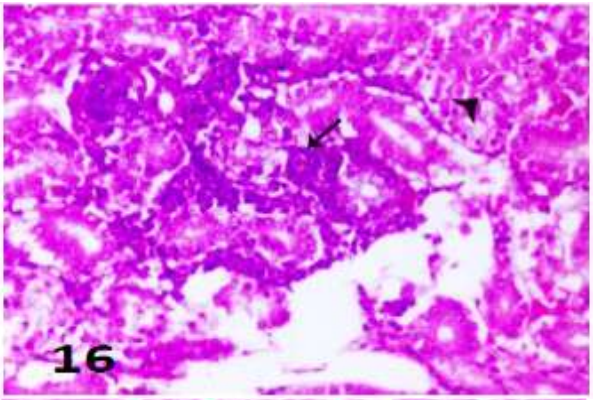


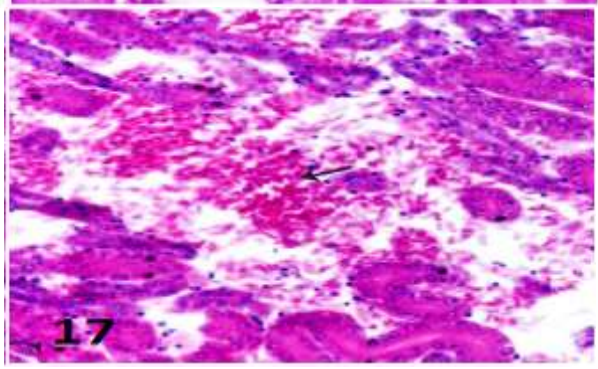
Figs.10-13: Sections of kidney of quail of normal animals showing normal renal cortex and medulla (10) and malathion treated group (11-13), showing focal mononuclear cell aggregation (arrow) (11), swelling of epithelial lining Bowman,s capsule and glomular tufts (arrow) (12) and haemorrhage (h) (13). [Figs. 10,11,13 X200; Fig.12 X 400].



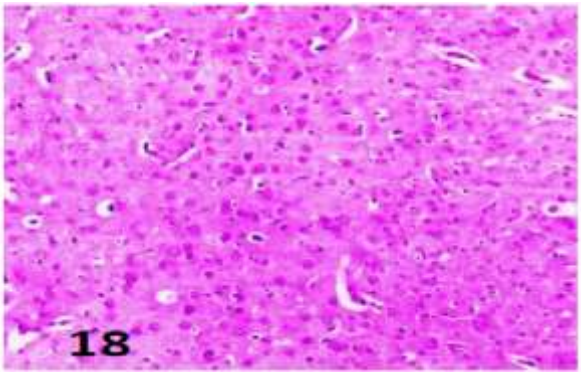


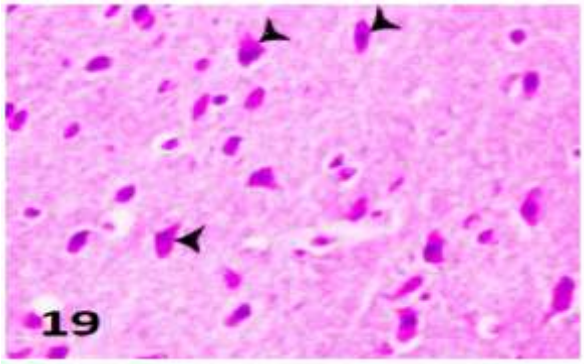


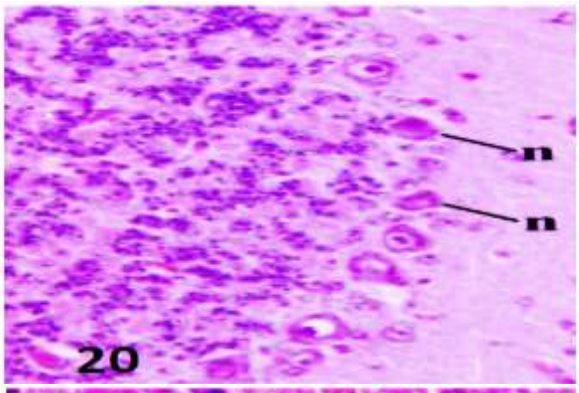


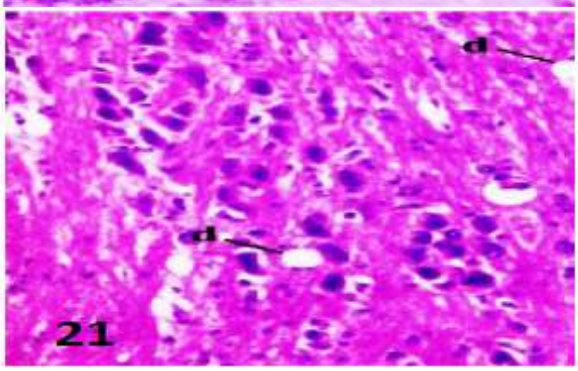


Figs. 14-17: Kidney sections of quail treated with PEG-600 (14,15), showing tubular karyopyknosis of the epithelial lining (arrow heads), focal mononuclear cell aggregation and vacuolation (15) (v), and malathion+PEG treated group (16,17) showing, peritubular leukocyte cell infiltration (arrow) together with karyopyknosis of renal tubular epithelium (arrow head) (16) and haemorrhage (17) (arrow). [Figs. X 200].

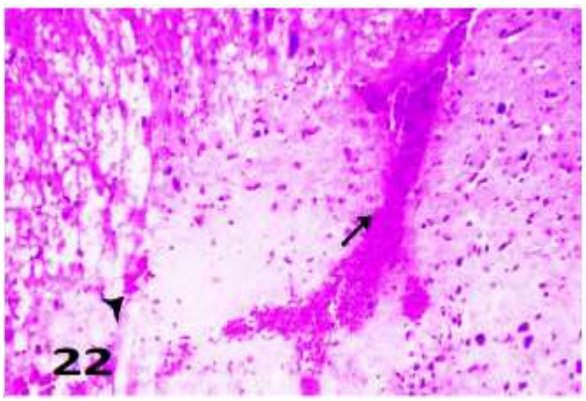


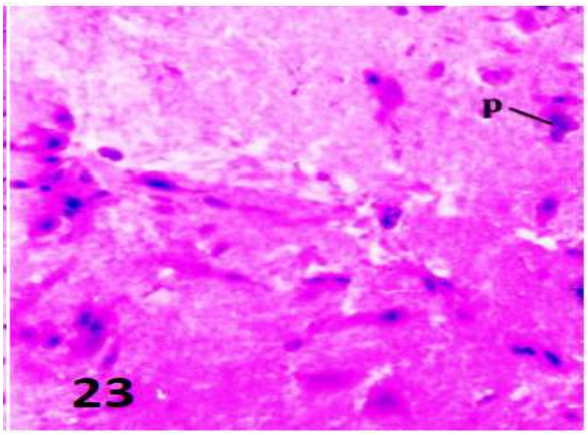






Figs. 18-21: Brain cortical sections of, normal bird showing normal neuronal cells (18); malathion-treated animals showing, neuronal degeneration and pyknosis (head arrow) (19) and necrosis of some purkinge cells (20) and PEG-600 treated animals (21) showing demylination of nerve fibers (gray matter) (d). [Figs. X200].





Figs. 22-23: Sections in cerebral cortex of quail treated with malathiion+PEG showing, marked congestion (arrow) as well as focal minute hemorrhage (arrow head) (22) and pyknosis of some neurons (23) (p). [Figs. X 200].

DISCUSSION

The present investigation shows that oral administration of malathion to quails at the dose level ¼ LD_{50} once a day for four days caused significant alterations in the plasma biochemical parameters. The activities of AST, ALT, ALP, and the levels of uric acid and glucose were statistically increased, while total protein, total albumin levels were significantly decreased. Moreover, the activities of brain and plasma AChE activity were also decreased in quails exposed to malathion. In addition, malathion induced severe hepatic, renal and neuronal histological damages as shown in histological examination. These results are in agreement with different previous researches which indicated that malathion exposure has been associated with severe metabolic disturbances (Wieder and Davis 1983), inflammation (Harith 2009), hepatotoxicity (Galal et al. 2015) and neurotoxicity (Salama et al. 2015). Otherwise, on the basis of the histopathological changes, severe damages has been observed in, liver, kidney and neuronal tissue sections. These observations are in accordance with that of Pourmirza, (2000), Bajgar (2004) and Harith (2009) who observed that malathion caused organ lesions in various species of birds. Moreover, as reported recently by Mamun et al. (2015), at different doses with malathion, intoxicated mice resulted in hepatic damage associated with, necrosis, vacuole formations, leukocytes infiltrations, and congestion of blood vessels with hemorrhage.

Malathion intoxication also caused several clinical CNS disturbances. These signs included reductions in flying, noticeable weakness, salivation, variable sequence of motor symptoms and wing-beat. These results are in agreement with Pal and Kushwah (2000) and Gada et al. (1999) who reported that, the exposure of hens to malation caused symptoms like tremors, convulsion, salivation and vomiting. These signs, however, are mainly attributed to AChE inhibition. As reported by many investigators the primary target action of OP pesticides, including malathion is through AChE inhibition (Čolović et al. 2013; Galal et al. 2015). In the present study relevant suggestion can be made about the relationship between AChE inhibition, which leads to accumulation of acetylcholine at the never ending and neuromuscular junctions and to cholinergic over stimulation manifested as muscarinic, nicotinic, and central nervous system effects. Moreover, It is concluded that, the histopathological alterations resulting from the exposure to malathion and/ or their metabolic derivatives may lead to malfunctioning of the quail body organs. This in turn will eventually lead to disturbances in the level of the studied biochemical and enzymes. According to Jokanovic (2001) malathion is converted to their metabolic derivatives in liver whose toxicity-is several times greater than that of the original compounds It has also been concluded that the decrease in protein and lipid contents at the tested acute dose may also suggest their possible utilization to meet the high energy demand under pesticide stress.

The present study also showed that, the toxicological characteristics of PEG-600 as adjuvant either administered individually or in combination with the tested pesticide substantially differ from those of malathion alone. This phenomenon is demonstrated not only in the determined LD_{50} value, but on the level of the studied haemato-biochemicals and the histological changes. From the present results, it is observed that, the toxicity of malathion was increased by 32.25% when administered in combination with PEG-600 and the tested mixture induced more decreased effect on total protein, albumin, and glucose levels and the activity of brain and plasma AChE was more inhibited. In addition, the tested mixture resulted in elevated GOT and ALP levels and induced more histological damage of liver, kidney and cerebral cortex than do malathion alone.

Till now the combined toxicity of adjuvant with pesticides has not been a focus of concern, and the data reported here will give resource guidance into the toxicity of PEG-600 as adjuvant when administered individually or in combination with malathion on birds. As reported by many investigators glycols have an irritant effect on the eyes and mucous membranes (Caravati et al., 2005), CNS depression (Ferguson and. Piccaro 2002), cardiotoxic effects (Park et al. 2009), renal and hepatic damage (Caliceti and Veronese 2003), lactic acidosis (QH et al. 2010) and serum hyperosmolality (Kraut and Kurtz 2008). These effects, however, are mainly attributed to their higher water-solubility and peak tissue levels which occur several hours after ingestion. According to Hall (1992) and Goldfrank (1998), ethylene glycols are metabolized in the liver by successive oxidations to a variety of mono-acids and di-acids toxic compounds. As reported by Herold et al. (1989), the di-acids

form stable, soluble complexes with calcium causing hypercalcemia that affect various parts of your body. Furthermore, the more enhanced histopathological toxicity of malathion in the presence of PEG can also be explained on the basis of their antioxidant effects. As reported by Wu et al. (2011), after application with different concentrations, malathion is known to produce a decrease in the GSH content or in the ratio of GSH/GSSG which may induce oxidative stress leading to the generation of free radicals and alterations in antioxidant and scavengers of oxygen-free radicals. Also, according to Bendjeddou and Khelili (2014), ethylene glycol causes a reduction of glutathione GHS in the liver, testes and epididymis. Otherwise, it may suggest that, the synergistic effects elicited by exposure to malathion and PEG are mediated by their toxic metabolites which elicit different toxic action than those elicited by either agent alone.

CONCLUSIONS

A summary of the present results indicates that the clinical responses of quails to malathion and PEG-600 exposure are greater than those elicited by either agent alone. Interestingly, each toxicant enhances the histopathological and biochemical alterations induced by the other. These studies provided some new insights into how glycols as adjuvant interact to initiate and promote alterations of pesticides effect. Further studies with other pesticides and glycols will help to define their true risk and mode of action to animals.

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