Toxicological Studies of Malathion on Japanese Quail (*Coturnix Japonica*)

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Abstract: Over the last few decades, ecotoxicological impacts of organophosphorus insecticides have been accentuated by sharp increase of their use in agriculture. In this study the effect of orally administrated Malathion on male Japanese quail was investigated. The acute oral 72-hrs LD_{50} of Malathion was found to be 146.06 mg/kg B.wt. Malathion was supplied at a dose of 1/20 of LD_{50} for eight weeks. The evaluation strategy of the current investigation used observation of clinical signs and stress related alterations which were assessed by evaluating relative organ weights; hematological; biochemical and histopathological investigations. The tendency of Malathion to accumulate in selected tissues and organs of male treated quails was evaluated by detecting its level in liver, kidney and muscle. Significant decrease in red blood cell counts RBCs, hemoglobin (Hb) and packed cell volume (PCV) of treated quails were observed in comparison to their controls. Significant alterations in total and differential leucocytes counts were also observed. Treated quails showed a significant increase in liver enzyme activities (AST, ALT and ALP) as well as in total bilirubin and glucose levels. Meanwhile, they showed significant decrease in total protein, albumin, and globulin. Regarding kidney function; serum creatinine, urea and uric acid of treated quails were significantly increased in comparison to their control. Cholesterol, triglycerides, VLDL-cholesterol and LDL-cholesterol levels of treated quails showed significant increase in comparison to their controls, while LDL-cholesterol levels showed a significant decrease. Malathion residue concentration in liver, kidney and muscle showed higher concentration in liver and kidney followed by muscles. Histopathological alterations were observed in treated quails. Safe use and all precautions should be followed during application of Malathion to minimize such undesirable effects.
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1. Introduction

Pesticides have contributed from one side to dramatic increase in crop yields, and from the other side they may induce adverse ecotoxicological and hazardous health effects on a variety of living organisms, including birds. Organophosphorus pesticides are widely used in agriculture and veterinary practice to control various pests. A number of long persistent organophosphates, which have been banned or severely restricted, are still used in many developing countries (De Silva *et al*., 2006). As birds have a high trophic level, they are vulnerable of accumulating large dosage of certain chemicals (Deka and Borah, 2008). Some sub-lethal effects of pesticides were studied in birds with a view to identify characteristic biochemical response that may be useful for the monitoring of exposure to sub-lethal levels in the field (Dahamna *et al*., 2004).

Organophosphates have a remarkable acute toxicity due to inhibition of the cholinesterase enzyme and inducing acute neurological effects. Malathion is one of the most commonly used organophosphorus insecticides and is the main cause of the most acute pesticide poisoning. Its contamination may occur in poultry following its application to fruit, vegetables, grain, fiber and other crops. Contamination of poultry birds may also result from ingestion of treated cereals (Moghadamina and Abdollahi, 2002) or from the use of Malathion in the control of external parasites (Rao and Yadgirkar, 2000). Malathion degrades into more toxic metabolites in the tissues like liver, kidney and brain and consequently poses a potential threat to public health due to the presence of pesticide residues in poultry meat (Garg *et al*., 2004b).

In the last few decades, the World wide attention is focusing on the environment and how to protect it. The fragmented and incomplete studies on the toxicological and histopathological action of Malathion necessitated this study to investigate the toxicological effects of subchronic exposure of male Japanese quail (*Coutrnix coutrnix japonica*) to Malathion after estimating its LD_{50}. These effects include the clinical signs, alterations in the relative weight of some organs (liver, kidney and spleen), hematological alterations, biochemical changes, and the tendency of Malathion to accumulate in liver, kidney and muscle during the treatment period. Histopathological alterations of liver, kidney and spleen were also investigated.

2. Materials and Methods

Experimental animals:

A total of 120 apparently healthy male Japanese quail (*Coturnix coturnix japonica*) weighing from 100 to 150 g, purchased from Faculty of Agriculture at Cairo University, applied for both LD_{50} and a treatment

study. Studied animals were kept under observation for two weeks before the onset of the experiment. Quails were divided into two groups; control group and malathion-treated group. 10 quails/cage was housed in wooden cages (100 x 80 x 60cm) under suitable hygienic free pathogens for 8 weeks.

Determination of the acute oral 72-hrs LD_{50} and dose determination:

The 72-hrs LD_{50} of Malathion was determined according to Finney (1964). The treated dose was calculated to be equivalent to 1/20 of the determined LD_{50}. Daily oral intubation of Malathion was extended up to 8 weeks, while control group was orally intubated by distilled water and was kept under the same laboratory conditions.

Sampling:

At the end of the 1st, 2nd, 4th and 8th week, 5 quails from each group were kept for about 12 hrs without water and feed. Quails **were slaughtered and blood was collected**. Blood was collected **sterile containers to avoid contamination**. Collected blood was divided into two portions. One was used for hematological examination after adding EDTA as an anticoagulant and the other portion was centrifuged to obtain serum that was stored at -20°C for further biochemical analysis. Post-mortem examination was carried out and relative weights of liver, kidney and spleen were recorded. Liver, kidney and muscle were stored at -20°C to detect concentration of Malathion residues. Parts of liver, kidney and spleen were fixed in 10% formalin buffer for histopathological examination.

Hematological parameters:

Total red blood cell (RBC) and white blood cell (WBC) counts (/µl) were determined by the Natt & Herrick (1952). Hemoglobin level and values of erythrocyte indices were evaluated as described by Wintrobe (1965), while haematocrite percentage (PCV%) and differential leukocytic percentage were determined according to Dacie and Lewis (1991).

Biochemical parameters:

Hepatic aspartate aminotransferase activity (AST) and alanine aminotransaminase activity were determined kinetically according to Schumann and Klauke (2003). Alkaline phosphatase activity (ALP) was determined according to Rec (1972), while total bilirubin was determined according to Jendrassik and Grof (1938). Glucose concentration was evaluated according to Young (2001). The serum albumin, and total protein values were estimated by Biuret and Dumas method as suggested by Dumas *et al. (1971)*. Serum globulin concentration and albumin/globulin ratio were calculated according to Rojkin *et al.* (1974). Serum concentration of creatinine, urea, and uric acid were determined according to Henry (1974), Patton and Crouch (1977) and Young (2001) respectively. Finally levels of serum cholesterol, triglyceride and HDL-cholesterol were determined according to Young (2001), while VLDL-cholesterol and LDL-cholesterol concentrations were evaluated according to Norbert (1995) and Friedwald (1972) formulas respectively.

Determination of insecticide residues:

Ten grams of tissue sample was mixed with Na_2SO_4 and packed in thimble of Soxhelt extraction and then the mixture was extracted and concentrated. The extract was cleaned up through alumina chromatography columns. Elution was done according to Erney (1983), and standard curve was constructed for Malathion by known concentration in known volume followed by injection of known volume into GLC-ECD.

Histopathological investigation:

Histopathological examination of liver, kidney and spleen was carried out according to Lillie (1969).

Statistical Analysis:

Data analysis was performed by using student ANOVA test and comparing between means using LSD as outlined by PC-STAT (1995).

3. Results and Discussion

Determination of the acute oral 72-hrs LD_{50}:

LD_{50} is commonly used to express the relative hazards associated with the acute toxicity of Malathion. The principle of safety evaluation studies is to define the potential of Malathion that cause damage. Gruzdyev *et al*. (1980) reported that the lower the absolute value of LD_{50} the higher is the toxicity characterizing the formulation. In the present study, the acute oral 72-hrs LD_{50} of Malathion was found to be 164.06 mg/kg. The mentioned results confirmed the moderate toxicity of Malathion to a variety of bird species that was reported by other authors.

Clinical signs:

After two weeks of treatment with Malathion treated quails have experienced some clinical signs including; roughened feathers, weakness, dropped wings, loss of balance, hyperexcitability incoordination, convulsions, thick mucoid discharge from the mouth, wheezing and dysnea. After four weeks, neurotoxic symptoms increased gradually till the end of the treatment period. Additionally, the study bird showed abnormalities in gait and behavior. Before death ataxia, muscular convulsion and comma have occurred in some cases. The treated bird showed signs of depression, and preferred to stand still followed by zigzag movements. Observed ataxia and incoordination were followed by hypersalivation, open mouth breathing and paralysis of legs. The observed clinical signs could be explained by the inhibitory effect of Malathion on acetylecholinesterase (Gultekin *et al*., 2006 and De Silva *et al*., 2006) that lead to abnormal acetylcholine build up. Similar signs were observed by other authors, including Varsik *et al*., 2005).

Organs relative weights:

Liver, kidney and spleen relative weight of treated quails showed a significant increase when compared to their controls. Such increase was recorded at P<0.05 from the 1st week of treatment for liver, meanwhile it was recorded at *P*<0.01 from the 4th week of treatment for kidney and from the 1st week of treatment for spleen . In all cases such significant increase showed a time dependent trend (Table 1). Such observed enlargement in the studied organs may indicate an initial effect of systemic toxicity that probably facilitates erythrocyte removal by the reticuloendothelial system (Mahmoud, 2000).

Hematological parameters:

From the 2nd week of treatment RBCs and Hb of treated quails showed a highly significant decrease (*P*<0.01), meanwhile MCV showed significant increase (*P*<0.05) when compared to their controls (Table 1). Such significant alterations of haematological parameters showed a time dependent trend, the most potent reduction or increase was recorded at the end of the experiment. The observed decrease in RBCs and Hb concentration could be due to the destruction of erythrocytes as a direct effect of Malathion treatment, or due to the indirect adverse effect of Malathion on the bone marrow (Nemi, 1993). Such decrease was concurred with splenomegally recorded in the current study. Destruction of red cells could be also due to mutagenic and hematotoxic effect of Malathion (El-Shater, 2003). After 8 weeks of treatment, a significant increase (*P*<0.01) of WBCs and neutrophils and a significant decrease (*P*<0.01) of eosinphils, monocytes and lymphocytes were observed when compared to their controls and all previously tested periods, in a time dependent manner (Table 1). The reported leukocytosis that began after the first week of treatment associated with lymphopenia and neutrophilia which began after the second week of treatment could be due to adverse effect of Malathion on the normal function of the bone marrow and/or lymphoid tissue (Rajini *et al*., 1987). In addition, the observed lymphopenia may reflect stress imposed on the immune system in response to Malathion intoxication (Garg *et al*., 2004a). It was interesting to correlate the recorded hematological changes with histopathological findings noticed in spleen of treated quails, such as hyperplastic proliferation in lymphoid tissue, angiopathy of follicular artery and depletion of lymphoid follicles.

Biochemical Changes:

Liver Function:

After 8 weeks of treatment AST, ALT and ALP activities of treated individuals showed a significant increase when compared to their activities of control groups and earlier tested periods. Such significant increase, observed after 8 weeks, was recorded at *P*<0.01 for AST and ALT and was recorded at *P*<0.05 for ALP. Significant differences were observed for the 3 enzymes in a time dependent trend as seen in table 1. Such noticed increase in AST and ALT activities could be used as an indicator of altered permeability of plasma membrane and/or cell damage (Hasheesh *et al*., 2002a). Meanwhile, the recorded increase in ALP activity may be due to the osteoblastic activity and general toxic damage to liver as reported by Garg *et al*. (2004b). Total bilirubin and glucose concentration of treated quails showed a significant increase (*P*<0.01) in comparison with their controls for all studied periods except bilirubin level of individuals treated for 1 week that sowed its significant increase at *P*<0.05. Time dependent significant increase of total bilirubin and glucose levels was also noticed at *P* <0.01 for total bilirubin of the 2nd, 4th and 8th week when compared to its value of the 1st week and at *P* <0.05 for glucose level of the 8th week when compared to its level of the 4th week (Table 2). The noticed increase in the bilirubin level may be due to haemolysis that could be caused by excessive rapid destruction of erythrocytes (Hasheesh *et al*., 2002a) and this was supported by the recorded low RBCs count observed in the present study. In general, the induced alteration of liver function could be explained by the formation of lipid peroxidation which is considered as one of the molecular mechanisms for Malathion-induced hepatic damage (Gokalp *et al*., 2003). Moreover, it was meaningful to correlate between the observed alterations of liver functions and the direct effect of Malathion on the histological features of liver of treated quails that showed focal lymphocytic infiltration, hyperplasia of the bile ducts, and desquamation of lining epithelial cells, proliferation of the fibrous tissue of the portal area, fatty changes and necrosis. The recorded increase in blood glucose concentration may be due to the accelerated glycogenolysis and increased level of lipid peroxidation (Abdollahi *et al*., 2004).

Protein profile:

From the 2nd week to the end of treatment period, total protein and albumin of treated quails showed a significant decrease (*P* <0.01) when compared to their controls (Table 2). Such significant decrease of total protein and albumin showed time dependent tendency. On the other hand significant decrease in globulin concentration of quails treated for 4 weeks was recorded at p<0.01 in comparison to their controls. Globulin concentration showed also a time dependent significant decrease (Table 2). Regarding A/G ratio, a significant decrease (*P* <0.01) was recorded for treated quails on the 2nd and 8th week of treatment in comparison to their controls. Such decrease of individuals treated for eight weeks showed a significant decrease at P<0.01 when compared to individuals treated for one week (Table 2). The noticed reduction in total protein could be related to the action of Malathion on nucleic acids (Devi, 1981) and it may indicate a physiological adaptability of quails to

compensate pesticide stress. As mentioned by Garg *et al*. (2004b), it may be concluded that the observed decrease in serum globulin could be due to reduction in synthesis by the plasma cells.

Kidney functions:

Serum creatinine and uric acid concentration of treated quails showed a significant increase (P <0.0) compared to their controls for all tested periods, with the exception of uric acid concentration of individuals treated for one week that showed non-significant increase (P >0.05) when compared to their controls. A time dependent significant increase was also recorded for both creatinine and uric acid when compared to their relevant concentrations of individuals treated for shorter time periods. Urea concentration of quails treated for two weeks showed a significant increase (P <0.05) in comparison to their controls. Such significant increase was recorded at P <0.01 on the 4th and 8th week of treatment (Table 2). The observed increase of serum creatinine may be attributed to renal insufficiency, urinary tract obstruction and impairment of renal function induced by Malathion (Hasheesh *et al*., 2002a). The same authors explained the increased level of urea by the increased nitrogen retention and/or due to corrupted renal function. The increase of uric acid usually occurs due to renal failure or toxemia induced by Malathion resulting in damage to the epithelium of the kidney tubules (Malik *et al*., 2004). In concurrent with the mentioned biochemical alterations, treatment with Malathion induced renal degenerative changes in renal tubules, progressive infiltration, degenerative changes in renal epithelial cells, in addition to atrophied renal corpuscles and focal interstitial nephritis.

Lipid profile:

Cholesterol, triglycerides, VLDL and LDL-cholesterol of treated quails showed a significant increase when compared to their controls during different treatment periods (Table 2) Such increase was recorded at P <0.01 with the exception of cholesterol that showed its increase at P <0.01. A time dependent significant increase (P <0.01) was also noted when cholesterol, triglycerides, VLDL and LDL-cholesterol levels of quails treated for 8 weeks compared to individuals exposed to Malathion for shorter periods of time. HDL-cholesterol concentration of the treated group showed a significant decrease (P <0.01) in comparison with their controls. Such decrease showed a time dependent trend. The recorded high level of cholesterol in blood of treated quails is a major risk factor as mentioned by AHA Science Advisory (2001). Such observed increase could be attributed to the blockage of liver bile duct causing reduction of its secretion (Kalender *et al*., 2005), and it could be also attributed the hyperadrenal activity that was induced because of Malathion treatment (Malik *et al*., 2004). The possible Malathion induced activation of serum enzyme activity could be considered for the observed HDL-cholesterol decrease (Ibrahim and El-Gamal, 2003). In accordance with Ibrahim and El-Gamal (2003), results of the current study suggested a change from HDL-cholesterol into LDL-cholesterol. Regarding the triglyceride level, the recorded increase in serum triglycerides of treated quails may be due to increased lipid mobilization from liver and its decreased removal from plasma (Slotkin *et al*., 2005).

Malathion residues in body tissues:

Concentration of Malathion in liver, kidney and muscle of quails treated for two and eight weeks showed a significant increase (P <0.01) when compared to their controls. Such significant increase showed a time dependent trend, as residue concentration detected in the mentioned organs showed a higher rate of increase as time of exposure extended (Fig. 9). The observed persistence of Malathion in studied tissues of treated quails could be attributed to its limited elimination and its biotransformation, despite the rapid hydrolysis of organophosphorus *in vitro* (Hasheesh *et al*., 2002b). The distribution of Malathion residues is likely to be correlated with the lipid content of the organ, being high in the organs with high lipid contents.

Histopathological Investigations:

Figure 2 (1-12) showed histopathological alterations of liver (1-5), kidney (6-8) and spleen (9-12) of quails treated with Malathion in comparison to their controls. From the first week of treatment, liver of treated quails showed focal lymphocytic aggregation and hyperplasia of bile ductules. As period of exposure extended, angiopathy and cholingitis associated with desquamation of epithelial cells and proliferation of the fibroblasts were noticed. In addition to that swollen endothelia of the branches of hepatic artery and degenerated tunica media were observed in addition to oedema. Moreover, numerous numbers of hepatic cells undergo fatty degenerative changes. Moreover, necrotic changes were observed in the hepatocytes. Kidney of quails treated with Malathion showed severe degenerative changes of the renal corpuscle and renal tubules with noticeable hydrpnephrosis and progressive infiltration of the inflammatory cells were observed after four weeks of treatment. As the period of exposure extended, focal interstitial nephritis was observed. Regarding spleen, the white pulp showed hypersensitivity of the reticular cells and the lymphoid elements showed depletion during the first four weeks of treatment. After the 8th week of treatment, the reticular cells were laden with haemosidrin. The subcapsular and medullary sinuses showed oedema and disorganization of lymphoid follicles appeared.

The observed histopathological alterations could be attributed to the direct effect of Malathion on the studied organs (Rodrigo *et al*., 2001).

Table (1): Body weight and hematological values of quails orally treated with Malathion (8.2 mg Kg b. wt $^{-1}$)

Parameter (unit)	1st week		2nd week		4th week		8th week		LSD at 5%	LSD at 1%
	C	MT	C	MT	C	MT	C	MT		
Liver (wt x 10^{-4})	156.00±6.00^{c}	178.00±3.00^{bcd}	158.00±6.00^{c}	182.00±7.00^{bc}	164.00±4.00^{cde}	190.00±9.00^{ab}	160.00±3.00^{de}	206.00±9.00^{a}	19.6	26.4
Kidney (wt x 10^{-4})	53.40±0.50^{c}	53.50±0.44^{c}	54.00±0.42^{c}	54.20±0.64^{c}	53.70±0.21^{c}	57.90±0.32^{b}	53.50±0.27^{c}	61.90±0.39^{a}	1.2	1.6
Spleen (wt x 10^{-4})	4.70±0.06^{d}	6.42±0.03^{c}	4.46±0.11^{d}	6.49±0.12^{c}	4.44±0.11^{d}	7.18±0.23^{d}	4.49±0.04^{d}	7.72±0.19^{a}	0.38	0.51
RBCs cells/mm^{3}	3.72±0.18^{a}	3.71±0.03^{a}	3.89±0.12^{a}	3.02±0.05^{b}	3.84±0.01^{a}	2.66±0.04^{c}	3.88±0.02^{a}	2.62±0.07^{c}	0.25	0.33
HB (g/dl)	11.80±0.38^{a}	11.46±0.14^{a}	11.46±0.27^{a}	9.54±0.12^{b}	11.74±0.25^{a}	8.72±0.07^{c}	11.60±0.15^{a}	8.60±0.11^{c}	3.05	4.11
PCV (%)	38.86±1.86^{ab}	35.94±1.10^{bc}	40.14±0.95^{a}	35.26±0.18^{c}	38.56±1.14^{ab}	30.84±0.44^{d}	40.24±1.23^{a}	30.44±0.60^{d}	3.05	4.11
MCV (Fl)	104.40±8.17^{bc}	96.85±3.53^{c}	103.14±1.39^{c}	116.72±3.39^{a}	100.34±2.81^{c}	115.96±3.19^{ab}	103.68±3.72^{c}	116.45±3.60^{a}	11.72	15.79
MCH (pg)	31.72±1.69	30.88±0.68	29.46±1.36	31.58±0.81	30.56±0.66	32.72±0.34	29.88±0.54	32.84±0.67	-----	-----
MCHC (%)	30.34±1.52^{ab}	31.90±1.06^{a}	28.58±1.04^{bc}	27.05±0.18^{c}	30.46±1.14^{ab}	28.61±0.57^{bc}	28.80±0.87^{bc}	28.26±0.30^{c}	2.67	3.6
WBCs (x10^{3})	3.42±0.06^{d}	4.54±0.19^{c}	3.39±0.13^{d}	5.84±0.21^{b}	3.52±0.06^{d}	6.30±0.26^{b}	3.43±0.04^{d}	7.72±0.10^{a}	0.47	0.63
Neutrophils (%)	2.60±0.24^{d}	2.40±0.24^{d}	3.60±0.24^{d}	7.60±0.24^{c}	2.40±0.24^{d}	15.00±0.45^{b}	2.60±0.24^{d}	20.40±1.18^{a}	1.41	1.9
Eosinophils (%)	2.60±0.24^{ab}	3.20±0.20^{a}	3.20±0.20^{a}	2.40±0.24^{b}	3.20±0.12^{c}	1.20±0.12^{c}	2.60±0.24^{ab}	00.00±0.00^{d}	0.75	1.01
Lymphocytes (%)	91.40±0.60^{a}	89.00±1.10^{ab}	90.00±2.94^{a}	85.60±0.75^{b}	91.20±1.17^{a}	81.60±0.75^{c}	92.00±1.43^{a}	78.60±0.60^{c}	0.67	0.91
Monocytes (%)	3.40±0.24^{c}	5.40±0.40^{a}	2.20±0.20^{c}	4.40±0.24^{b}	3.20±0.20^{c}	2.20±0.20^{d}	2.80±0.24^{cd}	1.00±0.00^{e}	0.67	0.91

Number of birds in each experiment was five. Data are expressed as mean ± SE.
LSD is the least significant difference.
Values which share the same superscript letters are not significantly different (P< 0.05).

Table (2): Serum Biochemical studies of quails orally treated with Malathion (8.2 mg Kg b.wt $^{-1}$)

Blood parameter (unit)	1st week		2nd week		4th week		8th week		LSD at 5 %	LSD at 1 %
	C	MT	C	MT	C	MT	C	MT		
Glucose (mg/dl)	101.80±2.06c	132.20±8030ab	103.40±4.87c	134.20±4.60ab	93.60±4.20c	122.00±1.01b	97.60±2.30c	142.00±12.50a	17.38	23.41
Protein (g%)	3.44±0.13ab	3.28±0.08b	3.54±0.06a	2.98±0.06c	3.54±0.06a	2.61±0.06d	3.50±0.05a	2.52±0.05d	0.2	0.28
Albumin (g%)	1.22±0.05ab	1.08±0.05b	1.34±0.02a	0.82±0.03c	1.20±0.02ab	0.78±0.05c	1.40±0.02a	0.68±0.03c	0.21	0.28
Globulin (g%)	2.22±0.18ab	2.20±0.03ab	2.20±0.07ab	2.10±0.03ab	2.34±0.07d	1.83±317.30ab	2.08±0.08bc	1.84±0.05cd	0.25	0.33
A/G (%)	0.57±0.03abc	0.48±0.02cde	0.60±0.03ab	0.41±0.01ef	0.51±0.03bcd	0.42±0.03def	0.66±0.04a	0.36±0.03f	0.01	0.13
Cholesterol mg%	139.80±1.03d	150.20±1.96c	135.00±1.15d	157.00±1.65c	137.60±3.10d	172.40±6.79b	141.00±2.52d	212.00±3.53a	9.19	12.37
Triglycerides mg%	99.20±3.29d	120.00±1.76c	105.40±0.75d	128.00±0.71bc	100.80±2.71d	135.60±1.04b	109.00±2.92d	153.00±8.17a	9.98	13.45
HDL- cholesterol mg%	101.3±1.29 a	65.80±0.52b	111.80±1.52a	60.50±0.22c	112.90±0.79a	59.50±1.25c	111.8±1.74a	55.50±1.52d	3.46	4.66
LDL-cholesterol mg%	19.84±0.65d	24.00±0.34c	21.08±0.15d	25.60±0.014bc	20.16±0.54d	27.12±0.20b	21.80±0.58d	30.60±01.63a	1.99	2.69
vLDL-cholesterol mg%	9.64±0.91d	60.66±5.50c	2.10±0.07d	70.8±5.15c	4.60±0.44d	85.58±6.94b	7.38±0.63d	125.90±3.45a	10.91	14.69
Creatinine mg%	0.27 ± 0.01c	0.37 ± 0.01ab	0.25 ± 0.02c	0.40 ± 0.03ab	0.22 ± 0.01c	0.36 ± 0.005b	0.26 ± 0.02c	0.43 ± 0.01a	0.06	0.08
Urea mg%	9.80 ± 0.25c	10.20 ± 0.52c	9.56 ± 0.32c	12.36 ± 0.35b	9.86 ± 0.34c	14.86 ± 1.33a	8.67 ± 0.45c	15.06± 1.37a	2.15	2.9
Uric acid mg%	2.28 ± 0.03cd	2.60 ± 0.12c	2.02 ± 0.17d	4.20 ± 0.33b	2.20 ± 0.05cd	4.66 ± 0.20ab	2.40±0.08cd	5.12±0.24a	0.52	0.7
AST (U/L)	204.0±4.10e	247.0± 9.10cd	209.0± 3.50e	255.2±21.20be	225.0±2.20de	278.6±3.10b	217.0±4.30e	325.0±20.50a	26.8	36.2
ALT (U/L)	40.0±2.80c	53.06 ± 2.41b	40.08± 3.90c	67.06 ± 6.30a	40.06± 2.90c	74.08 ± 4.70a	39.04±2.07c	73.06± 2.47a	10.57	14.24
Alkaline Phosphatase (U/L)	5667.4±201.9c	7845.60±250.50b	5637.2±324.10c	8480.4±17.50ab	5279.6±474.90c	8075.6±317.30ab	5705.8±207.60c	8838.6±157.50a	780.2	1050.7
T. bilirubin mg%	0.30±0.003b	0.33 ± 0.01b	0.33 ± 0.01b	0.44 ± 0.01a	0.30 ± 0.02b	0.43 ± 0.02a	0.31 ± 0.02b	0.48 ± 0.03a	0.06	0.08

Number of birds in each experiment was five. Data are expressed as means ± SE. LSD is the least significant difference.
Values which share the same superscript letters are not significantly different (P< 0.05).

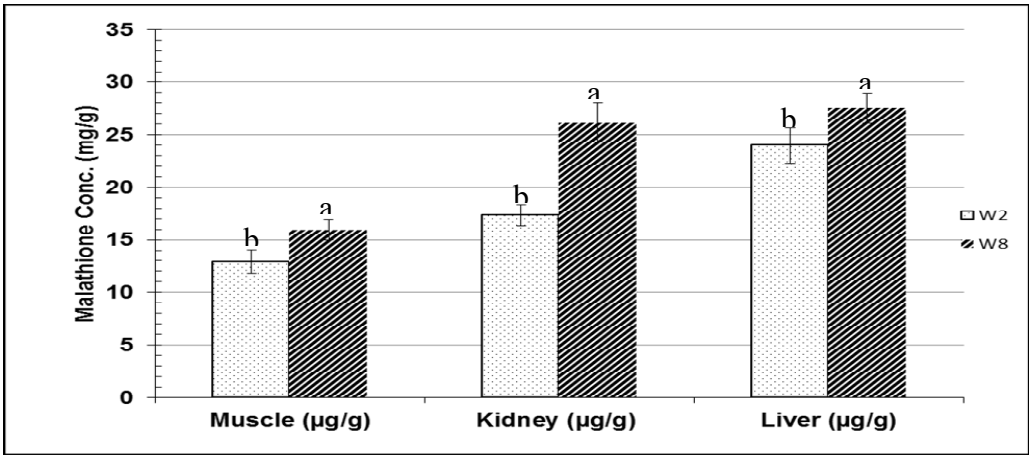

Fig. (1): Changes in residue of the Malathione concentration in toxicated tissues (g/g).

As mentioned by Gawish *et al*. (2006), the observed histopathological alterations may be due to the fact that organophosphorus insecticides generate free radicals in the biological system. Liver was reported as a target organ for Malathion toxicity (Yang *et al*., 2000 and Abdollahi *et al*., 2004). The necrotic condition observed in the liver of treated quails was in corroboration with the observed increase in AST, ALT and ALP that support the damage of liver cells observed with Malathion toxicity. Histopathological alterations of the kidney of treated quails could be caused during the course of excretion of these residues or it could be induced because of the cytotoxic effect of Malathion (Piramanayagam *et al.,* 1996). Alterations found in spleen could be based on the destructions of Vitamin A which is essential for normal growth and immunological functions (Adams *et al*., 1966).

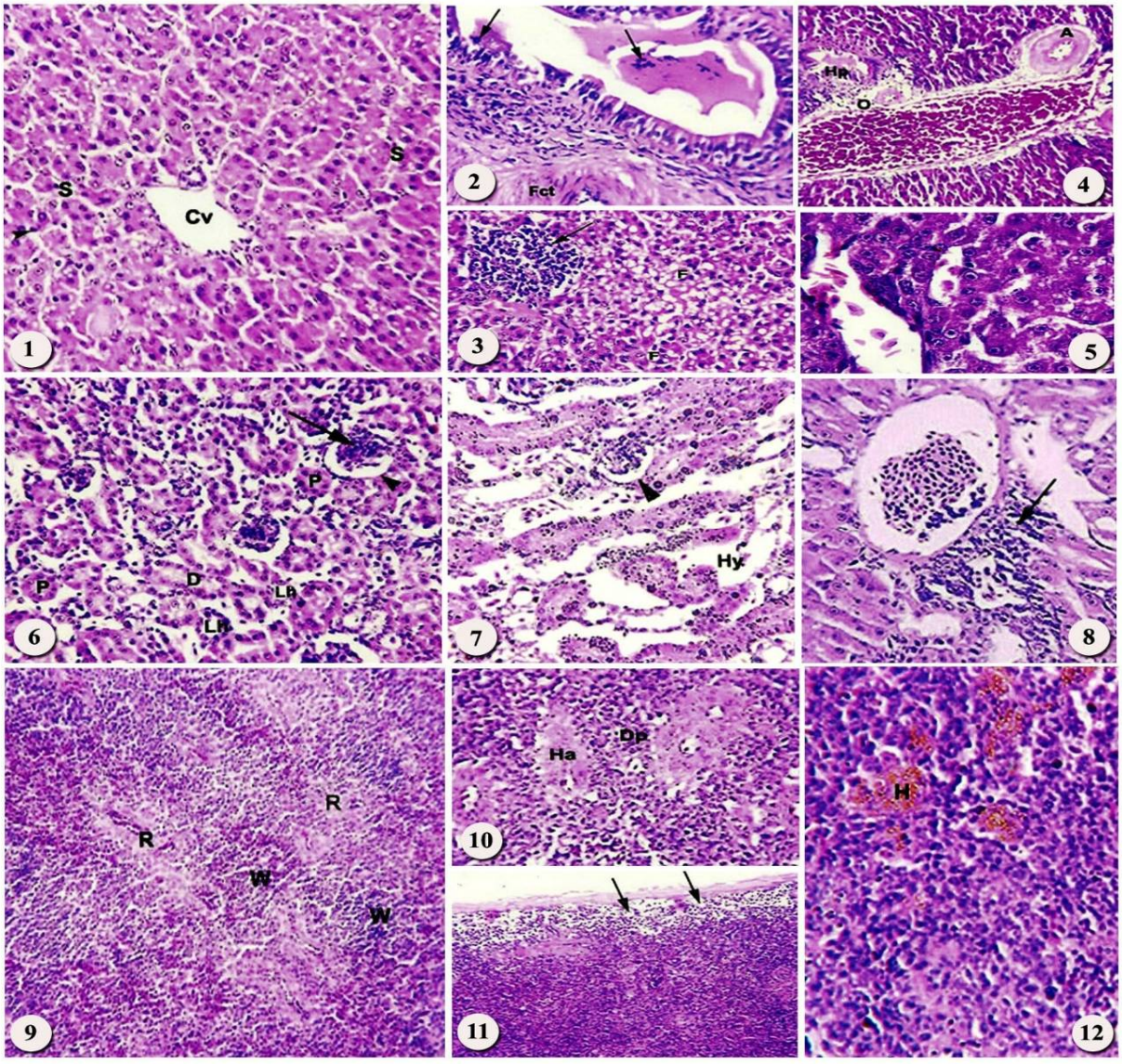

Fig. (2): Histopathological alteration of liver (1-5), kidney (6-8) and spleen (9-12) of control and treated quails.

1. Liver section of control quail showing a central vein (CV) surrounded by hepatic cords of normal hepatocytes. Notice the hepatic sinusoid (S) lined by endothelium (arrow head) and kupffer cells (H & E x400).
2. Two weeks-post treatment with malathion liver tissue section (8.2 mg Kg Bwt^{-1}) showing desqumation of the epithelial cells (arrows) of the bile ductless and fibrous connective tissues (Fct) formation around the branches of the hepatic artery (H & E x400).
3. Four weeks-post treatment with malathion liver tissue section (8.2 mg Kg Bwt^{-1}) showing angiopathy (A), hyperplasia (Hp) of the bile duct and edema (O) (H & E x200).
4. Four weeks-post treatment with malathion liver tissue section (8.2 mg Kg Bwt^{-1}) showing a number of hepatocytes undergo fatty changes (F) and focal aggregation of the lymphocytes (arrow) (H & E x200).
5. Eight weeks-post treatment with malathion liver tissue section (8.2 mg Kg Bwt^{-1}) multiple necrotic changes (H & E x1000).
6. Control kidney section showing normal renal corpuscle formed of Bowman's capsule (arrow head) and renal glomerulus (arrow). Note the proximal tubules (P), distal tubule (D) and loop of Henel's (Lh) (H & E x400).
7. Four weeks-post treatment with malathion kidney tissue section (8.2 mg Kg Bwt^{-1}) showing a degenerative renal capsule (arrow head) with noticeable hydronepherosis (Hy) with a progressive infiltration with inflammatory cells (H & E x200).
8. Eight weeks-post treatment with malathion kidney tissue section (8.2 mg Kg Bwt^{-1}) showing focal interstitial nephritis (arrow) (H & E x200).
9. Control spleen section showing normal aggregation of the white pulp (W) separated by red pulp (H & E x200).
10. One week-post treatment with malathion spleen tissue section (8.2 mg Kg Bwt^{-1}) showing hyperactivity (Ha) of reticular cells and depletion of lymphoid elements (Dp) (H & E x400).
11. Eight week-post treatment with malathion spleen tissue section (8.2 mg Kg Bwt^{-1}) showing edema (arrow) of the subcapsular sinuses (H & E x200).
12. Eight week-post treatment with malathion spleen tissue section (8.2 mg Kg Bwt^{-1}) showing edema mosidrosis (H) (H & E x400).

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