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PATHOLOGICAL AND SOME SERUM BIOCHEMICAL EFFECTS INDUCED BY MALATHION IN JAPANESE QUAIL *(Coturnix japonica)*

R. Hussain*, A. Khan and F. Mahmood

Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad 38040;
*University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur-63000, Pakistan.
Corresponding author e-mail: driazhussain@yahoo.com

ABSTRACT

In present experimental study gross, histopathological and different serum biochemical effects of malathion were observed in male Japanese quail. For this purpose apparently active and sexually mature male Japanese quail (approximately 5-6 weeks and each group 15 birds) were kept in six groups (A-F). Malathion @ 0, 25, 50, 75, 100 and 125 mg/kg b.w mixed in corn oil was administered orally for 51 days. Grossly, swollen, edematous and congested lungs, congested kidneys, pale to yellowish, fragile liver and regression of thymus in birds fed malathion (100 and 125 mg/kg b.w) was observed. Histopathological examination revealed mild to moderate cytoplasmic vacuolation in liver and severe pyknosis/condensation in nucleus of kidneys in treated quail (50 and 75 mg/kg b.w) at day 51 of the experiment. However these changes were severe in quail administered higher levels (100 and 125 mg/kg b.w) of organophosphate throughout the experiment. Severer hemorrhage in lungs, thymus and intestine was also observed in birds at higher doses. A significant decrease ($P \leq 0.05$) in serum albumin and serum total protein was recorded at higher levels (50, 75, 100 and 125 mg/kg b.w) of malathion. At day 17 of experiment serum creatinine and alanine transaminase was significantly increased in quail given higher levels (125 mg/kg b.w) of malathion. Significantly increased ($P \leq 0.05$) values of serum creatinine, aspartate transaminase and alanine transaminase at day 34 and 51 in treated quail (75, 100 and 125 mg/kg b.w) were also recorded. Serum lactate dehydrogenase was significantly increased throughout the experiment at different levels (50, 75, 100 and 125 mg/kg b.w). Malondialdehyde (MDA) contents were also significantly increased in quail. These results provide the first experimental evidence that prolonged and increased levels of malathion induces oxidative stress and tissue changes in birds.

Key words: Malathion, Quail, Toxicity, Pathology, Serum Biochemistry

INTRODUCTION

In agricultural settings different pesticides (insecticides, herbicides and fungicides) are frequently applied to numerous crops including wheat and corn which constitute the major source of potential environmental hazardous (Abd-Alla *et al.,* 2002; Ahmad *et al.,* 2012). Organphosphorous (OP) pesticides are abundantly used in domestic and garden applications to control fruit fly, mosquito eradication and as a topical treatment for head lice (Gervais *et al.,* 2009). Long term exposure to these chemicals induces countless abnormalities and shortens the life span of organisms (Naz *et al.,* 2011; Muhammad *et al.,* 2012). Due to moderate environmental persistence, high selectivity toward insects and relatively low toxicity to mammals, organophosphorus are extensively used in agro-production (Taylor, 2006). Malathion (1, 2-dicarbethoxyethyl) an important member of OP pesticides is extensively used over different cereal crops to control pests. Moreover, malathion is abundantly used for control of various ectoparasites in domestic animals (Naraharisetti *et al.,* 2009; Uzun *et al.,* 2009). Residues of malathion have been detected in different tissues of animals, vegetables, water and grains (Poet *et al.,* 2004). The animals, birds and human beings may be exposed to these pesticides by consuming contaminated food grains, water and different vegetables (Naraharisetti *et al.,* 2009). Various mechanisms of organophosphorus pesticide toxicity including induction of cellular proliferation, oxidative stress and immunotoxicity are well established in rats (Cabello *et al.,* 2001; Galloway and Handy, 2003; Abdollahi *et al.,* 2004). The toxic effects of malathion are related to its CYP-mediated oxidative desulfuration to bioactive malaoxon leading to accretion of acetylcholine in neuroeffector junctions. The accumulation of acetylcholine inhibits cholinesterase activity and causes cholinergic crisis through stimulation of the cholinergic receptors (Naraharisetti *et al.,* 2009). Organophosphate pesticides are well known to arrest acetylcholinesterase enzyme, can cause destructive and degenerative changes in blood vascular system (Kalender *et al.,* 2007), kidneys (Mossalam *et al.,* 2011), testes (Uzun *et al.,* 2009), liver (Kalender *et al.,* 2010) and various cellular changes (Uzunhisarcikli *et al.,* 2007). Various studies in rats have indicated that exposure to malathion (parathion) significantly increases the formation of lipid per oxidation product malondialdehyde (MDA) and increases the leakage of alanine transaminase (ALT), lactate

dehydrogenase (LDH), urea and creatinine (Mossalam *et al.,* 2011). Oxidative stress, an imbalance between oxidants and reductants at cellular level induces oxidative modification of cellular macromolecules, apoptosis or necrosis and structural changes in different tissue. Several pesticides and insecticides may damage the biological membranes especially phospholipid bilayers by inducing lipid peroxidation (Altuntas *et al.,* 2002). Scanty information is available about the oxidative and histopathological effects of malathion in avian species. Therefore the present study indicates experimentally induced pathological and some serum biochemical effects of malathion in visceral organs of sexually mature male Japanese quail (*Coturnix japonica*).

MATERIALS AND METHODS

The present experiment was conducted according to the guidelines and the strategies devised by the Advanced Studies and Research Board (ASRB) regarding protection of animals, University of Agriculture, Faisalabad.

Experimental design: A total of 90 sexually mature, clinically healthy male Japanese quail *(Coturnix japonica)* were purchased from local hatchery and were randomly divided into six equal groups after one week of acclimatization. The birds were kept in wire cages under similar housing and managemental conditions. All the quail were given fresh water and corn soybean meal based feed having 22 % protein (Naqi *et al.,* 2011) twice a day *ad libitum.* Malathion (95% technical grade) obtained from M/S Ali Akbar Enterprises, Pakistan was mixed in corn oil and given to birds orally via crop tube as follow: A (0), B (25 mg/kg b.w), C (50 mg/kg b.w), D (75 mg/kg b.w), E (100 mg/kg b.w) and F (125 mg/kg b.w) daily for 51 days.

Pathology procedures: At day 17, 34 and 51 of the present experiment, the blood samples with and without anticoagulant (EDTA; 1mg/ml); were collected from randomly selected five birds from each group by cutting jugular vein. Visceral organs including lungs, liver, kidneys, thymus, spleen, proventriculus, gizzard and intestine were removed and fixed in 10% neutral buffered formalin (Islam *et al*., 2012). About 4-5 µm thick tissue sections from these organs were cut with help of microtome and stained with hematoxylin and eosin for histopathological alterations (Mahmood *et al.,* 2012).

Serum Biochemical Changes: Serum was separated from blood samples without anticoagulant and stored at -20°C. Serum malondialdehyde (MDA) was determined spectrophotometrically (Hussain *et al.,* 2012a). Serum lactate dehydrogenase (LDH), aspartate aminotransferase, albumin, creatinine, alanine aminotransferase (ALT) and total protein were determined spectrophotometrically using commercially available kits (Hussain *et al.,* 2012).

Statistical Analysis: Data obtained in present study were subjected to statistical analysis using repeated measures analysis of variance (ANOVA) and the group means were compared by Tukey's test with $P \leq 0.05$.

RESULTS

Gross and histopathological abnormalities: In present experiment no mortality was recorded in birds of all groups. No gross changes were observed in visceral organs including lungs, liver, kidneys, gizzards, intestine, spleen and thymus in birds of control group throughout the experiment. However, at day 51 of the experiment the birds administered malathion (75 mg/kg b.w) showed mild gross changes in lungs, liver, kidneys and thymus. The lungs and kidneys were edematous, swollen and congested while liver was friable and pale to yellowish in appearance. The congestion in thymus was also observed. However, these gross changes were severe throughout the experiment in birds treated higher doses of malathion (100 and 125 mg/kg b.w). Histologically, the kidneys of different birds given lower doses (50 and 75 mg/kg b.w) exhibited mild congestion, increase urinary spaces and necrosis of renal tubular epithelial cells at day 51 of the experiment. Most of the tubular epithelial cells revealed pyknotic nucleus. In tissues sections from kidneys of quail received higher doses (100 and 125 mg/kg b.w), severe congestion, increase urinary spaces and necrotic changes in tubular epithelial cells (Fig.1) were observed. In some birds, hypertrophy of glomeruli and loss of circular shape containing few erythrocytes were also observed at higher doses throughout the experiment. Histologically, at day 17 mild cytoplasmic vacuolation in livers (10-15% cells) of quail given lower doses (50 and 75 mg/kg b.w) was observed. At day 34 and 51 of the experiment, mild cellular infiltration of leukocytes and nuclear condensation were also observed. Microscopic changes in hepatocytes of quail at day 17 administered malathion (100 and 125 mg/kg b.w) were moderate vacuolation of hepatocytes, granular appearance of nucleus (30-60%). However, at day 34 and 51 of the experiment, severe vacuolar degeneration in liver tissues (Fig.2), congestion and nuclear changes (fragmentation, condensation and disintegration) were observed in livers of all the birds. Hyperplasia of bile ducts and aggregation of lymphocytes were also evident at day 51 of the experiment. At higher concentrations, severe tissue changes including edema and congestion in lungs of quail were prominent throughout the experiment (Fig.3). Microscopically, congestion in thymus (Fig.4) and inflammatory reaction comprising of sloughing of the epithelium, hemorrhages and desquamation in intestine (Fig. 5) at higher doses (100 and 125 mg/kg b.w) was observed in quail throughout the experiment. Degeneration and hyalinization in muscle bundles of gizzard and pyknotic nucleus of proventriculus of quail (20-30%) was also observed at these doses.

Table 1. Serum biochemical profile for different parameters of Japanese quail administered different levels of malathion

Parameter/	Malathion (mg/kg/day)					
Days	0	25	50	75	100	125
Serum albumin (g/dl)						
17	6.66±0.03	6.47±0.09	6.36±0.02	6.07±0.02	5.29±0.05*	4.42±0.04*
34	6.59±0.02	6.36±0.01	6.17±0.02	5.59±0.07*	5.18±0.05*	4.29±0.06*
51	6.67±0.02	6.32±0.03	5.99±0.05	5.21±0.09*	4.15±0.02*	4.05±0.02*
Total protein (g/dl)						
17	3.37±0.01	3.26±0.05	3.24±0.09	3.14±0. 01	2.80±0.01*	2.55±0.05*
34	3.37 ±0.01	3.10±0.09	2.97±0.04	2.80±0. 04*	2.54±0.04*	2.30±0.03*
51	3.40 ±0.01	3.06±0.01	2.87±0.01*	2.24±0.02*	2.11±0.01*	2.08±0.01*
Creatinine (mg/dl)						
17	1.37±0.18	1.40±0.17	1.41±0.13	1.44±0.15	1.48±0.26	1.66±0.96*
34	1.39±0.13	1.45±0.32	1.50±0.63	1.66±0.73*	1.75±0.57*	1.91±0.38*
51	1.41±0.17	1.48±0.33	1.60 ±0.58	1.79±0.58*	1.94±0.56*	2.14±0.28*
Lactate dehydrogenase (unit/L)						
17	377.2±4.05	386.3±2.85	406.5±3.59*	418.9±2.76*	438.5±1.50*	460.1±1.70*
34	381.1±3.85	396.6±1.38	415.3±2.78*	429.9± 3.18*	447.2±2.50*	468.1±1.73*
51	384.7±4.05	401.1±1.77	421.7±5.11*	446.9±3.33*	464.5±3.32*	485.1±3.51*
Aspartate transaminase (unit/L)						
17	151.9±3.43	159.7±1.63	166.9±2.43	169.8±1.25	180.5±1.39*	183.5±1.74*
34	155.5±1.54	168.5±1.97	171.7±2.04	173.5± 1.65	187.7±1.91*	196.2±2.10*
51	154.5±1.68	170.6±2.14	176.5±1.91	182.1±2.29*	195.8±2.25*	202.5±2.33*
Alanine transaminase (unit/L)						
17	8.70±0.25	8.67±0.45	9.21±0.96	9.41±0.68	9.77±0.33	10.52±0.19*
34	8.79±0.26	8.92±0.15	9.76±0.49	9.81±0.11	10.94±0.12*	13.42±0.29*
51	8.55±0.27	9.50±0.19	10.76±0.13*	12.11±0.29*	13.86±0.21*	15.25±0.26*

Values (mean±SE) in rows bearing asterisk are significantly ($P \leq 0.05$) different from control group.

Table 2. Serum malondialdehyde concentration (µm/l) in malathion treated male Japanese quail

Malathion	Experimental days		
(mg/kg/day)	17	34	51
0	1.90±0.02	1.97±0.06	2.03±0.09
25	1.94±0.15	2.07±0.13	2.14±0.22
50	2.06±0.01	2.12±0.04	2.25±0.07
75	2.11±0.03	2.66±0.02*	2.70±0.02*
100	2.30±0.01*	2.70±0.01*	2.82±0.02*
125	2.53±0.04*	2.90±0.01*	3.17±0.05*

Values (mean±SE) in rows bearing asterisk are significantly ($P \leq 0.05$) different from control group

Serum biochemical parameters: The results on serum biochemical parameters are presented in Table 1. Serum albumin concentration was significantly reduced throughout the experiment in quail given higher doses 100 and 125 mg/kg b.w) of malathion as compared to control group. These values were also significantly decreased in birds received malathion (75 mg/kg b.w) at day 34 and 51. Serum total protein was significantly decreased at day 17 of experiment in quail given higher doses (100 and 125 mg/kg b.w), while at day 34 in birds (75, 100 and 125 mg/kg b.w) and at day 51 lower values of total protein were recorded in birds treated malathion (50, 75, 100 and 125 mg/kg b.w). Serum creatinine values at day 17 was significantly increased in birds administered malathion (125 mg/kg b.w) and in quail at day 34 and 51 given malathion (75,100 and 125 mg/kg b.w). Serum lactate dehydrogenase (LDH) concentration was significantly increased throughout the experiment in birds given (50, 75, 100 and 125 mg/kg b.w). Serum AST values in birds treated higher doses (100 and 125 mg/kg b.w) were significantly increased throughout the experiment. However, in birds treated malathion (75 mg/kg b.w) at day 51 also significantly increased. Serum ALT (ng/ml) concentration was significantly increased in

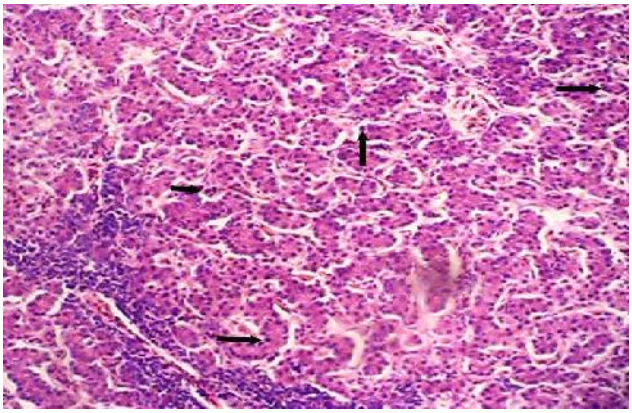

Fig. 1. Photomicrograph of kidneys showing necrotic (*arrows*) and vesicular nuclei. (200 X, H&E stain).

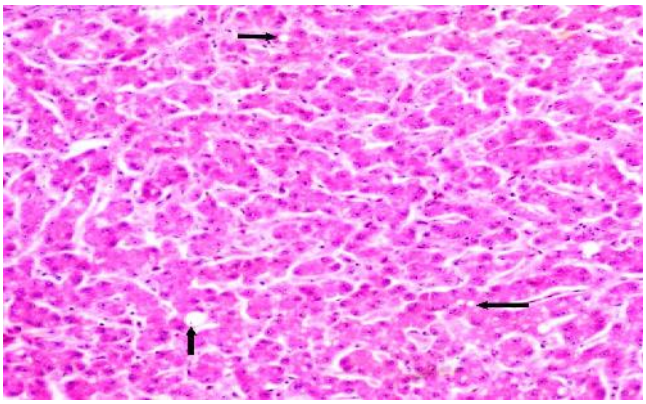

Fig. 2. Photomicrograph of liver showing the severe fatty vacuolation (*arrows)* in hepatocytes. (200 X, H&E stain).

birds given malathion (125 mg/kg b.w) at day 17, at day 34 in birds given malathion (100 and 125 mg/kg b.w) and at day 51 in birds given (50, 75,100 and 125 mg/kg b.w) of the experiment. Serum malondialdehyde (MDA) concentration (µm/l) in malathion treated male Japanese quail is presented in Table 2. Serum MDA levels were significantly increased in quail given higher doses of malathion (100 and 125 mg/kg b.w) at all the experimental days and in quail given malathion (75 mg/kg b.w) at day 34 and 51 of the experiment.

DISCUSSION

Malathion, a widely used pesticide in agro-production, ectoparasite control in livestock, grain storage and public health management is an important member of organophosphate pesticide and affects a variety of organs (Suresh *et al.,* 2009; Falicia *et al.,* 2011). In Pakistan most of the farmers are illiterate and extensively use pesticides without following the technical literature and guidelines provided with these chemicals. Moreover, they dispose of pesticide containers in the field and even use for watering

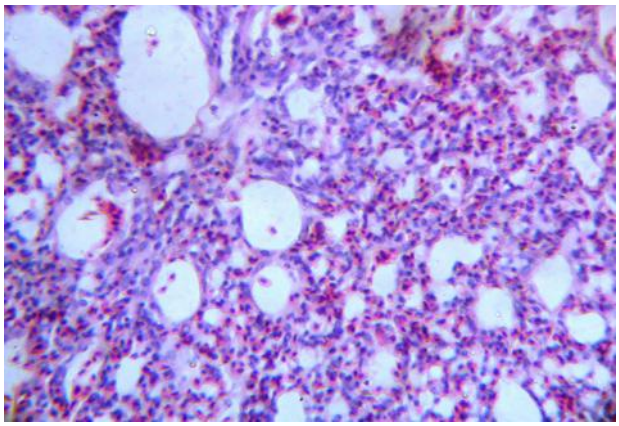

Fig. 3. Photomicrograph of lungs showing edema and congestion. (200X, H&E stain).

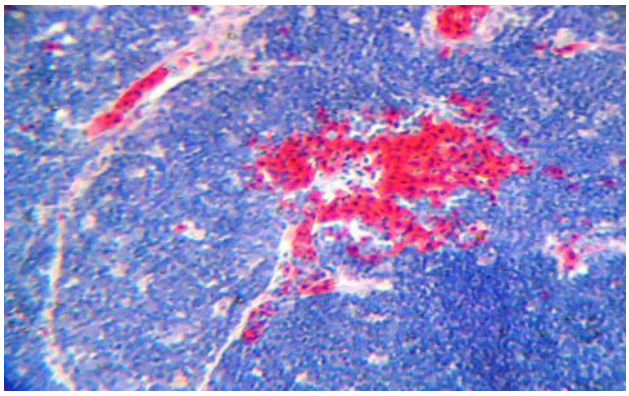

Fig. 4. Photomicrograph of thymus showing congestion. (200 X, H&E stain).

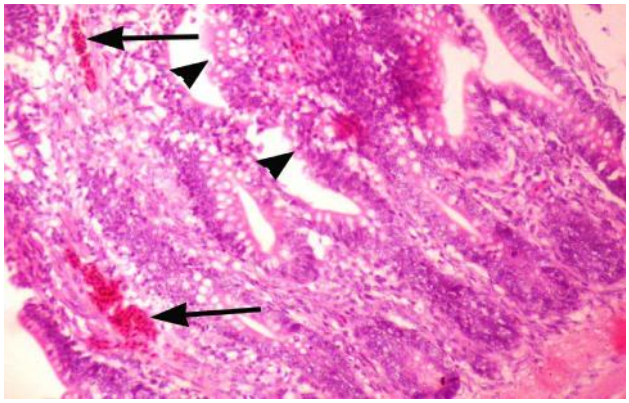

Fig. 5. Photomicrograph of intestine showing sloughing of epithelium (*arrow head*) and congestion (*arrows*). (200 X, H&E stain).

of birds and animals. Acute and chronic toxic effects of malathion on fish, mammals and non-target invertebrates are well established both under field and experimental conditions (Uzun *et al.,* 2009; Sodhi *et al.,* 2008; Moore *et al.,* 2009), but few studies have shown its toxic effects in birds (Maitra *et al.,* 2008; Kalipci *et al.,* 2010). Previously it has been determined that different pesticides

can induce various cytopathological and histopathological changes in reproductive tissues (Uzunhisarcikli *et al.,* 2007). In present study, gross changes in different visceral organs including swollen and congested kidneys, liver, lungs and thymus were linked with different levels of malathion and have not been previously reported. However, increased weight of liver, kidneys and spleen in quail exposed to malathion (Mahmoud *et al.,* 2012) and increased weight of kidneys in rats have been reported (Mossalam *et al.,* 2011). These macroscopic lesions are suggestive of systemic toxicity of malathion.

In present study, microscopic changes observed in lungs, liver, spleen, proventriculus, gizzard, intestine and kidneys tissues of different birds were severe degeneration, congestion, leukocyte infiltration and necrotic DNA. These necrotic changes observed in present study could be due to increased release of IL-1α and IL-33 from necrotic cells and are also linked with higher synthesis of intracellular DAMPS (HsP, N formal peptides and neuropeptides) and extracellular DAMPS (biglycan and hyloroune). Previously no reports could be found in accessible literature about the histopathological effects of malathion in lungs, proventriculus, gizzard, intestine and thymus tissues in birds. These histopathological lesions could be attributed to systemic toxicity. Similar light microscopic changes in liver, kidneys and spleen have been reported in birds and mammals (Mossalam *et al.,* 2011; Mahmoud *et al.,* 2012).The histopathological alterations in tissues could be due to accumulation and pathophysiological consequence of organophosphorus in visceral organs of the birds (Ahmad *et al.,* 2012; Mahmoud *et al.,* 2012). These pathological alterations suggest that organophosphorus insecticides manifest their toxic effects due to their ability to produce reactive oxygen species (ROS) (Altuntas *et al.,* 2002; Uzunhisarcikli *et al.,* 2007) a major cellular source of oxidative stress which damage biological membrane, carbohydrates, lipids and nuclear material of the cells (Gawish *et al.,* 2006; Sarabia *et al.,* 2009). Serum albumin and total protein concentration was significantly reduced in present study. These changes could be due to the toxic effects of pesticide on hemato-biochemical functions, impaired protein synthesis by hepatocytes in liver and leakage of protein from damaged kidneys. Decrease concentrations of serum albumin and total protein in rats (Mossalam *et al.,* 2011) and in quail (Mahmoud *et al.,* 2012) have been reported. Serum creatinine, AST, LDH and ALT levels were significantly increased in birds. The increased level of serum creatinine in the present study is suggestive of tubular renal insufficiency, impaired glomerular function, urinary tract obstruction and kidneys damage. These biochemical changes are evident of liver and kidney damage. Similar results have also been reported (Mossalam *et al.,* 2011; Mahmoud *et al.,* 2012, Malik *et al.,* 2004; Al-Attar, 2010). Increased levels of lipid peroxidation product and biomarker of oxidative stress, serum MDA in present experiment indicated injuries to membranes and cytoplasmic structures, impairment and dysfunction of biological mechanism. Previously no reports are available about the lipid per oxidation product in birds; however, similar results have been reported in rats (Mossalam *et al.,* 2011). The results of the present experimental study on the basis of gross, microscopic pictures of different visceral organs and serum biochemical investigation it can be concluded that malathion poses toxicity and induces oxidative stress to birds even at low levels.

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