Histopathological and Serum Biochemical Changes Induced by Sub-Chronic Doses of Triazophos in Quail

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The objective of this study was to evaluate the possible histopathological and some serum biochemical effects of frequently used triazophos (O, O-diethyl O-1-phenyl-1H-1, 2, 4-triazol-3-yl phosphorothioate), an organophosphate insecticide in avian species. For this purpose a total of 60 adult Japanese quail were randomly allocated to five equal groups (A-E) having 12 birds each. All the birds in experimental groups were orally administered triazophos @ 0, 2, 4, 6 and 8 mg/kg BW daily for 48 days. The blood and morbid tissues were collected at day 16, 32 and 48 of the post treatment. Grossly, kidneys, lungs, spleen and heart of birds received higher concentration of triazophos (8 mg/kg BW) were swollen and congested. No significant change was observed in relative weight of bursa, trachea and proventriculus. However, the relative weight of spleen, kidneys and lungs was significantly increased while the relative weight of heart significantly decreased as compared to control group. Serum urea, creatinine, cardiac enzymes (CPK and CK-MB) were significantly increased while total proteins decreased in treated birds. Histologically, mild congestion, hypertrophied glomeruli, detachment of renal tubules, tubular necrosis, atrophied Bowman’s spaces and pyknotic nuclei of epithelial cells in tissue sections of kidneys were observed. Heart tissues showed degenerating cardiac muscle fibers along with loss of transverse striations. Bursa of Fabricius in treated birds showed severe cytoplasmic vacuolation. Congestion in lungs and spleen was also observed at higher concentrations of insecticide. The present study revealed for the first time that triazophos causes histo-architectural and serum biochemical changes in birds in proportion to dose and duration.

INTRODUCTION

Pesticides (insecticides, herbicides and fungicides) are frequently employed in agriculture to prevent pathogens and unwanted weeds (Khan et al., 2012; Hundekari et al., 2013; Usman et al., 2013; Hussain et al., 2014). Due to low persistence and less toxicity, the organophosphorus compounds are commonly used in cereal crops (Abou-Donia, 2003; Kamath and Rajini, 2007; Gilden et al., 2010; Deka and Mahanta, 2012; Hussain et al., 2013; Ali et al., 2014). Excessive use of organophosphorus compounds in public health management and agro-production sector results in the accumulation of OPs residues in vegetables, cereal crops, soil, natural water systems and atmosphere (Schipper et al., 2008; Falicia et al., 2011; Chishti and Arshad, 2013). Organophosphorus pesticide poisoning results in long-term and major negative impacts directly or indirectly on birds, mammals and induces serious threats to biodiversity due to physicochemical changes (Smita et al., 2011; Ghazala et al., 2014). Organophosphorus pesticides are derivatives of phosphoric acid which severely affect central nervous system by inhibiting nicotinic and muscarinic acetyl cholinesterase (Kumar et al., 2010; Hundekari et al., 2011; Kazemi et al., 2012). After entry through inhalation or ingestion these insecticides are quickly accumulated in fatty tissues including, liver, salivary glands and kidneys (Vale, 1998). Organophosphorus can cause direct damage to cells and organs of the immune system and decrease the immune function. Different reports have indicated that pesticides may induce degenerative changes in different tissues both

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in animals and human beings (Hundekari et al., 2013). Hepatotoxic and nephrotoxic effects of triazophos have been observed in rats (Jain et al., 2010). Histopathological changes in immune tissues and organs, cellular pathology, altered maturation, changes in lymphocytes and functional alterations to immune competent cells are recognized after OPs exposure in mammals (Voccia et al., 1999; Ambali et al., 2011). Different experimental studies have revealed that exposure to triazophos in rats induces oxidative stress, increases the serum glutathione-S-transferase (GST), reduces erythrocyte glutathione (GSH) and degenerative changes in kidney, spleen and liver (Jain et al., 2010) and decreases total proteins in fish (Naveed et al., 2010). However, up to the best of our knowledge no report is available in published literature about the histopathological changes along with some serum biochemical alterations in birds due to triazophos insecticide. Therefore, the present experimental study was carried out to determine the histopathological effects of extensively used organophosphorus insecticide in different tissues of adult male Japanese quail (Coturnix japonica).

MATERIALS AND METHODS

Experimental birds: In the present experimental study adult male Japanese quail were purchased from the local hatchery and kept under similar housing conditions in laboratory of Zoology, Department of Life Sciences, The Islamia University of Bahawalpur. All the birds were given fresh clean water and basal diet having 20% protein (Olympia Feeds, Lahore Pakistan). All the birds were healthy and kept for 10 days in wire cages for acclimatization purpose.

Experimental protocol: After acclimatization a total of 60 adult male quail having approximately 4-5 weeks of age were randomly selected and kept in five equal groups (A-E) having 12 birds each. Triazophos (40% technical grade) was mixed in clean water and administered to each bird @ 0, 2, 4, 6 and 8 mg/kg/day, respectively using crop tube daily. The experiment lasted for 48 days.

Organ weight and histopathology: For gross and histopathological observations randomly selected four birds from each group were killed by cutting juguler vein at days 16, 32 and 48 of the experiment. After slaughtering different visceral organs including kidneys, heart, spleen, intestine, lungs, bursa and proventriculus were removed and examined for presence of any gross changes. The relative weight of these organs was determined as percentage of body weight. About 4-5 mm thick tissues were removed from the all visceral organs and were preserved in 10% buffered formalin. For histopathological observations about 5µm thick sections were processed using paraffin-wax embedding technique. The sections were stained with hematoxylin and eosin (Bancroft and Gamble, 2008).

Serum biochemical analysis: For serum biochemical parameters the birds were killed at days 16, 32 and 48 of the experiment. Blood was collected without anticoagulant. Serum was separated by placing the blood samples on ice and stored at -20°C for further analysis. Serum urea, creatinine, total proteins and cardiac enzymes (CPK and CK-MB) were analyzed spectrophotometrically by using commercially available kits.

Statistical analyses: All the collected data were subjected to statistical analysis. The means and their standard errors were also computed. For comparison the means were subjected to Tukey’s test with P<0.05.

RESULTS

Grossly the lungs, spleen, heart, kidneys, intestine, proventriculus and trachea of quail in groups A-C were normal in color and consistency throughout the experiment. However, lungs of quail in groups D and E were swollen, edematous and congested. Kidneys of birds in these groups were also swollen and congested as compared to group A. Grossly, spleen and heart of birds received higher concentration of triazophos (8 mg/kg BW) were swollen and congested.

No significant change was observed in relative weight of different organs such as bursa, trachea and proventriculus (Table 1) of treated birds when compared to the birds of control group. However, the relative weight of spleen, lungs and kidneys was significantly increased in groups D and E at day 48 of the experiment. The relative weight of heart in birds at higher values of triazophos was significantly reduced in groups D and E at day 48 of the experimental study (Table 1).

Extensive histological changes were observed in kidneys. These microscopic alterations included mild congestion, hypertrophied glomeruli, detachment of renal tubules, severe tubular necrosis, atrophied Bowman’s spaces and pyknotic nuclei of epithelial cells of quail given higher levels. Light microscopic analysis of heart sections showed degenerating cardiac muscle fibers along with loss of transverse striations. Histologically, pyknotic nuclei of proventriculus, moderate degeneration and hyalinization in muscle bundles of gizzard were observed at higher doses. Tissue sections from intestine indicated histoarchitectural changes such as sloughing of the epithelium, desquamation and hemorrhages at higher doses (6 and 8 mg/kg BW) throughout the present experiment. Bursa of Fabricius in treated birds showed cytoplasmic extensive vacuolation (Fig.1). Most of these vacuoles were pushing the nucleus toward the periphery. Congestion in lungs (Fig. 2) and spleen (Fig.3) was also observed at higher levels of insecticide.

The results on some serum biochemical parameters including different cardiac enzymes (Table 2) revealed that the serum urea and creatinine increased significantly, while serum total proteins decreased significantly in birds of groups D and E when compared to control group throughout the experiment. The concentrations of cardiac enzymes including CPK and isoenzyme CK-MB were significantly increased in birds of groups D and E as compared to control group.

DISCUSSION

Extensive and indiscriminate use of pesticides to protect different cereal crops poses serious threats to humans, birds and variety of other animals living in the
same ecosystem and the surrounding environment (Naveed et al., 2010; Hussain et al., 2012). Triazophos, a non-systemic insecticide, is broadly used in agro-production, livestock and public health (Mitra et al., 2011; Hundekari et al., 2013). Acute and chronic toxic effects of triazophos in different animals including non-target invertebrates are well established (Jain et al., 2010; Naveed et al., 2011) both under field and experimental conditions. However, the scanty information is available about its toxic effects in birds (Kumari et al., 2001).

In the present study, results showed gross abnormalities in different visceral organs at low levels of insecticide. Different gross changes such as swollen and congested lungs, kidneys, spleen and heart were prominent in birds given higher concentrations of triazophos which have not previously reported in birds. The relative weight of spleen, kidneys and lungs was increased significantly while the relative weight of heart was significantly decreased when compared to birds of control group. Previously no reports could be found in accessible literature about the macroscopic effects of triazophos in lungs, proventriculus, gizzard, and intestine. These gross lesions could be attributed to systemic toxicity. However similar results due to organophosphates poisoning in rats (Mossalam et al., 2011) and birds (Mahmoud et al., 2012) are available which indicate systemic toxicity of triazophos.

In the present study, extensive histological changes such as mild congestion, hypertrophied glomeruli, detachment of renal tubules, severe tubular necrosis,
atrophied Bowman’s spaces and pyknotic nuclei of epithelial cells of kidneys were observed at higher levels. Histologically, pyknotic nuclei of proventriculus, moderate degeneration and hyalinization in muscle bundles of gizzard, congestion in lungs and spleen were also observed at higher doses. These histarchitectural alterations in these tissues could be related to pathophysiological consequence due to accumulation of insecticide in visceral organs. These histopathological changes suggest that organophosphorus pesticides exhibit their harmful effects due to their ability to generate reactive oxygen species which injure the biological membrane, nuclear material, lipids and carbohydrates of the cells. Previously, histopathological alterations in brain, spleen and kidneys of rats treated with triazophos have been reported (Jain et al., 2010). In the present study histopathological analysis of heart tissues showed degenerating cardiac muscle fibers along with loss of transverse striations while in intestine tissues, sloughing of the epithelium, desquamation and hemorrhages were observed at higher doses (6 and 8 mg/kg BW). Bursa of Fabricius in treated birds showed extensive cytoplasmic vacuolation. These necrotic changes observed in different visceral tissues in our study could be due to increased release of IL-1α and IL-33 from dead cells. Moreover these changes can also be related to increased production of intracellular DAMPS (N formal peptides, HSP and neuropeptides) and extracellular DAMPS (biglycan and hyloroune).

The serum total proteins values were significantly decreased. The decreased in total proteins in current study could be due to impaired protein synthesis in liver, leakage of proteins from kidneys and altered hemato-biochemical functions. The toxicants may influence the hormonal balance which could directly or indirectly change the tissue protein levels. Decreased concentrations of liver proteins in fish (Naveed et al., 2010; Singh, 2013) have been reported. Serum creatinine and urea levels increased in birds suggesting tubular renal insufficiency, urinary tract obstruction, impaired glomelular function and kidneys damage (Mossalam et al., 2011; Mahmoud et al., 2012). The concentrations of cardiac enzymes including CPK and isoenzyme CK-MB were also significantly increased in treated birds. Previously in accessible literature no report is available about the toxic effects of triazophos on the concentrations of CPK and isoenzyme CK-MB in birds. Increased levels of these cardiac enzymes could be related to increased lipid peroxidation (oxidative stress) process due to increased generation of reactive oxygen species (ROS) causing injuries to biological membranes and cellular cytoplasmic structures. Previously increased status of oxidative stress due to organophosphate pesticides has been reported (Jain et al., 2010; Mossalam et al., 2011; Hundekari et al., 2013).

Form the findings of the present study it can be concluded that sub chronic exposure of triazophos induces various gross and serum biochemical changes in birds.

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