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HEMATO-BIOCHEMICAL AND TESTICULAR CHANGES INDUCED BY SUB-CHRONIC DOSES OF TRIAZOPHOS IN MALE JAPANESE QUAIL

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The present study was conducted to investigate the possible hemato-biochemical and histopathological changes induced by concurrent administration of triazophos in male Japanese quail. For this purpose, healthy and sexually mature 60 adult male Japanese quail were divided into five equal groups. The birds were kept in wire cages for six days for adaptation. The duration of the experiment was 48 days. All the birds in groups A-E were orally administered triazophos (O, O-diethyl O-1phenyl-1 H-1, 2, 4- triazol-3-yl phosphorothioate) an organophosphate insecticide @ 0, 2, 4, 6 and 8 mg/kg BW daily for 48 days. The blood/serum and morbid tissues were collected at post treatment days 16, 32 and 48. Significant ($P \leq 0.05$) decrease in relative weight of testes and liver, while an increase in relative weight of thymus was recorded in treated birds as compared to controls. Histologically, pyknotic nuclei with extensive vacuolation of hepatocytes and congestion in thymus was observed. Treatment also induced various testicular changes including admixture of necrotic cells, arrest of spermatogenesis, decreased seminiferous tubule diameter, germinal epithelial height and number of seminiferous tubules with normal sperms. Significantly increased activity of serum alanine transaminase, aspartate transaminase and lactate dehydrogenase with increased levels of cholesterol, triglycerides, high density lipids, low density lipids and malondialdehyde were also recorded in triazophos treated birds. Moreover, frequency of micronuclei formation, erythrocytes with lobed nuclei, blebbed nuclei, notched nuclei and pear shaped erythrocytes was significantly increased in treated birds. The present study revealed for the first time that triazophos induces histo-architectural and serum biochemical changes in birds.
Keywords: Triazophos, quail, micronucleus assay, nuclear changes, testis, histopathology

INTRODUCTION

Currently, the ecosystem is continuously exposed to different forms of pollution arising from various human activities. Over 80% of water pollution results from the mainland and originates from urban, agricultural and industrial activities (Barranger *et al*., 2014). Avian species in particular, have a distinctive place in ecosystem and are the best indicator of healthy state of the ecosystem. Different species of migratory birds from different biodiversities are exposed directly or indirectly to various pollutants due to extensive spray of insecticides/pesticides on agricultural crops which leads to physicochemical alterations on the mainland and poses serious threats to these birds (Mitra *et al*., 2011; Smita *et al.,* 2011; Aoun *et al*., 2014). Pesticides are synthetic organic compounds that have played imperative role in home and public health management all over the world (Mitra *et al*., 2011; Yang *et al*., 2014) and are a major cause of diseases and deaths in developing countries (Saxena and Saxena, 2010; Ali *et al*., 2014). Various pesticides include insecticides which are used to kill broad range of insects and rodents, fungicides to control molds (Mnif *et al*., 2011) and herbicides to remove various weeds present in agro-ecological system (Hussain *et al*., 2012; Hussain *et al*., 2014). Among different compounds frequently used in the field of agriculture, dairy industry and medicine, the organophosphates (OPs) are preferred due to low their persistence and less toxicity than organochlorine pesticides (Deka and Mahanta, 2012; Hundekari *et al*., 2013). Extensive application of OPs pesticides in agro-production sector, grain storage and public health management causes accumulation of their residues in different daily consumable food materials such as vegetables, cereal crops, water systems and act as the major sources of contamination (Falicia *et al*., 2013). These can cause direct damage to cells and organs of the immune system and decrease the immune functions. OPs compounds severely affect central nervous system, as they are the inhibitors of nicotinic and muscarinic acetyl cholinesterase (Mitra *et al*., 2011; Hundekari *et al*., 2011; Kazemi *et al*., 2012; Ahmad *et al*., 2015). Malondialdehyde (MDA), a lipid peroxidation product, is increased during different diseases associated with changes in biochemical and physiological functions of red blood cells caused by toxins. Determination of oxidative stress due to production of free radicals such as reactive oxygen species (ROS) has become an important pocus of studies in animals (Qin *et al*., 2014). Oxidative stress and tissue damage due to OPs pesticides has been reported (Sharaf *et al*., 2010; Hussain *et al*., 2013). Hematological and serum biochemical changes are the best indicators to determine oxidative stress in different species (Hussain *et al*., 2011; Shahzad *et al*., 2014).

Triazophos (O,O-Diethyl O-(1-phenyl-1H-1,2,4-triazol-3-yl) phosphorothioate), being an important member of Ops pesticides, is frequently used in agro-production sector to protect fruit plants and different crops such as vegetables, maize, cotton and sugarcane (Jain *et al*., 2010; Smita *et al*., 2011). Different reports have revealed that triazophos induces degenerative changes in different tissues of mammals (Hundekari *et al*., 2013). Reports are available about the hepatotoxic and nephrotoxic effects of triazophos in rats (Jain *et al*., 2010), pathological and functional changes in immune related cells in rats (Ambali *et al*., 2011). Various studies have indicated that triazophos induces oxidative stress in rats, reduces erythrocyte glutathione (GSH), increases serum glutathione-S-transferase (GST) and induces degenerative changes in kidneys, spleen and liver in fish (Jain *et al*., 2010; Naveed *et al*., 2010; Naveed *et al*., 2011).

Although different reports are available in published literature regarding the toxicological effects of triazophos in animals, but scanty information is available about its toxic effects on hemato-biochemical profile in birds (Kumari *et al*., 2001). Therefore, the present experimental study was executed to determine the hemato-biochemical and testicular effects of triazophos in adult Japanese quail (*Coturnix japonica*).

MATERIALS AND METHODS

***Experimental birds*:** In the present study, 60 sexually mature male Japanese quail, weighing 115-120 gm, were used. All the birds were kept in wire cages and maintained under similar physical housing conditions (daily photoperiod: about 12-14 h) in laboratory of Zoology, Department of Life Sciences, The Islamia University of Bahawalpur, with temperature of 30±2°C and relative humidity 50–60%. Clean fresh water and commercially available standard feed (Olympia Feeds, Lahore Pakistan) having 20% proteins was given to all the birds.

***Test material and experimental protocol*:** Triazophos (95%) was obtained from M/S Pak–China chemicals Lahore. Different concentrations of triazophos was mixed separately in corn oil and administered to male birds through oral intubations. After seven days of acclimatization, experimental quail were randomly divided into five equal groups (A-E) having 12 birds each. Triazophos was given to each bird daily in groups A-E @ 0, 2, 4, 6 and 8 mg/kg BW/day, respectively for 48 days.

***Gross and light microscopic investigations*:** For gross and light microscopic investigations four birds from each group were randomly selected and killed at day 16, 32 and 48 by cutting the juggler vein. After killing, the visceral organs including testes, thymus and liver were removed and examined for gross abnormalities. The relative weight of these organs as percentage of body weight was determined. Finally, for light microscopic analysis about 4-5µm thick sections from these organs were processed using paraffin–wax embedding and hematoxylin and eosin staining technique (Babińska *et al*., 2013; Jing *et al*., 2014). Blood samples were also collected from each bird, serum was harvested and stored at -20°C for further analysis.

***Measurement of seminiferous tubular diameter and epithelial height*:** For this purpose, histologically, 100 semi-rounds or round seminiferous tubules of each testis of mature quail were randomly selected and their images were obtained with the help of light microscope to measure the tubule diameter and epithelial height using image analysis software, Image J, version 1.41 (Rasband, 2008).

***Relative number of seminiferous tubules with normal and degenerated testicular germ cells*:** A total of 100 randomly selected seminiferous tubules from the each microscopic section observed from each testis of experimental bird were examined and counted for the presence of normal and degenerated germ cells. The mean values of these parameters were presented in percentage.

***Morphological and nuclear alterations in erythrocytes*:** For monitoring morphological and nuclear alterations in erythrocytes duplicate thin fresh blood smears were prepared from each quail and stained with Wright-Giemsa for at least 5-7 min. Finally, a total of 2000 erythrocytes/smear/bird were observed using oil immersion (100x) lens with the help of computer-assisted microscope (Nikon, Tokyo, Japan), as described earlier (Hussain *et al*., 2014).

***Serum biochemical parameters*:** Serum samples collected from experimental birds on day 16, 32 and 48 were analyzed for serum enzymes, cholesterol, triglycerides, high density lipids, low density lipids were analyzed by spectrophotometrically using commercially available kits (Ahmad *et al*., 2013; Khan *et al*., 2013). Serum malondialdehyde (MDA) was determined spectrophotometrically (Hussain *et al*., 2013).

***Statistical analyses*:** Data were subjected to statistical analysis by ANOVA. Mean±SE values were calculated for each parameter in each group. For multiple mean's comparison the mean values were subjected to Tukey's test with $P \leq 0.05$.

RESULTS

Physical parameters**:** Grossly the liver, thymus and testes of quail in groups A-C were normal in color and consistency. However, livers of birds in groups D and E were enlarged, lighter in color and revealed mild congestion. No gross changes were observed in thymus of quail of all experimental groups. However, the testes of quail treated with higher doses of triazophos (8 mg/kg BW) were smaller in size. Petechial hemorrhages were observed in thigh and pectoral muscles of quail in group E at day 48 of the experiment. A significant decrease in relative weight of testes of quail in groups D-E was recorded at days 32 and 48 of the experiment. Similarly, significantly lower relative weight of liver and thymus was recorded in birds of groups D and E at day 48 of the experiment (Table 1).

Histological observations**:** Histologically, pyknotic nuclei and extensive vacuolation of hepatocytes in quail of groups D-E given higher doses (6 and 8 mg/kg BW) of triazophos was observed throughout the experiment. In most of affected hepatocytes, the nucleus were pushed toward the periphery. Congestion in thymus was observed in quail of group D at day 48 and in group E throughout the experiment. Histopathological examination of the testes of quail in control group revealed the presence of all the successive stages of spermatogenesis such as spermatogonia, spermatocytes and spermatids. The testes of quail treated with higher doses of triazophos showed admixture of necrotic cells in the lumen of seminiferous tubules with arrest of the process of spermatogenesis. Seminiferous tubule diameter, germinal epithelial height and number of seminiferous tubules having normal sperms were significantly decreased in quail of groups D and E throughout the experiment when compared to control group. Seminiferous tubule diameter and number of seminiferous tubule having normal sperms were also significantly reduced at day 16 of the experiment in quail of group C as compared to control group (Table 2).

Serum biochemical variables**:** A marked increase in activity of different enzymes such as alanine transaminase, aspartate transaminase, lactate dehydrogenase and biochemical metabolites (cholesterol, triglycerides, high density lipids, low density lipids and malondialdehyde) was recorded in quail of groups D-E throughout the experiment (Table 3). Significantly increased level of these serum biochemical changes attributes also recorded in birds of group C at day 48 of the experiment as compared to control group.

Cellular abnormalities in erythrocytes**:** In present experimental study, the frequency of different nuclear and morphological alterations in erythrocytes of quail was significantly increased at all the experiment days. Among these cellular changes, the frequency of formation of micronuclei was the highest in group E quail given higher level of triazophos. The frequency of erythrocytes with lobed nuclei, blebbed nuclei and notched nuclei was significantly increased in groups D and E as compared to control group, while the frequency of erythrocytes with blebbed nucleus, vacuolated erythrocyte, notched nucleated erythrocytes and pear shaped erythrocytes was significantly increased in quail of groups C-E (Table 4).

DISCUSSION

Over the last several years, countless human illness and deaths have occurred as a result of extensive and indiscriminate use of pesticides in the field of agriculture, livestock and public health management (Mitra *et al*., 2011; Hussain *et al*., 2012; Hundekari *et al*., 2013). Pesticides are randomly used in agro production to protect various cereal crops which not only damages environment but also poses serious threats to humans, birds and variety of other animals living in the same ecosystem (Hussain *et al*., 2011; Joshi *et al*., 2012). Various toxic effects of triazophos in exposed and non-target species both under field and experimental

Table 1. Relative weight of various visceral organs of Japanese quail given different doses of triazophos

Parameters /Days	Groups A	B	C	D	E
Testes					
16	3.30±0.11	3.29±0.085	3.23±0.09	3.14±0.12	2.79±0.06
32	3.36±0.14	3.26±0.07	3.18±0.11	2.57±0.07*	2.43±0.03*
48	3.35±0.12	3.24±0.06	3.16±0.13	2.44±0.05*	2.38±0.05*
Thymus					
16	0.15±0.07	0.14±0.05	0.12±0.05	0.12±0.00	0.9±0.01*
32	0.15±0.08	0.13±0.03	0.13±0.01	0.12±0.01	0.8±0.02*
48	0.14±0.04	0.12±0.02	0.12±0.07	0.8±0.05	0.7±0.01*
Liver					
16	2.92±0.17	2.89±0.14	2.85±0.09	2.82±0.07	2.81±0.08
32	3.11±0.17	2.97±0.09	2.89±0.17	2.82±0.10	2.79±0.06
48	3.12±0.16	2.96±0.10	2.88±0.21	2.44±0.03*	2.44±0.03*

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

Table 2. Various testicular parameters of Japanese quail given different doses of tiazophos

Parameters /Days	Groups A	B	C	D	E
Seminiferous tubule diameter (µm)					
16	325.00±1.22	321.50±3.19	318.50±0.96	289.50±4.09*	268.25±3.20*
32	328.50±1.04	319.00±4.91	317.00±3.29	284.00±2.92*	257.25±4.80*
48	321.25±1.80	316.75±2.75	290.00±4.14*	275.25±2.95*	248.50±5.17*
Epithelial height (µm)					
16	89.25±0.85	83.50±1.85	82.25±0.85	74.75±0.48*	71.50±0.65*
32	88.75±1.89	86.00±1.47	82.75±1.63	71.00±0.82*	66.25±0.85*
48	84.50±1.85	83.75±1.31	80.12±0.91	67.50±0.65*	62.25±1.11*
Number of seminiferous tubules having normal sperms					
16	86.25±1.55	85.50±1.29	81.00±2.91	76.50±0.65*	71.25±0.85*
32	87.50±0.65	85.75±2.5	78.50±1.20	72.75±1.25*	68.75±0.25*
48	86.75±0.95	84.75±1.5	75.50±1.85*	68.25±0.85*	56.25±1.38*

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

Table 3. Various serum biochemical parameters of Japanese quail given different doses of tiazophos

Parameters /Days	Groups A	B	C	D	E
Alanine transaminase (U/L)					
16	7.93±0.33	7.96±0.09	8.17±0.17	9.94±0.13*	10.96±0.27*
32	8.11±0.29	8.56±0.12	8.74±0.10	9.90±0.09*	12.25±0.32*
48	8.54±0.08	8.62±0.07	10.99±0.27*	12.30±0.42*	14.57±0.19*
Aspartate transaminase (U/l)					
16	124.95±0.63	125.60±2.25	127.87±1.36	149.70±1.68*	159.98±1.21*
32	130.38±0.79	135.40±1.94	139.77±1.72	154.25±2.99*	167.48±2.06*
48	131.73±0.87	135.00±2.34	145.53±1.51*	156.70±2.89*	172.55±2.01*
Lactate dehydrogenase (U/L)					
16	349.93±2.97	354.83±2.20	355.20±4.63	388.38±3.44*	410.80±3.31*
32	353.73±2.30	363.55±3.08	378.98±1.13	392.20±2.27*	425.10±3.85*
48	354.50±1.29	368.05±3.44	384.83±1.62*	398.83±1.72*	432.00±3.65*
Cholesterol (mg/dL)					
16	116.38±2.43	118.33±1.04	119.00±1.45	133.98±1.83*	141.20±1.04*
32	117.18±3.08	119.88±0.54	120.58±0.82	136.80±1.71*	142.88±1.25*
48	116.70±1.75	120.45±0.49	131.03±0.80*	139.90±1.65*	145.82±0.23*
Triglycerides (mg/dL)					
16	63.03±1.40	67.03±1.44	69.33±0.89	76.85±0.97*	82.18±0.91*
32	64.58±1.13	68.58±1.45	70.53±0.85	78.65±0.64*	84.18±1.14*
48	66.23±2.07	69.13±2.21	78.13±0.86*	80.85±0.79*	86.70±0.26*
High density lipids (mg/dL)					
16	34.88±0.63	36.55±0.50	37.10±0.40	40.90±0.26*	43.25±0.50*
32	35.90±0.42	37.45±0.42	40.68±0.60	42.60±0.48*	45.50±0.46*
48	35.18±0.36	38.23±0.40	41.73±0.65*	43.55±0.52*	47.73±0.65*
Low density lipids (mg/dL)					
16	28.33±0.56	30.73±0.40	31.28±0.25	34.33±0.32*	38.78±0.61*
32	29.68±0.30	31.75±0.48	32.25±0.19	36.15±0.57*	42.13±1.41*
48	30.60±0.24	32.78±0.82	36.90±0.28*	38.95±0.68*	45.00±0.91*
Malondialdehyde concentration					
16	67.03±1.40	70.03±1.44	72.33±0.89	76.85±0.97*	82.18±0.91*
32	68.58±1.13	72.58±1.45	74.53±0.85	78.65±0.64*	84.18±1.14*
48	69.23±2.07	74.13±2.21	77.13±0.86*	80.85±0.79*	86.70±0.26*

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

conditions are well-known (Naveed *et al*., 2010; Jain *et al*., 2010; Singh, 2012). However, few reports are available about its toxic effects in birds (Kumari *et al*., 2001; Ghaffar *et al*., 2014).

The present study showed that the administration of triazophos to quail at higher dose levels induced different gross changes in visceral organs in time dependent manner. The relative weight of liver and thymus triazophos in treated

Table 4. Various morphological and nuclear changes in erythrocytes (%) of Japanese quail given different levels of triazophos

Parameters /Days	Groups				
	A	B	C	D	E
Erythrocyte with micronucleus					
16	0.11±0.01	0.13±0.03	0.15±0.03	0.74±0.05*	1.61±0.03*
32	0.12±0.01	0.14±0.02	0.15±0.01	1.69±0.07*	1.69±0.02*
48	0.11±0.03	0.15±0.02	0.16±0.02	1.59±0.11*	1.68±0.01*
Erythrocytes with Lobed Nucleus					
16	0.28±0.03	0.32±0.01	0.31±0.01	0.33±0.03	0.47±0.02*
32	0.26±0.02	0.30±0.01	0.35±0.05	1.42±0.03*	1.42±0.07*
48	0.29±0.02	0.34±0.02	0.37±0.02	1.45±0.01*	1.47±0.02*
Erythrocyte with Blebbed Nucleus					
16	0.19±0.01	0.22±0.04	0.23±0.03	0.85±0.01*	0.96±0.01*
32	0.48±0.05	0.52±0.09	0.63±0.09*	1.87±0.012*	1.94±0.01*
48	0.49±0.03	0.54±0.06	0.87±0.01*	1.95±0.01*	1.98±0.08
Notched Nucleated Erythrocyte					
16	0.05±0.05	0.07±0.04	0.08±0.02	0.63±0.01*	0.77±0.01*
32	0.06±0.02	0.07±0.06	0.31±0.29*	1.13±0.02*	1.24±0.02*
48	0.07±0.03	0.09±0.01	0.35±0.12*	1.34±0.06*	1.19±0.02*
Binucleated Erythrocyte					
16	0.08±0.03	0.09±0.04	0.09±0.08	0.75±0.06*	0.98±0.06*
32	0.07±0.03	0.08±0.07	0.27±0.03*	1.38±0.04*	1.45±0.07*
48	0.09±0.05	0.11±0.08	0.28±0.11*	1.46±0.02*	1.52±0.05*
Pear Shaped Erythrocytes					
16	0.29±0.01	0.30±0.03	0.34±0.05	0.83±0.01*	0.95±0.01*
32	0.27±0.02	0.30±0.04	0.35±0.03	1.43±0.01*	1.47±0.01*
48	0.29±0.03	0.33±0.01	0.42±0.06*	1.49±0.01*	1.54±0.02*

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

quail was significantly decreased as compared to control birds. Significantly decreased relative weight of testes and liver can be considered as classical indicator for pesticide toxicity. Previously, no reports could be found in accessible literature about these pathological effects of triazophos in quail which could be attributed to systemic toxicity. The decreased relative weight of these organs in the current study could also be due to deficiency or inhibition of protein synthesis which is crucial for tissue development and its deficiency can result in metabolic disorder and decrease the parenchyma of these organs. Similar observations due to OPs poisoning in rats (Mossalam *et al*., 2011) and birds (Mahmoud *et al*., 2012) are available. The decreased weight of testes could be due to less production of steroid hormone by Leydig cells, decrease in size of seminiferous tubules and arrest of spermatogenesis. Similar results have also been reported due to pesticide poisoning in mammals (Maitra *et al*., 2008).

Histologically, pyknotic nuclei and extensive vacuolation of hepatocytes with congestion was observed. The testes of treated quail showed admixture of necrotic cells in the lumen of seminiferous tubules, arrest of spermatogenesis, decreased seminiferous tubule diameter, decreased height of germinal epithelial and number of seminiferous tubules with normal sperms. Previously, no report is available about the liver and testicular changes induced by triazophos in avian species. These histological changes could be related to pathophysiological consequences following the accumulation of insecticide. Moreover, production of ROS may have injured the nuclear material, lipids and carbohydrates of the tissues. The necrotic changes observed in testicular tissues in the current study could also be due to increased release of IL-1α and IL-33 from dead cells (Hussain *et al.,* 2012). Moreover, these changes can also be related to increased production of intracellular damage associated molecular patterns (N formal peptides, HsP and neuropeptides) and extracellular DAMPS (biglycan and hyloroune).

In the current study, various serum biochemical metabolites arameters enzyme such as alanine transaminase, aspartate transaminase, lactate dehydrogenase, cholesterol, triglycerides, high density lipids, low density lipids and malondialdehyde were significantly higher in triazophos treated birds. The increase concentrations of different enzymes could be attributed to generation of free radicals that damage the biological membrane and cause labialization of lysosomal system. The necrotic changes in liver tissue observed in present study are also suggestive of liver damage which increased levels of liver enzymes. Previously, increased status of oxidative stress due to OPs pesticides has been reported (Mossalam *et al*., 2011; Hundekari *et al*., 2013; Hussain *et al*., 2014). However, in accessible literature no report is available about the triglyceride status in triazophos exposed birds. The increased level of the triglycerides in triazophos treated quail could be due to their decreased removal from the blood vascular system and increased lipid mobilization from liver (Slotkin *et al*., 2005). The increased level of cholesterol in present study may be due to the increased activity of adrenal gland in association to triazophos OPs insecticide (Malik *et al*., 2004).

In the present experimental study, the frequency of different nuclear alterations in erythrocytes such as micronuclei, lobed, blebbed and notched nuclei and morphological changes including vacuolated and pear shaped erythrocytes was significantly increased in treated quail. Previously, in published literature no report is available as far as our knowledge is concerned about the incidence of these nuclear and morphological changes in erythrocytes of quail exposed to different concentrations of triazophos. These pathological alterations could be due to over production of intracellular ROS and nitrogenous species as result of increased oxidative stress to mitochondrion (Campos-Pereira *et al*., 2012; Gad and El-Maddawy, 2014). The higher incidence of erythrocytes with micronuclei and lobed nuclei reflecting genotoxic and mutagenic damages in our experiment could be related to the increased production of caspase activated DNase responsible for the cleavage of cytoskeletal and nuclear proteins (vimentin gelsolin and fodrin) and Aneuploidy (Hussain *et al*., 2012). Previously, different workers reported that high frequencies of various nuclear alterations are related to cytotoxic effects of different chemicals which induce chromosomal abnormalities such as acentric chromosomic fragments (Lim *et al*., 2009). The vacuolated, notched, lobed, blebed and binucleated cells in the present study might be related to the process of Aneuploidy and failure of tubuline polymerization due to the toxic effects of OPs insecticide (Fernandes *et al*., 2007).

Conclusions: The results of the present study on the basis of data of hemato-biochemical and tissue changes revealed that triazophos has genotoxic and different histopathological effects even at lower concentrations in Japanese quail.

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