Endosulfan (Thiodan® EC 35) induced toxico-pathological effects in male Japanese quails (*Coturnix japonica*)

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Abstract: The objective of the present study was to determine the toxico-pathological effects of endosulfan in Japanese quails. Male Japanese quails (n=120) of 4 weeks of age were administered endosulfan in feed at 0 (control), 5, 25, 50, 100, and 500 mg/kg feed, respectively for 90 consecutive days. The quails of the 5 mg/kg group did not show clinical signs and had significantly lower values of feed intake, testes relative weight and leukocyte number than those of the control group. The quails of the 25 and 50 mg/kg groups had mild depression while those of the 100 and 500 mg/kg groups showed nervous excitation following ingestion of endosulfan. There was a dose related delay in onset of crowing, appearance of foamy material in the droppings and mounting upon the pen mates. The feed intake, body weight, erythrocyte and leukocyte counts, haematocrit values, and serum total proteins of endosulfan fed quails were significantly ($p < 0.05$) lower than that of the control group. Serum testosterone levels did not change significantly in all the treatment groups. The liver of endosulfan fed birds exhibited fatty change of hepatocytes and necrotic foci in parenchyma. Testes of the endosulfan fed quails had lower weight, volume and diameter of seminiferous tubules compared with control birds. Degenerated spermatids and multinucleated cells were present in the germinal epithelium. Severity of haematological, biochemical and pathological alterations in quails increased with the increase in dietary endosulfan levels. In conclusion, endosulfan in Japanese quails had toxic effects upon liver, nervous, haemopoietic and reproductive system. Moreover, feed intake and body weight also decreased significantly suggesting general toxicity of endosulfan.

Keywords: Body weight, *Coturnix japonica*, endosulfan, haematological values, histopathology, Japanese quail.

INTRODUCTION

Pesticides have become omnipresent contaminants of our environment and have been found in soil, air, water and also in human and animal tissues worldwide (Ahmad *et al*., 2009). The range of the adverse health effects of pesticides include and are not limited to acute and persistent injury to the nervous, respiratory, reproductive, immune and endocrine systems and may also lead to birth defects and cancer.

Among the pesticides, endosulfan ($C_9H_6Cl_6O_3S$), an organochlorine of the cyclodiene group is a broad-spectrum agricultural insecticide. It is moderately persistent in the soil environment with a reported average field half-life of 55–277 days (Shalini *et al*., 2000). Endosulfan gains entry into the body *via* oral, pulmonary and dermal routes. Its toxicity is high in fish (Naqvi & Vaishnavi, 1993) and high to moderate in rats (Reuber, 1981). In acute toxicity in rats, endosulfan causes stimulation of the nervous system, dyspnea and cyanosis (Naqvi & Vaishnavi, 1993). Possible mechanism by which neurotoxicity develops could be that organochlorine intoxication produces neural damage (Slotkin *et al*., 2007). Organochlorine interact with ɣ-amino butyric acid (GABAA) channels (Brannen *et al*., 1998) for the development of nervous signs.

Adverse effects of endosulfan upon the male reproductive system of rats include reduction in the weight of testes and accessory sex organs and inhibition of testicular functions (Chitra *et al*., 1999). Endosulfan residues have been detected in a variety of different wild and domestic mammalian species, fish and birds. Different species of birds collected from three agricultural areas with long histories of pesticide use in northwestern Mexico showed residues of endosulfan along with other pesticides (Mora & Anderson, 1991).

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Endosulfan and other organochlorine pesticides have been detected in tissues of two fish-eating and one omnivore bird species in Argentina (Cid *et al*., 2007) and in vultures in India (Muralidharan *et al*., 2008). It is also assumed that endosulfan exposure of free-range poultry and wild bird population may occur through searching for food in endosulfan treated fields and ingesting contaminated grains, plant components or insects. The presence of residues in avian species suggests the plausibility of endosulfan to induce toxico-pathological effects in these species. Garg *et al.* (2004a, b) conducted experimental studies in broiler chicks using low levels of dietary endosulfan (2mg/kg) and reported its adverse effects upon the immune system, and some biochemical parameters including blood glucose, serum globulin and acetyl cholinesterase, alkaline phosphatase activity and total protein levels. This study also reported atrophy/hypoplasia, depletion of lymphocytes and haemorrhages in the thymus. In the femur, the zones of proliferating, maturing and degenerating, and calcifying cartilage cells were reduced in width. The metaphysis in treated birds showed a reduced number of cartilage cells. An experimental study reported immunotoxicological effects in broiler chicks induced by concurrent subchronic exposure to arsenic and endosulfan (Aggarwal *et al*., 2009). A poultry lymphocyte cell culture model has been described to determine zero-immunotoxic effect of endosulfan and chlorpyrifos and other pesticides in animals (Mehta *et al*., 2008).

In previous studies a wide range of endosulfan has been administered, ranging from 2−30 mg/kg feed to broiler chicks (Garg *et al*., 2004a,b; Aggarwal *et al*., 2009) and up to 4000 mg/kg body weight in quails (Prakash *et al*., 2009). However only scanty information was available in literature about dose and duration related endosulfan induced toxico-pathological effects in avian species. Therefore, dietary levels varying from low (5 mg/kg) to high (500 mg/kg) were tested in the present experiment to observe the possible pathological effects in quails. Information about endosulfan induced clinical toxicity and toxico-pathological effects upon the male reproductive system, liver and kidneys of birds is scarce. With these observations, the present study was designed to evaluate the toxico-pathological effects of endosulfan in avian species. The Japanese quails (*Coturnix japonica*) were selected for this study because this species is extensively used as a model for different experimental studies and the results obtained may be extrapolated to other avian species.

METHODS AND MATERIALS

Experimental design: A total of 120 male Japanese quails (*Coturnix japonica*), 25 d old (95 to 105 g body weight) and apparently free from any clinical ailment were procured from a quail farm, randomly divided into 6 groups (20 birds in each group) and kept in metal wire cages at ambient temperature (26 ± 2 °C) in an animal house. The birds were fed on a corn soy basal feed containing 21 % total protein (Khan *et al*., 2010). Five experimental feeds were prepared by the incorporation of 5, 25, 50, 100 and 500 mg/kg endosulfan (Thiodan ®35 EC, 32.9 % w/w endosulfan: Aventis, Pakistan). After 1 wk of acclimatization, the birds of the different groups were randomly offered experimental feeds. One group was kept on basal feed as the control. The food and water was given *ad libitum*. To observe the age and dose related changes in organs and serum, 5 birds from each group were humanely killed on day 15 and 30 while the remaining birds were killed on day 90 of the experiment. Killing of the birds was performed as per the Ethics Committee rules, University of Agriculture, Faisalabad, Pakistan, considering the national and institutional legislations regarding animal protection and welfare.

Clinical signs and behavioural alterations: As envisaged from literature, the salient features of endosulfan toxicity include neurotoxicity (Anand *et al*., 1986) and reproductive toxicity (Jaiswal *et al*., 2005). Therefore, for the present study, signs suggestive of nervous derangement (depression, excitation, attraction to feed) and those indicating onset of sexual maturity (crowing, passing of foamy material in the faeces and mounting upon pen mates) were taken into account and were subjectively evaluated twice daily. Based upon the severity and frequency, each clinical sign was subjectively scored from 0 to + 4. The body weight of birds of each group was determined on day 15, 30 and 90 of the experiment.

Pathological parameters: The birds sacrificed on day 15, 30 and 90 were examined for the presence of gross lesions on different organs. Organs including liver, kidneys, spleen, heart and testes were weighted and their relative weights were calculated as percent of the body weight. Testes volume was determined by fluid displacement method (Khan *et al*., 2008). Part of the liver and a cross sectional slice of about 5 mm thickness from mid portion of testes of each sacrificed quail was fixed in 10 % neutral buffered formalin and processed for histopathological studies using the routine method of paraffin embedding (Awaad *et al*., 2010). Sections of 5 μm thickness were stained with haematoxylin and eosin for microscopic study.

Haematological and serum biochemical parameters: The blood of each bird was collected prior to killing for haematological and serum biochemical studies. The

haematological parameters included erythrocyte counts, total leukocyte counts, haematocrit and haemoglobin concentration (Jalees *et al*., 2011). The serum total protein concentration was determined by Biuret method and albumin by bromocresol dye binding technique. The serum testosterone levels were determined by an ELISA method (Testosterone ELISA, DRG® Instruments, GMBH, Germany).

***Statistical Analysis*:** The results obtained were statistically analyzed by analysis of variance using a computer software package (MSTATC). Different group means were compared by Duncan's multiple range tests. The level of the significance was p ≤ 0.05.

RESULTS

Clinical signs and behavioural changes

A comparison of the clinical signs based on subjective evaluation is presented in Table 1. In the control and 5 mg/kg groups, no clinical signs and behavioural changes were observed. Crowing, appearance of foamy substance in faeces and mounting upon pen mates started on day 10 of the experiment. In the 25 mg/kg group, a few birds showed a mild degree of depression in the 1st week.

Table 1: Effect of different dietary levels of endosulfan upon clinical signs and behavioural alterations in male Japanese quails

Parameters	Groups					
	A	B	C	D	E	F
Depression	0	0	+1	+1	0	0
Excitation	0	0	0	0	+2	+4
Decreased attraction towards feed	0	+1	+1	+2	+3	+3
Delayed crowing	0	0	+1	+2	+3	+4
Delayed mounting upon pen-mates	0	0	+1	+2	+3	+4
Delayed appearance of foamy material in faeces	0	0	+1	+2	+3	+4

Variation from 0 to + 4 denotes an increase in the severity of clinical signs (average of morning and evening score). Endosulfan levels in feed of groups A, B, C, D, E and F were 0, 5, 25, 50, 100 and 500 mg/kg, respectively.

Overall attraction towards feed was less than that of the control group. In this group, crowing, presence of foamy material in the faeces and mounting upon their pen mates started from day 14 of the experiment. In the 50 mg/kg group, 3 to 4 quails showed mild degree of depression in the 1st week of the experiment. Attraction towards feed was less than the control group. Crowing, presence of foamy material in the faeces and mounting upon their pen mates was observed from day 18 of the experiment. There were no difference in response to external stimuli in groups recieving 5, 25 and 50 mg/kg compared to the control.

In the 100 mg/kg group, all birds showed excitation and some birds attempted to fly in the cage. The birds responded intensely and nervously towards external stimuli compared to the control, 5, 25 and 50 mg/kg groups. Attraction towards feed was less than that of the control. Crowing, presence of foamy material in the faeces and mounting upon their pen mates started from day 21 of the experiment. In the 500 mg/kg group, nervous excitation of quails was more severe than that of the100 mg/kg group. Birds attempted to fly in the cage and hit the ceiling and walls. Five birds in this group showed torticollis. Response towards stimuli was intense and nervous. Attraction towards feed in this group was lower than that of all the other groups. Crowing, presence of foamy material in the faeces and mounting upon their pen mates was observed from day 26 of the experiment.

Body weights and endosulfan intake

The body weights of quails (Figure 1a) in the 5 and 25 mg/kg groups on day 15 of the experiment showed no significant change while those in the 50, 100 and 500 mg/kg groups showed significantly (p < 0.05) lower body weights compared to the control. On day 30 of the experiment, the quails in the 5 mg/kg group showed no significant (p > 0.05) change in body weight while all other groups showed significant (p < 0.05) lowering of the body weight when compared with that of the control group. On day 90, the quails in all treatment groups showed a significant (p < 0.05) decrease in body weight in comparison with the control group.

Relative weight of the liver and testes and volume of testes

On day 90, the relative weights of liver in the 5, 25, 50 and 100 mg/kg groups were significantly (p < 0.05) higher than that of the control group. The differences in the relative weights of the testes (Figure 2a) on day 15 of the experiment were not significant among all the
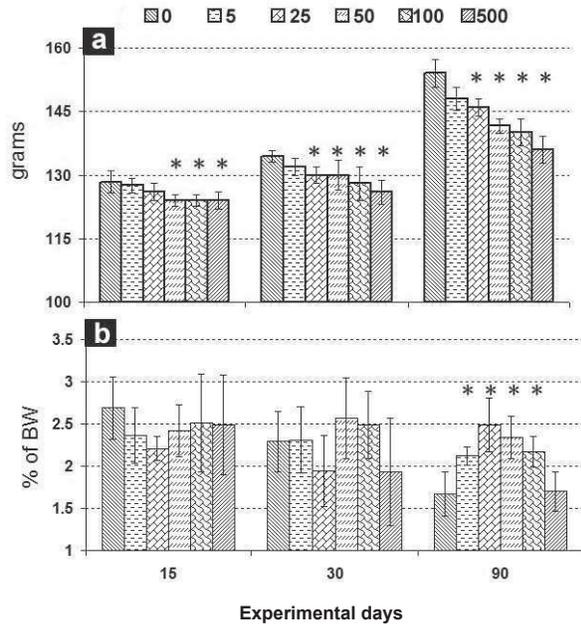
Figure 1. Effect of different exposure levels of endosulfan in feed on a) the body weight and b) the relative weight of liver, in male Japanese quails

groups. On day 30, the 50, 100 and 500 mg/kg groups had significantly ($p < 0.05$) low relative weights of testes than the control group. On day 90, all treatment groups had significantly ($p < 0.05$) low relative weights of testes than that of the control.

The volume of the testes (Figure 2b) on day 15 did not show significant differences among the groups. On day 30, the testes volume in the 50, 100 and 500 mg/kg groups were significantly ($p < 0.05$) low while the 5 and 25 mg/kg groups were not significantly different from the control. On day 90, all treatment groups (5 to 500 mg/kg) showed non significant lowering of testes volume compared to the control group.

Diameter of seminiferous tubules

On day 15, diameter of the seminiferous tubules (Figure 2c) of quails in all treated groups (5 to 500 mg/kg) showed non-significant differences from that of the control. On days 30 and 90, all treated (5 to 500 mg/kg) groups had significantly ($p < 0.05$) lower values than the control group except the 5 mg/kg group, which showed a non-significant difference at 90 days.

Haematological and biochemical parameters

Total leukocyte counts and erythrocyte counts on day 15 were significantly ($p < 0.05$) lower in the 25 and
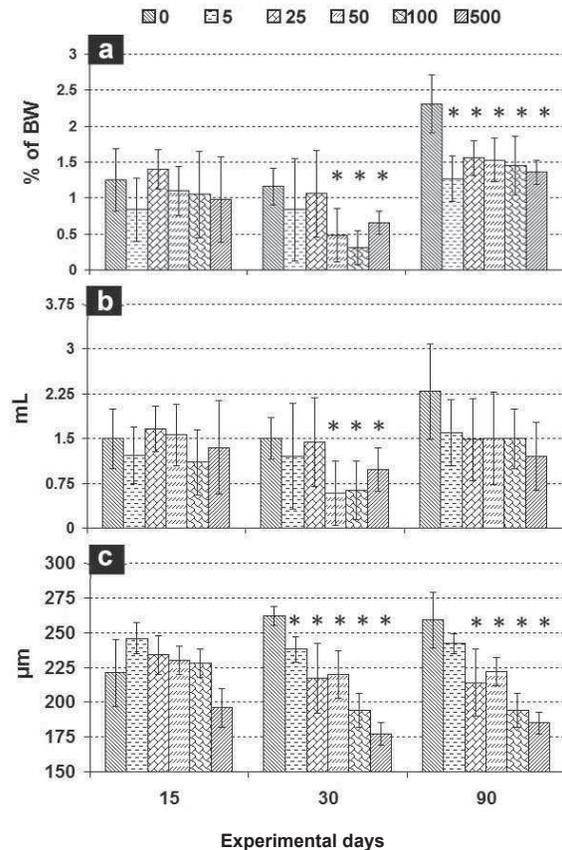

Figure 2. Effect of different exposure levels of endosulfan in feed on a) the relative weight of testes, b) testes volume and c) diameter of seminiferous tubules, in male Japanese quails

50 mg/kg groups compared to the control (Table 2). On days 30 and 90, erythrocyte counts in the 50, 100 and 500 mg/kg groups were significantly ($p < 0.05$) lower than those in the control. On day 30, all the experimental groups had significantly ($p < 0.05$) lower leukocyte counts than the control group. On day 90, the leukocyte and erythrocyte counts in the 50 and 500 mg/kg groups were significantly ($p < 0.05$) lower than that of the control group. Haematocrit values (Table 2) decreased in the 100 and 500 mg/kg groups compared to the control on all the days, whereas haematocrit values of 25 and 50 mg/kg groups were significantly ($p < 0.05$) decreased on days 30 and 15. Differences in the haemoglobin concentration on all occasions were not significant in all treatment groups compared to the control.

Serum total protein (Table 3) of 25 and 100 mg/kg groups was significantly ($p < 0.05$) lower than that of the other groups on day 15. On day 30 the 25, 100 and 500 mg/kg groups had significantly ($p < 0.05$) lower values than the control group. On day 90, only the 25,

Table 2: Effect of different dose levels of endosulfan in feed on different haematological parameters (mean ± SD) in male Japanese quails

Parameters/			Groups			
Period (days)	A	B	C	D	E	F
Erythrocytes (10^6/µL)						
15	4.67 ± 0.25	4.45 ± 0.31	4.64 ± 0.21	4.21 ± 0.27*	4.47 ± 0.26	4.49 ± 0.36
30	4.78 ± 0.32	4.66 ± 0.11	4.61 ± 0.17	4.40 ± 0.27*	4.07 ± 0.16*	4.20 ± 0.28*
90	4.86 ± 0.08	4.68 ± 0.12	4.46 ± 0.23	4.33 ± 0.21*	4.17 ± 0.199*	3.94 ± .39*
Leukocytes (10^3/µL)						
15	8.26 ± 0.88	8.11 ± 1.79	7.16 ± 1.02*	7.36 ± 0.42	6.91 ± 0.62	6.78 ± 0.54
30	8.90 ± .850	7.49 ± 1.01*	6.99 ± 0.81*	7.55 ± 0.83*	6.81 ± 0.42*	6.80 ± 0.43*
90	9.08 ± 0.46	9.29 ± 1.15	8.97 ± 1.29	7.95 ± 0.47*	8.27 ± 0.59	8.01 ± 0.19*
Haematocrit (%)						
15	33.00 ± 1.79	31.67 ± 2.73	31.17 ± 0 .93	30.67 ±1.03*	29.33 ± 1.21*	28.50 ± 1.52*
30	32.83 ± 1.83	32.00 ± 2.10	29.50 ± 0.84*	31.00 ± 1.79	29.50 ± 0.84*	29.00 ± 1.79*
90	32.33 ± 1.86	31.17 ± 0.98	31.33 ± 1.21	31.00 ±1.26	29.83 ±1.33*	29.00 ± 1.79*
Haemoglobin (g/dL)						
15	13.99 ± 1.65	12.30 ± 1.93	11.49 ± 1.19	12.53 ± 2.32	12.25 ± 0.99	11.55 ± 1.06
30	15.22 ± 0.84	14.65 ± 0.86	13.95 ± 0.93	11.33 ± 1.02	14.40 ± 1.05	15.06 ± 0.78
90	14.52 ± 0.95	14.48 ± 0.82	14.01 ± 0.97	14.14 ± 0.82	14.14 ± 0.86	14.01 ± 0.58

Values (mean ± SD) bearing asterisk in a row differ significantly ($p \leq 0.05$) than that of the control (0 mg/kg group). Endosulfan levels in feed of groups A, B, C, D, E and F were 0, 5, 25, 50, 100 and 500 mg/kg, respectively.

Table 3: Effect of different dose levels of endosulfan in feed on biochemical parameters (mean ± SD) in male Japanese quails

Parameters/			Groups			
Period (days)	A	B	C	D	E	F
Serum testosterone (IU)						
90	4.43 ± 2.11	3.66 ± 2.37	3.43 ± 1.79	3.01 ± 1.20	3.15 ± 1.97	2.58 ± 2.15
Serum total proteins (g/dL)						
15	4.04 ± 0.33	3.84 ± 0.43	3.19 ± 0.12*	3.48 ± 0.36	3.44 ± 0.29*	3.50 ± 0.44
30	4.08 ± 0.39	4.28 ± 0.30	2.84 ± 0.91*	3.28 ± 0.67	2.68 ± 0.41*	2.82 ± 0.66*
90	4.12 ± 0.33	3.88 ± 0.41	3.50 ± 0.22*	3.46 ± 0.26*	3.12 ± 0.23*	3.48 ± 0.59
Serum albumin (g/dL)						
15	1.05 ± 0.22	1.36 ± 0.26	1.46 ± 0.42	1.41 ± 0.41	1.61 ± 0.01	0.90 ± 0.23
30	0.69 ± 0.35	0.76 ± 0.25	1.23 ± 1.14	0.71 ± 0.25	1.36 ± 0.39	0.92 ± 0.33
90	0.83 ± 0.07	0.65 ± 0.20	0.89 ± 0.26	0.84 ± 0.23	1.43 ± 0.36	0.72 ± 0.37

Values (mean ± SD) bearing asterisk in a row differ significantly ($p \leq 0.05$) than that of the control (0 mg/kg group). Endosulfan levels in feed of groups A, B, C, D, E and F were 0, 5, 25, 50, 100 and 500 mg/kg, respectively.

50 and 100 mg/kg groups had significantly ($p < 0.05$) lower values while the other groups showed differences that were not significant when compared to the control group. The serum albumin concentration of all treatment groups showed no significant differences from the control group on days 15, 30 and 90 of the experiment. The serum testosterone concentration of all treatment groups decreased with the increase in dietary levels of endosulfan. However, the differences compared to the control were non significant (Table 3).

Gross and histopathological lesions

No gross lesions were observed in the different visceral organs of quails in the groups that were given endosulfan at different dietary dose levels. The histological pattern of liver tissues of quails of the control group did not show any deviation from the normal on day 15, 30 and 90 of the experiment. Livers of quails in all endosulfan treated groups exhibited fatty change of hepatocytes and congestion of blood vessels in hepatic parenchyma on day 15 and 30 (Figure 3). In addition to these, livers of the quails in the 50, 100 and 500 mg/kg groups also showed hepatocyte necrosis on day 30. On day 90 of the experiment, livers of these groups exhibited areas of hepatic necrosis.

Seminiferous tubules of the testes of birds in the control group sacrificed on days 15, 30 and 90 had normal histological pattern (Figure 4). In the 5 mg/kg group, on day 15 and 30, the micros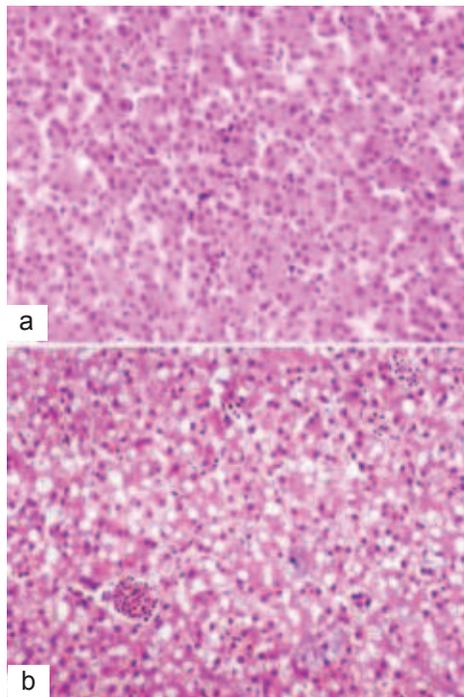copic picture of the testes was the same as in the control. On day 90 of the experiment, semeniferous tubules of 50 % of the birds in the 5 mg/kg group exhibited degenerated spermatids characterized by dark shrunken nuclei. Multinucleated cells having 2−4 nuclei were present in the lumen of some seminiferous tubules. In the 25 mg/kg group on day 15, multinucleated cells and desquamated apoptotic spermatids were present in the germinal epithelium in 50 % of the birds. On day 30, all birds in this group exhibited spermatozoa with rounded heads and condensed, dark nuclei in spermatids. On day 90, degenerated spermatids and
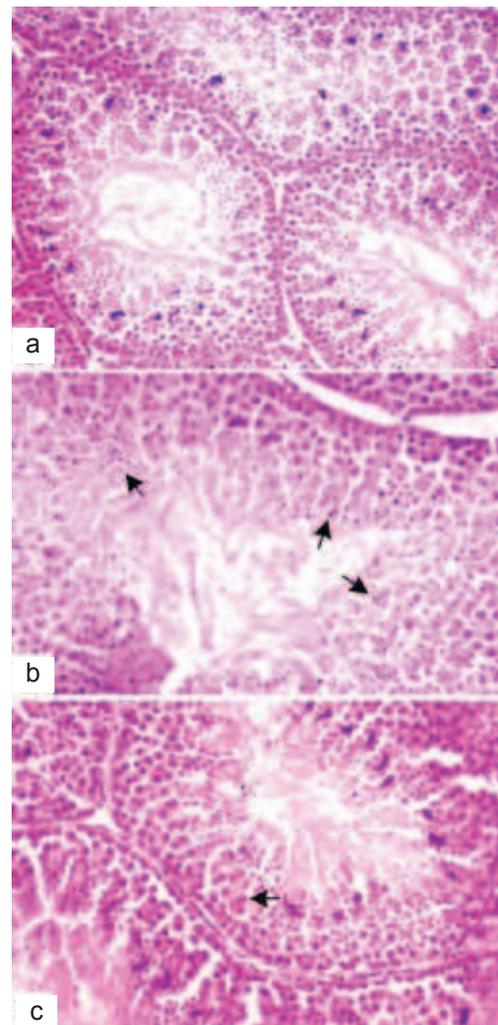

Figure 3.   Photomicrograph of liver of a male Japanese quail. Hepatocytes a) without any pathologic lesion (control) and b) hepatocytes showing fatty change at 50 mg endosulfan/kg for 15 days exposure level. H & E stain, X200.

Figure 4.   Photomicrograph of testis of a Japanese quail showing a) normal spermatogenesis (control group), b) condensed and dark nuclei of spermatids (arrows) and c) multinucleated cells (arrow) in germinal epithelium layer of seminiferous tubule. Endosulfan exposure level was 25 mg/kg feed for 30 (b) and 90 days (c). H & E stain, X200.

spermatozoa having rounded heads and multinucleated cells were observed in the germinal epithelial layer of the seminiferous tubules (Figure 4). All the birds in 50, 100 and 500 mg/kg groups had histological alterations similar to those in the 25 mg/kg group. Testes in the 100 and 500 mg/kg groups at day 90 of the experiment also exhibited increased cellular debris and multinucleated cells in the lumen of the tubules.

DISCUSSION

Nervous excitement of the quails was a prominent clinical sign in the 100 and 500 mg/kg groups. Nervous excitement following administration of endosulfan has been reported earlier in pigeons (Anand *et al*., 1986) and rats (Dikshith *et al*., 1988). In human beings nervous excitement, tonic and clonic seizures have also been described following ingestion of endosulfan (Yavuz *et al*., 2007). Japanese quails given 1000, 2000 and 4000 mg/kg of endosulfan containing feed have shown lethargy, weakness and diarrhoea (Prakash *et al*., 2009). The mild depression in low dose groups (25 and 50 mg/kg) could be a consequence of general toxic effects associated with organochlorine pesticides. Bhalla and Thami (2004) mentioned dizziness, seizures and nervous system damage after accidental ingestion or industrial exposure of lindane (an organochlorine).

In the present study, dose-related significant reduction in the intake of feed treated with endosulfan was observed. The decrease in feed consumption may be attributed to either the biological effect of endosulfan or due to low palatability of the treated feed (Prakash *et al*., 2009). A significant endosulfan dose-related decrease in body weight was observed in the present study. The reduction in body weight could be due to decreased feed consumption (Prakash *et al*., 2009) or due to general toxicity of endosulfan. A decrease in body weight has also been reported in rats (Chitra *et al*., 1999). However, in broiler chicks feeding on 2 mg/kg chlorinated pesticide for 8 weeks did not affect the body weight (Garg *et al*., 2004a), which might be due to low dietary levels of endosulfan or species variation. Pathological changes observed in the liver in the present study have also been reported in rats (Choudhary *et al*., 2003) and fish (Nowak, 1996) but not in avian species.

Haematological parameters responded to endosulfan toxicity by a significant decrease in erythrocyte and total leukocyte counts along with a decrease in haematocrit levels at and above 100 mg/kg endosulfan treated feed. These observations suggest the inhibitory effect of endosulfan on haematopoietic systems in Japanese

quails. A dose of 2 ppm dietary endosulfan for 8 weeks had no effect upon haematological parameters in broiler chicks (Garg *et al*., 2004a). Similarly, no significant difference has been detected in blood parameters of 44 persons accidentally intoxicated by ingestion of endosulfan contaminated food (Venkateswarlu *et al*., 2000). The possible reason for no effect of endosulfan on haematological parameters as reported by some studies could be due to the low dose of endosulfan (2 ppm) compared to the present study (at and above 100 mg/kg endosulfan/kg feed).

A decrease in serum total proteins and albumin, observed in quails following endosulfan feeding has also been reported in fish (Gill *et al*., 1991). Serum protein synthesis mainly takes place in the liver and is impaired due to many pathological conditions of liver (Latimer *et al*., 2003). The degenerative pathomorphological changes observed in the liver of endosulfan fed quails in the present study might have resulted in low serum protein concentration.

In the present study, harmful effect of endosulfan upon the male reproductive system of Japanese quail was evident by pathomorphological alterations in testes characterized by a low relative weight, low volume, decreased diameter of seminiferous tubules, and presence of degenerated round spermatids and multinucleated cells in germinal epithelium and lumen of the seminiferous tubules. A decrease in spermatogenesis by exposure of rats to endosulfan has been reported (Dalsenter *et al*., 1999). In male Japanese quails, crowing behaviour is strictly androgen dependent (Chiba & Hosokawa, 2006). Crowing, foamy droppings and mounting are the indications of onset of sexual maturity in male Japanese quails (Adkins-Regan, 1999). Delayed onset of these signs in Japanese quails suggested delayed onset of puberty or slowing of timing of reproductive maturation. An endosulfan induced delay in onset of puberty has been reported in boys (Saiyed *et al*., 2003; Roy *et al*., 2009). In the present study, serum testosterone concentration did not show significant differences among different treatment groups, indicating that delayed onset of sexual maturity might not be related to testosterone levels. Decreased testosterone and steriodegenic enzyme activity in rats have also been reported (Singh & Pandey, 1990).

Although there are no reports of endosulfan induced pathological effects upon male reproductive system of avian species, the gonadotoxic effects of endosulfan upon male reproductive system of rats have been documented. These changes included a decrease in

testes weight, spermatogenic cell population, total sperm count, sperm motility and increase in the number of abnormal spermatozoa (Pandey *et al*., 1990; Rao *et al*., 2005). In addition to the above alterations, absence of spermatids and presence of multinucleated cells in germinal epithelium of endosulfan treated rats have also been reported (Jaiswal *et al*., 2005). Smaller weights and volumes of testes, smaller diameter of seminiferous tubules, necrosis of spermatids and multinucleated cells in the germinal epithelium observed in Japanese quails in the present study fairly corresponds with the findings reported in testes of rats administered endosulfan from 2.5 to 10 mg/kg body weight for variable periods of time (Rao *et al*., 2005).

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