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Authors' Affiliation:
1. Faculty of Veterinary Science, University of Agriculture Faisalabad, Pakistan
2. Shandong Vocational Animal Science and Veterinary College, Weifang, 261061, China
3. Institute of Animal and Dairy Sciences, University of Agriculture Faisalabad Pakistan
4. PARC National Agricultural Research Center, Islamabad, Pakistan
5. Poultry Research Institute, Murree Road Rawalpindi, Pakistan
6. Subcampus Toba Tek Singh, University of Agriculture Faisalabad Pakistan
7. Department of Microbiology Cholistan University of Veterinary and Animal Sciences, Bahawalpur Pakistan

***Corresponding Author:**
Muhammad Kashif Saleemi
Email: drkashif313@gamil.com

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Toxicopathological effects of endosulfan in female Japanese Quails (*Coturnix japonica*)

Tahir Hussain[1], Muhammad Kashif Saleemi[1*], Muhammad Zargham Khan[1], Ahrar Khan[1,2], Rao Zahid Abbas[1], Muhammad Qamar Bilal[3], Farrah Deeba[1], Hamid Irshad[4], Zahida Fatima[4], Farhan Afzal[5], Umar Farooq[6], Muhammad Moazam Jalees[7]

Abstract

Background: The current study was planned to investigate the toxicopathological effects of endosulfan in female Japanese quails.

Methods: A total of 120 quail of 4 weeks old were divided into six equal groups (A-F) and administered endosulfan in feed at dose rate of 0, 5, 25, 50, 100, and 500 mg/kg feed, respectively for 90 days. Parameters studied included clinical signs, feed intake, body weight and mortality. Hematology, serum biochemistry, hatchability and fertility were also determined. Gross and microscopic changes on different organs were recorded.

Results: The quails of the group B did not show any clinical signs and had significantly lower values of feed intake, testes relative weight and leukocyte number than those of the control group A. The quails of group C and D had mild depression while those of the group E and F showed nervous excitation following ingestion of endosulfan. There was a dose related delay in onset of crowing, appearance of foamy material in the droppings. The feed intake, erythrocyte and leukocyte counts, hematocrit values, and serum total proteins of endosulfan fed quails were significantly ($p < 0.05$) lower than that of the group A. The total egg production in groups A, B and C was significantly higher from group D, E and F. The hatchability in group A and B was significantly higher from groups C, D, E and F. The difference of dead in shell % and early dead among different groups was nonsignificant. Infertile egg percentage was significantly higher in group E compared with all other groups except group F. The necrotic changes were observed in all parts of oviduct in high dose groups, similarly necrotic changes and vacuolar degeneration was observed in hepatic parenchyma in high dose groups D-F.

Conclusion: It may be concluded that endosulfan leads to dose dependent changes in the quails.

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Introduction

Pesticides, heavy metals and mycotoxins contamination is a global issue effecting badly to our environment [1,2,6,21]. Endosulfan, a chlorinated hydrocarbon pesticide of the cyclodiene subgroup is used to protect the crops from a wide variety of insects and mites. The chemical formula for endosulfan is $C_9H_6Cl_6O_3S$. It is effective against a wide range of insects and certain mites on cotton, cereals, grains, coffee, fruits, oil seeds, potatoes, tea and vegetables. Technical endosulfan is made up of a mixture of alpha- and beta-isomers. In Pakistan, endosulfan is available in agricultural market with trade names like Thiodan, Hektionex, Technofan, Fentox etc.

Endosulfan residues persist in the soil with an average half-life of 55-277 days [26]. This period of half-life develops a toxicological hazard for nontarget species including wild birds, free range chicken and livestock kept by the farmers. Endosulfan gained entry into the body through oral route, inhalation or directly through skin. It causes endocrine disruption, can either imitate or block hormones like estrogen, testosterone and thyroid hormones. These are extremely powerful chemicals normally present in the body in micro quantities. Therefore, an even very low level of the pesticides that imitate or block the function of these hormones significantly alters their effects [12,13,16,22,25]. In human beings endocrine disrupters act more subtly, resulting in birth defects, infertility, and learning disabilities. Endosulfan is also toxic to reproductive system and its reproductive toxicity and teratogenic effects have been reported in female and male mice and rats. In acute toxicity it causes stimulation of nervous system, dyspnea, cyanosis, tremors, and convulsions [20]. In chronic toxicity there is reduced growth, liver enlargement, and change in blood and organ chemistry [11].

Natural cases of endosulfan toxicities as well as its experimental production have been reported in different species including rats, mice, rabbits, fish, chicken, cow, human etc. [22]. Free-range poultry in villages and wild birds' population is exposed to endosulfan by feeding on the contaminated grains or other plant components as well as by eating contaminated insects died of endosulfan application. Toxic effects of endosulfan are well documented in different species including rats, however, information regarding toxic effects and morphological changes induced in different organs particularly female reproductive system in avian species is not available in the accessible literature. With these intensions this project has been designed to study the endosulfan toxicity in Japanese quails with particular reference to female reproductive system. The results of this study would be helpful to diagnose and differentiate the clinical syndromes of obscure etiologies in these species.

Methods

Birds' management

A total of 120 female Japanese quails (Coturnix japonica) at the age of 25 days were purchased from a local farm. Birds were kept in metal wire cages at ambient temperature in a poultry house. The birds were fed on commercial broiler starter feed containing 21 % total protein (Ani® Feeds, Gujranwala) as basal feed. All the birds were given feed and water *ad libitum*.

Experimental design

The birds were divided randomly into six equal groups A-F having 20 birds in each group. These groups were kept in separate cages. After one week of acclimatization, the birds were administered endosulfan (Thiodan® 35 EC, 32.9 % w/w endosulfan and 67.1% solvent w/w, Aventis, Pakistan) incorporated in the feed at different levels (table 1).

Groups	Treatment Endosulfan mg/kg BW	No of birds
A	0	20
B	5	20
C	25	20
D	50	20
E	100	20
F	500	20

Table 1: Experimental Design

The duration of the experiment was 90 days. Five birds were slaughtered on day 15 and 30 of the experiment from each group and remaining birds at the end of experiment day 90.

Parameters studied

Clinical signs and behavioral alterations twice daily. A subjective score range 0-4 was selected for clinical signs. For no signs score was 0 and most severe sign the score was 4. These included attraction towards feed and water, foaminess in droppings etc. Feed intake on daily basis, bodyweights weekly, mortality on daily and % mortality was calculated.

Hematology and serum biochemistry

The six birds from each group were sacrificed on 15th, 30th day and remaining all at the end of experiment of experiment to collect blood for hematobiochemical analysis. Microhematocrit method [5] was used to determine hematocrit and hemoglobin concentration was measured by cyanmethemoglobin method. Total protein was measured by Biuret method and albumin by Bromocresol Green Dye Binding method using commercially available colorimetric kits of Merck Company following instructional manual.

Hatchability parameters

Eggs were collected on daily basis and hatch was set weekly in automatic incubator. After every hatch, hatch analysis was conducted and record of the total eggs set, hatched, early dead, dead in shell and infertile eggs were collected.

Gross and microscopic pathology

On each slaughtering, organs were collected, weighed and examined for gross pathology liver, kidneys and oviduct tissues were fixed in 10% neutral buffered formalin for histopathology [16].

Statistical analysis

The data thus obtained were analyzed statistically by analysis of variance test (ANOVA) and group means

were compared by Duncan's multiple range test. The level of the significance was $p \leq 0.05$.

Results

Clinical signs and behavioral alterations

The results of clinical signs and behavioral alterations have been presented in table 2.

Feed intake

Feed intake of Japanese quails in different groups has been presented in Table 3. In 1st week of the experiment the feed intake of group E was significantly higher from group C and D. In second week of experiment the feed intake of group D was significantly higher from all other groups. In 3rd week the feed intake of group A was significantly higher from group C and D and nonsignificantly higher than group B, E and F. In 4th week the feed intake of group A was significantly higher from group C and D and nonsignificantly higher than group B, E and F. In 5th week the feed intake of group A and B was significantly higher from all other groups. In 6th week the feed intake of group A and B was significantly higher from all other groups. In 7th week feed intake of group A was significantly higher from all other groups. Group A was significantly higher in feed intake from all other groups. In last week of the experiment the feed intake of group A and B was significantly higher from all other groups.

Body weight

Body weights of the birds in different groups have been presented in the Table 4. Difference in body weight among different groups was nonsignificant throughout the length of the experiment.

Weeks	Behavioral changes	Score range	Groups (Endosulfan mg/kg)					
			A (0)	B (5)	C (25)	D (50)	E (100)	F (500)
1	Alertness of the bird	0-4	4	4	4	4	3	3
	Mating behavior	0-4	1	0	0	0	0	0
	Foaminess in the droppings	0-4	1	0	0	0	0	0
	Total		6	4	4	4	3	3
2	Alertness of the bird	0-4	4	4	4	3	3	3
	Mating behavior	0-4	2	2	1	1	1	0
	Foaminess in the droppings	0-4	2	1	1	1	0	0
	Total		8	7	6	5	4	3
3	Alertness of the bird	0-4	4	4	3	3	2	2
	Mating behavior	0-4	3	2	2	1	1	0
	Foaminess in the droppings	0-4	3	1	1	1	0	0
	Total		10	7	6	5	3	2
4	Alertness of the bird	0-4	4	4	3	2	2	2
	Mating behavior	0-4	3	2	2	1	1	0
	Foaminess in the droppings	0-4	4	2	1	1	0	0
	Total		11	8	6	4	3	2
5	Alertness of the bird	0-4	4	4	3	2	2	1
	Mating behavior	0-4	4	3	2	1	1	0
	Foaminess in the droppings	0-4	4	2	1	1	0	0
	Total		12	9	6	4	3	1
6	Alertness of the bird	0-4	4	3	3	2	2	1
	Mating behavior	0-4	4	3	2	1	1	0
	Foaminess in the droppings	0-4	4	2	1	1	1	0
	Total		12	8	6	4	4	1
7	Alertness of the bird	0-4	4	3	3	2	2	1
	Mating behavior	0-4	4	3	2	1	1	0
	Foaminess in the droppings	0-4	4	3	1	1	0	0
	Total		12	9	6	4	3	1
8	Alertness of the bird	0-4	4	4	3	2	2	1
	Mating behavior	0-4	4	3	2	1	1	0
	Foaminess in the droppings	0-4	4	3	2	1	0	0
	Total		12	10	7	4	3	1
9	Alertness of the bird	0-4	4	3	3	2	2	1
	Mating behavior	0-4	4	2	2	2	1	0
	Foaminess in the droppings	0-4	4	3	2	1	0	0
	Total		12	8	7	5	3	1
Grand total		108	95	70	54	39	29	16

Table 2: Clinical signs & behavioral alterations in different groups administered with endosulfan (Mean±SD)

Weeks	Groups					
	A 0 mg/kg	B 5mg/kg	C 25 mg/kg	D 50mg/kg	E 100mg/kg	F 500mg/kg
1	34.7 ± 2.6ab	33.1±1.9abc	32.1 ±1.9bc	31.4 ±2.2c	35.0 ± 3.7a	34.7±1.7ab
2	36.0 ±5.1b	31.1 ±1.9 c	28.9 ±2.5c	39.3 ±1.4a	34.6 ±1.4a	29.9 ±1.7c
3	37.4 ±2.76a	35.1 ±1.9abc	34.1±1.9bc	32.71±2.6c	35.5 ±3.4ab	35.4±1.7abc
4	38.1 ±2.9a	36.0±1.7abc	34.7±1.8bc	33.2±1.2c	36.1±2.7ab	33.5±1.9bc
5	39.4 ±2.7a	38.1±1.5a	34.8±1.6b	34.5±2.1b	34.7±2.3b	32.5±2.5b
6	40.8 ±2.1a	37.2±1.6b	35.2±1.7bc	32.7±1.5c	32.8±2.6c	28.8±2.6b
7	41.5 ±2.4a	37.4±1.7b	35.5±1.9bc	33.5±2.1c	29.2±3.0d	27.0±2.7d
8	40.5 ±1.7a	37.8±2.0b	36.8±1.5b	33.8±3.2c	27.8±3.7d	27.0±2.1d
9	39.4 ±1.9a	39.5±2.4a	35.8±1.5b	31.7±3.6c	28.0±2.1d	24.0±2.1e

Values in different rows followed by different letters are statistically significant ($P \leq 0.05$)

Table 3: Feed intake (g/bird/day) of female Japanese quails administered different doses of endosulfan in feed (Mean±SD)

Weeks	Groups					
	A 0mg/kg	B 5mg/kg	C 25 mg/kg	D 50mg/kg	E 100mg/kg	F 500mg/kg
1	144±13.9	132±13.5	138±10.4	136±9.2	134±10.2	133±11.3
2	146±9	145±8.3	144±7.9	142±7.4	141±8.8	143±7.3
3	147±4.7	144±6.4	145±6.4	143±5.7	145±7.4	142±7.3
4	152±5.9	149±4.6	146±4.3	145±4.0	144±4.0	143±4.8
5	158±5.3	148±4.6	146±3.0	146±5.2	143±4.9	143±5.9
6	158±4.9	156±4.5	150±4.9	146±7.0	144±6.3	143±5.7
7	161±4.5	161±5.8	155±6.9	155±6.9	151±6.2	148±6.0
8	167±6.0	161±5.1	158±5.2	155±4.8	152±6.2	149±7.9
9	174±4.4	170±3.2	164±3.8	163±3.7	153±5.6	154±5.7

Values followed by different letters are statistically significant (P ≤ 0.05)

Table 4: Body weights of female Japanese quails administered different doses of endosulfan in feed (Mean± SD)

Parameters	Groups					
	A control)	B (5mg/kg)	C (25mg/kg)	D (50mg/kg)	E (100mg/kg)	F (500mg/kg)
Total eggs	16.5±6.2a	13.8±6.2a	13.0±7.4a	7.0±2.1b	6.5±5.9b	5.3±3.9b
Hatched (%)	50.3±13.0a	45.4±6.6a	32.5±7.5b	24.3±8.9bc	20.2±17.0cd	9.9±15.6d
Dead in shell (%)	20.6±7.8	20.3±6.7	20.9±3.8	27.9±9.1	21.9±15.5	19.8±15.9
Early Dead (%)	10.4±4.3	9.1±6.0	10.5±5.7	13.9±7.1	4.1±8.3	13.5±16.0
Infertile (%)	18.7±6.9d	25.7±5.8cd	32.1 ±11.1bcd	33.9±7.9bc	53.7±20.7a	45.6±21.3ab

Values in rows followed by different letters are statistically significant (P ≤ 0.05)

Table 5: Effect of different dose levels of endosulfan in feed on hatchability parameters of female Japanese quails. (Mean±SD)

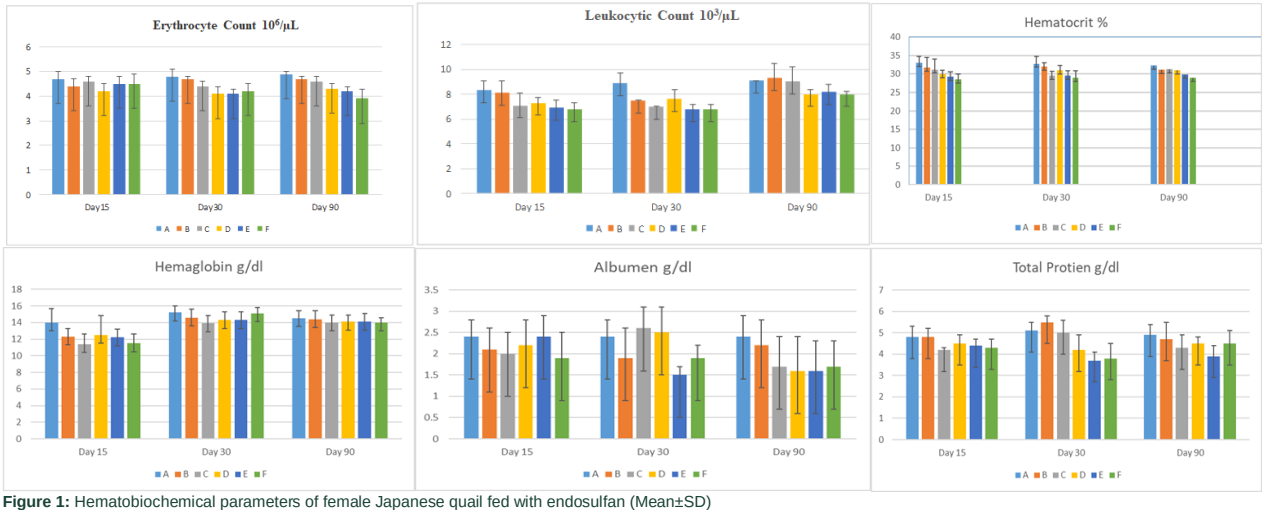

Figure 1: Hematobiochemical parameters of female Japanese quail fed with endosulfan (Mean±SD)

Figure 2: Relative organ weights of female Japanese quail fed with endosulfan (Mean±SD)

Effect of endosulfan on relative organ weights
The difference in weight of ovary among different groups was nonsignificant up to 30 days (table 5). From day 30 to 90 the ovary weight of group A was significantly higher from group C, D, E and F. The difference in weight of oviduct among different groups was nonsignificant up to 30 days. From day 30 to 90 the oviduct weight of group A to D was significantly higher from groups E and F but nonsignificant difference among each other. The difference in weight of kidney among different groups was nonsignificant throughout the length of experiment. The difference in weight of liver among different groups was nonsignificant up to day 60. From day 30 to 90 the liver weight of group C was significantly higher from groups A, B and F but it was nonsignificantly higher from group D and E. The difference in weight of heart among different groups was nonsignificant throughout the length of experiment. The difference in weight of spleen among different groups was nonsignificant throughout the length of experiment.

Effect of endosulfan on hematological parameters
There was a significant difference in erythrocyte count noted among control group and group D, E and F on day 15, 30 and 90 of experiment as shown in Fig. 1. Leukocytes count of group A was significantly higher from all other groups on day 30. From day 30 to 90 leukocytes count was significantly higher in group A and B from group D and F. The difference between group A and B was nonsignificant. There was a significant difference in hematocrit value observed among control group and group E and F on day 15. The group A and B were significantly higher in hematocrit value from all other groups. From day 30 to 90 group A had significantly higher value from group E and F, while nonsignificantly higher from groups B, C and D. There was a significant difference observed in hemoglobin concentration among control group and all other groups on day 15 of the experiment. However, nonsignificant difference was observed on day 30 and 90 of experiment as shown in fig. 1

Effects of endosulfan on serum biochemistry
Serum biochemistry
The difference in serum total protein values among different groups throughout the experiment were nonsignificant (Fig. 1). The difference in serum albumin values among different groups throughout the experiment was nonsignificant.

Effect of endosulfan on egg production and hatchability parameters
Different parameters related hatchability were shown in table 5.

Total Egg production:
The total egg production in groups A, B and C was significantly higher from group D, E and F. The difference in the total egg production among group A, B and C was nonsignificant.

Hatchability (%): The hatchability in group A and B was significantly higher from group C, D, E and F.

Dead in Shell (%): The difference of dead in shell % among different groups was nonsignificant through the length of experiment.

Early dead (%): The difference of early dead in shell % among different groups was nonsignificant throughout the length of experiment.

Infertile (%): Infertile egg percentage was significantly higher in group E compared with all other groups except group F.

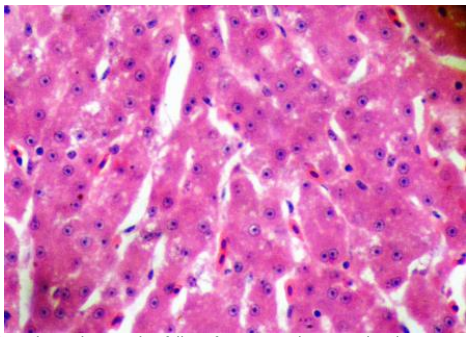

Figure 3a: Photomicrograph of liver from control group showing normal hepatic parenchyma (H & E, Staining 200X)

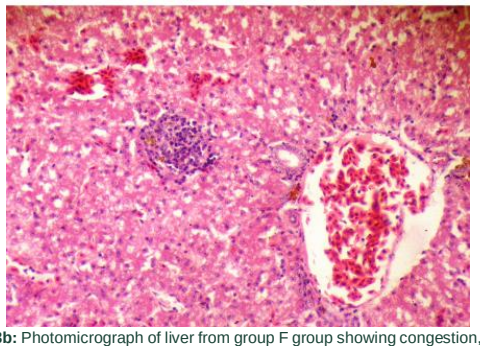

Figure 3b: Photomicrograph of liver from group F group showing congestion, cellular infiltration and vacuolar degeneration with necrotic changes in hepatic parenchyma (H & E, Staining 200X)

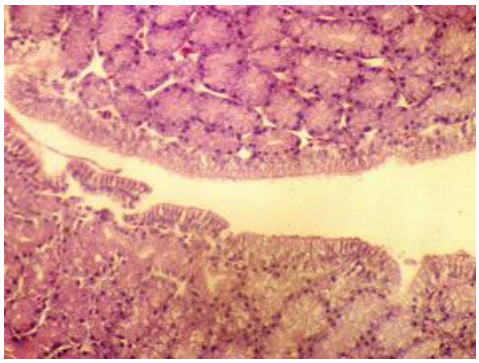

Figure 3c: Photomicrograph of magnum from control group showing intact epithelium and albumin globules (H & E, 400X)

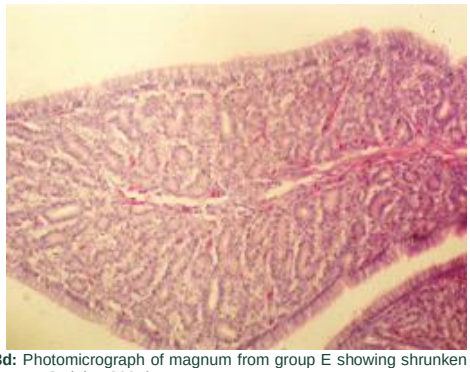

Figure 3d: Photomicrograph of magnum from group E showing shrunken albumen glands (H & E, Staining 200X)

Gross pathology

No gross lesions were observed in all the groups that were administered endosulfan at different dose levels.

Microscopic pathology

Oviductal size was normal in group A and B (Fig. 3 c). The tubular glands atrophy and patent lumen with decreased mucosal folds height in magnum and isthmus with epithelium only in magnum were noted in oviduct of quails treated with endosulfan from C-F groups (Fig. 3 d). At few places degenerated nuclei were showing necrotic changes. There were also cellular infiltration in stroma of folds of magnum showing inflammatory response there. In the liver in group D, E and F necrotic changes were present in the hepatocytes and biliary hyperplasia was also present in the portal area (Fig. 3 b). No such changes were present in the group A and B (Fig. 3 a). In kidneys in the group D, E and F tubular necrosis was present.

Discussion

Food security is still a significant Millennium Development Goal in most of the developing countries [29]. Endosulfan is an important member of organochlorine group of pesticides. Endosulfan is persistent in the soil and its average field half life is 55-277 days [26]. Residues of endosulfan present in soil environment, on fruits and on different plant parts pose a high risk to the domestic and wildlife species living in agricultural areas. Domestic and wild birds may also be intoxicated by eating dead insects contaminated by endosulfan.

Reports describe the acute [20] and chronic [11] effects of endosulfan in different species. However a little information is available regarding endosulfan toxicity in domestic and wild birds. This study describes the pathological changes, experimentally induced in female Japanese quails by endosulfan mixed in their feed.

Female Japanese quail (*Coturnix japonica*) is a domesticated bird which grows rapidly and reaches at puberty in a short period. It therefore acts as model bird for experimental studies in avian species. The results obtained from experiments from female Japanese quails may be extrapolated, domesticated birds like chicken as well as wild birds.

Neurological excitement was an important sign observed in endosulfan treated quails. The nervous signs in the present study appeared in quails of given 25 mg endosulfan/kg feed gradually became more prominent with increase in dose level, highest in F group that was given 500 mg endosulfan/kg feed. This indicates dose related neurological excitement due to endosulfan fed to them. Neurological excitement characterized by excitability, and transient torticollis. Nervous excitement following administration of endosulfan have been reported in pigeons [3], human beings, [7] and rats [10].

A significant reduction in feed intake was observed in birds fed endosulfan 25mg / kg feed or above. Feed intake was dose related, decrease being reciprocal to endosulfan intake by the birds. A decrease in feed intake has also been reported by [8] in rats. It appears that apart from other pathological manifestations, endosulfan also induced decreased feed intake.

Many authors have reported the nephrotoxic effects of endosulfan. Earlier, studies reported the highest concentrations of alpha and beta isomers of racemic endosulfan in rats [4,22]. The toxicity of endosulfan on kidneys of male rats in relation to drug metabolizing enzymes has already been established [27]. Histological changes have been reported earlier in which an acute tubular necrosis following endosulfan insecticide poisoning was observed [18]. A difference in species LD50 has also been reported in rats after oral administration [28] from 18 to 160 mg/kg. The reported 5-day dietary LC50 is 2906 ppm in Japanese quail [14]. In fish species, the reported 96-hour LC50 value was 1.5ug/L in rainbow trout [17].

Hematological parameters responded to endosulfan toxicity by a significant decrease in erythrocyte and leukocyte counts along with decrease in hematocrit level at higher doses (100mg endosulfan/kg feed and above). These observations suggest inhibitory effect of endosulfan on hematopoietic organs. However, research reported nonsignificant difference on complete blood count induced by endosulfan administration [30].

A decrease in serum total proteins and albumin has been observed in the lateral stages of endosulfan toxicity. This reduction has also been reported in literature [11]. This decrease could be due to degenerative changes produced by endosulfan in the liver.

A significant degenerative effect of endosulfan was observed in female Japanese quails. This degenerative effect was characterized by delayed and low egg production in groups that were administered endosulfan. A gradual delay in onset of puberty, low egg production, more number of unhatched eggs and increase in number of infertile eggs with increase in endosulfan level in feed of quails suggests a dose related increase in degenerative effects on female reproductive system.

In present study, endosulfan also exerts injurious effects on female reproductive system. Reduction in weight of ovaries and oviduct, a smaller number of eggs early embryonic deaths have been observed in the quails administered endosulfan in feed in the present study. Endosulfan induced gonadotoxicity has been reported by many authors. Michael reported the reproductive effects in birds exposed to pesticides and industrial chemicals

[19]. Effects on breeding adults as well as developmental effects on embryos were studied. Papov studied embryo and genotoxic effects of endosulfan on rat embryo [24]. Dalsenter *et al.,* reported reproductive effects of endosulfan on male off-springs of rats exposed during pregnancy and lactation. Microscopic picture of reproductive organs revealed pyknotic nuclei, foamy cytoplasm with excessive vacuolation, decreased size of glands. These histomorphological changes were also documented [23]. This decrease could be due to degenerative changes produced by endosulfan in the reproductive organs.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authors' Contribution

TH, MKS, MMJ, UF and MZK plan the study and executed the experiment and performed lab work, RZ, AK, HI helped in the data analysis and MZK, TH, MKS, MJ, FD, QB, FA and ZF were involved in manuscript preparation.

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