



## RESEARCH ARTICLE

### Toxico-pathological and Reproductive Effects of Concurrent Oral Administration of Ochratoxin A and Endosulfan in Pregnant Rabbits (*Oryctolagus cuniculus*)

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#### ABSTRACT

The present study was designed to investigate the potential toxicological interactions of concurrent oral administration of ochratoxin A (OTA) and endosulfan in the pregnant rabbit and developing embryos. For this purpose, 45 female rabbits were divided into nine equal groups (A-I). The rabbits in group A were kept as control, while those in group B to I were given OTA (0.03, 0.06mg/kg BW) and endosulfan (1.0 to 1.5mg/kg BW) for 15 day (day 6 to 20 of gestation). Half of the rabbits were euthanized at the end of experimental feeding period and embryos were collected, weighed and their crown to rump lengths were recorded. Blood samples for hematological and serum biochemical profiling and tissue for histopathological studies were collected. From the remaining sets of rabbits litter size and weight were recorded. Serum urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were higher in the groups of rabbits treated with either OTA or endosulfan. A significant reduction in the length and weights of fetuses and birth weight was recorded in the treated groups. A significant reduction in hematological parameters, including cell counts was noted in the groups given both chemicals in combination. Histopathological alteration included fatty change in hepatocyte, hemorrhage and congestion in the liver parenchyma, glomerular shrinkage, tubular degeneration and accumulation of protein rich fluid in the renal parenchyma were recorded. The intestine showed sloughing, degeneration and decrease in villi length. All these changes showed dose dependent increase and more prominent in the co-contaminated groups.

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#### INTRODUCTION

Mycotoxins and pesticides are major contaminants of agricultural products, and thus of important concern for human and animal health. In the developing world, unchecked use of pesticides, and poor agricultural practices makes the occurrence of these toxic substances above the permissible levels in edible food and feed. In a long list of >400 known mycotoxins, aflatoxins, ochratoxins, deoxynivalenol (DON) and zearalenone are most frequently detected in the agricultural commodities (Bennett and Klich, 2003; Gabarty and Abou, 2016).

Ochratoxins are produced by several species of toxigenic fungi from genus *Aspergillus* and *Penicillium* (Ostry *et al.*, 2013; Saleemi *et al.*, 2015; Valtchev *et al.*,

2015). These toxigenic fungi have potential to attack on the cereals crops at their growing, harvesting and most importantly during storage; where humidity and temperature plays a significant role in their propagation and spread. Ochratoxin A (OTA), the most toxic among ochratoxins, is well known nephrotoxic, reprotoxic, hepatotoxic, immunosuppressive, mutagenic, cardiovascular toxic, teratogenic and possible human carcinogen (Hassan *et al.*, 2012; Ahmad *et al.*, 2012; Hussain *et al.*, 2016). In animals, studies proved it as one of the major profit limiting factor in the livestock productivity, due to decreased fertility, growth inhibition and posing the intoxicated population at risk to opportunistic pathogens.

Endosulfan, an organo-chlorides pesticide, was partially banned in USA due to its acute toxicity,

bioaccumulation and endocrine disrupting activity. In the year 2011, Stockholm convention negotiated a global ban on its use, by which 80 countries, including European Union (EU) members, announced the phase out. However, it is being still manufactured and used in India, China, Pakistan and some other developing nations. In Pakistan, many food items including river fish (Akhtar *et al.*, 2014), vegetables (Randhawa *et al.*, 2014), fruits (Latif *et al.*, 2011) and cattle milk (Muhammad *et al.*, 2012) have been found to be contaminated with endosulfan. In experimental and natural exposure study, endosulfan have been reported to be reproductive toxic compound in rabbits (Rastogi *et al.*, 2014) and human (Saiyed *et al.*, 2003), respectively.

In keeping the contamination levels and toxicological aspects of OTA and endosulfan, it is reasonable to suspect, that these chemicals at times, may be present in the human and animal food and feed at higher than permissible limits. So this study was designed by using pregnant mammalian modal (rabbit), to get insight of the interaction amongst test chemicals upon concurrent oral administration.

## MATERIALS AND METHODS

**Ochratoxin and feed:** Ochratoxin A was produced from *Aspergillus ochraceus* (CECT 2948, Culture Collection Center, University De Valencia, Valencia, Spain) by culturing on wheat grain (Trenk *et al.*, 1971) and quantified using HPLC and fluorescent detection methods (Bayman *et al.*, 2002).

Prior to use, each batch of the basal feed was analyzed for ochratoxin, aflatoxin, and zearalenone to ensure that the levels of each were  $<1.0\mu\text{g}/\text{kg}$ . OTA-contaminated feeds were prepared by incorporation of known quantities of OTA (Hassan *et al.*, 2012). Prior to being used for feeding, the OTA concentration in each experimental diet was verified by HPLC.

**Animal management and experimental protocol:** The study was conducted jointly at the Department of Animal Health, the University of Agriculture Peshawar and Centre of Animal Nutrition, Directorate of Livestock Research and Development, Peshawar, Pakistan. A total of 45 females and 10 male rabbits (~1.5kg BW) were procured from the local rabbit market. An approval for the study was granted by faculty ethical committee, Faculty of Animal Husbandry and Veterinary Sciences, the University of Agriculture, Peshawar. These animals were acclimatized for one month, in order to monitor their reproductive health and stage. After confirmation of the non-pregnancies, a random natural mating with healthy males was allowed. The mating was confirmed by vaginal swab method (Ochiogu *et al.*, 2006), and then animals were divided into nine (A-I) groups of five rabbits in each. The animals in group A were kept as control and feed normal rabbit feed, while those in group B and C were given OTA at 0.03 and 0.06mg/kg BW respectively. In the feed of groups D, E and F, endosulfan was added at 1.0mg/Kg BW, along with OTA at 0, 0.03 and 0.06mg/kg BW, respectively. To the rabbits in groups G, H and I, endosulfan was fed at 1.5mg/kg feed, along with 0, 0.03 and 0.06mg/kg BW OTA, respectively. These animals were kept on experimental feed for 15 days, i.e., day 6 to 20 of gestation.

## Parameters studied

**Hematology and serum biochemistry:** At the end of experimental feeding period, blood was collected for hematological studies, including Hb, PCV, RBC count, MCV, MCH, MCHC, TLC and DLC. From the other sets of blood samples, serum was separated to determine liver and kidney function test including AST, ALT, Creatinine and Urea. All these tests were performed on semi-automatic chemistry analyzer (Procan-520, China) using commercially available kits.

**Teratology:** Half the animals in each group were killed at day 20 of the experiment, and their abdomen was excised to collect the fetuses. The fetuses were washed in saline solution, weighed and were observed for developmental anomalies under stereo-microscope. The number and crown to rump length of each fetus was recorded.

**Histopathology:** Immediately after killing samples from liver, kidneys and intestine of the female rabbits were collected and preserved in 10% buffered formalin. After dehydration in ascending grades of alcohol tissue samples was processed following routinely histological procedures for the H&E staining (Bancroft and Gamble, 2008). The stained section was studied under microscope. The remaining animals were allowed to continue full term pregnancies, and were monitored for the health and body conditions till kitting. The litter size and birth weight of each kid was recorded.

**Statistical analysis:** Data was analyzed using two-way analysis of variance (ANOVA), means in different groups were compared for any significance difference by Duncan's Multiple Range Test (DMRT). For this purpose, Sigma Plot (version 12.5) statistical software was used.

## RESULTS

**Serum biochemical profile:** Serum urea levels were significantly higher in the groups fed higher levels of OTA as compared to the rabbits in group A (control). Feeding endosulfan at any level tested, had non-significant on the serum urea concentration (Table 1). In co-fed groups, the values were more significant as compared that noted in the groups given each toxin alone.

Serum creatinine levels were higher in the group D, E, F, G, H and I, as compared to levels shown in the rabbits from group A. Feeding both the toxicants in combination resulted a significantly higher level as compared to the levels noted in the alone fed groups.

Serum AST activity was higher in the groups of rabbits given OTA alone (at both doses) or in combination with endosulfan. Feeding endosulfan alone to the pregnant rabbits had non-significant effect on the serum AST level. In the group of rabbits given higher dose of OTA along with endosulfan resulted in highly significant increase than all the other groups.

Serum ALT activity was significantly higher in all the exposed groups, compared with the rabbits in control group. A significantly higher values were noted in the groups of rabbit given both the chemicals in combination as compared to the groups fed each toxin alone. Rabbits in group I, given higher doses of both the toxicants in

combination showed a significantly higher ALT activity compared to all studied groups.

**Hematological profile:** WBC's count was non-significantly different in the groups given endosulfan and OTA alone or in combination (both chemicals at low doses). However, a significant reduction was noted in the group H and I, where both the toxicants were fed in higher doses (Table 2).

The red cells count of the rabbits exposed to different dietary concentration of endosulfan and OTA showed a significant reduction in all experimental groups as compared to the values noted in the control group. Feeding OTA alone showed a non-significant difference in the RBC counts as compared to the groups given low doses of endosulfan along with OTA, i.e., E and F. A most significant reduction was noted in the rabbit fed higher doses of both the toxicants together.

Blood Hb levels of the rabbits in C, F, H and I was significantly lower than the values noted in control group. Feeding OTA at higher dose (0.06mg/kg feed), alone or in combination with endosulfan, showed a significant reduction in Hb levels. However, the rabbits kept on endosulfan (alone) at both experimental doses (1.0 and 1.5mg/kg) resulted a non-significant difference with control.

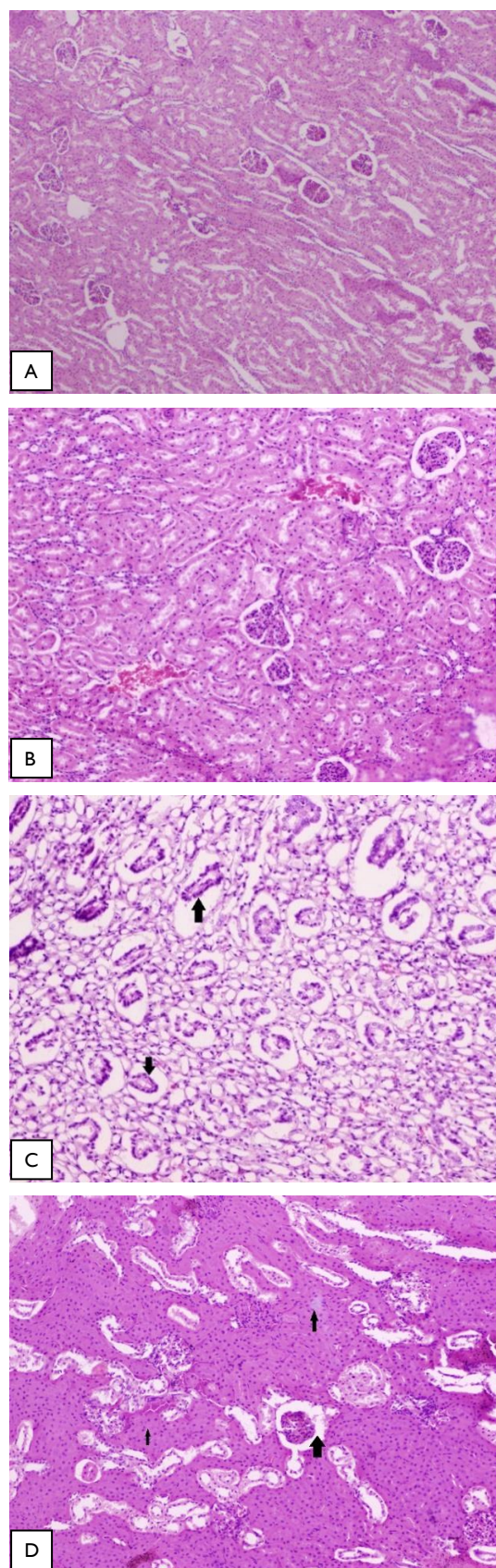
Hematocrit (PCV), in the rabbits showed a non-significant difference as compared to control, only in the rabbits exposed to endosulfan and OTA at low doses alone. However, a significant reduction was noted in the PCV values of the rabbits given two test chemicals alone or in combination.

An inconsistency was noted in the values of MCV in the groups of rabbits exposed to different dietary concentrations of endosulfan and OTA. Feeding OTA alone or in combination with endosulfan (at higher doses) resulted a significant higher MCV values, while in the groups co-exposed to OTA along with low dose of endosulfan (1mg/kg) showed a non significant difference, compared to the control group.

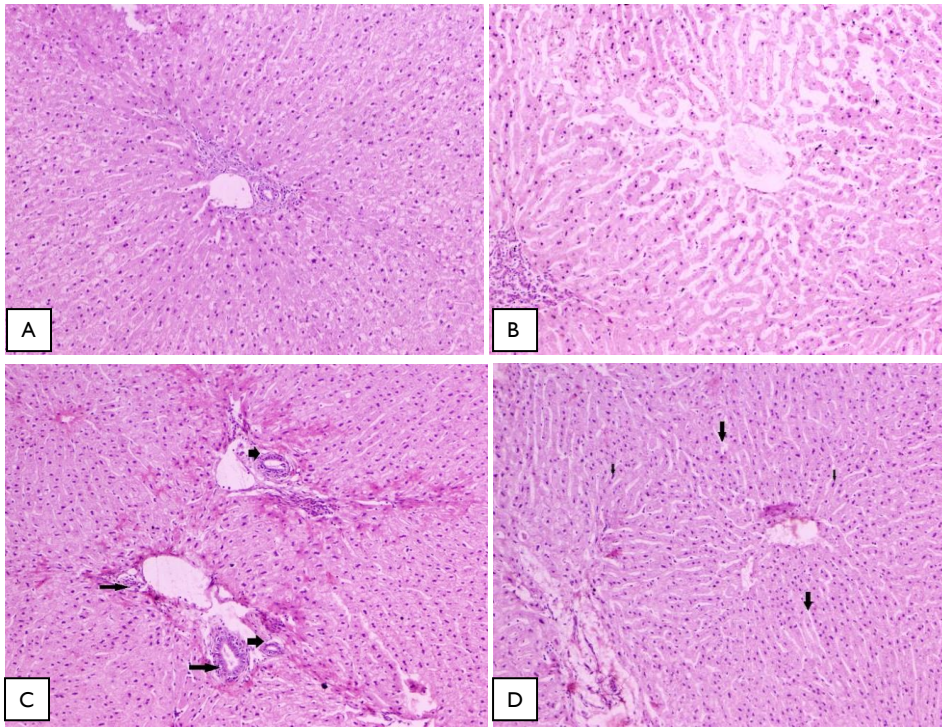
MCH values were non-significantly difference in all the groups except C and D, as compared to the control. MCHC values of the rabbits in group E and G were significantly higher as compared to control, while all the other groups showed a non-significance difference.

**Fetal weight and length:** Feeding OTA alone at the two levels had significant effect, in weight gain (decrease weight gain) as compared to the rabbits in control group. In group D, the weight of fetuses was non-significantly different from the control group fetuses (Table 3). In the group of rabbits given endosulfan along with OTA at 0.03mg/kg feed, a non-significant difference was noted in the fetus weight. Although, feeding OTA alone at the same levels showed a significant reduction in the weight.

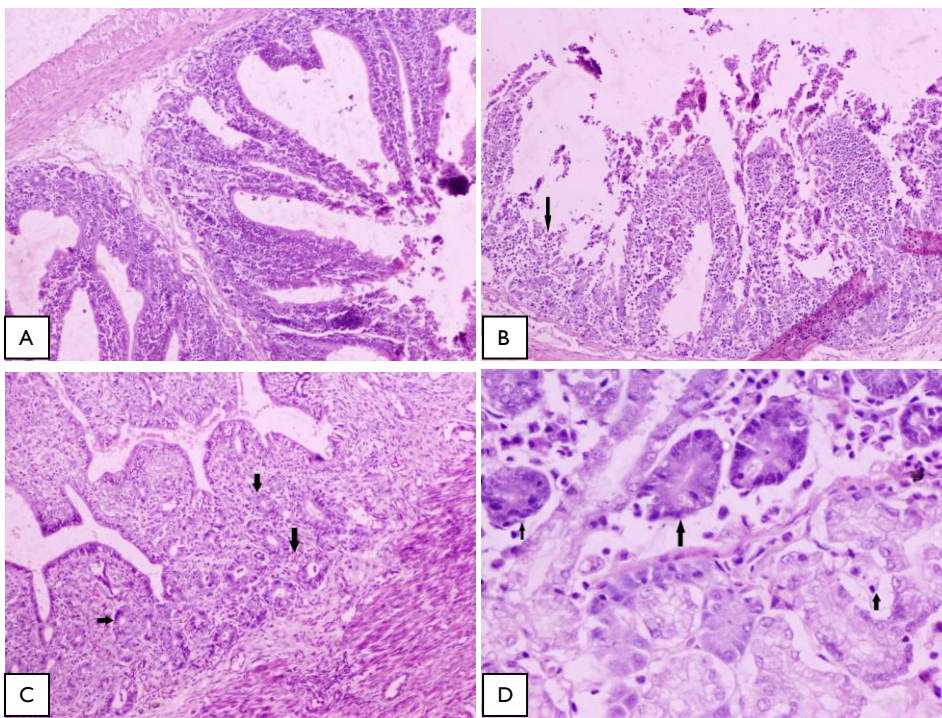
The length of fetus recorded at day 20 of gestation, showed a non-significant difference in group D, while in all other groups there was significant reduction as compared to the values noted in group A (control). The co-administration of endosulfan and OTA to the pregnant rabbits resulted in the significant reduction as compared to the values noted in alone fed group.



**Fig. 1:** Photo-micrograph of the kidneys of rabbits intoxicated with OTA and endosulfan. A) Section from control group. B) Rabbits given endosulfan and OTA 1.0+0.03mg/kg BW showing severe depletion of tubular epithelial cells. C) Severe glomerular and tubular degeneration in rabbits treated with endosulfan 1.0+OTA 0.06mg/kg BW. D) Degeneration of kidney parenchymal cells and accumulation of protein rich fluid in rabbits treated with endosulfan 1.0+OTA 1.5mg/kg BW. H&E; 200X.



**Fig. 2:** Photomicrograph of the liver of the rabbits treated with OTA and endosulfan. A) Liver of rabbit control group. B) Liver of rabbits given OTA 0.06mg/kg BW, hepatocytes showing nuclear degeneration and polarization. C) Bile ducts hyperplasia in rabbits given endosulfan 1.0+OTA 0.03mg/kg BW. D) Cellular shrinkage along with mild congestion and hemorrhages in rabbits fed endosulfan @ 0.06mg/kg BW. H&E; A, C and D: 100X; B: 200X.



**Fig 3:** Histological photographs of the intestine of the rabbits given OTA and endosulfan at various doses. A) Intestine of control rabbit. B) Disruption of tissue architecture and villi in the group fed endosulfan 1.0+OTA 0.03mg/kg BW. C) Increased glandular cell population in group fed endosulfan 1.0+OTA 0.06mg/kg BW. D) Depletion of glandular cells in the submucosa of rabbits fed endosulfan 1.0+OTA 1.5mg/kg BW. H&E; A-C: 100X; D: 400X.

**Table 1:** Serum Biochemical profile of the rabbits exposed to different dietary concentrations of OTA and Endosulfan

Group [Endosulfan+OTA (mg/kg BW)]	Urea (mmole/L)	Creatinine ( $\mu$ mol/L)	AST (U/L)	ALT (IU/L)
A (0+0)	8.67 $\pm$ 1.55a	22.98 $\pm$ 5.23a	129.32 $\pm$ 15.66a	60.23 $\pm$ 2.00a
B (0+0.03)	9.65 $\pm$ 1.89a	25.19 $\pm$ 4.56a	175.00 $\pm$ 8.36b	74.19 $\pm$ 0.63b
C (0+0.06)	11.93 $\pm$ 1.50b	32.44 $\pm$ 5.23a	183.95 $\pm$ 11.87b	82.24 $\pm$ 7.19bc
D (1.0+0)	9.20 $\pm$ 1.25a	38.27 $\pm$ 6.59b	135.00 $\pm$ 26.81a	79.23 $\pm$ 1.00b
E (1.0+0.03)	10.60 $\pm$ 0.87a	50.12 $\pm$ 5.56b	190.00 $\pm$ 15.00b	91.66 $\pm$ 2.48bc
F (1.0+0.06)	12.00 $\pm$ 1.06b	81.26 $\pm$ 8.23c	199.00 $\pm$ 17.71bc	106.78 $\pm$ 3.69c
G (1.5+0)	9.60 $\pm$ 1.01a	55.00 $\pm$ 9.45b	145.19 $\pm$ 7.24a	93.60 $\pm$ 1.63c
H (1.5+0.03)	10.57 $\pm$ 0.72a	120.84 $\pm$ 10.56c	198.00 $\pm$ 24.78b	116.32 $\pm$ 4.00c
I (1.5+0.06)	13.23 $\pm$ 0.99b	123.76 $\pm$ 12.26c	212.00 $\pm$ 13.00c	127.32 $\pm$ 4.29d

Values (Mean $\pm$ SD) in each column followed by different letters are significantly different from each other ( $P\leq 0.05$ ).

**Litter size and birth weight:** A non-significant difference was noted in average litter size among different experimental groups (data not shown). Birth weights were significantly lower in all the groups given endosulfan at

two levels and OTA alone and/or in combination (Table 3). In the interaction groups E, F, H and I, the values were significantly lower as compared to feeding these chemicals alone.

**Table 2:** Hematological profile of the rabbits exposed to different dietary concentrations of OTA and Endosulfan

Group [Endosulfan + OTA (mg/kg BW)]	WBC ( $\times 10^3/\mu\text{L}$ )	RBC ( $\times 10^6/\mu\text{L}$ )	Hb (g/dl)	PCV (%)	MCV (fL)	MCH (pg)	MCHC
A (0+0)	8.45 $\pm$ 1.50a	7.36 $\pm$ 0.47a	13.60 $\pm$ 0.62a	42.00 $\pm$ 3.00a	57.04 $\pm$ 5.00a	18.48 $\pm$ 2.23a	32.39 $\pm$ 1.25b
B (0+0.03)	6.92 $\pm$ 1.51a	5.30 $\pm$ 0.33b	12.00 $\pm$ 1.19ab	38.00 $\pm$ 2.31ab	70.50 $\pm$ 4.23b	22.26 $\pm$ 1.96ab	31.57 $\pm$ 2.36b
C (0+0.06)	7.12 $\pm$ 1.60a	4.36 $\pm$ 0.18c	10.23 $\pm$ 0.45b	35.00 $\pm$ 2.67b	80.14 $\pm$ 6.25b	23.42 $\pm$ 1.84b	29.22 $\pm$ 3.25b
D (1.0+0)	5.50 $\pm$ 1.58b	5.33 $\pm$ 0.41b	12.45 $\pm$ 2.00a	39.00 $\pm$ 6.07a	73.14 $\pm$ 7.23b	23.35 $\pm$ 2.26b	31.93 $\pm$ 2.53b
E (1.0+0.03)	6.30 $\pm$ 1.66ab	5.65 $\pm$ 0.35b	11.23 $\pm$ 1.57a	31.00 $\pm$ 3.95b	54.84 $\pm$ 5.15a	19.86 $\pm$ 0.92a	36.22 $\pm$ 1.23a
F (1.0+0.06)	6.02 $\pm$ 1.41ab	4.15 $\pm$ 0.71c	8.23 $\pm$ 0.80c	28.00 $\pm$ 3.74b	67.35 $\pm$ 6.58ab	19.79 $\pm$ 2.36a	29.39 $\pm$ 3.26b
G (1.5+0)	7.15 $\pm$ 1.45a	5.50 $\pm$ 0.3b	11.58 $\pm$ 1.14a	32.00 $\pm$ 3.07b	58.12 $\pm$ 7.23a	21.03 $\pm$ 1.25a	36.18 $\pm$ 4.26ab
H (1.5+0.03)	5.19 $\pm$ 0.60b	4.06 $\pm$ 0.63c	7.75 $\pm$ 1.23c	27.50 $\pm$ 1.29bc	67.73 $\pm$ 5.23b	19.08 $\pm$ 1.56a	28.18 $\pm$ 2.35c
I (1.5+0.06)	4.80 $\pm$ 0.60b	3.44 $\pm$ 0.22d	6.23 $\pm$ 1.50c	23.25 $\pm$ 2.06c	67.48 $\pm$ 4.15b	18.08 $\pm$ 1.32a	26.79 $\pm$ 1.98c

Values (Mean $\pm$ SD) in each column followed by different letters are significantly different from each other ( $P\leq 0.05$ ).

**Table 3:** Weight and length of fetus (recovered at day 18), litter size and birth weight of rabbit kids exposed to different dietary concentrations of OTA and Endosulfan

Group [Endosulfan + OTA (mg/kg BW)]	Fetus weight (g)	Fetus length (cm)	Birth weight (g)
A (0+0)	7.52 $\pm$ 0.46a	5.43 $\pm$ 0.40a	71.75 $\pm$ 5.38a
B (0+0.03)	4.13 $\pm$ 0.15c	4.25 $\pm$ 0.05b	56.50 $\pm$ 5.80b
C (0+0.06)	2.40 $\pm$ 0.52d	3.45 $\pm$ 0.39c	52.75 $\pm$ 0.96b
D (1.0+0)	8.17 $\pm$ 1.35a	5.70 $\pm$ 0.26a	52.75 $\pm$ 0.96b
E (1.0+0.03)	6.83 $\pm$ 0.38a	4.15 $\pm$ 0.12b	44.00 $\pm$ 6.73bc
F (1.0+0.06)	4.55 $\pm$ 0.58b	3.18 $\pm$ 0.077c	39.75 $\pm$ 3.20c
G (1.5+0)	5.01 $\pm$ 0.28b	4.27 $\pm$ 0.20b	41.25 $\pm$ 0.96c
H (1.5+0.03)	1.58 $\pm$ 0.36e	2.77 $\pm$ 0.59c	32.00 $\pm$ 2.16d
I (1.5+0.06)	0.87 $\pm$ 0.26f	2.03 $\pm$ 0.57cd	27.00 $\pm$ 3.65d

Values (Mean $\pm$ SD) in each column followed by different letters are significantly different from each other ( $P\leq 0.05$ ).

### Histopathological findings

**Kidneys:** Kidneys of rabbits in group B and C showed a mild to moderate degree of degenerative changes including, pyknosis and necrosis of glomeruli, congestion and sloughing of the tubular epithelial cells, against the normal histological structure in group A (Fig. 1). Additionally, accumulation of eosinophilic pinkish material, indicating protein loss from the glomeruli and clumping up of the epithelial cells at collecting ducts was noted in the group fed higher dose of OTA. Kidneys of the rabbits in groups D, E and F, showed dose dependent severity in lesions. Feeding endosulfan in combination with OTA in group E and F, showed a significant damage in the both cortical and medullary region. In the group of rabbits given endosulfan at higher dose (1.5 mg/kg BW) alone or in combination with OTA showed similar lesions, but the with higher intensity.

**Liver:** The histological sections of the liver of the rabbits in group A (control) showed normal appearance with foamy cytoplasm of the hepatocytes (Fig 2). In group B, no pathological lesions other than a mild cellular swelling was noted. Likewise, rabbits in group C, showed a moderate degree of degeneration as depicted by cellular shrinkage and widening of sinusoidal spaces. Additionally, a moderate degree of cellular vacuolation suggesting fatty change was also observed at some places. The groups of rabbits given endosulfan at low dose (1 mg/kg BW) alone (D) or in combination with OTA (group E and F), showed similar changes but higher in severity. In group G, the liver sections showed vacuolated hepatocytes and small patches of congestion. In group H and I, given two chemicals together, the microscopic lesions were similar but with dose dependent increasing trends.

**Intestine:** The histological appearance of the intestine was normal in the rabbits fed basal diet with normal length of

villi, cellular proportion and vascular network (Fig. 3). Feeding OTA resulted in sloughing of villus epithelial cells which varied with the dose of toxin in feed. Likewise, feeding endosulfan alone in group D and G, resulted no alteration in the intestinal histology. However, in the groups of rabbits given endosulfan at 1.0mg/kg BW with OTA at 0.03 and 0.06mg/kg BW, resulted in sloughing in villus epithelial cells, shortening of villi, moderate congestion and degeneration of mucosal glands. In group H and I, fed higher dose of endosulfan at 1.5mg/kg BW along with OTA at 0.03 and 0.06mg/kg BW, the lesions were similar with higher intensity.

### DISCUSSION

A significant increase in the serum levels of urea and creatinine in the groups of rabbit maintained on OTA alone or in combination with endosulfan may be attributed to the nephrotoxic activities of the test chemicals. In animals body OTA (~99%) is bound to plasma proteins, mainly albumin, which can't be excreted by glomerular filtrate. However, unbound portion (~1%) can be found in the urinary filtrate. The remaining OTA is only excreted via organic Anion Transporter (OAT) route, which prone the proximal tubular epithelial cells to damage, by virtue of depletion of indigenous dicarboxylic acid (glutarate, ketoglutarate) on expense of OTA internalization (Sekine *et al.*, 2006; Khatoon *et al.*, 2016). The increase in the serum biomarkers of kidneys damage, and the mechanistic nephrotoxicity associated with the OTA, has also been augmented by histological alteration in the tubular cells of nephrons. Several natural and experimental OTA exposure studies have recorded similar changes in the kidney function (Khan *et al.*, 2014). In human at least three important kidney disease, including Balkan endemic nephropathy (BEN), chronic interstitial nephritis, and karyomegalic interstitial nephritis are partially linked to elevated dietary levels of OTA. A potentiation in the renal toxicities noted in the present study may be linked to the oxidative damage associated with OTA and endosulfan in the co-fed groups (Kannan *et al.*, 2000).

Mechanistically, OTA at low doses influences energy metabolism such as carbohydrate, amino acid, cofactors and vitamins. However, in the high doses pathways influenced by OTA are associated with the different body systems including circulatory, digestive, endocrine, excretory system. In the present study liver damage depicted by enzyme chemistry and histological alteration can be linked to OTA mediated direct toxicity or altered metabolism. Endosulfan is potent free radicals' generator in hepatocytes and the injury to these cells results in elevated

levels of serum ALT and AST. The damage to hepatocytes and accumulation of lipids in the cells, presumably fatty change (due to decreased apo-protein synthesis) can be linked to endosulfan mediated hepatotoxicity. The more severe response in the groups of rabbits, co-fed the two contaminants indicates the synergistic toxic effects. In an experimental study, Qamar *et al.* (2012), reported endosulfan as potent toxic compound for hemopoietic, nervous, reproductive system and livers of the male Japanese quails. Other than direct cytotoxic effects of endosulfan on reproductive cells, Takhshid *et al.* (2012) reported a reduction in the sperm motility, viability, daily sperm production (DSP) and increased the number of sperm with abnormal chromatin condensation. Similarly, testicular MDA and plasma LDH are increased in the endosulfan exposed rats.

In the present study, feeding OTA alone at the two levels had significant effect on weight gain of developing fetus. However, a sort of competitive interaction was noted when both the toxins were fed at low doses. In the higher doses (group H and I) when both chemicals were fed together to the pregnant rabbits, led to significant reduction in the fetus weight. The teratogenic and embryocidal effects of OTA have been well documented in avian (Hassan *et al.*, 2012) and mammalian spp (Wangikar *et al.*, 2005). These developmental changes and teratogenic effects are associated with chromosomal abrasions during fetal developmental (Kumar *et al.*, 2014) in the endosulfan and OTA treated pregnant rats. The genotoxic effects of OTA can be attributed to the DNA binding potential which leads to important errors during DNA replications. These errors may range from point mutation to segment deletion, resulting genetic defects or developmental anomalies in the developing embryos/fetuses. Another important effect of genomic abrasion is the missing of some key enzymes coding gene, which leads to impaired growth of the developing fetuses. The decrease in the crown to rump length in the developing embryos noted in the present study can be linked to aforementioned effects.

In addition to nephrotoxic, hepatotoxic and teratogenic effects of OTA, it is also considered as potent myelotoxic agent (Moura *et al.*, 2004). In the present study, a significant alteration in the hematological profile of the rabbits was noted in the treatment groups. Effects of OTA and endosulfan on the hematopoietic system have been widely investigated in the mammalian and avian modal (Qi *et al.*, 2014).

**Conclusions:** Based on the findings of this study, we can conclude that there are dose dependent toxicological effects of OTA and/or endosulfan in the rabbits. There exists an interaction in terms of synergism in the toxicological effects when both the chemicals are fed together. However, no effects on litter size indicated, no embryocidal activities at the tested doses and their interaction groups, which could be linked to time of treatment.

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