CHAPTER 3

BIOCHEMICAL AND HISTOPATHOLOGICAL ALTERATIONS RENDERED BY COMBINATION INSECTICIDE (CHLOROPYRIFOS + CYPERMETHRIN) IN TWO GENERATIONS OF *GALLUS DOMESTICUS*

INTRODUCTION

Pesticides play a key role in the agricultural industry as well as in the household to counter the pest havoc. Ergo, injudicious use of the pesticides is leading to environmental pollution and thereby causing health hazards to various non-target species, which even includes severe cases of human poisoning. The inapt use of pesticides has been related to many health issues ranging from headache and nausea to chronic impacts like that affecting the reproductive and endocrine system of an individual, and cancer too has been reported (Eskenazi *et al*., 1999; Jones *et al*., 2015).

The researchers at the Public Health Institute, California (2006) found a six fold increase in risk factor for autism spectrum disorders (ASD) in the children of women who were exposed to organochlorine pesticides. The Agency for Toxic Substances and Disease Registry (CDC/ATSDR, 2009) published a study which found that children who live in homes where their parents use pesticides are twice as likely to develop brain cancer versus those who live in residences in which no pesticides are used. There is also increasing evidence that exposure to pesticides disrupts the endocrine system, creating havoc within the complex regulation of hormones, the reproductive system, and embryonic development. Endocrine disruption can produce infertility and a variety of birth defects and developmental defects in offspring, including hormonal imbalance and incomplete sexual development, impaired brain development, behavioural disorders, and many others (Waldbillig *et al.,* 1998).

Evaluation of the toxic effects induced by pesticide exposure is done by several methods. The physiological status of the organism gives a preliminary picture of a toxic manifestation. The analysis of blood indices has proven to be a valuable approach for analysing the health status of an organism. These indices provide reliable information on metabolic disorders, deficiencies and chronic stress status. However, an evaluation of the biochemical parameters can be related to the prevailing physiological condition of the organism, which monitors the activity of various enzymes like ALT, AST, ALP etc. in the serum. Assessment of these

manifestations in the serum would either reveal a normal condition or impairment to the detoxifying organs, *viz*., the liver and kidney. Dysfunction in the activities of the enzymes or leakage of enzymes from the vital organs can also arise from damage to the structural integrity of the organs, which can be seen through microscopic examination of the tissue sections. Hence, histological observation over and above biochemical parameters can help clarify the mechanism by which pesticide is affecting or causing damage to the body.

Objective

The various teratogenic and hematologic effects imposed by Nurelle D 505 EC on two generations of RIR chicks have been clearly depicted in the earlier chapters. Apart from these manifestations, the investigation of levels of certain enzymes in the blood, serve as useful indices to evaluate the damage to certain vital organs of the body like the liver and kidney. Also, in order to reconfirm the damage to the internal organs if any, histopathological studies were conducted on two generations of RIR chicks after an in-ovo exposure of Ci was made to the F1 generation. The current chapter therefore, highlights the evidences to toxicosis through serum enzyme activities and the histomorphological changes, to deliver important preliminary clues as to which internal tissue function has suffered a setback and to what extent.

MATERIAL AND METHODS

Test Chemical

Commercially available insecticide Nurelle D 505 EC (chlorpyrifos 50% EC + cypermethrin 5% EC, Dow AgroSciences, India) was used in the study.

Egg Procurement

The fertilized Rhode Island-Red (RIR) eggs were collected from Poultry Science Division of Anand Agricultural University, Anand, and refrigerated at 4^{o}C until used. Eggs were swabbed with povidone-iodine to avoid infection. The investigation covered four groups of fifty fertilized RIR eggs each; three for experimental and one control. Each egg was marked to receive the respective dosage treatment of the Ci for experimental groups and corn oil for control group. The study was conducted on the F1 (Parents) and F2 (Chicks) generations as the animal model.

Insecticide Injection

Combination insecticide was diluted in corn oil and three sub-lethal doses namely low, mid and high were made as described previously (Uggini *et al*., 2012). A single dose of 0.01, 0.05 and 0.1 µg/egg of Ci diluted in corn oil was administered to the airspaces of fertilized eggs from low, mid and high group respectively on day zero of incubation. The eggs from the control received corn oil in the similar manner. The injected portion of each egg was sealed by molten paraffin wax. The whole experiment was done in a sterile environment under a laminar air flow. The dose volume of Ci and corn oil was maintained at 25µl/egg.

Incubation

A cleaned, disinfected and fumigated automatic egg incubator and hatcher were used. Incubator regulates the factors such as temperature of 37.5 ± 0.5^{o}C, 75-80% relative humidity and periodic turning of the eggs. Immediately after treatment, both the treated and control eggs were kept in the incubator with proper marking for 18 days. On 18th day the egg candling was done and the viable eggs were transferred to hatcher till the day of hatch. On day 21 after hatching, the hatchling groups (F1 generation) from the treated and control eggs were tagged with wing bands with respect to their dosage treatment and were housed in separate pens with immediate supply of water. After three hours starter mash feed was provided to the chicks. To promote the survival of the chick, assisted hatching was involved in case of those chicks who failed to progress normally at hatching stage due to deformities or weakness caused by Ci.

The F1 generation chicks when reached 25 weeks old were randomized within the respective treatment groups and the similar weighed ones were selected for breeding. Twenty hens and two roosters from each of the four groups were selected and pen mated for a week. The eggs collected from the F1 generation were marked as per the treatment groups of F1 parental generation and incubated. On hatch out the F2 generation chicks were wing banded with respect to their parent's group and reared on standard diet for 4 weeks. The biochemical analyses were performed on 25 week old parents and 4 week old second generation chicks.

Biochemical Estimations

Blood samples were drawn from brachial vein of 25 week old parent and 4 week old F2 chick using 2ml disposable syringes and transferred to eppendorf tubes. Subsequently, allowed the

blood to clot at room temperature for 15-30 minutes. Centrifuged the tubes at 1500g for 10 minutes at 4^{o}C. The supernatant was collected and refrigerated till further use.

Versatile Bio-Chemistry analyzer RX-50V (Micro Lab Instruments, India) was used to evaluate biochemical parameters which included Serum glucose, Serum albumin, Serum globulin, Serum protein, Urea, Blood Urea Nitrogen (BUN), Creatinine, Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST),lactate dehydrogenase (LDH) and nucleic acid (DNA, RNA) content.

Histopathological Investigation

The control and treated birds belonging to F1 and F2 generations were terminally sacrificed at the age of 25 weeks and 4 weeks respectively. Liver and kidney of each bird were processed for histopathological examination, fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 5 microns and stained with haematoxylin and eosin for microscopic observation.

Statistical Analysis

The data were analyzed by one-way ANOVA followed by Dunnet's multiple comparison test. Results were expressed as mean ± standard error. The threshold of statistical significance was set at $P \leq 0.05$. Statistical analysis was performed using GraphPadPrism, version 5.

RESULTS

Biochemical estimations

Analysing the results of the biochemical tests in the F1 and F2 generations, significant variations were found in the Ci treated group when compared to the control group of birds. In F1 generation birds, the level of random blood sugar showed a significant increase in the group which received 0.10µg/egg of Ci. Similar result was displayed by the F2 chicks, where the random blood glucose values showed a significant increase only in the high dose group descendants (Tables 3.1 and 3.2).

The F1 generation has shown a dose dependent decrease in albumin, globulin and protein levels in all the three Ci treated groups (Figure 3.1-A, Figure 3.2-A, (Figure 3.3-A). In the F2 generation, the albumin (Figure 3.1-B) and globulin (Figure 3.2-B) decreased in all the three

progeny groups of Ci treated parents, while protein showed a decrease in the progeny of only the highest dose i.e. 0.10µg/egg of Ci treated group. (Figure 3.3-B)

The values of blood urea (Figure 3.4-A) and BUN stayed unaltered, while the creatinine levels (Figure 3.5-B) have shown a significant increase in all the three groups of Ci treated F1 generation. In F2 generation, blood urea showed an increase in progeny of groups treated with 0.05 and 0.10 µg/egg of Ci (Figure 3.4-B), while BUN showed no significant changes in any of the three groups. Creatinine levels were increased in all the treatment groups in F2 generation (Figure 3.5-B).

In F1 generation, ALT was found elevated in groups which received 0.05 and 0.10 µg/egg of Ci (Figure 3.6-A), whereas the AST levels were significantly high in all three treated groups (Figure 3.7-A). ALP showed a raise in 0.05 and 0.10 µg/egg of Ci treated groups (Figure 3.8-A). LDH showed an increase in all the three Ci treated groups (Figure 3.9-A). In the F2 generation too, the ALT (Figure 3.6-B) and AST (Figure 3.7-B) values elevated in progeny of 0.05 and 0.10 µg/egg of Ci treated groups, while ALP (Figure 3.8-B) and LDH (Figure 3.9-B) showed increase in all the three treated groups.

The DNA (Figure 3.10-A) and RNA (Figure 3.11-A) content showed a decrease in all the treated F1 generation birds except for 0.01 µg/egg of Ci treated group which showed no significant variation in RNA content. In the F2 generation the DNA (Figure 3.10-B) decreased in all the three groups while RNA decreased in descendents of 0.05 and 0.10 µg/egg of Ci treated groups (Figure 3.11-B).

Histopathology

The control group birds of both the F1 and F2 generations showed normal architecture in the hepatocytes showing clear cytoplasm and nucleus and the central veins (Figure 3.12). The kidney also showed normal histo-morphological architecture of renal tubules and the glomeruli (Figure 3.13). Microscopic observation of liver and kidney of Ci treated birds showed various histopathological lesions in the F1 generation as well as in the F2 generation. The microscopic observations of liver tissue of F1 generation showed moderate inflammatory changes in 0.01 µg/egg of Ci treated groups (Figure 3.14), while the 0.05 µg/egg of Ci treated groups showed chronic focal inflammation in the periportal area (Figure 3.15). The eggs with

0.10 µg/egg of Ci treatment were showing chronic inflammation, paranuclear clean space and fibrosis of hepatic parenchyma (Figure 3.16). The kidneys of these birds showed pathological changes only at 0.05 and 0.10 µg/egg of Ci treatment, where the renal tubules showed hyalinization, atrophied glomeruli and interstitial fibrotic changes (Figure 3.17, Figure 3.18).

Among the F2 generation, the livers of chicks descended from the birds which received 0.01µg/egg of Ci, showed mild changes like sinusoidal epithelium in the hepatic parenchyma, inflammatory reactions as well as fibrotic changes (Figure 3.19). The chicks belonging to 0.05 and 0.10 µg/egg of Ci treated parents showed more intense changes in the liver in the form of chronic inflammation and infiltration of mononuclear cells, cytoplasmic rarefractions of the hepatocytes and multifocal vacuolar degeneration (Figure 3.20, Figure 3.21). The kidney of F2 birds of the 0.05µg/egg of Ci treated parents showed intratubular capillary congestion and cystic transformation of the renal tubules (Figure 3.22), mild renal epithelial vacuolar degeneration; while those of 0.10 µg/egg of Ci treatment showed mild epithelial renal vacuolar degeneration and hyaline cast in places of tubular lumen (Figure 3.23).

Table 3.1: Biochemical parameters of F1 generation RIR domestic fowl subjected to various doses of combination insecticide during their embryonic development.

Parameters	Control (Corn oil)	Low (0.01µg/egg)	Medium (0.05µg/egg)	High dose (0.1µg/egg)
Random Blood Sugar (mg/dl)	$204.33 \pm 15.24^{a,b}$	$223.50 \pm 19.77^{a,b}$	$228.67 \pm 20.25^{a,b}$	251.33 ± 21.91^{b}
Albumin (g/dl)	1.73 ± 0.06^{a}	1.50 ± 0.093^{b}	1.07 ± 0.086^{c}	0.82 ± 0.041^{d}
Globulin (g/dl)	3.87 ± 0.218^{a}	2.67 ± 0.216^{b}	2.08 ± 0.204^{c}	1.30 ± 0.125^{d}
Protein (g/dl)	3.50 ± 0.275^{a}	$3.00 \pm 0.292^{b,c}$	$2.92 \pm 0.212^{b,c}$	1.75 ± 0.172^{d}
Blood Urea (mg/dl)	23.50 ± 1.15^{a}	24.50 ± 2.22^{a}	25.33 ± 1.63^{a}	26.00 ± 1.63^{a}
Blood Urea Nitrogen (mg/dl)	9.76 ± 0.76^{a}	9.81 ± 1.32^{a}	10.02 ± 1.08^{a}	10.83 ± 1.05^{a}
S. Creatinine (mg/dl)	0.50 ± 0.031^{a}	$0.62 \pm 0.049^{b,c,d}$	$0.68 \pm 0.051^{b,c,d}$	$0.70 \pm 0.063^{b,c,d}$
SGPT (IU/L) or ALT	32.67 ± 2.74^{a}	$36.17 \pm 2.93^{a,b,c}$	$38.50 \pm 3.47^{b,c}$	$39.00 \pm 3.65^{b,c}$
SGOT (IU/L) or AST	35.50 ± 2.83^{a}	43.00 ± 3.44^{b}	$53.33 \pm 4.68^{c,d}$	$58.83 \pm 5.61^{c,d}$
ALP (IU/L)	183.17 ± 11.69^{a}	185.33 ± 13.55^{a}	188.00 ± 16.07^{b}	190.17 ± 15.44^{c}
LDH (IU/L)	758.67 ± 13.72^{a}	$885.83 \pm 14.29^{b,c}$	$963 \pm 32.04^{b,c}$	1067.83 ± 105.00^{d}
DNA (mg/g)	4.75 ± 0.31^{a}	$3.92 \pm 0.34^{b,c,d}$	$3.72 \pm 0.37^{b,c,d}$	$3.63 \pm 0.36^{b,c,d}$
RNA (mg/g)	8.34 ± 0.73^{a}	7.35 ± 0.69^{a}	6.08 ± 0.63^{b}	4.85 ± 0.45^{c}

Values are expressed in mean ± SD; n = 6; Values with same superscript are not statistically significant for each parameter.

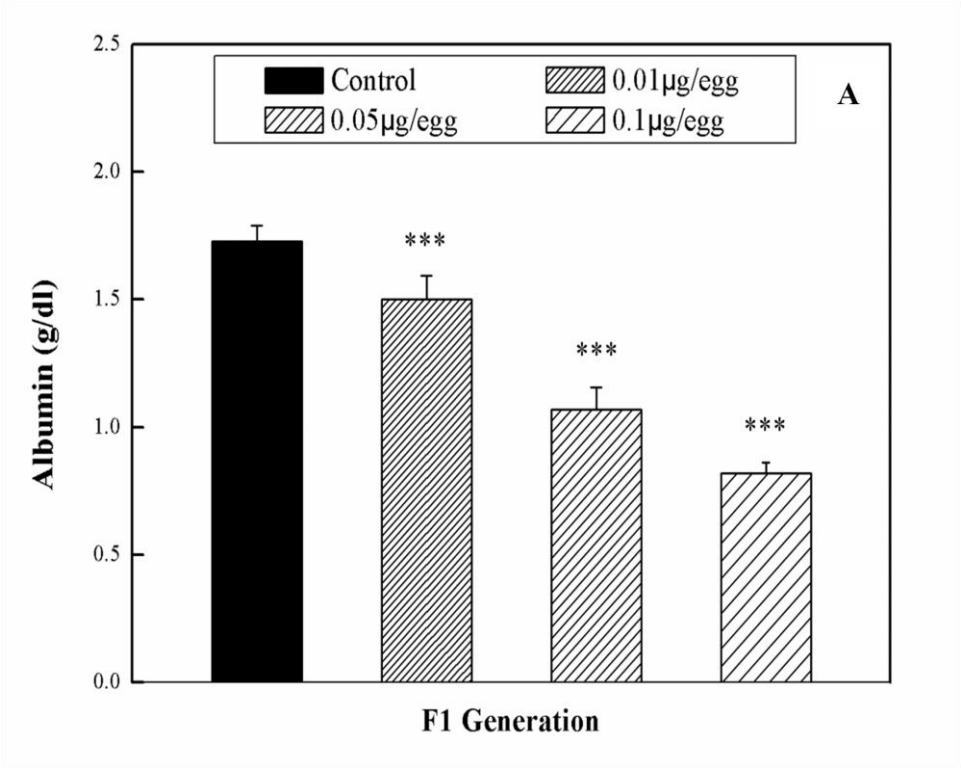
`

Table 3.2: Biochemical parameters of F2 generation RIR domestic fowl subjected to various doses of combination insecticide during their embryonic development.

Parameters	Control (Corn oil)	Low (0.01µg/egg)	Medium (0.05µg/egg)	High (0.1µg/egg)
Random Blood Sugar (mg/dl)	200.00 ± 7.21^{a,b}	212.83 ± 14.97^{a,b}	220.00 ± 12.26^{a,b}	223.17 ± 12.21^{b}
Albumin (g/dl)	1.70 ± 0.081^{a}	1.42 ± 0.098^{b}	1.15 ± 0.10^{c}	0.83 ± 0.082^{d}
Globulin (g/dl)	3.86 ± 0.27^{a}	2.83 ± 0.24^{b}	2.17 ± 0.211^{c}	1.38 ± 0.12^{d}
Protein (g/dl)	3.07 ± 0.26^{a}	2.92 ± 0.27^{a}	2.83 ± 0.275^{a}	1.67 ± 0.154^{b}
Blood Urea (mg/dl)	20.14 ± 1.46^{a}	21.00 ± 2.03^{a,b}	24.00 ± 2.18^{b}	24.83 ± 2.24^{b,c}
Blood Urea Nitrogen (mg/dl)	9.41 ± 0.68^{a}	9.81 ± 0.72^{a}	10.19 ± 1.07^{a}	10.25 ± 1.02^{a}
S. Creatinine (mg/dl)	0.23 ± 0.014^{a}	0.50 ± 0.031^{b}	0.70 ± 0.054^{c}	0.72 ± 0.062^{c,d}
SGPT (IU/L) or ALT	45.29 ± 3.70^{a}	47.00 ± 4.47^{a,b}	53.00 ± 4.86^{b,c}	57.67 ± 4.69^{c}
SGOT (IU/L) or AST	31.14 ± 3.01^{a}	37.33 ± 3.12^{a}	51.67 ± 4.25^{b,c}	52.50 ± 4.57^{c}
ALP (IU/L)	173.43 ± 16.13^{a}	182.00 ± 16.88^{b}	186.00 ± 16.66^{b,c}	189.67 ± 17.51^{c,d}
LDH (IU/L)	859 ± 18.44^{a}	970.50 ± 23.75^{b,c}	1011.67 ± 43.44^{b,c}	1093.33 ± 53.16^{d}
DNA (mg/g)	4.96 ± 0.44^{a}	4.21 ± 0.49^{b,c}	3.80 ± 0.36^{b,c}	3.55 ± 0.34^{b,c,d}
RNA (mg/g)	8.49 ± 0.61^{a}	7.55 ± 0.57^{a}	6.23 ± 0.78^{b,c}	5.07 ± 0.91^{b,c}

Values are expressed in mean ± SD; n = 6; Values with same superscript are not statistically significant for each parameter.

Figure 3.1: Albumin content in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



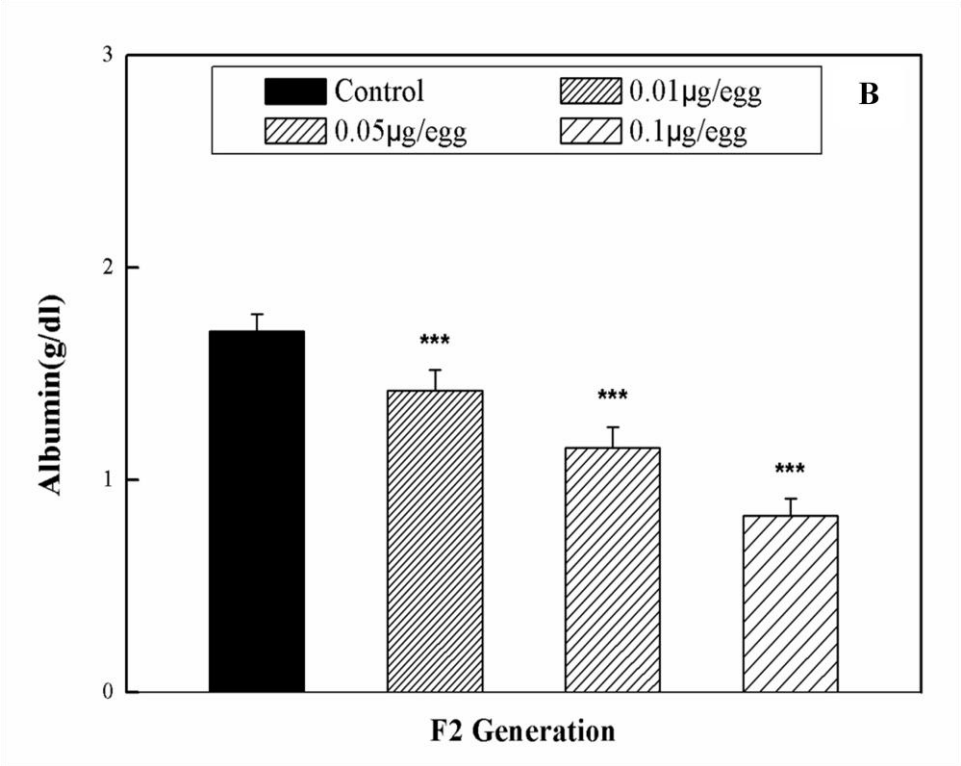
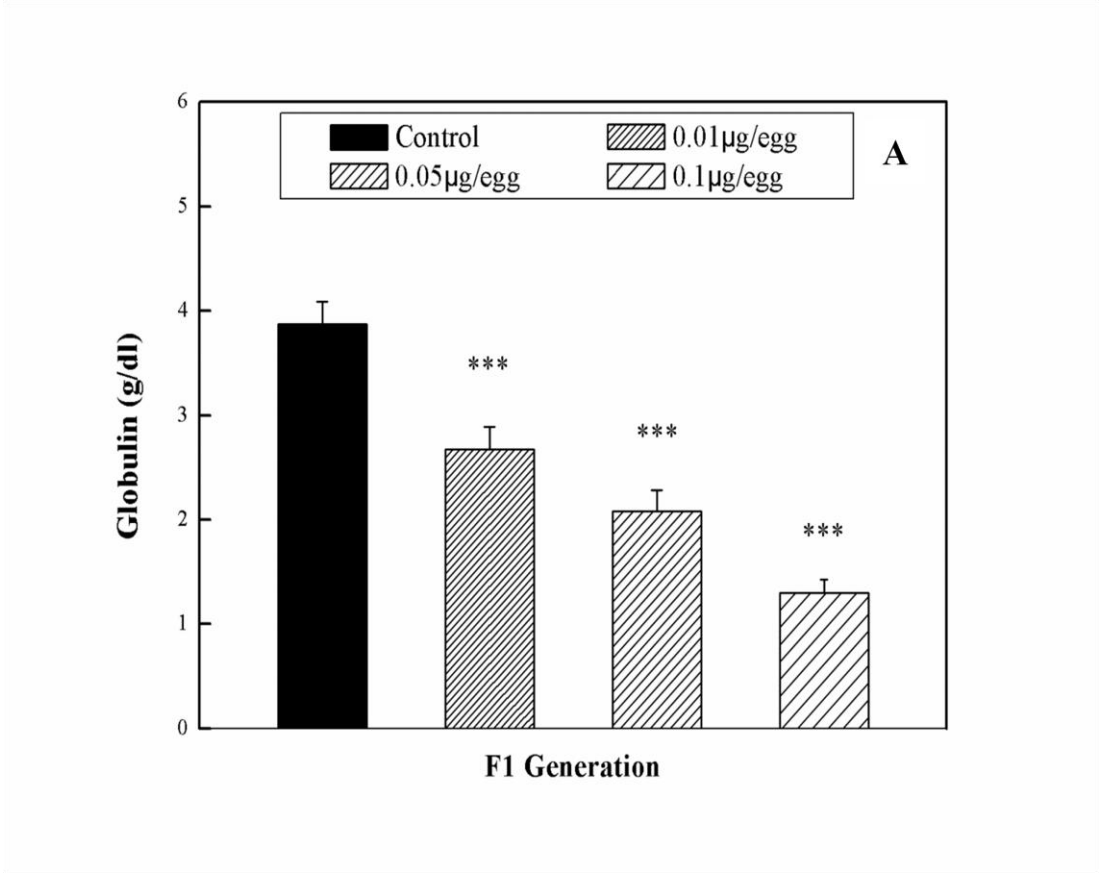


Figure 3.2: Globulin content in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



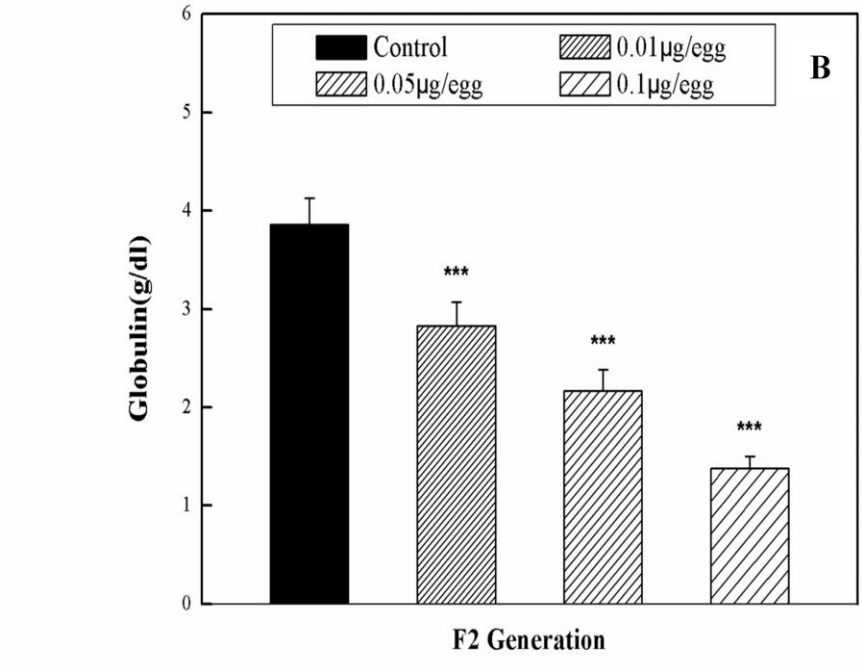
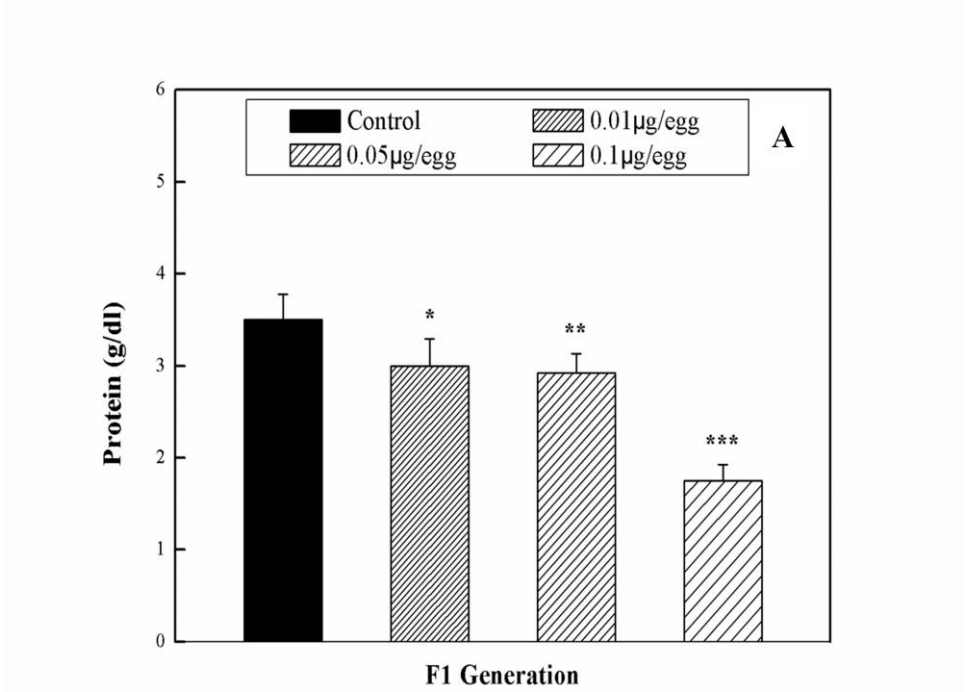


Figure 3.3: Protein content in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



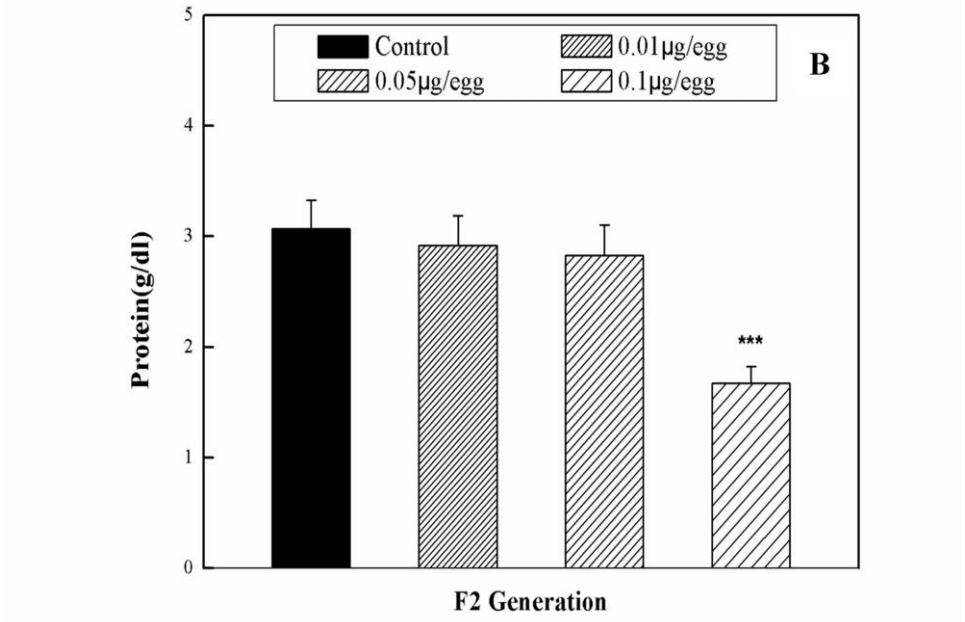
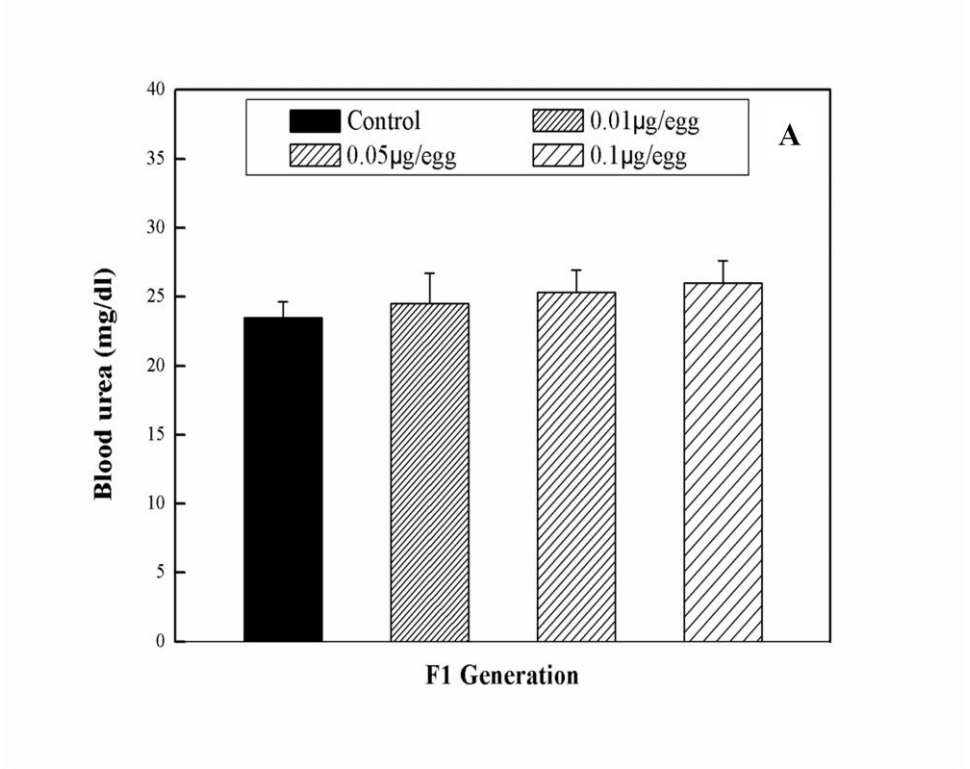
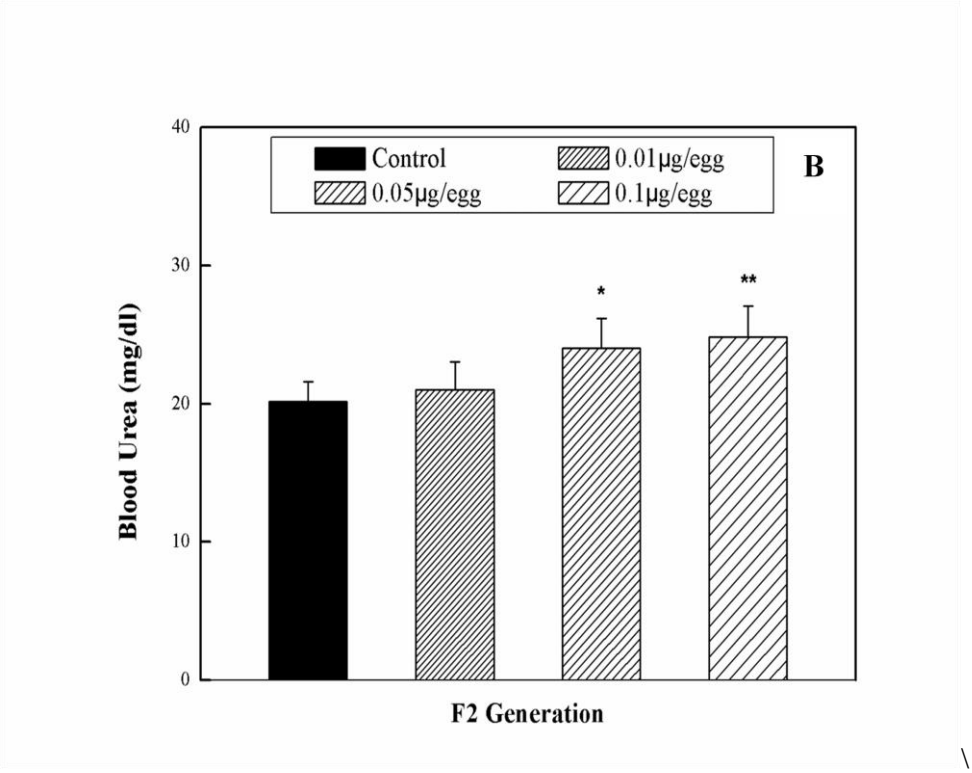


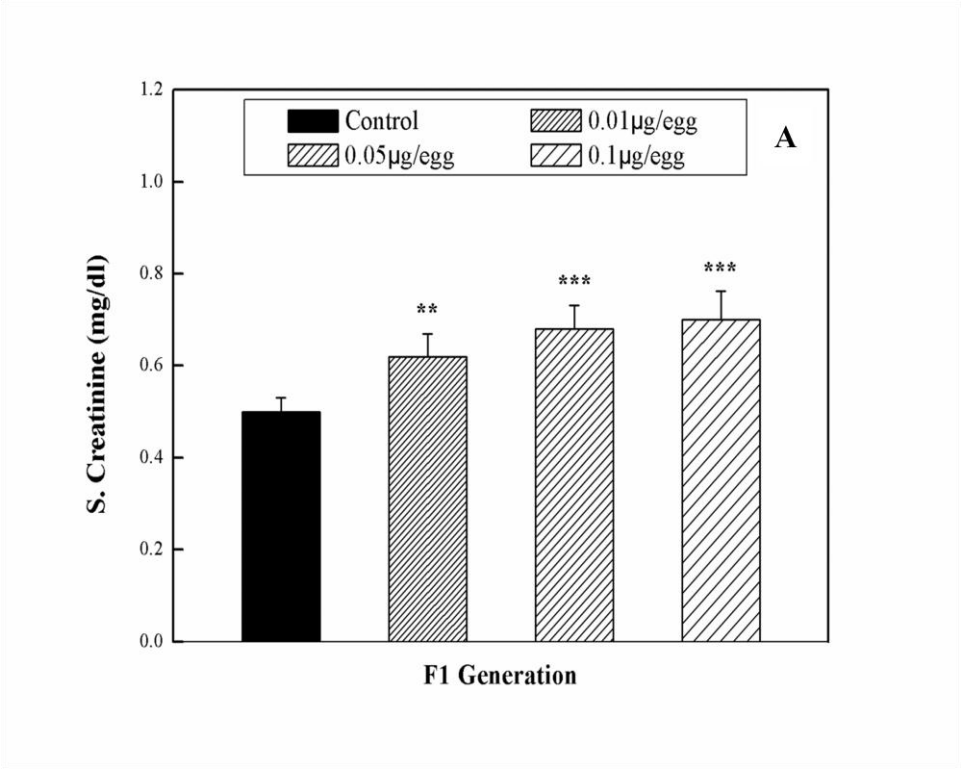
Figure 3.4: Blood urea in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci





\

Figure 3.5: S. Creatinine in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



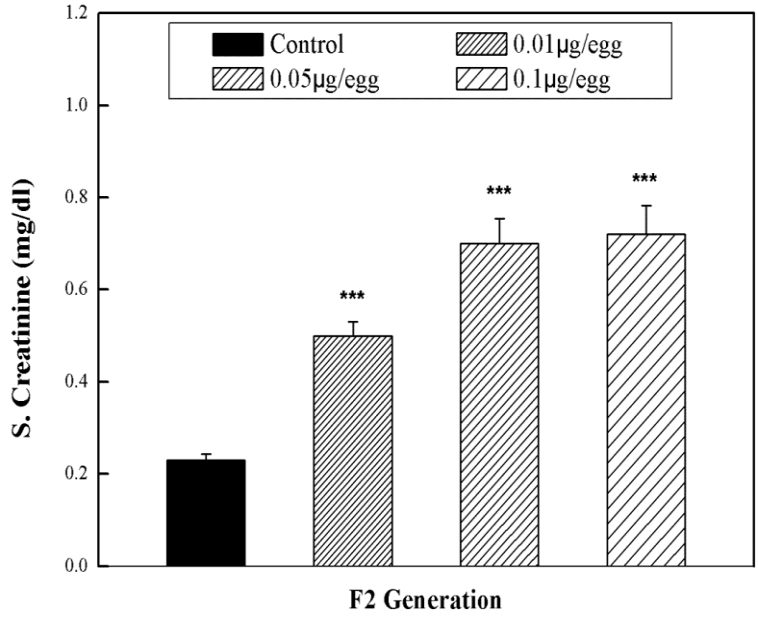
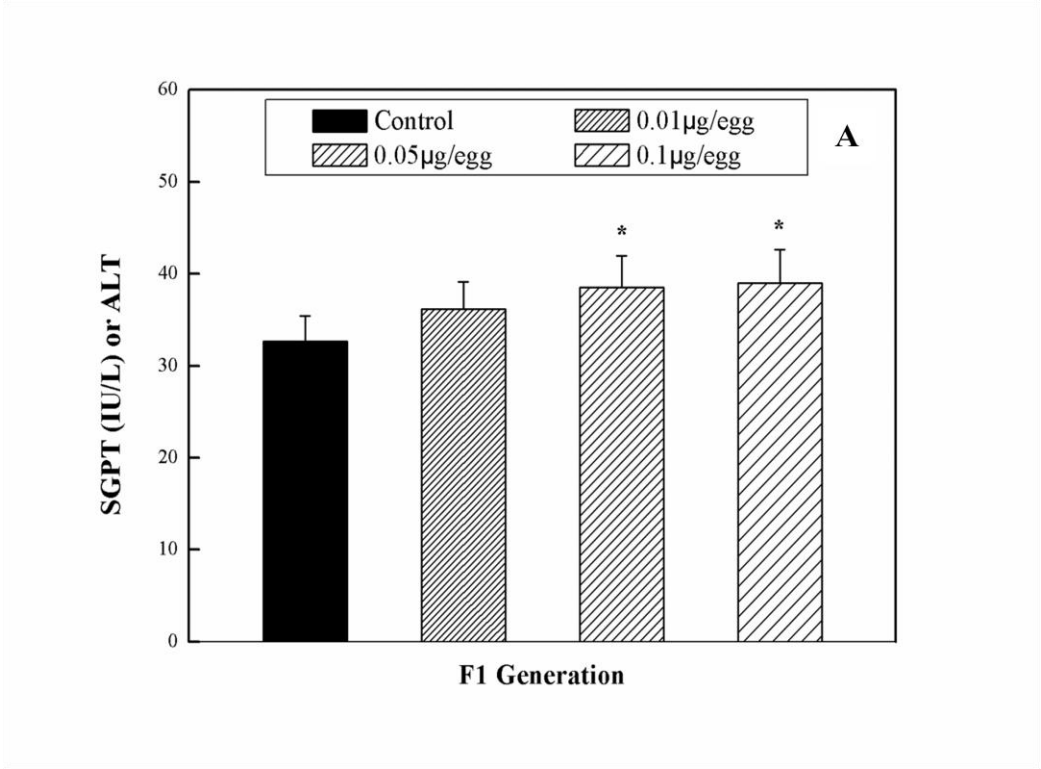


Figure 3.6: SGPT in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



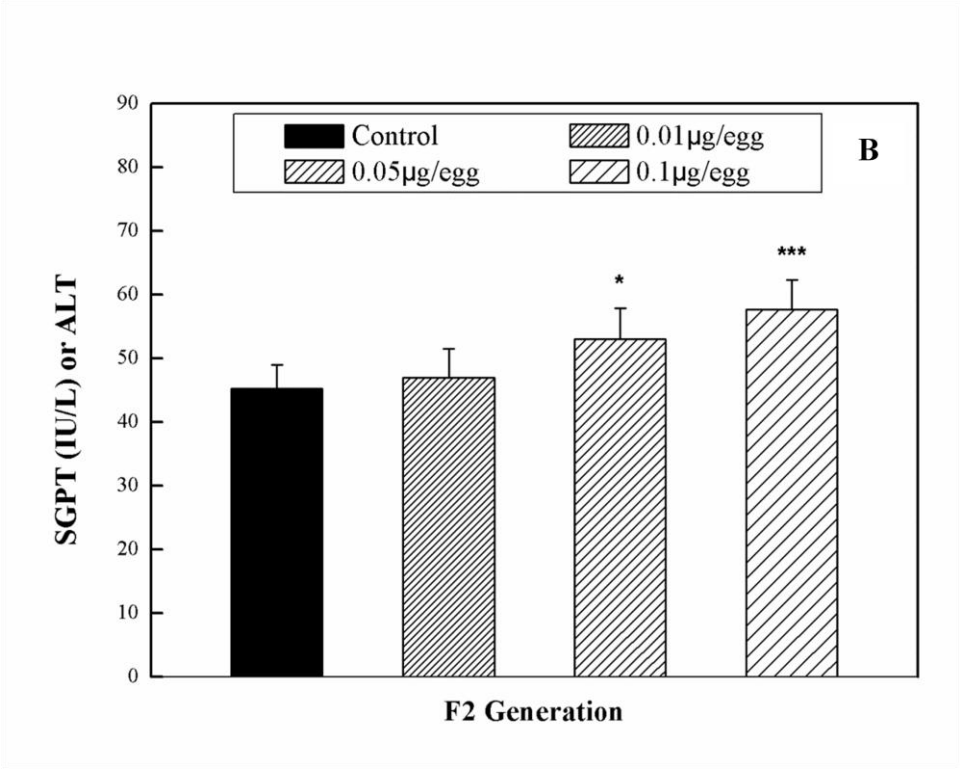
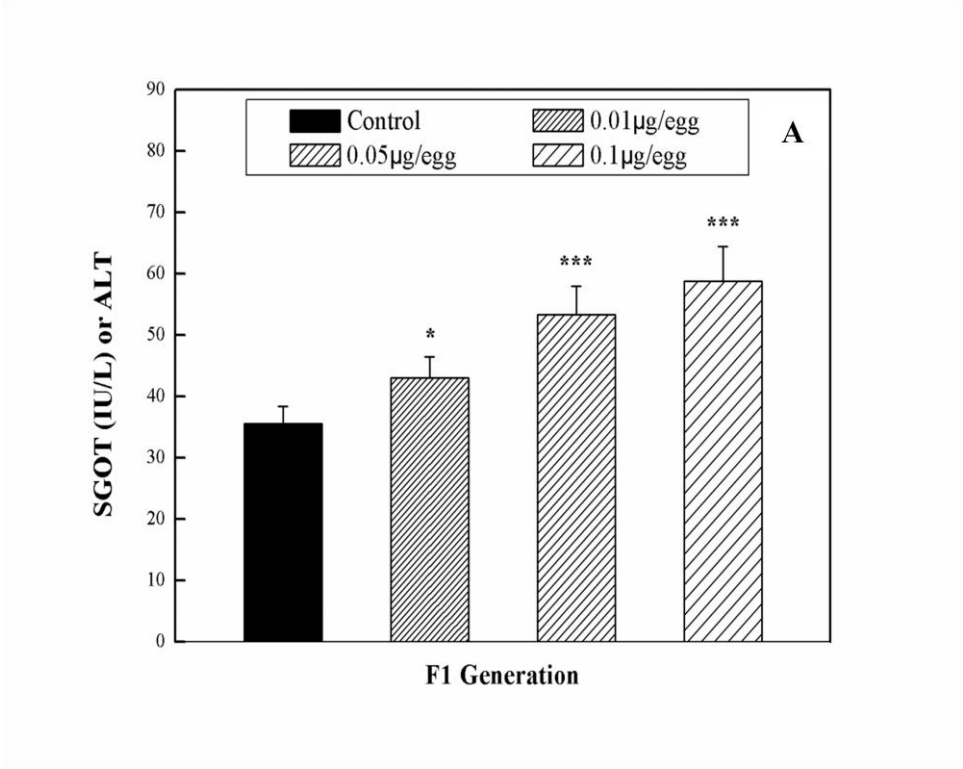


Figure 3.7: SGOT in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



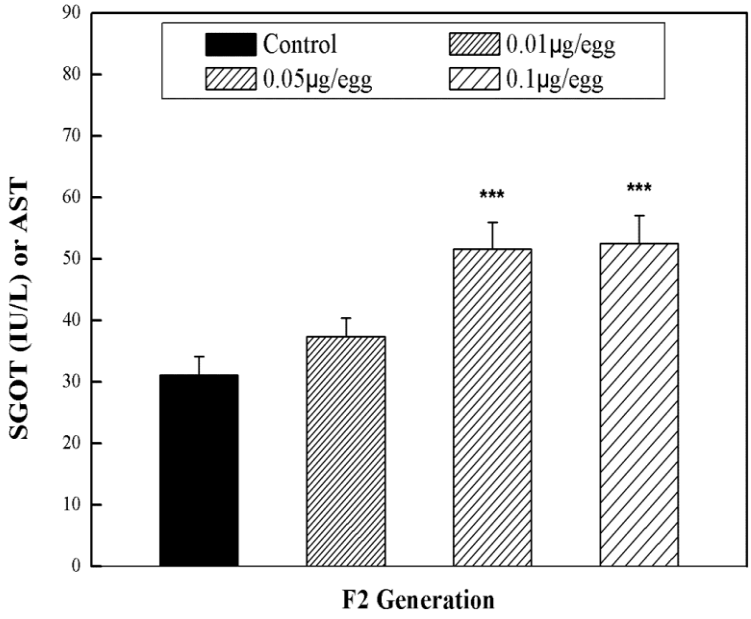
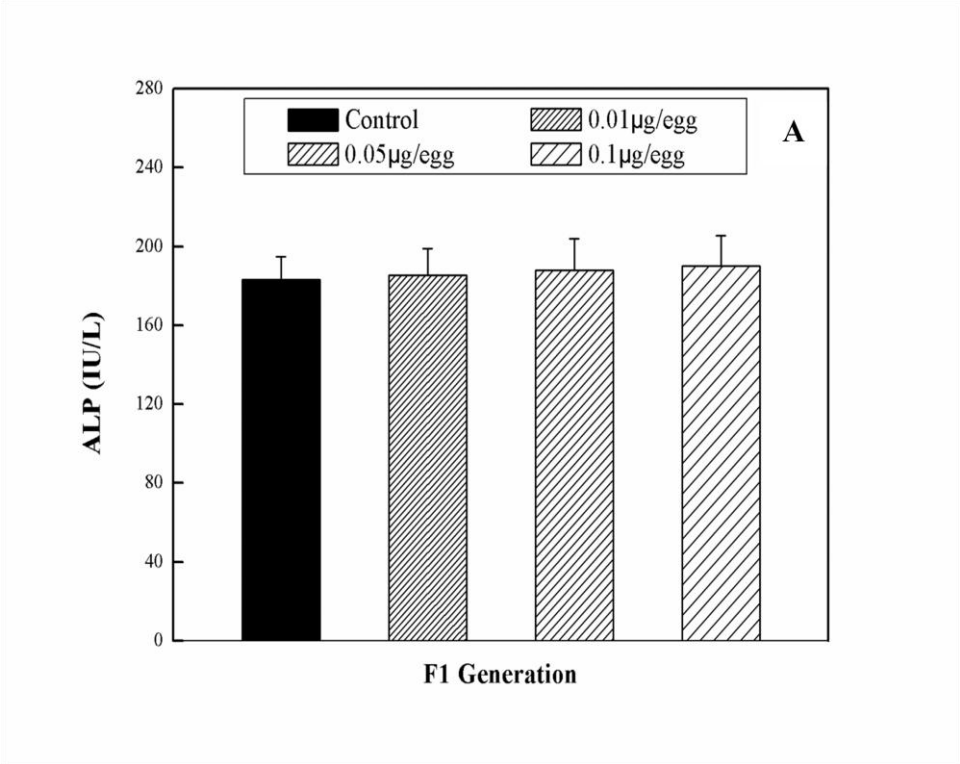


Figure 3.8: ALP in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



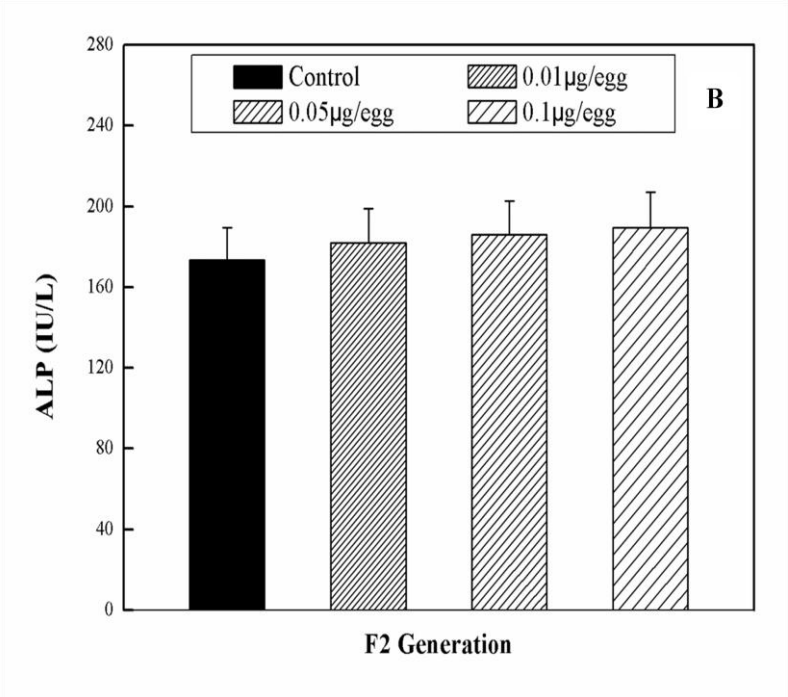
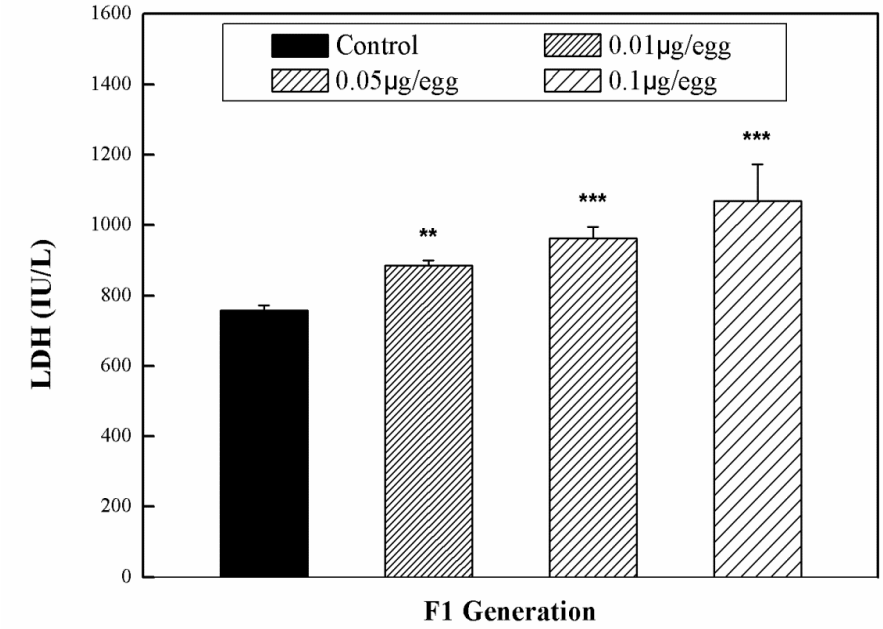


Figure 3.9: LDH in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



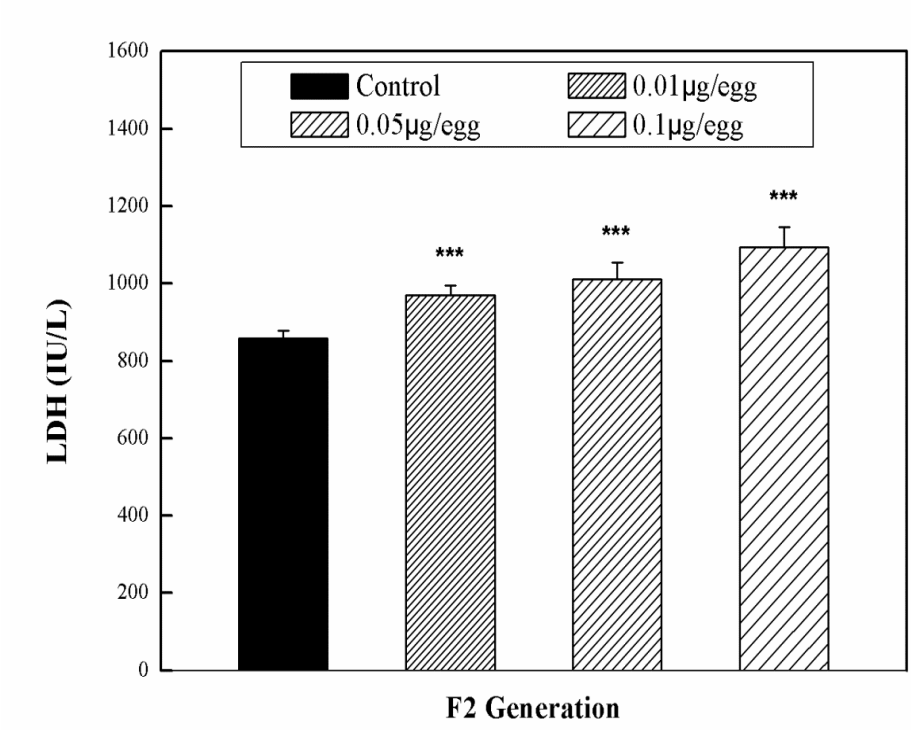
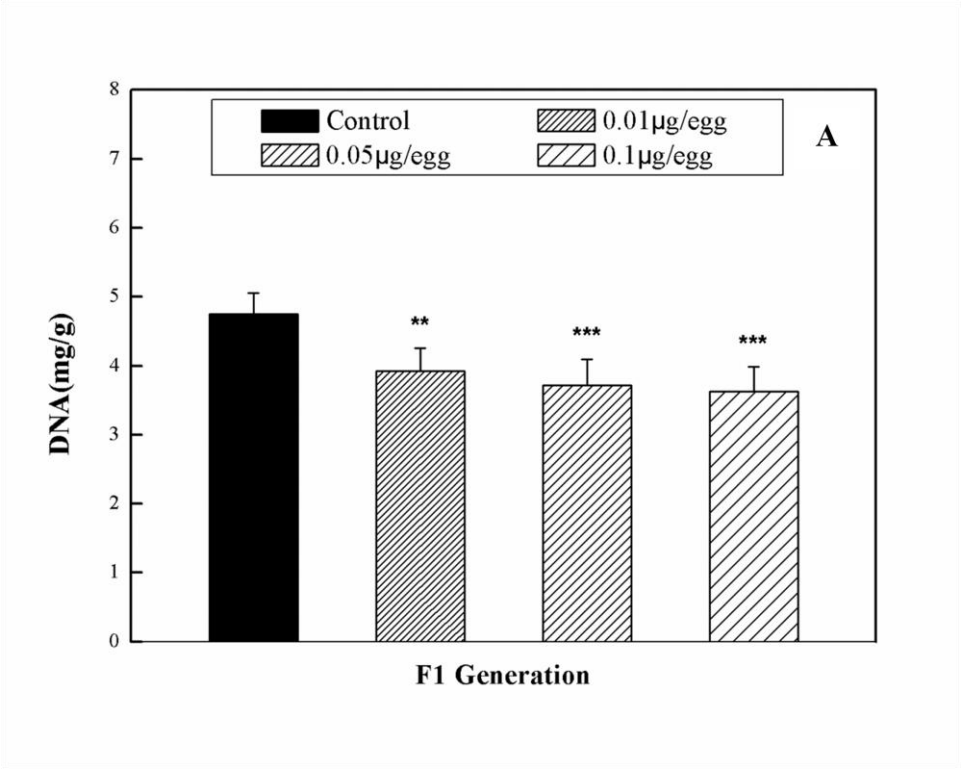


Figure 3.10: DNA in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



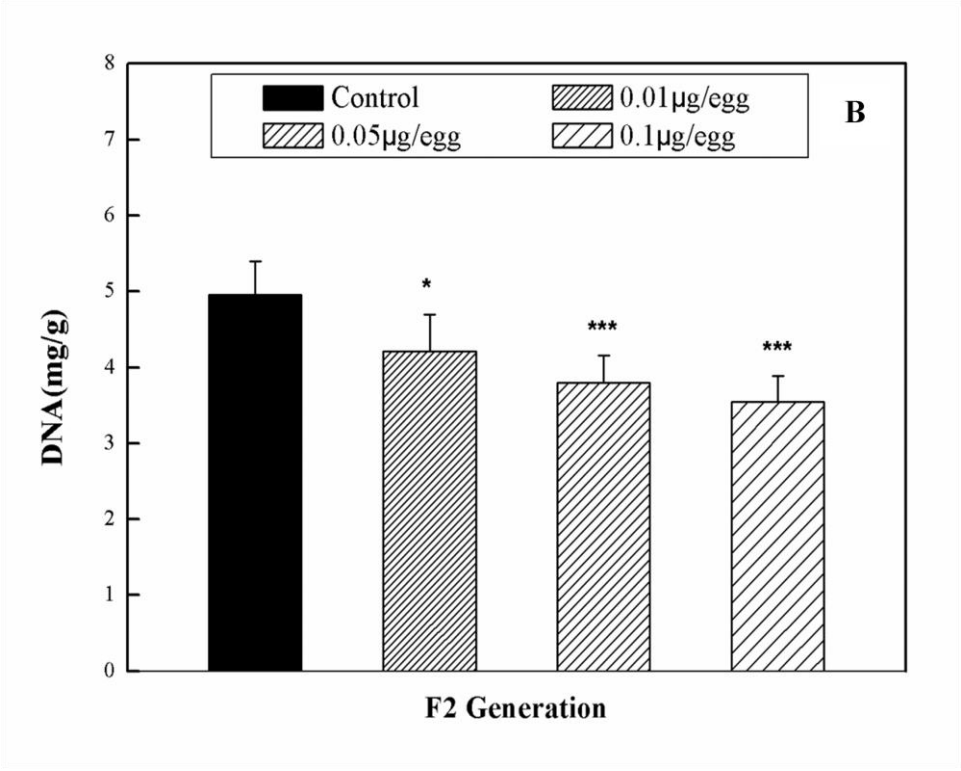
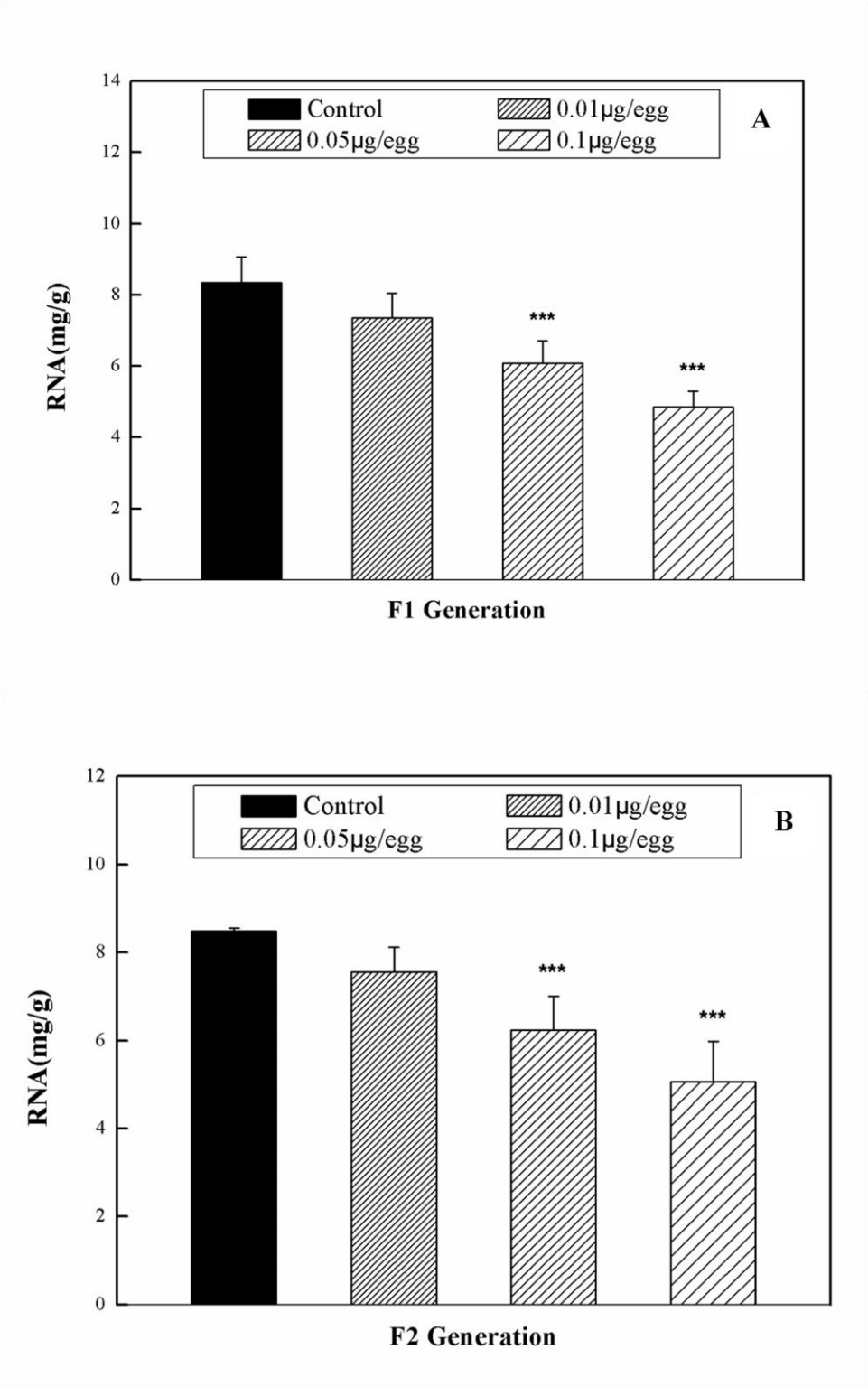


Figure 3.11: RNA in F1 (A) and F2 (B) generation fowls subjected to various doses of Ci



DISCUSSION

The Ci treatment (0.10 µg/egg) has lead to an increase in the levels of blood glucose in both F1 and F2 generations. This result is in agreement with the earlier studies showing hyperglycemia as a result of exposure to Chlorpyrifos (Ambali, 2009), other organophosphates (Ceron *et al*., 1997; Luskova *et. al*. 2001, Rahimi and Abdollahi, 2007) and Cypermethrin (Rahman *et al*., 1990; Chernaki *et al*., 2013). Blood glucose is the major metabolite that is closely associated with the sustainability of energy supply for the execution of the physiological and biochemical functions in the body (Klasing, 2000). It is needed as a substrate for the production of energy through Krebs cycle (Klasing, 2000) and used by most of the body's cells to gain energy (Hazelwood., 2000). At a high dose of 0.10µg/egg, Ci is causing significant hyperglycemia, which might be associated to physiological stress (Clement, 1985; Fletcher, 1988), oxidative stress, inhibition of cholinesterase of the central or peripheral synapses that act in endocrine regulation of glucose metabolism (Matin and Siddiqui, 1982) and also by causing disturbances in the metabolic machinery of liver (Joshi and Rajini, 2009).

The in-ovo Ci exposure has led to a significant decrease in the albumin, globulin and total protein levels in the F1 and F2 generation birds, clearly indicating that the intoxication has an effect on protein synthesis. Both stimulatory and inhibitory factors that selectively alter the protein synthesis and secretions are present in hepatocyte fluids. Earlier reports also showed a decrease in serum total protein due to Chlorpyrifos (Ojezele and Abatan, 2009) and Cypermethrin (Lakkawar *et al*., 2006; Chernaki *et al*., 2013) treatments. It could be postulated that the change may be either due to low level of anabolic activity of cell or higher levels of degradative activities. Rivarola and Balegno, 1991, ascribed this decrease of protein content to changes in protein and free amino acid metabolism and their production in the liver. The depletion in soluble protein contents has been reported by various authors under insecticidal stress (Murthy *et al*., 1986; Sancho *et al*., 1992; Khattak and Hafeez, 1996; Chitra *et al*., 1999). It has been reported that pyrethroid treatment decrease total serum proteins and also decrease concentration (as compared to that of the control) of serum albumin and globulin (Khan *et al*., 2009).

The estimations concerning the kidney function i.e. the blood urea, BUN and creatinin levels did not show any statistically significant variation in any of the treated F1 birds, however the

increase in the blood urea levels in mid and high treatment group progeny allows us to come to a conclusion that the derailment of physiological functions in Ci treated F1 generations, also gets manifested in the F2 generation through a molecular cue involved in maternal imprinting , though the finer mechanism and the transcripts involved remain to be identified.

Urea is a nitrogenous waste product and creatinine is metabolic product of creatine phosphate dephosphorylation in muscle. They are transported in the blood to the kidneys where they are excreted in the urine (Ravel, 1995; Ahmad *et al*., 2011). Blood urea values may rise as a result of intense nitrogen catabolism during weight loss (Khan *et al*., 2012) or very high urea and creatinine values may be due to anemia (Harcourt- Brown, 2002). Earlier studies by (Yousef *et al*., 2003, 2006; Ahmad *et al*., 2011) reported increased urea and creatinine concentration in blood of pyrethroid treated animal. Yousef *et al*., 2006 opined that increase in plasma creatinine and urea results could be due to declining ability of the kidneys to filter these waste products from the blood. This explanation can hold true for the current observation too wherein we also noticed damaged renal tubules.

The increase of ALT , AST, ALP and LDH levels in all the treated birds or at mid and high dose level treatment in F1 generation and the repetition of almost alike results in the F2 generation too, shows that the test compound interferes with the liver metabolism and the distress created is passed on to the F2 generation. Aminotransferases (ALT and AST) are synthesized in the liver and are good markers of damage to hepatocytes and muscles (Lumeij, 1997). Significant increase in ALT and AST indicates hepatocellular degenerative changes. The same was reported earlier as an effect of treatment by chlorpyrifos, (Sharma *et al*., 2013; Kaur *et al*., 2000; Krishna *et al*., 2009; Wang *et al*., 2009a) and cypermethrin (Khan *et al*., 2009) in exposed animals. The results indicate the necrosis in vital organs like liver which results in enzyme leakage in to the blood (Deepak *et al*., 2000). Various studies (El-Tawil and Abdel- Rahman, 1997; Manna *et al*., 2004; Khan *et al*., 2009; Aslam *et al*., 2010; Ahmad *et al*., 2011) have revealed increased serum activities of leakage enzymes including alanine transaminase (ALT) and aspartate transaminase (AST) in pesticide intoxicated animals. Increase in serum level of AST as observed in birds dosed with Ci may reflect damaged liver cells as opined by Obaineh and Matthew (2009).

Increase in LDH level indicates tissue damage. Various pesticides cause an increase in lactate dehydrogenase after inflicting a tissue necrosis. Earlier reports showed an elevated LDH as a result of organophosphate exposure in tilapia (Rao, 2006), pyrethroid exposure in broiler chicks (Jayasree *et al*., 2003) and also in various animals treated with cypermethrin (Saleem *et al*., 1988; Das and Mukherjee, 2003; Manna *et al*., 2004).

ALP is membrane bound hydrolase enzyme, it is found on all cell membranes where active transport occurs. The highest concentrations of ALP are found in the liver, biliary tract epithelium, bone and intestinal mucosa (Ravel, 1995). Serum ALP activity increases in case of damage to hepatic cells and obstruction of bile duct (cholestasis) through proliferation of hepatocytes (Pande 2001; El-Demerdash *et al*., 2003; Bhushan *et al*., 2013). ALP increase has been reported by cypermethrin by several workers (Gupta *et al*. 1991; Manna *et al*., 2004; Yousef *et al*., 2006; Yavasoglu *et al*., 2006; Yadav 2010; Bhushan *et al*., 2013).

There was a decrease in DNA and RNA content in the liver of 0.05 and 0.10 µg/egg of Ci treated groups while in F2 progeny the DNA decreased in all the three treated groups while RNA decreased only in descendents of mid and high doses of Ci treated groups. Deoxyribonucleic acid (DNA) is confined to the nucleus. Its variation in tissue is of clinical significance. In present study depletion in total DNA contents might be due to slight hypotrophy of tissue under insecticidal stress. Decrease in total DNA contents in testis has been reported by (Chitra, 1999) in liver of rats treated with chlorinated insecticide. Concentration of RNA in cell reflects the rate of transcription in the cell. Liver showed lower levels of RNA in treated exposed groups. Also, as discussed in the earlier part of this study, Ci has induced a decrease in protein levels in both F1 and F2 generation birds, which might be due to the arrest of the said protein synthesis at translation stage. Similar observations made by Arshad *et al*., (2007) give credence to the present finding.

Further, the histological details when examined microscopically showed major changes like chronic inflammation, vacuolation and fibrotic changes in the liver of 0.05 and 0.10 µg/egg of Ci treated F1 as well as the F2 generation birds; and pathological changes were observed in the renal tubules and glomeruli of the kidney of these birds. These results of structural damage to the liver and kidney which arose as a result of Ci treatment, contribute to a better understanding, as well support the results of biochemical analysis.

The overall results of the present study show that the organism, during its embryonic developmental stage, is highly vulnerable to the toxicity of external agents. After analysing the results of the biochemical parameters in the early embryos and in the hatchling, it can be derived that the Ci treatment at a concentration 0.05 and 0.1µg/egg lowered the DNA and RNA content, thereby indicating a hampered transcription followed by stalled protein synthesis. The median and high doses caused terminal changes in the structure and functioning of liver and kidney of both F1 and F2 generations, as evidenced by the histological and biochemical findings. The disturbed structural integrity of liver and kidney give clues to noticed alterations in the activity of various enzymes.

The toxic mechanisms not only inflicted the birds which were directly exposed during their embryonic period, but also these inflictions were passed to their next generation in spite of absence of direct insecticidal dosage. The two generation evaluation of biochemical analysis and histopathological studies, after an initial in-ovo Ci intoxication in different doses, therefore, heightens the concerns regarding a toxic exposure during the developmental phase of an organism. The study therefore substantiates the elusive nature of developmental mechanisms which on slightest disturbance might tend to develop long-lasting effects which could get accumulated in the future generations too.