Effect of Carbofuran on Hematology, Serum Biochemistry, and Histopathology of Immune Organs of Cattle Egret (*Bubulcus ibis coromandus*)

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ABSTRACT

This study analyzed the impact of carbofuran (CF) on blood parameters, serum biochemistry, and histopathological examination of immune organs of cattle egret. No significant difference ($P>0.05$) was observed in RBC count, PCV (%), Hb, MCH, MCHC, MCV, basophils (%), and monocytes (%) in CF exposed cattle egret. Lymphocytes (%) of CF exposed cattle egret were significantly ($P<0.05$) high, while lymphocytes (%), eosinophils (%) were significantly ($P<0.05$) low. Significantly high ($P<0.05$) values of ALT, AST, and creatinine were found in serum samples of CF exposed cattle egret, but they have significantly ($P<0.05$) low value of blood urea nitrogen. In CF exposed cattle egret mild lymphocytic depletion in some bursal follicles, mild to moderate necrosis and lymphocytic depletion in spleen and plasmacytosis in the cortical region followed by mild degenerative changes in immune cells were found in the thymus. It can be

concluded that exposure to CF has a significant impact on hematological, biochemical parameters while causing hepatic perturbation and histological changes in immune organs of cattle egret.

INTRODUCTION

Contamination by heavy metals is a great threat to the functional and structural integrity of the ecosystem. In agricultural land, a higher level of heavy metals (Cu, Pb, Zn, and Cr) occurs due to excess use of fertilizers (Kamboj et al., 2006). Pesticides are a great threat to aquatic species. Many birds including egrets, herons, sparrow, myna, and many other species are widely used as bio-indicators and serve to prove the exposure of these contaminants. The accumulation of these contaminants in different body organs, eggs, and prey samples of avian species has been reported all over the world (Kaur et al., 2012).

Carbofuran has been used extensively as insecticides in the past 40 years and forms an important category of insecticides. They are more acceptable than many other insecticides due to their capability of very low persistence in the environment (Hamed and Osman, 2017). Carbofuran is generally known for its neurotoxic effects particularly in insects but they also induce some other conditions including rhabdomyopathy, delayed neuropathy, immunotoxicity, and teratogenicity. Historically, several ecological experiments in wildlife were mostly restricted to the measurement of reproductive variables and death (Vithanage et al., 2016). Due to recent advances in diagnostic techniques, a great variety of endpoint experiments are now available including immune parameters. Researchers have focused on the immune system's role in maintaining homeostasis(Purushothaman and Kuttan, 2017). Any damage to the immune system may cause an increased rate of diseases and deaths resulting from some neoplasia and opportunistic pathogens. In this view, organophosphates adverse effects may range above neurotoxicity resulting increased level of mortality and morbidity(Schöntag et al., 2019).

Egrets are water birds belonging to the Ardeidae family. Environmental pollutants greatly affect them as they have the potential to accumulate these pollutants, thus making them a suitable biomonitor of environmental pollution (Kioko et al., 2016). Several factors are responsible for the decline in

Table 1. *Effect of carbofuran on hematological parameters of cattle egret*

Parameters	Carbofuran exposed cattle egret	Non-exposed cattle egret	P-value
RBC (x106/µL)	2.463 ± 0.11	2.734 ± 0.09	0.069
PCV (%)	45.7 ± 0.15	46.0 ± 0.14	0.166
Hemoglobin (g/dL)	8.1 ± 0.11	8.3 ± 0.07	0.074
MCH (pg)	30.11 ± 0.36	30.94 ± 0.49	0.190
MCHC (g/dl)	24.13 ± 0.06	24.14 ± 0.05	0.886
MCV (fl)	160.0 ± 0.09	160.0 ± 0.09	0.689
Heterophils (%)	23.43 ± 0.27	38.65 ± 0.028	< 0.0001
Lymphocytes (%)	67.35 ± 0.77	45.88 ± 0.17	< 0.0001
Basophils (%)	0.58 ± 0.013	0.61 ± 0.017	0.139
Monocytes (%)	4.177 ± 0.014	4.208 ± 0.014	0.137
Eosinophils (%)	3.743 ± 0.139^{a}	4.648 ± 0.200^{b}	0.0009
White Blood Cells (x106/µL)	8.879 ± 0.379^{a}	13.91 ± 0.391^{b}	< 0.0001

Note: $^{a-b}$ within a row indicate significant differences (P<0.05) between the groups

Table 2. *Effect of carbofuran on serum biochemical parameters of cattle egret*

Parameters	Carbofuran exposed cattle egret	Non-exposed cattle egret	P-value
ALT(U/L)	34.40 ± 0.565[b]	23.03 ± 0.223[a]	< 0.0001
AST(U/L)	153.4 ± 2.137[b]	94.94 ± 3.248[a]	< 0.0001
ALP(U/L)	184.5 ± 6.989	181.3 ± 6.633	0.7385
Creatinine mg/dL	0.781 ± 0.0134[b]	0.4180 ± 0.004[a]	< 0.0001
Blood urea nitrogen mg/dL	3.121 ± 0.096[a]	4.226 ± 0.044[b]	< 0.0001

Note: [a-b] within a row indicate significant differences (P<0.05) between the groups

Table 3. *Effect of carbofuran on immune organs of cattle egret*

Organs	Carbofuran exposed cattle egret	Non-exposed cattle egret	P-value
Bursa of Fabricious	1.75 ± 0.190[a]	0.40 ± 0.112[b]	< 0.0001
Spleen	2.20 ± 0.186[a]	0.75 ± 0.099[b]	< 0.0001
Thymus	0.65 ± 0.109[a]	2.15 ± 0.166[b]	< 0.0001

Note: [a-b] within a row indicate significant differences (P<0.05) between the groups

their population, but the most important one is pesticides, which affected almost 87% of threatened avian species. They have adverse effects on the bird's reproduction and immune system(Miño et al., 2017). Researches use immunopathology to study different diseases of immune organs along with some other parameters like lymphoid histomorphometry, hematology, lymphoid organ histopathology, lymphoid organs, and body weight ratio (Dendrou et al., 2015). Several types of research have revealed that histopathological studies can be useful for the assessment of immunotoxicity caused by chemicals. Histopathology can be useful in the examination of cells of lymphoid organs affected in vivo conditions. Many tests are available for the assessment of immunotoxicity and immunopathology, which arean important part of this diagnostic system (Khurana and Chauhan, 2005).

Studies reported that populations of many birds are declining in areas where pesticides are being used on a large scale. To our knowledge, there is no reported study available to evaluate effects of pesticides contaminations on cattle egret (Bubulcus ibis coromandus), hence this study is designed to elaborate the effects of pesticides contaminations on egg quality of cattle egret and to investigate the immune status of the cattle egret in areas highly exposed to pesticides.

MATERIALS AND METHODS

Study Area

This study was conducted for 1 year and sampling was done from May 2018 to March 2019 from different regions of Punjab, Pakistan. Cattle egret (n=100) were captured from both carbofuran exposed area (n=50) and non-exposed area (n=50). For this a mist-netting technique was used as defined by the Food and Agriculture Organization (FAO).

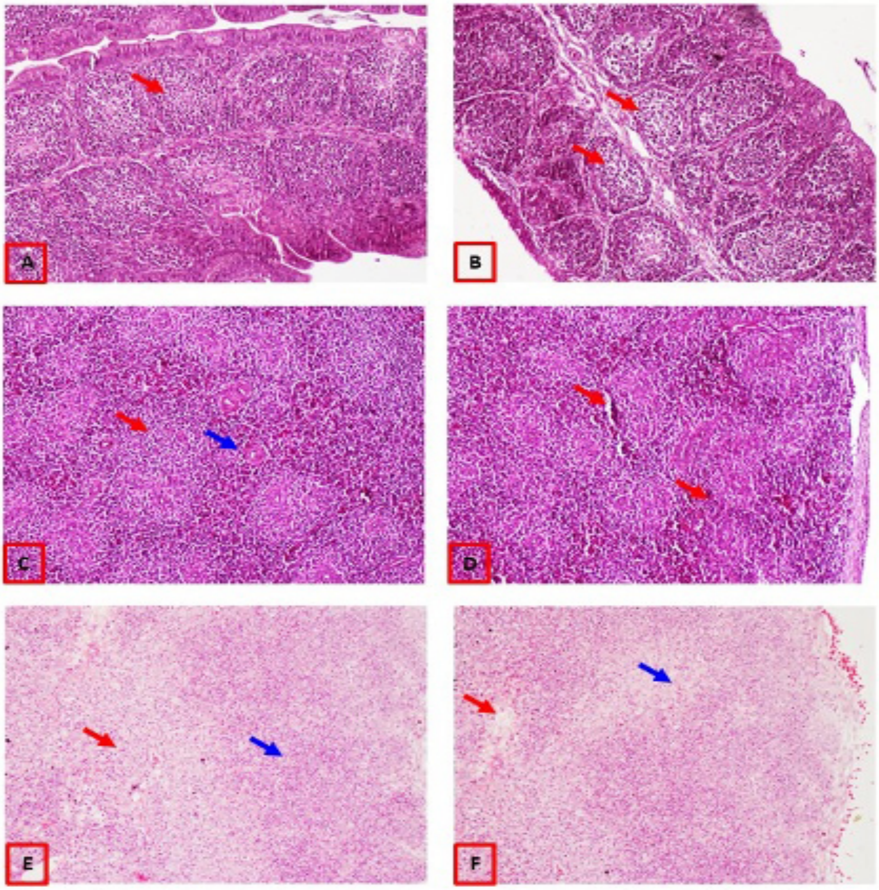
Blood Sample Collection

Blood samples (6 mL) of each bird were collected from a brachial wing vein and divided into EDTA containing vacutainer and without EDTA vacutainer, centrifuged (3,500 rpm for 5 min), and processed for serum chemistry analysis.

Hematological Indices

The hemoglobin concentration (Hb) was assessed using the cyanmethemoglobin technique. Red blood cell (RBCs) and white blood cell (WBCs) counts were determined using a Wintrobe hematocrit method as

Figure 1. *Photomicrographs of immune organs (H&E; 10X) A:Normal bursa of Fabricius-with intact bursal follicles (red arrow) B:Mild lymphocytic depletion in bursal follicles (red arrow) of CF exposed cattle egret C:Spleen with normalwhite pulp (red arrow) and red pulp (blue arrow) D:Mild to moderate necrosis and lymphopenia in the spleen of CF exposed cattle egret (red arrow) E: Thymus with the normal cortex (red arrow) and medulla (blue arrow) F:Mild degenerative changes in immune cells in cortical region (red arrow), lymphopenia in the medulla (blue arrow) of CF exposed cattle egret*



described by Oso et al. (2017). Differential leucocyte count (DLC) was determined using the blood smears stained with the May-Grunwald-Giemsa stain. Mean cell volume (MCV), mean cell volume (MCH), mean cell hemoglobin concentration (MCHC) were calculated as described by Lala et al. (2015).

Serum Chemistry

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine, and urea levels were determined as described by Nazir et al. (2020)with the help of commercial kits (Roche COBAS testing Kits, Roche, Basel, Switzerland).

Histopathological Examination

Cattle egret (n=50 each) from both carbofuran exposed and the non-exposed areas were sacrificed by cervical dislocation so that the bird feels the minimal pain. Immune organs (thymus, spleen, and bursa of Fabricius) were fixed in 10% neutral buffered formalin for 48 hours, following the tissue sectioning

(3-4 mm size), tissue processing, embedding in paraffin wax, sectioning with a microtome (3-4 μL), and staining with H&E stain. The mounting the slide with DPX was observed under a light microscope at 10X and 40X (Iqbal et al., 2015). Histopathological lesion scoring of the spleen, bursa, and thymus were determined as described by Gariglio et al. (2019). Briefly, organs were scored 0, 1, 2, and 3 based onthe absence or severity of lesions (mild, moderate, or severe). Degenerative changes, necrosis, atrophy, congestion, hemorrhages, and depletion of cells were observed.

Statistical Analysis

The obtained data were subjected to a non-parametric t-test on IBM SPSS (version 23.0). The level of significance was accepted at P<0.05.

RESULTS AND DISCUSSION

Table 1 showed the hematological parameter values of both carbofuran exposed and non-exposed cattle egret. Data depicted that there was no significant difference in both types of cattle egret in the relation of RBC count, PCV (%), Hb, MCH, MCHC, MCV, basophils (%), and monocytes (%). A significant difference (P<0.05) was found in terms of heterophils (%), lymphocytes (%), eosinophils (%), and WBC count. Our results are in agreement with Hossen et al. (2017) who reported that CF treated rats had low RBC count as compared to the control group. Likewise, the HB level was also low non-significantly (P>0.05) in CF fed rats. But CF did not affect significantly (P>0.05) on MCH, MCHC, and MCV values of rats.

Table 2 revealed the effect of carbofuran on serum biochemical parameters, which depicted that carbofuran has a significant (P<0.05) impact on ALT, AST, creatinine, and BUN levels in serum, but no significant difference (P<0.05) was present for ALP values. The elevated levels of serum ALT, AST, and ALP indicate CF-induced hepatic injuries and disturbance in the biosynthesis of these enzymes. These enzymes leak into the blood circulation from liver cytosol due to alterations in membrane permeability of hepatocytes caused by CF intoxication, indicating necrosis and inflammatory reactions. These findings are in line with findings of Jaiswal et al. (2015). In rats, co-treated with turmeric, a significant increase in AST, ALT, ALP, and LDH levels can be attributed to the presence of high levels of flavonoids, phenolics, and curcumin in turmeric extracts. These compounds have inhibitory effects on membrane peroxidation along with robust free radical scavenging abilities. Furthermore, the elevated TC level in CF-treated rats could be due to blockage of the liver bile ducts, which causes a significant decrease in its secretion into the duodenum subsequently leading to cholestasis. The data substantiated with the results described by Jaiswal et al. (2013), in which rats were exposed to CF in sublethal doses for 30 days.

Table 3 shows that carbofuran has a significant effect (P<0.05) on immune organs with regard to microscopic studies. Carbofuran induced the depletion of lymphocytes in bursal follicles of BF, resulting in atrophy of BF (Figure 1B). Carbofuran induced mild to moderate necrosis in RBC and WBCs in the spleen (Figure1D). Similarly, thymus of cattle egret exposed to carbofuran had mild degenerative changes in immune cells in the cortical region and increase the number of lymphocytes in response to inflammation (Figure1E). No microscopic lesions were detectable in BF (Figure 1A), spleen (Figure 1C), and thymus (Figure 1E) of normal cattle egret.

CONCLUSION

Exposure to CF has a significant impact on some hematological, biochemical parameters while causing hepatic perturbation and histological changes in immune organs of cattle egret.

Disclosure Statement

We declare that there is no conflict of interest.

REFERENCES

1. Immunopathology of multiple sclerosis. Dendrou C A, Fugger L, Friese M A. 2015, *Nat. Rev. Immunol*, pp. 545–558.
2. Effects of the dietary inclusion of partially defatted

black soldier fly (Hermetia illucens) meal on the blood chemistry and tissue (spleen, liver, thymus, and bursa of fabricius) histology of muscovy ducks (cairina moschata domestica). Gariglio M, Dabbou S, Crispo M, Biasato I, Gai F, Gasco L, Schiavone A. 2019, *Animals*, pp. 1–13.

3. Modulatory effect of lycopene against carbofuran toxicity in African catfish, Clarias gariepinus. Hamed H S, Osman A G M. 2017, *Fish Physiol. Biochem,* pp. 1721–1731.
4. Protective mechanism of turmeric (Curcuma longa) on carbofuran-induced hematological and hepatic toxicities in a rat model. Hossen M S, Tanvir E M, Prince M B, Paul S, Saha M, Ali M Y, Karim N. 2017, *Pharm. Biol*, pp. 1937–1945.
5. Replacement effect of vitamin E with grape polyphenols on antioxidant status, immune, and organs histopathological responses in broilers from 1-to 35-d age. Iqbal Z, Kamran Z, Sultan J, Ali A, Ahmad S, Shahzad M, Sohail M. 2015, *J. Appl. Poult. Res,* pp. 27–134.
6. Hepatoprotective Effect of Citrus limon Fruit Extract against Carbofuran Induced Toxicity in Wistar Rats. Jaiswal S K, Gupta V K, Siddiqi N J, Pandey R S, Sharma B. 2015, *Chinese J. Biol*, pp. 132-137
7. Carbofuran induced oxidative stress in rat heart: ameliorative effect of vitamin C. Jaiswal S, Siddiqi N, Sharma B. 2013, ISRN *Oxidative Med* pp. 1–10. Retrieved from http://downloads.hindawi.com/journals/isrn.oxidative.medicine/2013/824102.pdf
8. Carbofuran-induced neurochemical and neurobehavioral alterations in rats: Attenuation by N-acetylcysteine. Kamboj A, Kiran R, Sandhir R. 2006, *Exp. Brain Re*s, pp. 567–575.
9. Attenuation of cellular antioxidant defense mechanisms in kidney of rats intoxicated with carbofuran. Kaur B, Khera A, Sandhir R. 2012, *J. Biochem. Mol. Toxicol*, 393–398.
10. Immunopathological effects of carbofuran on humoral immune response in sheep. Khurana R, Chauhan R. 2005, *J. Immunol. Immunopathol*, pp. 54–57.
11. Cattle Egret Bubulcus ibis interactions with large mammals in the Tarangire-Manyara Ecosystem, Northern Tanzania. Kioko J, Boyd E, Schaeffer E, Tareen S, Kiffner C. 2016, *Scopus J. East African Ornithol*, pp. 15–20. Retrieved from https://www.ajol.info/index.php/scopus/article/view/129676
12. Effect of supplementation with molecular or nano-clay adsorbent on growth performance and haematological indices of starter and grower turkeys fed diets. Lala A, Oso A, Ajao A, Idowu O, Oni O. 2015, *Livest. Sci*, pp. 209–215.
13. Use of noninvasive 'bug-eggs' to enable comparative inferences on genetic mating system with and without parental information: A study in a cattle egret colony. Miño C I, De Souza E D, Moralez-Silva E, Valdes T A, Cortiço Corrêa Rodrigues V L, Del Lama S N. 2017, *PLoS One*, 153-183.
14. Effects of Dietary Fish Oil Replacement by Soybean Meal on Performance and Physiology of Rainbow Trout, Oncorhynchus mykiss. Nazir M A, Anjum K M, Naseer J, Anjum A, Durrani A, Usman S,Usman M. 2020, Pak. *J. Zool*, pp. 1–7.
15. Effect of dietary supplementation with arginine on haematological indices, serum chemistry, carcass yield, gut microflora, and lymphoid organs of growing. Oso A, Williams G, Oluwatosin O, Bamgbose A. 2017, *Livest. Sci*, pp. 58–64.
16. Protective effect of curcumin against carbofuran-induced toxicity in Wistar rats. Purushothaman B, Kuttan R. 2017, *J. Environ. Pathol. Toxicol. Oncol*, pp. 73–86.
17. Coating polystyrene beads with iron oxide for the adsorption of carbofuran from the water supply. Schöntag J M, Alves A A, Romero Esquivel L G, Sens M L. 2019, *Environ. Technol*, pp. 2833–2839.
18. Kinetics, thermodynamics and mechanistic studies of carbofuran removal using biochars from tea waste and rice husks. Vithanage M, Mayakaduwa S S, Herath I, Ok Y S, Mohan D. 2016, *Chemosphere*, pp. 781–789.