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Effects of Carbofuran Exposure on the Great Coucal (*Centropus sinensis*): A Comparative Study in Wild and Captive Population

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Abstract

Carbofuran is a very toxic carbamate insecticide commonly used in agriculture to control insects, mites, and nematodes. Although it works well against pests, non-target creatures such as birds, animals, and aquatic life are at serious risk due to its high acute toxicity, which can have detrimental physiological and behavioral impacts even at low exposure levels. This study investigated the toxicological effects of carbofuran exposure on populations of Great Coucals (*Centropus sinensis*) in Pakistan's Lodhran and Bahawalpur areas. The study evaluated both wild and captive-reared birds by examining carbofuran residues in tissues, eggs, and prey items, as well as by assessing hematological and biochemical responses. Across all seasons, dietary analysis showed a strong preference for animal-based components, with fruits, vegetables, and caterpillars all ranking highly. The highest residue amounts were found in eggs (0.68 mg/kg), leg muscle (0.14 mg/kg), liver (0.21 mg/kg), stomach contents (5.28 mg/kg) and blood (2.71 mg/kg) of captive-reared birds subjected to 5 mg/kg carbofuran. Hematological indices such red blood cells count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were significantly reduced in birds treated with dose-dependent incremental carbofuran. Dose-dependent mortality rates were 30% at 0.5 mg/kg, 65% at 2.5 mg/kg, and 85% at 5 mg/kg. A sharp drop in probability of survival for birds subjected to 0.5 mg/kg and 2.5 mg/kg carbofuran recorded between 10 and 20 weeks. These results reveal the serious physiological and pathological effects of carbofuran exposure in Great Coucals, underscoring the possible harm to ecosystem health and bird populations.

Keywords: Carbofuran; Great Coucals; Hematology; Dietary preferences; Mortality rates; Serum biochemistry

Introduction

Over the past several decades, a wide range of pesticides have been extensively utilized in agriculture to control pest populations and mitigate the spread of vector-borne diseases. Apart from pests, this approach poses a serious risk to non-target species in surrounding environment (Ali *et al.* 2021). Many nations have banned the use of certain organochlorines in their agricultural sectors; yet, it is impossible to overlook their production because of sanitary use (Eqani *et al.* 2012). In Europe, North America, and Australia, organochlorines have mostly been replaced by carbamate and organophosphate (Carvalho 2017). Carbamates are exactly as dangerous as organophosphates, although they are less harmful and have a much shorter half-life. However, the use of carbamate as an organophosphate alternative has caused them to accumulate in the environment (Ramesh *et al.* 2015).

Since carbofuran (carbofuran) is a systemic pesticide, it enters the plant and poisons the insect when it consumes it. Food, water, and air pollution from the widespread use of carbofuran in agriculture has a detrimental impact on the health of people and animals (Garg *et al.* 2004). Chromatographic and spectrophotometric methods can be used to determine the amount of carbofuran present in environmental samples. According to Kamboj *et al.* (2006), a value of 0.21 mg/kg body weight has been used for risk assessment study in avian species. Due to their adverse effects on the reproductive system, the already declining wild bird population has stunted growth and breeding failure (Ibáñez *et al.* 2010). Pesticides including organochlorines and neonicotinoids, impair avian brain function and immunological

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responses, disturb calcium homeostasis, resulting in eggshell thinning, and cause genotoxic effects, including as DNA damage and heritable mutations (Khan *et al.* 2014).

Particularly in the United Kingdom, where birdwatchers have been counting birds for decades, the number of birds is used as an indicator for environmental degradation (Fernández *et al.* 2005). The Great Coucal, endemic to the Indus basin and South Asia, belongs to the order Cuculiformes and the Family *Cuculidae*, adapting to habitats ranging from dense rainforests to agricultural fields near canals and rivers (Fayyaz and Hussain 2023). This species is often used to determine the pollution profiles in terrestrial and aquatic environments (Shimshoni *et al.* 2012). Despite their adaptability, Great Coucal face challenges from habitat loss and pesticide exposure in agricultural landscapes, potentially exposing them to carbofuran residues (Surender *et al.* 2013; Munir *et al.* 2022). Pesticide contamination affects their food sources and disrupts ecological balance by reducing insect populations vital to their diet (Baharudin *et al.* 2024). Carbofuran's impact extends beyond mortality to include physiological and behavioral changes that could jeopardize their survival. Residues in prey items and the environment can alter feeding behavior, reduce food availability, and lead to nutritional deficiencies and compromised health (Elfis *et al.* 2020). As a result, the purpose of this study was to look the residual carbofuran in muscle tissues, eggs, and prey items, as well as its toxicological impact on the hemo-biochemical response in Great Coucals in both wild and captivity.

Materials and Methods

Ethical consideration

This study was approved from the Islamia University Bahawalpur, the Institute of Forest Sciences, Faculty of Agriculture and Environment, and was conducted in complete compliance with the established guidelines stated in Pakistan's Prevention of Cruelty to Animals Act (1890), Punjab Wildlife Protection, Preservation, Conservation and Management Act (1974).

Experimental station

The study was conducted over a three-year period, from January 2022 to December 2024, across nine sampling sites: three in Lodhran and six in Bahawalpur districts. Each site was divided into four quadrants, with one quadrant randomly selected for survey, using the line transect method. Detailed information on the sampling sites, including their GPS coordinates, is provided in Table 1.

High-quality binoculars (Nikon™ Aculon A211, 12 x 50 model) were used for distant bird observation and identification. An Android smartphone with integrated GPS functionality was employed for precise location tracking of bird sightings, while a laser rangefinder (Nikon™ Prostaff 7i, range: 1200 m) was utilized to measure the distance between the observer and avian subjects.

Table 1: Sampling sites in Lodhran and Bahawalpur districts

District	Site Number	GPS Coordinates
Lodhran	1	29.488313 N, 71.517158 E
	2	29.643371 N, 72.083792 E
	3	29.45512 N, 71.39036 E
Bahawalpur	4	29.276986 N, 71.121465 E
	5	29.291128 N, 71.627507 E
	6	29.456716 N, 72.009622 E
	7	29.112879 N, 71.781970 E
	8	29.765541 N, 72.423878 E
	9	28.767507 N, 71.339243 E

Capture of adult great Coucals and collection of egg samples

Study sites were selected based on areas with dense foliage, forest edges, or abundant food sources. Necessary permissions were obtained from wildlife authorities, and all procedures adhered to ethical guidelines for wildlife research. High-quality, fine nylon mist nets nearly invisible to birds were deployed at strategic heights along flight paths of Great Coucals, supported by lightweight, sturdy poles. Nets were set during peak activity periods (early morning or late afternoon) and concealed using surrounding vegetation. Trained personnel monitored nets continuously, and entangled birds were carefully extracted by experienced handlers to avoid injury to wings, legs, or feathers. Sampling avoided the breeding season peak to minimize disruption to nesting and stress to parent birds and chicks. Over the study period, 90 adult birds (5 males and 5 females per site) were captured, while juveniles were immediately released. Captured birds were placed in soft, breathable holding bags to reduce stress and prevent injury. Additionally, 120 eggs were collected from nests across the nine sites, wrapped in aluminum foil, and transported to the laboratory. Of these, 60 eggs were selected for incubation, while the remaining was stored at 4°C for subsequent carbofuran residue analysis.

Experimental design

Over a three-year period, a total of 90 birds were captured from the wild at different sites, with captures conducted intermittently rather than all at once, 60 were immediately processed for blood sampling from the wing veins to assess the hemato-biochemical effects of carbofuran in wild populations. From these, 30 birds were released back into the wild, while the remaining 30 were humanely euthanized and dissected to analyze carbofuran accumulation in muscle tissues and prey items. The other 30 birds were reared in a specialized aviary alongside 47 successfully hatched eggs, which were transferred to brooder boxes. Hatchlings were fed a protein-rich diet of finely chopped insects (mealworms, crickets), minced meat, and a specialized bird-rearing formula (Kaytee™ Exact Hand Feeding Baby Bird Formula) *via* feeding syringe every 2–3 hours during the day for the first two weeks. At 4–6 weeks old, fledglings were moved to the aviary with the 30 wild-captured birds.

The 77 captive-reared birds were divided into four treatment groups based on carbofuran exposure levels: Treatment 0 (0 mg/kg, n = 17), Treatment 1 (0.5 mg/kg, n = 20), Treatment 2 (2.5 mg/kg, n = 20) and Treatment 3 (5 mg/kg, n = 20). The control group consists entirely of captive-hatched birds, while the other treatment groups each include 10 wild-caught birds and 10 captive-hatched birds to ensure statistical robustness. The trial spanned six months, with birds provided ad libitum access to food and water throughout the study.

Assessment of feeding preferences of great coucal in wild

To investigate the feeding habits and predation patterns of Great Coucals on invertebrate and vertebrate species, we conducted detailed dissections of 30 euthanized birds, focusing on the crop and proventriculus. Standard dissection tools were used, and dissections were performed over several months to account for seasonal dietary variations. The crop and proventriculus were excised, weighed to the nearest 0.1 g using an electronic balance, and opened to examine food contents under a binocular microscope. Six crops were empty and excluded from analysis. Food items were categorized into five groups: cereal grains, vegetables and fruits, tree material, animal matter (invertebrates and vertebrates), and unidentified items, including grit.

Assessment of carbofuran toxicity in great coucal

Carbofuran residues in eggs, stomach contents, blood, muscle, and liver samples from both wild and captive-reared birds were quantified using gas chromatography-tandem mass spectrometry (GC-MS/MS) (Shimadzu GC-MS-QP2010 Ultra™) following derivatization with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Sigma-Aldrich®, USA). Additionally, carbofuran levels were analyzed in captive birds that died during the experimental period to determine lethal toxicity thresholds. Chromatographic separation was performed on an Agilent 7890A™ GC system (Agilent Technologies®, USA) equipped with an HP-5ms capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies®, USA). Mass spectrometric analysis was conducted using an AB Sciex QTRAP 4500™ instrument (AB Sciex®) in multiple reaction monitoring (MRM) mode, with data processed using AB Sciex Analyst software (version 1.6.2). The detection limit (DL) ranged from 0.1 to 1 ng/mL, equivalent to 0.1–1.0 μg/kg for biological samples (wet weight, assuming a density of ~1 g/mL) (Petropoulou *et al.* 2006). The Codex Alimentarius maximum residue limit (MRL) for carbofuran in meat, including avian meat, is 0.01 mg/kg, which was adopted as the limit of quantification (LOQ); any detectable concentration exceeding this threshold was deemed unsafe.

Hematology analysis

Sixty captured birds from the wild and all of the surviving captive reared birds were sampled for blood and serum at the end of study period. A small, sterile needle was used to draw blood from the wing vein in a quick and minimally invasive manner. For the hematology analysis, blood samples were prepared by gently mixing the blood in the EDTA tube by inverting it several times. An automated hematology analyzer (Paramedical Italy®, PKLPPC 610 H) was used to measure parameters such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (Kuzmina *et al.* 2021).

Serum biochemistry analysis

For the serum biochemistry analysis, blood samples were processed by centrifuging the blood in the serum separator tube at 3000 rpm for 10 min to separate the serum. The serum was carefully transferred to a clean tube using a pipette. A biochemistry analyzer (Vet Scan VS2® analyzer, ABAXIS, USA) was then used to measure various serum biochemical parameters such as glucose, total protein, albumin, globulin, AST (aspartate aminotransferase), ALT (alanine aminotransferase), calcium, phosphorus, Blood Urea Nitrogen (BUN) and creatinine, following the manufacturer's instructions for the specific assays and reagents used (Hoppes *et al.* 2015).

Statistical analysis

The Kruskal-Wallis test was used to analyze the food preference data of wild Great Coucal and a one-way ANOVA was used to evaluate seasonal differences in eating preferences. carbofuran residues in several tissues (eggs, leg, breast, liver, proventriculus and blood), as well as hematological and serum biochemical parameters, were compared between bird groups using standard one-way ANOVA. For all statistical analyses, GraphPad Prism® (version 8.4.3) was used.

Results

Feeding ecology

As shown in Table 2, the median stomach content weight was quantified at 9.6 ± 5.90 g, and a Kruskal-Wallis test confirmed significant variability in the mass of different ingested food categories ($P < 0.0001$). Additionally, the relative feeding preferences of *C. sinensis* across distinct seasonal periods specifically summer, monsoon, and winter were quantitatively assessed. Statistical analysis revealed significant variations in the consumption of different food items among seasons, with an average significance level of $P < 0.0001$ (Table 3).

Table 2: Composition of food items identified from stomach samples (crop and proventriculus) of wild Great Coucals

Food Constituents	Average Weight (g)	Average Percentages	Kruskal-Wallis's Statistic	Mean Rank	*P* value
Moths	1.01 ± 0.60	10.48	217.1	411.3	*< 0.0001*
Caterpillars	0.71 ± 0.42	7.41		376.5	
Scarabaeidae	2.74 ± 1.63	28.45		467.7	
Cerambycidae	0.53 ± 0.31	5.51		349.9	
Carabidae	0.27 ± 0.16	2.80		318.6	
Elateridae	0.06 ± 0.06	0.65		224	
Stenopelmatidae	0.71 ± 0.91	7.36		283.3	
Rhaphidophoridae	0.46 ± 0.67	4.77		246.7	
Gryllidae	0.02 ± 0.03	0.17		163.2	
Cicadidae	0.05 ± 0.08	0.49		184.2	
Phasmatodea	0.08 ± 0.14	0.88		190.7	
Mantodea	0.03 ± 0.03	0.29		190.3	
Diplopoda	0.01 ± 0.01	0.11		184.2	
Oligochaeta	0.02 ± 0.02	0.25		197.7	
Cereal Grains	1.31 ± 0.77	13.55		433.7	
Vegetables & fruits	0.78 ± 0.46	8.13		386.2	
Tree material	0.06 ± 0.11	0.59		172.6	
Grit	0.09 ± 0.05	0.90		271	
Unidentified items	0.7 ± 0.41	7.22		372.9	

*Feed items belonging to Kingdom Animalia have been identified to the family level. $P < 0.05$ was considered statistically significant

Table 3: Seasonal dietary preferences based on stomach contents (crop and proventriculus) of the Great Coucal

Food items	Summer	Monsoon	Winter
Animal Material	5.61 ± 3.58^a	8.52 ± 4.78^a	5.98 ± 4.02^a
Cereal Grains	1.17 ± 0.71^b	1.4 ± 0.78^b	1.35 ± 0.89^b
Vegetables & Fruits	0.7 ± 0.42^c	0.84 ± 0.47^c	0.81 ± 0.53^c
Tree Material	0 ± 0.00^c	0 ± 0.00^c	0.17 ± 0.13^c
Grit	0.08 ± 0.05^c	0.09 ± 0.05^c	0.09 ± 0.06^c
Unidentified Items	0.62 ± 0.38^c	0.74 ± 0.42^c	0.72 ± 0.47^c

*Means ± SD with different superscripts within a column are statistically significant ($P < 0.05$)

Table 4: Hematological parameters of wild and captive-reared Great Coucals exposed to different carbofuran concentrations (Control = 0 mg/kg, Treatment 1 = 0.5 mg/kg, Treatment 2 = 2.5 mg/kg, Treatment 3 = 5 mg/kg)

Parameters	Wild Birds	Treatment 0	Treatment 1	Treatment 2	Treatment 3
RBC (10^6/µL)	3.7 ± 1.05^a	4.33 ± 1.11^a	3.29 ± 0.91^{ab}	2 ± 1.41^b	0.83 ± 0.29^c
Hb (g/dL)	10.93 ± 1.15^a	11.37 ± 0.99^a	10.94 ± 1.02^a	7.43 ± 0.73^b	4.77 ± 3.69^c
HCT %	45.4 ± 5.55^a	46.67 ± 5.51^a	44.71 ± 6.81^a	28.43 ± 2.15^b	19.33 ± 11.93^c
MCV (fL)	113.27 ± 14.09^a	117.47 ± 16.26^a	114.93 ± 17.56^a	90 ± 10.60^b	70.67 ± 14.29^c
MCH (pg)	41.83 ± 6.23^a	43.93 ± 6.23^a	39.21 ± 5.74^a	20.43 ± 7.59^b	20 ± 5.29^b
MCHC (g/dL)	32.38 ± 2.73^a	34.6 ± 2.64^a	32.43 ± 2.87^a	24.57 ± 3.21^b	15.33 ± 2.08^c
WBC (10^3/µL)	12.87 ± 2.99^b	10.93 ± 3.69^b	9 ± 3.21^b	16.29 ± 1.38^a	3.33 ± 1.53^c
Heterophils %	62.1 ± 9.26^b	56.67 ± 10.38^b	59.5 ± 11.70^b	64.29 ± 6.95^a	68 ± 7.00^a
Lymphocytes %	31.53 ± 7.63^a	34.07 ± 8.63^a	30.07 ± 8.24^a	12.43 ± 8.94^b	15.67 ± 12.50^b
Monocytes %	5.80 ± 3.18^b	6.40 ± 2.77^a	7.36 ± 1.95^a	6.43 ± 3.51^a	5 ± 3.46^b
Eosinophils %	2.42 ± 1.79^a	2.87 ± 1.73^a	2.93 ± 1.54^a	3.14 ± 1.95^a	2.33 ± 2.31^a
Basophils %	1 ± 0.92^a	0.67 ± 0.62^a	0.79 ± 0.80^a	1.29 ± 0.76^a	0.33 ± 0.58^b

*Means with different superscripts within a row indicated significant difference at $P < 0.05$
RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentrations; WBC, white blood cells

Carbofuran toxicity

The bioaccumulation of carbofuran was evaluated in both captive-reared Great Coucals subjected to dose-dependent carbofuran exposure and wild-captured individuals. Concentrations of carbofuran were analyzed across various tissues, revealing statistically significant differences ($P < 0.05$) in eggs, leg muscle, liver, stomach contents, and blood samples. In contrast, no significant differences were detected in breast muscle tissue (see Fig. 1 for detailed results). The highest carbofuran residue levels were consistently observed in captive-reared birds exposed to 5 mg/kg (Treatment 3), demonstrating a clear dose-dependent relationship in bioaccumulation. Additionally, carbofuran concentrations in eggs, stomach contents, and blood samples were significantly higher in captive-reared birds exposed to 2.5 mg/kg (Treatment 2) compared to those exposed to 0.5 mg/kg (Treatment 1), highlighting distinct accumulation patterns associated with varying exposure levels.

Notably, the Codex Alimentarius has established a maximum residue limit (MRL) for carbofuran in bird meat at 0.01 mg/kg. As illustrated in Fig. 1, the findings raise

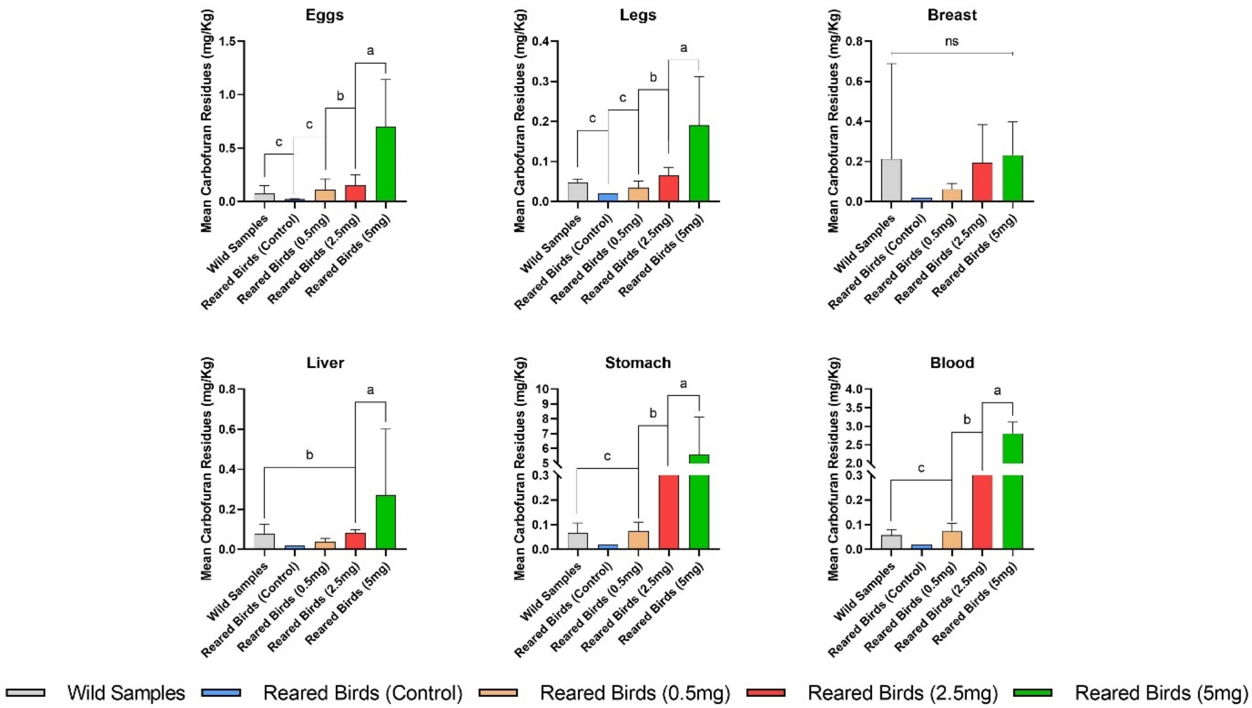

Fig. 1: Interleaved bar charts illustrating carbofuran levels detected in the eggs, muscles (leg and breast), liver, stomach contents and blood of captured wild as well as farm reared birds. Different superscripts (a, b, c) indicated significant difference when $P < 0.05$

significant food safety and environmental concerns. carbofuran levels in eggs from all groups including wild samples, control, and treated reared birds exceeded the MRL, indicating that both eggs and meat were unsafe for human consumption. Among all tissues analyzed, the highest carbofuran residues were detected in stomach contents, followed closely by blood samples. These results underscore the potential risks posed by carbofuran contamination in avian species and its implications for food safety and ecosystem health.

Hematology

The hematological parameters, including RBC count ($10^{-6}/\mu L$), hemoglobin (Hb, g/dL), hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dL), exhibited the lowest values in captive-reared birds exposed to 5 mg/kg of carbofuran, as shown in Table 4. In contrast, control birds and wild individuals remained within the normal physiological range for these parameters. These findings suggest that carbofuran exposure significantly altered hematological profiles, with higher exposure levels correlating with more pronounced hematological disturbances. The observed changes were indicative of anemia, immune suppression, and inflammatory responses. Moreover, the severity of these alterations increased proportionally with carbofuran concentration, with statistically significant differences detected between exposed birds and the control groups.

Serum biochemistry

The mean values of serum biochemical parameters for wild and captive-reared Great Coucals exposed to varying concentrations of carbofuran are presented in Table 5. The data indicate that the lowest mean serum glucose levels were observed in the Treatment 3 group (149.67 ± 67.87 mg/dL), suggesting potential hypoglycemia, which may be attributed to stress or organ dysfunction. In contrast, no significant differences were detected in calcium and phosphorus levels across the experimental groups. Similarly, total protein concentrations remained unaffected by incremental carbofuran exposure across all treatment groups. However, hepatic enzyme activities were significantly elevated in both the Treatment 2 and Treatment 3 groups, indicating potential liver stress or damage associated with higher carbofuran concentrations. These findings highlight the differential physiological responses to carbofuran exposure, particularly in glucose metabolism and hepatic function.

Mortality rate

Throughout the carbofuran toxicity trial in captive-reared Great Coucals, mortality data was recorded as presented in Table 6. The highest mortality rate (85%) was observed in birds exposed to 5 mg/kg (Treatment 3) of carbofuran during the rearing period, followed by 65% mortality in the 2.5 mg/kg (Treatment 2) group, 30% in the 0.5 mg/kg (Treatment 1) group and 11.76% in the control group (Treatment 0).

Table 5: Serum biochemical parameters in wild Great Coucals and captive-reared groups exposed to different carbofuran levels

Parameters	Wild Birds	Treatment 0	Treatment 1	Treatment 2	Treatment 3
Glucose (mg/dL)	244.73 ± 30.25 [a]	252.8 ± 28.17 [a]	209.07±29.39 [b]	155.29 ± 24.77 [c]	149.67 ± 67.87 [c]
Calcium (mg/dL)	9.98 ± 1.42[a]	10 ± 1.46[a]	9.43 ± 1.22[a]	7.29 ± 1.38[a]	5.67 ± 3.06a
Phosphorus (mg/dL)	4.4 ± 1.21[a]	4.6 ± 1.24[a]	3.93 ± 0.92[a]	2.71 ± 0.76[a]	2.67 ± 1.15[a]
Total Protein (g/dL)	4.52 ± 1.08[a]	4.53 ± 1.30[a]	3.29 ± 1.20[a]	3.71 ± 0.49[a]	3 ± 1.00[a]
Albumin (g/dL)	1.98 ± 0.81[a]	1.67 ± 0.72[a]	1.46 ± 1.01[a]	1.46 ± 0.30[a]	0.73 ± 0.25[a]
Globulin (g/dL)	3.18 ± 0.85[a]	3.13 ± 0.83[a]	2.75 ± 1.00[a]	2.23 ± 0.61[a]	2.03 ± 0.21[a]
AST (U/L)	199.02 ± 58.79 [c]	216.67 ± 54.27 [c]	391 ± 56.19 [b]	485.86 ± 94.16 [a]	494.67 ± 108.19 [a]
ALT (U/L)	23.93 ± 9.60 [b]	25.93 ± 7.82 [b]	69.14 ± 7.13 [a]	57 ± 10.54 [a]	77.33 ± 10.97 [a]
ALP (U/L)	119.17 ± 48.84 [b]	134.73 ± 46.22 [b]	276.64 ± 36.68 [a]	279.43 ± 49.23 [a]	309.67 ± 38.07 [a]
Bilirubin (mg/dL)	0.58 ± 0.26[b]	0.59 ± 0.26[b]	1.55 ± 0.27[a]	1.87 ± 0.48[a]	1.97 ± 0.35[a]
BUN (mg/dL)	12.9 ± 4.58 [c]	12.27 ± 3.79 [c]	22.14 ± 4.35 [b]	31.71 ± 7.04 [a]	39 ± 6.24 [a]
Creatinine (mg/dL)	0.87 ± 0.22[ab]	0.91 ± 0.20[ab]	1.47 ± 0.24[a]	1.6 ± 0.28[a]	2.03 ± 0.25[a]

*Different superscripts (a, b, c) within a row indicated significant difference when $P < 0.05$
AST, aspartate aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen

Table 6: Mortality rates of captive Great Coucals exposed to varying levels of carbofuran pesticide

Parameters	Treatment 0	Treatment 1	Treatment 2	Treatment 3
Total Numbers	17	20	20	20
Numbers of Deaths	2	6	13	17
Numbers of survivors	15	14	7	3
Mortality Rate (%)	11.76	30	65	85
Kruskal-Wallis's statistic	23.62			
P value	$P < 0.0001$			

Over the 24-week experimental period, weekly mortality rates differed significantly ($P < 0.001$) among treatment groups. Birds exposed to 5 mg/kg of carbofuran experienced the highest mortality rate, averaging 0.708 deaths per week, underscoring the dose-dependent lethality of carbofuran in captive-reared Great Coucals.

Discussion

In different species, carbofuran oral median lethal dose (LD50) varied from 1 to 50 mg/kg body weight; in birds, the LD50 values varied from 0.24 to 7.2 mg/kg (Purushothaman and Kuttan 2017). It caused poisoning in wildlife through both proper and improper use, and particularly through illegal use in toxic baits intended for predator control (Munir *et al.* 2022). The bioaccumulation of carbofuran in wild birds remains poorly understood; however, the present study revealed the highest carbofuran concentrations in the stomach, followed by leg muscle and eggs, in Great Coucals exposed to 5 mg/kg and wild-captured specimens. Comparable findings include Tongo *et al.* (2022), who reported carbofuran bioaccumulation levels of 0.00001–0.00150 μg/kg in pelagic fish (*Tilapia zilli*) from the Ikpoba River, Nigeria and Hasan *et al.* (2022), who detected 0.68 ± 0.45 μg/kg in cultured Pangas Catfish (*Pangasius pangasius*), underscoring tissue and species-specific accumulation patterns. A previous study administered 2.5 mg/kg body weight of carbofuran to nine 6-week-old Arbor Acres broiler chickens, demonstrating significant tissue accumulation of carbofuran residues that exceeded safety thresholds set by regulatory standards such as Codex Alimentarius (Lehel *et al.* 2010). These findings highlight the potential for carbofuran to bioaccumulate and pose risks to avian health at levels above established safety limits. In France, one of the main causes of red kite (*Milvus milvus*) poisoning is insecticide exposure (Berny and Gaillet 2008). It makes up 33% of the baits found in Bulgaria. carbofuran coated grains such as pea led to high mortality in pigeon (Lelièvre *et al.* 2001). Eleven red foxes (*Vulpes vulpes*) poisoned with carbofuran were discovered dead in the Dadia forest in northeastern Greece, along with seven vultures and an eagle. This suggests that fox eating may have caused subsequent poisoning of birds of prey (Antoniou *et al.* 1996). Upon ingestion, it accumulates in tissues, including egg yolk and reproductive organs, leading to acute poisoning, organ damage and impaired embryo development, which can cause growth abnormalities, defects, or mortality in birds (Mccorry 2007). Furthermore, cases of significant poisoning with carbofuran pesticides in wild birds, ruminants, and other mammals were reported in Bulgaria between 2018 and 2020 (Petrichev 2022). To ensure the embryo's and the organs' healthy and normal development, it is crucial to avoid exposure to carbofuran and other harmful compounds during embryonic development (Khan *et al.* 2021).

Great Coucals raised in captivity and exposed to different levels of carbofuran showed noticeably lower blood parameter values than both control and wild-captured birds. The decrease in these hematological indicators points to the inflammatory reactions, immunological suppression, and beginning of anemia, which is most likely caused by carbofuran's detrimental effects on bone marrow function. This hematopoietic system dysfunction resulted in decreased cell production, weakened immunity, and increased inflammatory responses. Similar lower hematological parameter values have been reported by Gupta (1994) in albino mice, Adhikari *et al.* (2004) in fish, Mahmoud *et al.* (2014) in chickens, Ramesh *et al.* (2015), Munir *et al.* (2022) in cattle egret and all of which are consistent with the current findings. Because carbofuran can directly poison blood vessel cells, necrosis, or cell death, takes place in blood vessels (Arbel *et al.* 2014). Great Coucal treated to carbofuran showed significant variation in leukocyte, heterophil, monocyte, eosinophil, and basophil counts as compared to control and wild-captured birds. Similar findings has already been determined in cattle egret (Munir *et al.* 2022) and African catfish (Harabawy and Ibrahim 2014). The overall immune response and inflammatory conditions may be the cause of the elevated leucocyte count (Dick and Dixon 1985; Singh and Srivastava 2010). To ensure the embryo's and the organs' healthy and normal development, it is crucial to avoid exposure to carbofuran and other harmful compounds during embryonic development (Khan *et al.* 2021). carbofuran disrupts the nervous system, can affect embryonic development by altering vascular control and generating inflammation, which leads to blood vessel damage and necrosis (Chen *et al.* 2017).

Carbofuran-exposed Great Coucal showed a dose-dependent rise in ALT, AST, ALP, creatinine and urea in comparison to the control group and wild capture. This research clearly indicates that carbofuran negatively affects the liver's structure and function. Moreover, ALT may have been released outside of cells due to degenerative alterations in hepatocytes, raising blood ALT levels (Sultan *et al.* 2017). This was probably produced by enzymes that leaked into the circulation from the liver cytosol as a result of carbofuran poisoning, which undermined the integrity of the hepatocyte membrane (Hamed and Osman 2017; Baharudin *et al.* 2024) . These results aligned with those of Binev *et al.* (2016), who documented comparable impacts in quails. Munir *et al.* (2022) determined similar comparable dose-dependent liver damage in cattle egrets exposed to carbofuran. According to Faisal *et al.* (2008), birds with liver damage may have higher enzymatic activity. Furthermore, our findings are consistent with the nephrotoxic effects observed in cattle egret and rats intoxicated with carbofuran, as described by Kaur *et al.* (2012) and Munir *et al.* (2022). While exposure to carbofuran at 0.0127 mg/0.1 mL/egg during the embryonic period significantly increased liver cell necrosis in the central vein and Kiernan's triangle of one day and two week old chicks (Huda *et al.* 2024). Carbofuran induces cellular toxicity and disrupts nervous system function, with low-dose or short-term exposures showing minimal accumulation. However, higher concentrations or prolonged exposure lead to toxin accumulation in organs such as the liver, exacerbating damage and impairing metabolic processes, with the severity of metabolic disruption and liver dysfunction being dose and duration dependent (Yan *et al.* 2023). Significant organ damage in birds is more likely to occur when high dosages or prolonged exposure to carbofuran are used (Zhao *et al.* 2005).

Great Coucals exposed to 5 mg/kg of carbofuran have a significant death rate, because of direct cellular damage, including rupture of organelles and cell membranes, which results in morphological and functional abnormalities that can eventually lead to necrosis. Additionally, carbofuran causes vasoconstriction, which impairs blood flow, lowers the availability of oxygen and nutrients, and exacerbates cellular malfunction and death (Ruano *et al.* 2022). carbofuran exposure to non-target species is minimized and the potential of food contamination is successfully reduced when it is used in livestock settings under strict adherence to established safety regulations and suitable management practices. This method guarantees environmental preservation and food safety.

Conclusion

Carbofuran exposure produces dose-dependent bioaccumulation and hemato-biochemical changes in Great Coucals, however the specific mechanism is unknown. Particularly at high concentrations, systemic pesticides like carbofuran provide serious hazards to both humans as well as the environment. To reduce exposure to non-target species, public health education and strict carbofuran application monitoring are crucial. Whether intentional or unintentional (such as through hunting or baiting), poisoning endangers endangered animals and necessitates concerted worldwide efforts to reduce risks. Reducing morbidity, mortality, and ecological consequences requires an understanding of clinical indicators of poisoning and improved information sharing.

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Author Contributions

Experimental design was conceived by AA and JN. Data were collected by AA and MAY. Statistical analysis was conducted by AA, JN, TH, AS and SH. Original draft was written by AA and MAY. All authors have contributed to the revision and final proof-reading of the manuscript.

Conflict of Interests

The authors declare that they have no competing interests.

Data Availability

The authors declare that the experimental data supporting the present study findings have been made available to the corresponding author (MAY).

Ethics Approval

All experimental protocols were approved by the Animal Ethics Committee (AEC) of the Institute of Forest Sciences, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur. All procedures involving animals, including field surveys, trapping, and behavioral monitoring, were conducted in accordance with the Punjab Wildlife Protection, Preservation, Conservation, and Management Act (1974). The necessary permissions were obtained under Permit No. 69.

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