Research Article

The Haemato-Pharmaceutical Studies of Cyproterone Acetate in *Coturnix coturnix* (Common Quail)

Hassan Raza[1], Nayab Fatima[2], Kiran Aftab[3*], Muhammad Akbar Khan[1], Yusra Ashfaq[4], Hasnain Raza[5] and Memoona Mehndi[3]

[1]Institute of Zoology, University of the Punjab, Quid-e-Azam Campus, Lahore 54590, Punjab, Pakistan; [2]Department of Zoology, Ghazi University, Dera Ghazi Khan, Punjab, Pakistan; [3]Department of Zoology, University of Gujrat, Gujrat, Pakistan; [4]Jiangsu Key Laboratory for Microbes and Genomics, School of Life Sciences, Nanjing Normal University, 1 wenyuan Road, Nanjing 210023, China, China; [5]Department of Environmental Sciences, Ghazi University, Dera Ghazi Khan, Punjab, Pakistan.

Abstract | The hematological and biochemical effects of cyproterone acetate/ethinylestradiol (CPA/EE) in adult male common quail (*Coturnix coturnix*) in ex situ conditions were assessed in this work. Ten of the fifty birds were used as untreated controls, and forty of them were given CPA/EE orally twice a day for five, ten, fifteen, or twenty days at a dose of 1 mg/kg body weight (n = 10 per duration; two duplicates of five birds). One-way ANOVA with Tukey's post hoc test was used to measure and analyze hematological parameters (total red blood cells (TRBC), total white blood cells (TWBC), packed cell volume (PCV), mean corpuscular volume (MCV), and serum biochemical markers (urea, creatinine, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, total protein, albumin, creatine kinase-MB (CK-MB). After a 20-day exposure to CPA/EE, treated quails exhibited significantly higher levels of urea (63.6±10.2 mg/dL; control 10.25 mg/dL), uric acid (30.10±2.50 mg/dL; control 2.20 mg/dL), ALT (31.0±3.47 U/L; control 3.47 U/L), AST (50.2±6.61 U/L; control 8.44 U/L), and cholesterol (141.0±40.1 mg/dL; control 50.51 mg/dL), CK-MB (3,029.9 ± 375.9 U/L; control 499.9 U/L), TWBC (174.6 ± 44.9 U/L; control 60.14 U/L), TRBC (2.70 ± 0.47 × 10 µ cells/µL; control 0.05 × 10 µ cells/µL), PCV (36.59 ± 5.84 fL; control 6.58 fL), MCV (79.59 ± 10.56 fL; control 3.99 g/dL), and decreased total protein (2.56 U/L) and albumin (2.56 ± 0.98 g/dL; control 5.54 g/dL). Clinically, quails treated with CPA/EE showed depression, organ enlargement, limb swelling, feather loss, and partial mortality, while control birds showed no abnormalities. These results highlight the significance of using steroidal hormonal compositions in avian research and management with caution and show that repeated oral administration of CPA/EE at a therapeutic dose substantially alters hematological and biochemical homeostasis in common quail.

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***Correspondence** | Kiran Aftab, Department of Zoology, University of Gujrat, Gujrat, Pakistan; **Email:** dr.kiran@uog.edu.pk
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Keywords | Blood cells, Cyproterone acetate, *Coturnix coturnix*, Serum, Ex-situ, Quail

Introduction

Hematologic testing and other preclinical factors are important tools for evaluating the avian patient because birds often lack clear clinical symptoms of disease (Capitelli and Crosta, 2013). Avian species, especially transgenic birds, are being researched as bioreactors for the production of recombinant biopharmaceuticals, such as vaccines and antibodies, because of their high protein yield in eggs and human-like glycosylation patterns (Kawabe and Kamihira, 2011). The study of avian models has led to an increase in vaccine production, especially for zoonotic diseases such as influenza in birds (Song and Han, 2011). For evaluating avian health and diagnosing illnesses, standard hematologic techniques such as hemoglobin concentration and packed cell volume measurements are crucial (Ochoa *et al.*, 2019). However, the ethical concerns surrounding the use of animal models in research underscore the need for a comprehensive strategy that gives equal weight to both scientific advancement and animal welfare.

Cyproterone acetate (CPA) and in vitro fertilization techniques (IVF-C) are increasingly being used in avian medical and therapeutic examination, primarily regarding reproductive health and developmental biology. While CPA, an antiandrogen, has been evaluated for its effects on sexual differences and early development in birds, IVF-C techniques are being investigated for their potential in avian breeding and genomic research. According to Dee Cooman *et al.* (2011), CPA is used to treat testicular neoplasia, egg-laying continuity, and reproductive issues in birds. CPA may be used as a model to investigate endocrine disruptors because, according to research, it can decrease embryonic development without interfering with gonadal isolation (Jessl and Oehlmann, 2023). Increased levels of CPA have a negative impact on the sperm parameters of local cocks, suggesting that it could be used to control fertility (Mohan *et al.*, 1990). Hirsutism, prostate cancer, and acne are among the complications that CPA proficiently cures as an anti-androgen by hindering the work of androgens (Neumann and Kalmus, 1991; Majid and Kareem, 2022). Because of its progestin properties, it can be used in conjunction with estrogen for hormonal treatments, primarily for gender-affirming treatments in transgender women (Kuzior *et al.*, 2023). However, the use of CPA is associated with serious side effects, such as hypertension and meningioma, especially when taken in excess (Majid and Kareem, 2022; Kuzior *et al.*, 2023).

Without using IVF-C methods, current research primarily examines the effects of CPA on sexual differentiation and embryonic development (Jessl and Oehlmann, 2023). IVF-C is essential for comprehending reproductive technologies, but its effects in combination with exposure to CPA in avian models have not been investigated (Jammes *et al.*, 2010). Because of their unique biological and developmental characteristics, the *Coturnix coturnix* (common quail) is a valuable model organism for hemato-pharmaceutical research and the effects of environmental pollutants on the physiological systems of specific organs (Sadiq *et al.*, 2024). Because quail embryos exhibit human-like developmental patterns, they can be used to study developmental biology and human genetic syndromes (Baer *et al.*, 2015). Research on the effects of steroids on quail tissues has shed light on the pharmacokinetics of medications (Nadeem *et al.*, 2022). Their role in evaluating the safety and efficacy of treatment is highlighted by the fact that they are used to investigate the effects of numerous drugs, such as bromocriptine and estradiol valerate (Nadeem *et al.*, 2022). The combined effects of CPA and IVF-C on avian hematological parameters and general health, however, are not well documented in the literature.

In order to fill this important research gap and advance our knowledge of the effects of these substances in non-mammalian models, this study intends to assess the hemato-pharmaceutical effects of *Cyproterone acetate* in common quail.

Materials and Methods

Adult male *Coturnix coturnix* (common quails) (n= 50) were taken in Barkhan area in Baluchistan, Pakistan, to assess the physiological impact of cyproterone acetate/ethinyl estradiol (CPA/EE) in birds. Body weights of all birds were 60 to 80 g and were verified to be healthy and disease-free. The quails were taken to the Zoological Laboratory of the Ghazi University in Dera Ghazi Khan and made to a 3-4week acclimatization to laboratory conditions before being experimented. The number of birds listed in Table 1, made up each group.

Table 1: *Experimental design and group allocation for the assessment of cyproterone acetate/ethinyl estradiol (CPA/EE) effects in Coturnix coturnix.*

Group	Number of Birds	Treatment	Dosage (CPA/EE)	Administration	Duration	Observation parameters	Remarks
Control	10	Untreated	—	—	20 days	Normal monitoring	No abnormalities observed
Group I	10 (2 sub-groups of 5)	CPA/EE	1 mg/kg body weight	Oral, twice daily	5 days	Body weight, temperature, clinical signs	Mild physiological changes
Group II	10 (2 sub-groups of 5)	CPA/EE	1 mg/kg body weight	Oral, twice daily	10 days	Body weight, temperature, clinical signs	Moderate physiological changes
Group III	10 (2 sub-groups of 5)	CPA/EE	1 mg/kg body weight	Oral, twice daily	15 days	Body weight, temperature, clinical signs	Marked clinical signs
Group IV	10 (2 sub-groups of 5)	CPA/EE	1 mg/kg body weight	Oral, twice daily	20 days	Body weight, temperature, clinical signs	Severe signs and partial mortality

A total sample was 50 birds (10 untreated controls and 40 evenly divided in four treatment groups; n=10/group; 2 replicates of five birds each). The biological replication and statistical accuracy was maintained by treating each replicate separately. CPA/EE (1 mg/kg body weight) was administered orally twice daily on five, ten, fifteen or twenty days, respectively, to treatment groups. Five birds were euthanized in each sub group after the time period.

Accurate CPA/EE administration was made achievable by employing a calibrated micropipette whereby the steroidal suspension was made fresh in 0.5 mL sterile corn oil prior to administration. A syringe was used to collect blood samples in the brachial vein during euthanasia. Hematological testing involved EDTA tubes whereas serological analysis was done by separating serum in gel tubes. The EDTA tubes were well shaken to avoid clotting. The hematological parameters (total red blood cells, total white blood cells, packed cell volume, mean corpuscular volume) were determined through the conventional methods: PCV centrifugation at 14,000 RPM during 10 minutes and counting of the RBC and WBC through the Neubauer chamber and centrifugation at 14,000 RPM during 10 minutes respectively. The diagnosis was done with diagnostic kits (Diasys, Germany) and a Micro Lab 200 (Merc, Germany) by measuring serum biochemical markers (urea, creatinine, uric acid, ALT, AST, cholesterol, total protein, albumin, CK-MB) with the corresponding wavelength of each assay.

The results were all expressed in the form of mean ± standard error of mean (SEM). The effect of treatment duration (control, 5, 10, 15, and 20 days CPA/EE exposure) on hematological and biochemical variables was evaluated by one-way analysis of variance (ANOVA). The Shapiro-Wilk and Levene tests were used to test the assumptions of normality and homogeneity of variance, respectively. A post hoc test of the Tukey honestly significant difference (HSD) was applied to the pairwise comparisons in the event that ANOVA showed a significant main effect ($P < 0.05$). Any P-value > 0.05 was taken to be significant.

Results

A total of fifty adult male *Coturnix coturnix* (wild common quails) were studied. Results are presented seasonally (winter), by sampling day (5-, 10-, 15-, and 20-day intervals), and by medication (Cyproterone acetate or Diane). Cyproterone acetate (CPA) administration produced several adverse clinical effects including gizzard swelling and discoloration, liver enlargement and destruction, internal hemorrhage, decreased food and water intake, diarrhea, and loss of feathers on the head, neck, belly, and other body regions.

Seasonal analysis revealed non-significant variations ($P > 0.05$) for all biochemical and hematological markers, suggesting that winter circumstances by themselves had no effect on these variables shown in Figures 1, 2, 3. Day-wise analysis revealed significant differences in all hematological parameters across 5-, 10-, 15-, and 20-day intervals ($P < 0.05$) as shown in Tables 2, 3, 4 and 5. This demonstrates that treatment duration had a strong effect on hematological profiles, with parameters such as PCV and MCV showing progressive increases over time shown in Figures 1, 2, 3.

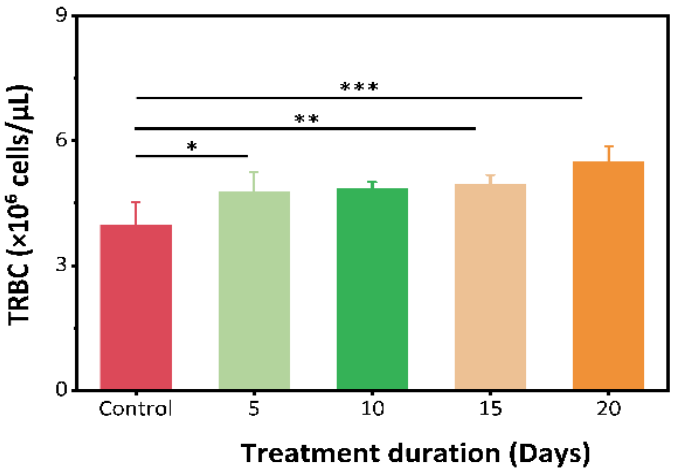

Figure 1: *Total TRBC count (mean ± SEM) in Coturnix coturnix following 5-, 10-, 15-, and 20-day CPA/EE exposure. Error bars indicate SEM (n = 5).*

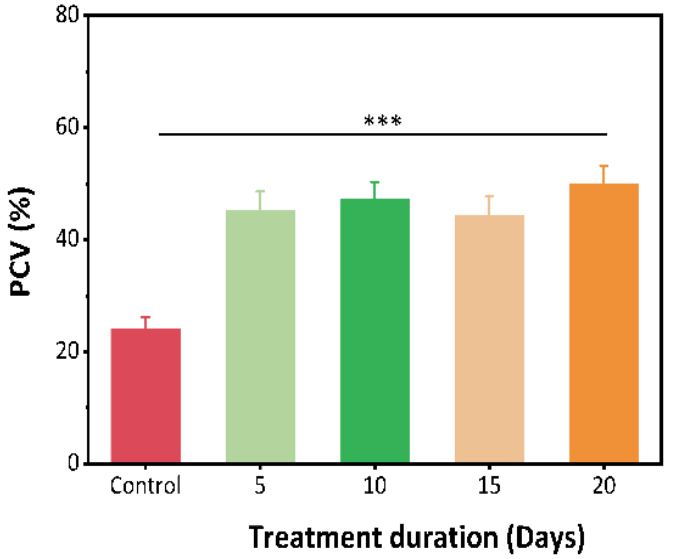

Figure 2: *Total PCV % in Coturnix coturnix following 5-, 10-, 15-, and 20-day CPA/EE exposure. Error bars indicate SEM (n = 5).*

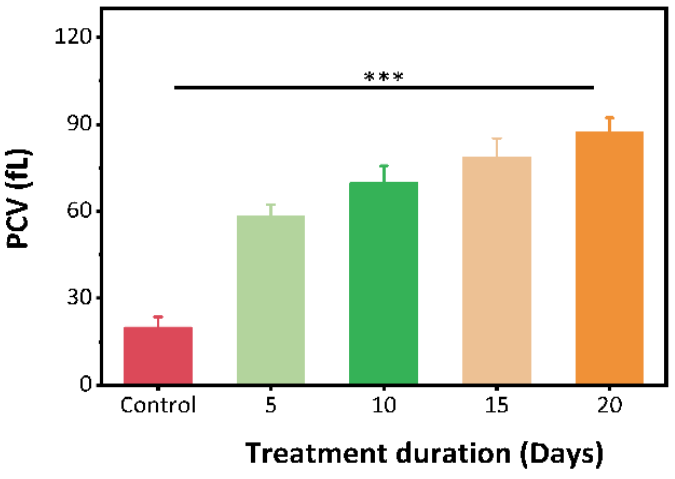

Figure 3: *Total MCV count (mean ± SEM) in Coturnix coturnix following 5-, 10-, 15-, and 20-day CPA/EE exposure. Error bars indicate SEM (n = 5).*

Table 2: *Hematological and serological values after 5 days of CPA/EE (n = 5 treated birds).*

Parameter	Control	Mean ± SEM
Urea (mg/dL)	5	47.6 ± 8.06
Creatinine (mg/dL)	0.36	0.59 ± 0.07
Uric acid (mg/dL)	11.23	16.13 ± 6.11
ALT (U/L)	15	21.6 ± 5.04
AST (U/L)	29	43.0 ± 10.53
Cholesterol (mg/dL)	102	153.2 ± 38.00
Total protein (g/dL)	5.67	3.71 ± 0.69
Albumin (g/dL)	4.89	2.38 ± 0.44
TWBC (cells/µL)	10,000	24,260 ± 4.565
TRBC (×10⁶ cells/µL)	3.10	4.17 ± 0.57
PCV (%)	23	45.2 ± 5.77
MCV (fL)	84.0	91.81 ± 11.39

Table 3: *Hematological and serological values after 10 days of CPA/EE (n = 5 treated birds).*

Parameter	Control	10-day CPA/EE
Urea (mg/dL)	10	51.4 ± 8.21
Creatinine (mg/dL)	0.30	0.71 ± 0.09
Uric acid (mg/dL)	14.0	21.19 ± 4.19
ALT (U/L)	30	35.0 ± 6.14
AST (U/L)	150	61.4 ± 12.25
Cholesterol (mg/dL)	96	131.2 ± 46.53
Total protein (g/dL)	5.76	4.56 ± 0.86
Albumin (g/dL)	1.74	2.66 ± 0.39
TWBC (cells/µL)	11,000	2,776 ± 460.6
TRBC (×10⁶ cells/µL)	3.70	4.06 ± 0.43
PCV (%)	40	39.20 ± 5.07
MCV (fL)	122.0	89.92 ± 9.12

Table 4: *Hematological and serological values after 15 days of CPA/EE (n = 5 treated birds).*

Parameter	Control	15-day CPA/EE
Urea (mg/dL)	20.0	51.0 ± 7.96
Creatinine (mg/dL)	0.21	0.59 ± 0.08
Uric acid (mg/dL)	9.60	17.96 ± 5.33
ALT (U/L)	3.50	36.6 ± 5.28
AST (U/L)	8.90	63.2 ± 9.69
Cholesterol (mg/dL)	76.5	137.2 ± 45.27
Total protein (g/dL)	4.11	4.63 ± 0.86
Albumin (g/dL)	3.93	3.77 ± 0.56
CK-MB (U/L)	1,021.1	2,908.11 ± 547.9
TWBC (cells/µL)	51.2	270.2 ± 39.94
TRBC (×10⁶ cells/µL)	0.49	3.56 ± 0.34
PCV (%)	5.20	34.4 ± 5.59
MCV (fL)	10.28	96.83 ± 10.93

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Table 5: *Hematological and serological values after 20 days of CPA/EE (n = 5 treated birds).*

Parameter	Control	20-day CPA/EE
Urea (mg/dL)	10.25	63.6 ± 10.23
Creatinine (mg/dL)	0.10	0.68 ± 0.09
Uric acid (mg/dL)	2.20	30.10 ± 2.50
ALT (U/L)	3.47	31.0 ± 3.47
AST (U/L)	8.44	50.2 ± 6.61
Cholesterol (mg/dL)	50.51	141.0 ± 40.07
Total protein (g/dL)	3.99	3.09 ± 0.34
Albumin (g/dL)	5.54	2.56 ± 0.98
CK-MB (U/L)	499.9	3,029.93 ± 375.9
TWBC (cells/µL)	60.14	174.6 ± 44.87
TRBC (×10^6 cells/µL)	0.05	2.70 ± 0.47
PCV (%)	6.50	36.0 ± 5.84
MCV (fL)	16.58	79.59 ± 10.56

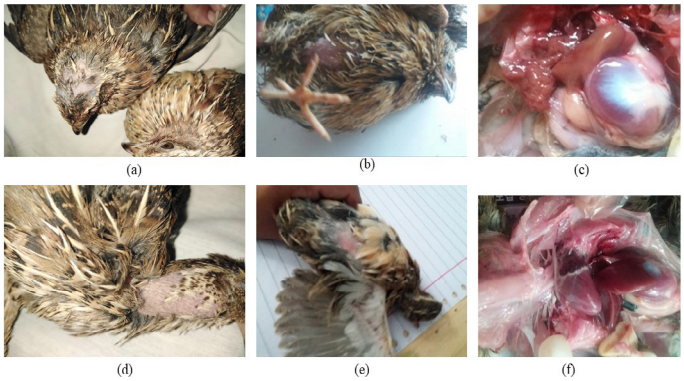

Figure 4: *Loss of feathers and other side effects in birds after the treatment of Cyproterone acetate (a) Loss of feathers from head; (b) Loss of feathers from belly; (c) Swelling in gizzard; (d) Loss of feathers from the neck; (e) Loss of feathers below the wings; (f) Enlargement of heart.*

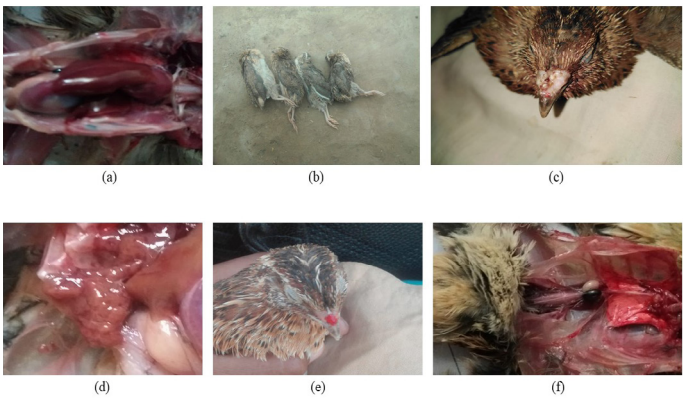

Figure 5: *Effect of Cyproterone acetate on different body organs of birds (a) Enlargement of liver; (b) Sudden deaths due to CPA after 15 days of giving dose (c) Swelling and pus in the nose (d) Destruction of liver (e) Nose bleeding (f) Blacking of 1 testis.*

One-way ANOVA revealed no significant difference among groups for the measured parameter at 5 days ($F(1, 11) = 1.039$, $P = 0.05$), 10 days ($F(1, 11) = 1.379$, $P = 0.05$), 15 days ($F(1, 11) = 1.520$, $P = 0.05$), or 20 days ($F(1, 11) = 1.424$, $P = 0.05$), indicating that CPA/EE treatment duration did not significantly affect this variable across the experimental groups. The Figures 4, 5 and 6 show the effect of *Cyproterone acetate*.

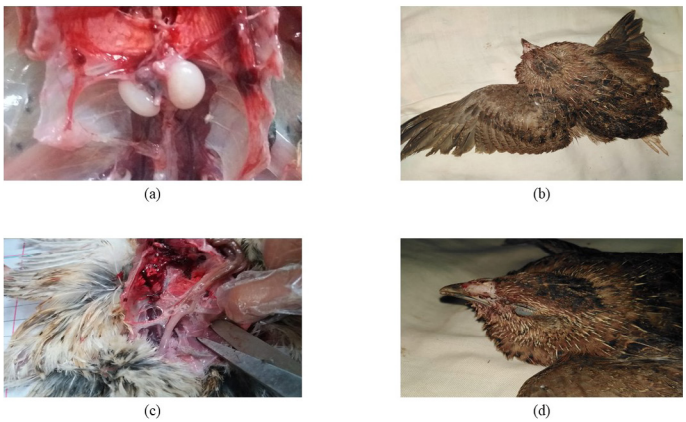

Figure 6: *Side effects on the body of Cyproterone acetate treated birds a) Enlargement of testis; (b) Loss of control of 1 wing; (c) Webbing on all internal body organs; (d) Fails to open 1 eye.*

Discussion

In the domains of biology, chemistry, and medicine, steroids play a crucial role, influencing processes such as reproduction, metabolism, and development (Covey, 2006; Sultan and Raza, 2015). Steroid hormones, including sex hormones, molting hormones in insects, and bile acids, are vital for animal reproduction and the development of secondary sexual characteristics (Diotel *et al.*, 2018; Von Engelhardt and Groothius, 2011). However, excessive exposure to these hormones can lead to tissue damage and physiological dysfunction.

Our study demonstrates that repeated administration of cyproterone acetate/ethinylestradiol (CPA/EE) in common quail significantly alters hematological and serological profiles, with elevated levels of urea, uric acid, creatinine, ALT, AST, cholesterol, total red and white blood cells, packed cell volume, and mean corpuscular volume, alongside decreased albumin and total protein compared to control as shown in Tables 2, 3, 4 and 5. These findings suggest that CPA/EE disrupts multiple physiological systems, including liver and kidney function, and hematopoiesis. The

observed increases in ALT and AST are indicative of hepatocellular damage, likely due to oxidative stress and altered lipid metabolism induced by steroid exposure (Batukan *et al*., 2007; Casagrande *et al*., 2012). Furthermore, the reduction in albumin may reflect impaired hepatic protein synthesis, while elevated urea and creatinine levels suggest nephrotoxicity or increased protein catabolism (Hussain *et al*., 2017).

The increase in PCV and MCV observed in treated quails points to erythropoietic stress or hemoconcentration, possibly resulting from hormonal imbalance or tissue injury (Sultana *et al*., 2020). These changes are consistent with previous studies showing that steroid hormones can modulate hematological parameters and immune responses in avian species (Xie *et al*., 2018; Crouch *et al*., 2022). The morphological and behavioral abnormalities noted in our study, such as feather loss, organ enlargement, and increased aggression, align with reports of hepatic and renal toxicity, as well as behavioral alterations following steroid exposure in other avian models (Ahmad *et al*., 2022; Quinn *et al*., 2007; Nirenberg, 2013).

The observed clinical and biochemical changes in our study are supported by previous findings in avian and mammalian models, highlighting the conserved effects of steroid hormones on physiological systems. However, the precise mechanisms underlying these effects, including the role of oxidative stress, receptor-mediated signaling, and immune modulation, warrant further investigation.

The study was limited by a small sample size (n= 10 per group), a relatively short 20-day exposure period, and the absence of post-treatment recovery assessment. Environmental variables and individual metabolic differences may also have influenced the results. Future research should incorporate dose-response designs, hormonal assays, and histopathological analyses to better elucidate the mechanisms and long-term effects of CPA/EE in avian species.

Conclusion

The hematological and serological parameters of common quail were adversely affected by cyproterone acetate. This drug's physical adverse effects included swelling of the feet, nasal bleeding, loss of feathers and wings, and enlargement of the liver, heart, and testicles. Hematological changes included elevated TWBC, TRBC, MCV, and PCV levels. Serological studies, however, revealed an unusual rise in urea, uric acid, creatinine, and cholesterol and a reduction in albumin and total protein. These findings imply that long-term or high-dose cyproterone acetate treatment is detrimental to common quails. A short term of treatment and careful dose control are recommended to prevent physiological and fatal adverse effects.

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Novelty Statement

This study provides the first detailed hemato-biochemical characterization of cyproterone acetate/ethinylestradiol (CPA/EE) induced toxicity in adult male common quail under ex situ conditions, demonstrating dose duration dependent disruption of hepatic, renal, and hematological homeostasis. The findings highlight common quail as a sensitive avian model for evaluating steroidal contraceptive mixtures and emphasize the need for cautious use of CPAEE in avian management and research.

Author's Contribution

Hassan Raza: Designed and conducted the research experiments.
Nayab Fatima: Conducted statistical analysis and drafted initial manuscript.
Kiran Aftab: Contributed in literature review and data visualization.
Muhammad Akbar Khan: Assisted in methodology optimization, provided technical supervision and critical insights.
Hasnain Raza and Yusra Ashfaq: Contributed in results interpretation.
Memoona Mehndi and Yusra Ashfaq: Contributed in literature review and reviewed formatted and finalized the paper.

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Ethics approval

Study was conducted after taking approval from the ethical committee of Department of Zoology, Ghazi University, Dera Ghazi Khan.

Generative AI and AI-assisted technology statement

The authors declare that no generative artificial intelligence or AI-assisted technologies were used in the conception, data collection, data analysis, interpretation of results, or writing of this manuscript.

Conflict of interest

The authors have declared no conflict of interest regarding the publication of this article.

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