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Original article

Biochemical and morphological attributes of broiler kidney in response to dietary glucocorticoid, dexamethasone

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ABSTRACT

Glucocorticoids (GCs) initiate oxidative stress and cause renal damage which lead to hypertension, heart failure and ultimately death. The current study aimed to investigate the alterations in serum biochemical parameters i.e. HDL and LDL; gross anatomy, histomorphology and histomorphometry of broiler kidney in response to dietary GC, dexamethasone (DEX). Day old chicks (DOCs) were randomly assigned into four groups: control and three treatment groups (T1, T2 and T3). The control group was fed commercial broiler type ration and the treated groups were fed commercial broiler type ration containing GC (Dexamethasone @ 3, 5 and 7 mg/kg in T1, T2 and T3 group respectively). To measure the biochemical parameters, blood samples were collected on days 7, 14, 21, and 28 of the experiment. For histological investigation, kidney (left) samples were collected from the individual birds after sacrificing on days 7, 14, 21, and 28 of the experiment. Histomorphological alterations of the kidney were assessed by routine hematoxylin and eosin (H&E) staining. Biochemical analysis showed significantly increased serum HDL and LDL level compared to the control. In gross study, dark congested kidney was found with significantly decreased weight, length and width. Treatment with DEX augmented congestion, inflammation and fibrosis in kidney, as evidence by histomorphometric study. Extensively degenerated and atrophied glomeruli, degenerated tubular epithelium with distorted tubules and inter tubular empty spaces were seen. Percentage of atrophied glomeruli increased significantly and maximum percentage of glomerular atrophy was seen at day 28. These changes were found more explicitly in the higher dose group. Histomorphometric study also revealed significant decrease in the diameter of glomerulus. The findings of this study suggest that DEX may alter the serum biochemical parameters as well as kidney gross and histomorphology.

1. Introduction

The demand of animal protein for human consumption is currently uprising with the ascent of population growth. A large portion of animal protein is supplied from different farm animals. To meet up this progressively uprising demand of animal protein, various growth promoting agents are frequently utilized in livestock unlawfully, either alone or mixing with other prohibited substances (Cannizzo et al., 2010). In veterinary clinical practice, glucocorticoids (GCs) like dexamethasone (DEX) have a wide scope of remedial applications. Moreover, they are often added with broiler feed as growth promoters for boosting the hereditary potentiality, to promote feed conversion ratio (FCR), survival rate and to reduce fatality in broilers (Mostafa et al., 2016). According to Khatun et al. (2016) total production of steroids and growth promoters in Bangladesh are 0.64 & 1.2 tons respectively. Different types of antibiotics, vitamins, minerals and steroids are used as growth promoters (GPs) to enhance growth of meat producing animals and birds. It has already been reported by Afrose et al. (2018)

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that supplementation of steroid growth promoters like GCs with diet do not improve overall growth rate (i.e. body weight and dressed weight) of broilers. Besides that, long term use of corticosteroids is more problematic because it creates an atmosphere for potential risk of serious irreversible health conditions (Buchman, 2001).

The effects of GCs are brought about by glucocorticoid receptor (GR) almost in all organs, including the brain, heart, lung, and kidney (Ackermann et al., 2010). Primarily, GCs enter into the target cells and bind with inactive cytoplasmic GR and form active ligand–receptor complexes. These complexes are then translocated into the nucleus and govern gene transcription by both direct and indirect mechanism (Stahn et al., 2007). Both GC and high density lipoprotein (HDL) have anti-inflammatory property (Navab et al., 2011; Oh et al., 2017). But oxidative stress and inflammation resulting from renal tissue damage reduce HDL's anti-oxidant and anti-inflammatory activities as well as convert HDL to pro oxidant and pro inflammatory (Navab et al., 2011). In such cases, low density lipoprotein (LDL) spontaneously gets oxidized and HDL's ability to defend against lipid oxidation declines. Moreover, systemic inflammation decreases the efficiency of HDL in this field as well (Vaziri et al., 2010). Tissue damage triggers inflammation and inflammation is involved both in tissue regeneration and development of fibrosis (Mack, 2018). These abnormalities can augment the tendency of patients to develop atherosclerosis with end-stage renal disease (Moradi et al., 2009).

The application of DEX on poultry has a high and long term impact. Such as weight loss of immune organs (Vicuña et al., 2015) and depleted blood lymphocytes were also recorded in DEX treated broiler chicks (Aengwanich, 2007; Afrose et al., 2018). Besides liver enlargement, both weight and function of lymphoid organs including thymus, bursa of Fabricius and spleen were found to decline by corticosteroid treatment in birds (Mehaisen et al., 2017). Dietary application of DEX in broiler can easily be a feasible, controlled, and flexible tool in the search of host adaptation process to stressors. Prolonged exposure to DEX may lead to untoward effects on kidney development (Nicod et al., 2003). GCs influence both kidney development and function by influencing the cardiovascular system and by exerting their effects on glomerulotubular function (Smets et al., 2010). They can also actuate mineralocorticoid receptor (MR) and thus incite renal injury (Rafiq et al., 2011). In the kidneys, GCs initiate oxidative stress, alter the renin–angiotensin system (RAS) and decrease the number of nephrons, which ultimately lead to renal damage and increased blood pressure (Moisiadis and Matthews, 2014). DEX damages kidney by vacuolation of kidney tissue and shrinking the tubules (Salman and Hassooni, 2020). It can also lead to chronic progressive glomerulonephritis along with severe fibrosis, glomerulosclerosis, atrophy and some regeneration of the tubular system (Kamphuis et al., 2007). Prenatal administration of GC, DEX causes significant reduction of glomeruli number (Ortiz et al., 2002). Albeit, some advantageous outcomes of GCs in sepsis-associated kidney injury have also been reported (Johannes et al., 2009).

The kidneys are involved in a wide range of vital functions. Hence, it is not surprising that kidney dysfunction can lead to major drawback for the broiler industry. Effects of dietary DEX on different organs i.e. liver (Sultana et al., 2020a), immune organs (Sultana et al., 2020b) and hematological parameters (Afrose et al., 2018) of broiler were studied. In a study Moonen et al., (2018) found that treatment with dexamethasone attenuated development of fibrosis, which persisted for a long period of 3 weeks evidenting by stagnation of collagen I deposition in the ischemic kidney in mice. On the other hand, Hussain et al., (2008) tried to find out the effect of corticosteroid, diclofenac on four broiler chicks (*Gallus gallus*, 15 days old), pigeons (*Columba livia*, 3 months old), Japanese quail (*Coturnix japonica*, 4 weeks old) and mynah (*Acridotheres tristis*, independent young) poultry species. They found clinical signs in all species included depression, somnolence, decreased body weight and mortality. Histologically, uric acid crystal aggregates (tophi) and associated lesions were exhibited in the parenchyma of kidneys and liver. But the dose and age dependent impact of dietary DEX on kidney of broiler is still obscure. Therefore, we hypothesized that dietary DEX supplementation may lead to alterations in serum biochemical properties and gross and histological characteristics of kidney. To test this hypothesis, we compared the mentioned parameters in DEX treated broilers at different dose with the control. The aim of the current study is to identify the changes in serum biochemical parameters i.e. HDL and LDL and to predestine the gross morphological, histomorphological and histomorphometric alterations in broiler kidney treated with DEX.

2. Materials and methods

2.1. Ethical approval

This study was conducted in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Bangladesh. Selection of animal model, rearing and all other experimental procedures of the current study were performed with the approval (Ethical Approval No- [AWEEC/BAU/2021(3)]) of Bangladesh Agricultural University institutional 'Animal welfare and Experimentation Ethics Committee'.

2.2. Experimental design

2.2.1. Animal model

In this experiment, 80 day old chicks (DOCs, Cobb-500) were purchased from local commercial hatchery (Provita Hatchery Ltd., Bangladesh). Upon arrival, the DOCs were supplemented with vitamin-C for alleviation of stress. Then they were divided into four groups (n = 20 for each group) through blind randomized procedure and housed into separate cages (5 × 4 square feet). The groups were then randomly assigned to various experimental conditions such as control group (C), treatment group-1 (T1), treatment group-2 (T2) and treatment group-3 (T3). The DOCs were kept at brooding temperature (95°F) for the first 3 days. Then the temperature was decreased gradually (5°F each day) and stabilized at 70 ± 2°F throughout rest of the experiment period. Fifty to sixty percent relative humidity was maintained throughout the experiment period

2.2.2. Experimental feeding trial

Both the control and treatment groups were fed (ad libitum) commercial broiler type ration purchased from renowned local poultry feed suppliers (Nourish Poultry and Hatchery Ltd., Bangladesh). The purchased feed was free from any kind of antibiotic, growth promoter or other drug that may alter the real outcomes. Size of the pellets and composition (Sultana et al., 2020b) of the pre- starter, starter and finisher feed was chosen to fulfill the nutritional requirements at different ages. Dietary glucocorticoid (Dexamethasone, BP 0.5 mg, Opsonin Ltd.) was supplied to the treated groups at the rate of 3 mg/kg (T1), 5 mg/kg (T2), and 7 mg/kg (T3). Extra care with continuous monitoring was ensured so that each bird of the treatment groups receives desired rate of DEX with the diet.

2.2.3. Sample collection

To investigate the effects of dietary DEX on serum HDL and LDL level, blood samples were collected from five birds of each group. Blood sampling was performed through cardiac puncture. Then the birds were sacrificed by manual cervical subluxation method

and dissected immediately. To investigate the effects of dietary DEX on kidney, tissue samples were collected from five birds of each group. Intact left kidney was collected carefully from their original anatomical location at day 7, 14, 21 and 28 from each group. The samples were then washed with physiological saline (0.9%) and preserved in 10% neutral buffered formalin.

2.3. Biochemical study

Collected blood samples were transferred into sterile glass test tubes without anticoagulant and left undisturbed in a slanting position at room temperature for proper clotting. The tubes were then placed into the refrigerator at 4 °C for overnight. The blood samples were centrifuged at 1000 rpm for 15 min for serum separation. The supernatant serum samples were collected, transferred into screw-capped vial and preserved at −20 °C in a screw-capped vial until further use. The measurement of HDL and LDL was performed by spectrophotometer using a Hyman type Humalyzer 2000 analyzer (Wiesbaden, Germany).

2.3.1. Measurement of HDL

One ml of serum sample and 100 μL precipitating reagents, phosphotungstic acid (14 mmol/L) and magnesium chloride (2 mmol/L) were pipetted into a centrifuge tube and mixed properly. The mixture was allowed to stand for 10 min at room temperature. Then it was centrifuged at 4000 r.p.m for 20 min and the supernatant was collected. A reagent, mixture of cholesterol esterase (1000U/L), cholesterol oxidase (300U/L), peroxidase(650U/L), 4-aminophenazone (0.4 mmol/L) to 100 ml cholesterol buffer solution was used in this process. The assay condition of the spectrophotometer was set as 505 nm wave length, 1 cm light path for cuvette and 37 °C temperature. The instrument was adjusted to zero with distilled water. Then three mixtures were prepared in separate cuvettes as blank (1 ml enzyme solution), standard (1 ml reagent solution and 10 μL cholesterol aqueous primary standard(200 mg/dL) and test sample (1 ml reagent solution and 10 μL supernatant sample) and incubated at 37 °C for five minutes. Then the absorbance (A) value of the samples and standard was recorded against the blank. Then the concentration of the HDL (mg/dL) in the sample was calculated using the following formula- [{(A) sample − (A) blank}/(A) standard − (A) blank}] × standard concentration.

2.3.2. Measurement of LDL

Serum LDL level was measured using two reagents. Reagent-1 was a mixture of piperazine-N, Nbis [2-ethanesulfonic acid] (50 mmol/L), cholesterol esterase (600U/L), cholesterol oxidase (500U/L), catalase (600KU/L) and trinder's reagent (2 mmol/L) whereas reagent-2 was a mixture of piperazine-N,N bis [2-ethanesulfonic acid] (50 mmol/L), 4-aminoantipyrine (4 mmol/L) and peroxidase (4KU/L). The assay condition of the spectrophotometer was set as 600 nm wavelength, 1 cm light path for cuvette and 37 °C temperature. The instrument was adjusted to zero with distilled water. Then three mixtures were prepared in separate cuvettes as blank (300 μL reagent-1), standard (300 μL reagent-1and 4 μL standard solution) and test sample (300 μL reagent-1 and 4 μL sample). Then they were mixed properly and incubated at 37 °C for five minutes. 100 μL of reagent-2 was then added to each cuvette and again incubated at 37 °C for five minutes. Finally, the absorbance value of the samples and standard was recorded against the blank and the concentration of the LDL (mg/dL) was calculated using the formula mentioned earlier.

2.4. Gross morphometric study

For gross study- color, weight of left kidney, length and width of cranial lobe of the left kidney were considered. The color of the kidney was compared within the control and treatment groups by visual inspection. Weight was measured using high precision balance (FGH Series, AND Company Ltd, Korea) and length, width were measured by graded scale.

2.5. Histomorphological study

For histological investigation, the tissue samples were processed and stained (Hematoxylin and Eosin; H & E) according to the protocol described by Sultana et al., 2020. All stained tissue sections were examined by single person to avoid individual variation and analyzed blindly to avoid any biasness. The histological characteristics of kidney tissues were examined under light microscope (Leica DMR; Leica Microsystems, Wetzlar, Germany) at 100× and 400× magnifications. For counting the number of glomeruli, five randomly selected focuses from each tissue sections were studied at 400× magnification.

2.6. Histomorphometric study

For histomorphometric study, a total of 25 randomly selected glomeruli were considered for measurement from each section

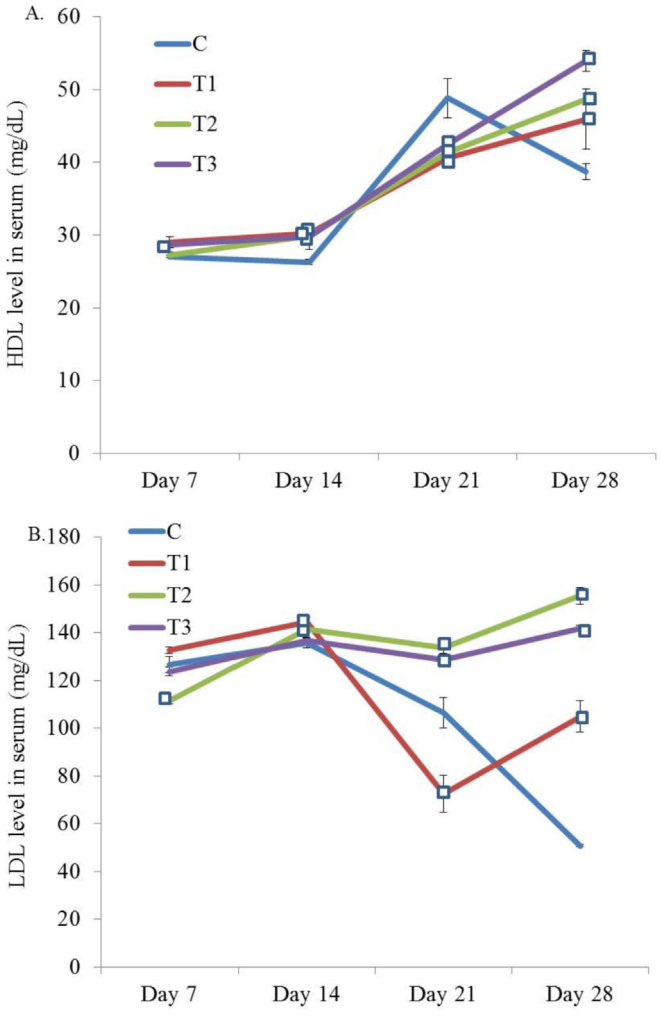

Fig. 1. Dynamics of HDL and LDL in serum in relation to age of broiler chicken treated with dexamethasone. Data were expressed as mean ± standard error and differences among the groups of birds were compared using one-way ANOVA with post-hoc Duncan's multiple range test. Lines with squares at different days are significantly different ($p < 0.05$) from control of the respective day.

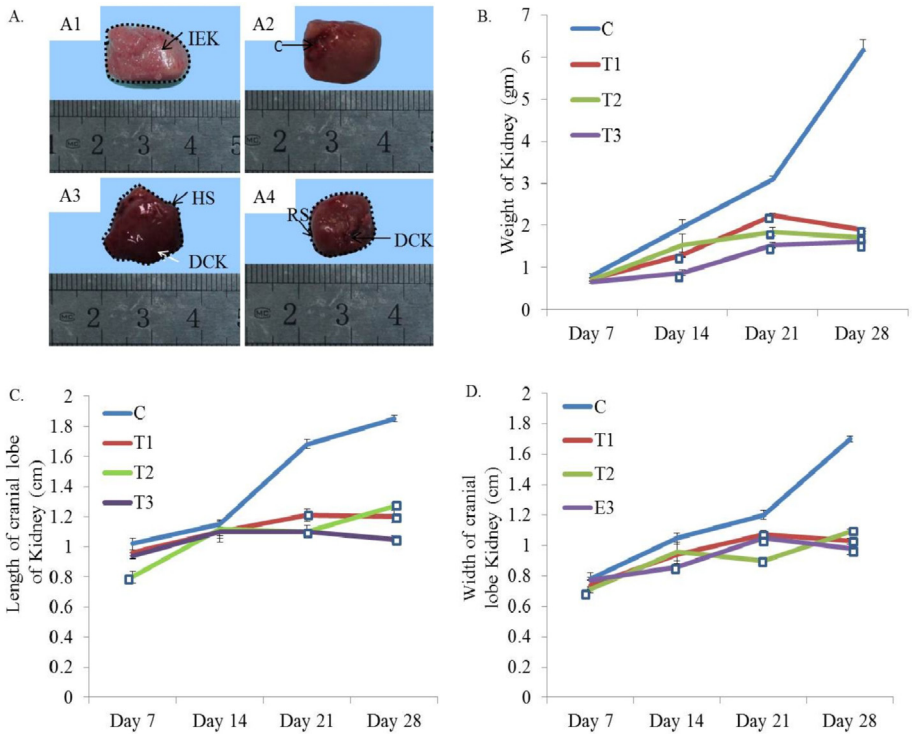

Fig. 2. A. Gross view of broiler left kidney (cranial lobe) during collection of sample at day 28. A1- Control group; A2- Treatment group-1, T1; A3- Treatment group-2, T2; A4- Treatment group-3, T3. IEK- Irregular and elongated kidney, HS- Hexagonal shape, RS- Somewhat rounded shape, C- Congestion, DCK- Dark and congested kidney. Effects of dietary glucocorticoid on the weight (gm) of left kidney, length (cm) and width (cm) of cranial lobe of left kidney in DEX treated broiler is shown in figure B, C and D respectively. Data were expressed as mean ± standard error and differences among the groups of birds were compared using one-way ANOVA with post-hoc Duncan's multiple range test. Lines with squares at different days are significantly different ($p < 0.05$) from control of the respective day.

(five sections for each group). The longest and shortest diameter of glomeruli was measured in micrometer (μm) using calibrated stage micrometer.

2.7. Photomicrographs

Necessary photographs were taken from 10 randomly selected focuses at 100X and 400X magnifications for better illustration of the obtained results. All the pictures were captured by photomicroscope (Model: CX41U-LH50HG, Olympus Corporation, Tokyo, Japan).

2.8. Statistical analyses

All the data obtained from biochemical analyses, gross and histomorphometric studies were analyzed using SPSS software (IBM SPSS Statistics 22). The normality of the data set was evaluated by Shapiro-Wilk test. In all trials, data were expressed as mean ± standard error of mean (SEM). Differences among the groups of birds were compared using one-way ANOVA with post-hoc Duncan's multiple range test where $P < 0.05$ were considered significant.

3. Results

3.1. Alterations in biochemical parameters

Serum HDL and LDL level was almost similar with minor variation in both the control and treated group except T1 and T2 groups at day 7 of the experiment. DEX induced a significant rise in serum HDL and T2 group showed a significant decrease in LDL as compared to the control (Fig. 1). At day 14, HDL level increased significantly in all the treated groups from the control. Serum LDL level was also increased in the treated groups but this increase was significant only in T1 and T2 groups. HDL level significantly decreased in the treated groups at day 21. LDL level significantly decreased in T1 group whereas increased significantly in both T2 and T3 groups. At day 28, both HDL and LDL level increased significantly in all the treated groups than the control. The HDL level in T3 group was increased significantly in comparison to that of the T1 and T2 groups. The LDL level in T2 and T3 groups also increased significantly compared to the T1 group (Fig. 1).

3.2. Gross morphometric alterations

The kidney of both control and treated groups showed normal anatomical structure and located immediately caudal to the lungs in each side of median plane occupying the synsacral fossa. The control group exhibited dark brown color kidney with its irregular elongated shape. The treated groups revealed various shapes like hexagonal or somewhat rounded shape and dark reddish or blackish color with moderate to severe congestion (Fig. 2A).

The control and DEX treated groups showed no significant variation in weight of left kidney though it was slightly decreased numerically with the increase of dose at day 7 of the experiment (Fig. 2B). The length and width of cranial lobe of left kidney was also numerically decreased in the treated groups but significant difference was found only in T2 group (Fig. 2C & D).

At day 14, the weight was significantly decreased in T1 and T3 group whereas in T2 group the weight decrease was not significant (Fig. 2B). The length and width was numerically decreased in all the treated groups as compared to the control. Statistically significant difference was found only in width of T3 group (Fig. 2C & D).

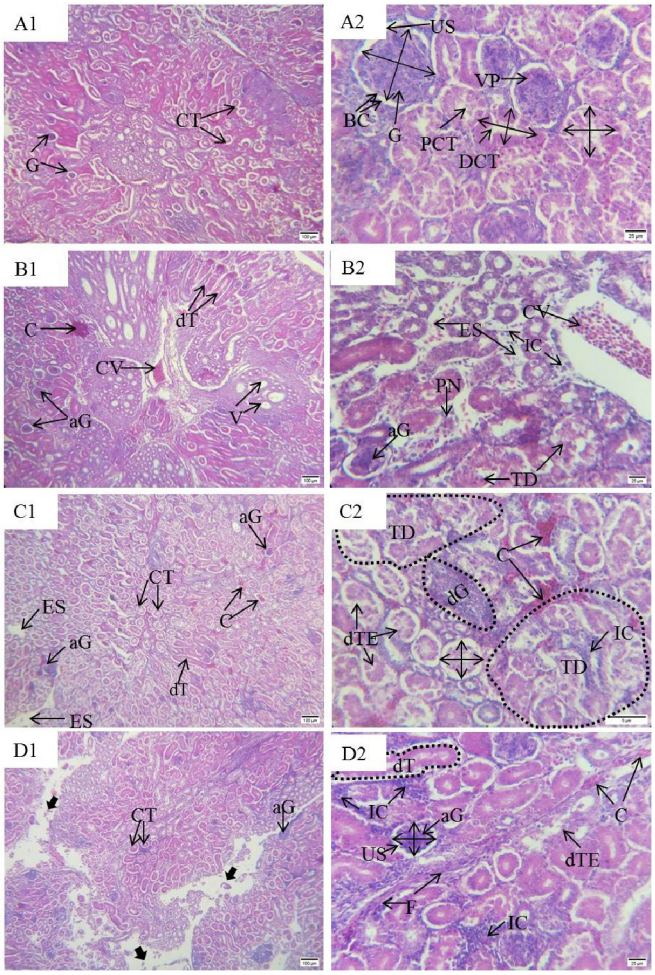

Fig. 3. Representative photomicrographs of transverse section (H & E stained) of kidney in the 7 day old broiler of control (A1, A2), treatment group-1 (B1, B2), treatment group-2 (C1, C2) and treatment group-3 (D1, D2) at 100x and 400x magnification. BC- Bowman capsule, G- Glomerulus, aG- Atrophied glomerulus, dG- Distorted glomerulus, US- Urinary space, CT- Convoluted tubules, PCT- Proximal convoluted tubule, DCT- Distal convoluted tubule, dT- Dilated tubules, TD- Distorted and degenerated tubules, dTE- Degenerated tubular epithelium, VP- Vascular pole, C- Congestion, CV- Congested vein, ES- Empty space, F- Fibrosis, IC- Inflammatory cells, V- Vacuolation, Thick arrows- Loss of tubules, PN- Pyknotic nuclei, Cross arrows- Diameter.

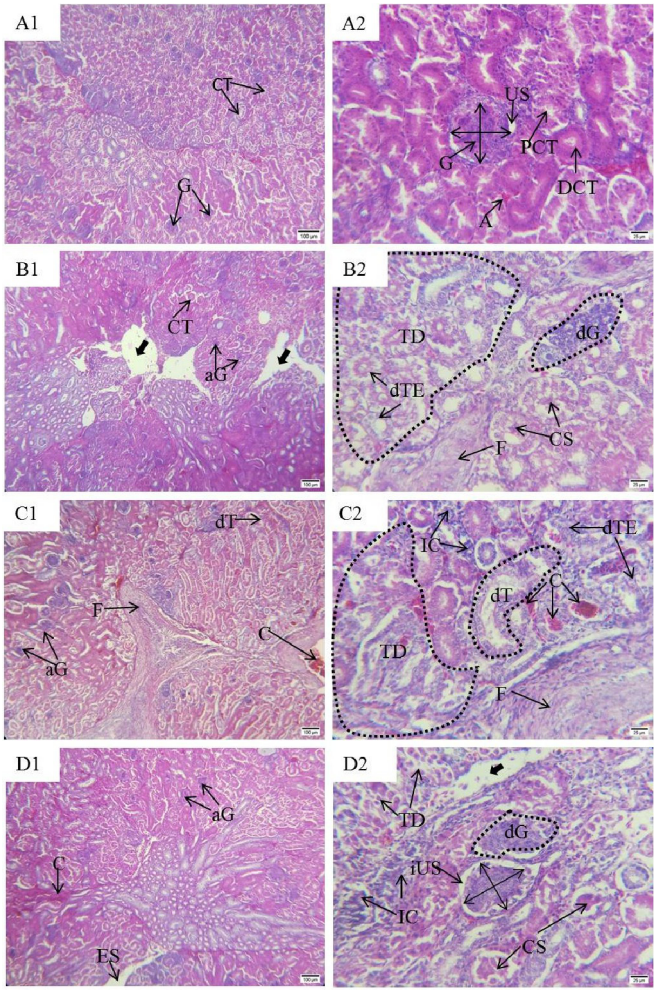

Fig. 4. Representative photomicrographs of transverse section (H& E stained) of kidney in the 14 day old broiler of control (A1, A2), treatment group-1 (B1, B2), treatment group-2 (C1, C2) and treatment group-3 (D1, D2) at 100x and 400x magnification. BC- Bowman capsule, G- Glomerulus, aG- Atrophied glomerulus, dG- Distorted glomerulus, US- Urinary space, CT- Convoluted tubules, PCT- Proximal convoluted tubule, DCT- Distal convoluted tubule, dT- Dilated tubules, TD- Distorted and degenerated tubules, dTE- Degenerated tubular epithelium, C- Congestion, CS- Tubular cast formation, ES- Empty space, IC- Inflammatory cells, Thick arrows- Loss of tubules, F- Fibrosis, A- Artery, Cross arrows- Diameter.

The weight, length and width of the kidney revealed significantly lower values in all the treated groups as compared to the control at both day 21 and 28. These values gradually decreased with the increase of dose of DEX with some exceptions (Fig. 2B, C and D).

3.3. Histomorphological alterations

In the control group, the kidney exhibited general histology with regularly distributed glomeruli throughout the indistinctly divided cortex and medulla. The glomeruli were surrounded by Bowman's capsule with urinary space in between them. The proximal convoluted tubules were lined by simple cuboidal epithelium with apical brush border which formed hazy luminal appearance. The distal convoluted tubules showed smooth apical surface with relatively wide and clear lumen (Figs. 3–6).

At day 7 (Fig. 3), the glomeruli showed some extent of degeneration which was seen more explicitly at day 14, 21 and 28 (Figs. 4–6). Glomerular atrophy and shrunken glomerular tuft was seen predominantly in the cortical region of kidney in all the treated groups at different days. Percentage of atrophic glomeruli increased with the progression of both age and DEX dose. Maximum percentage (73.1%) of atrophic glomeruli was seen at day 28 in T3 group (Table 1). Degenerating tubular epithelia were seen at day 7 in T2 and T3 groups (Fig. 3). At day 14 and 21, tubular epithelial degeneration was found ameliorated with distorted tubular structure (Figs. 4 and 5). At day 28, tubular architecture was almost compromised with severe atrophy. Formation of cast within the tubular lumen by degenerated tubular epithelia was found at day 14 (Fig. 4).

Wide empty spaces between the tubules were seen at day 7 (Fig. 3) which were found more extensive in T3 groups at day 14, 21 and 28 (Figs. 4–6). In lower dose groups, these empty spaces were filled with inflammatory cells and pyknotic nuclei. More inflammatory cells and fibrosis were observed in the higher dose group. Varying degree of venous and inter tubular congestion were found in the treated groups, especially in T1, T2 groups at day 21 and T2 group at day 28 (Figs. 5 and 6). Extensive area of fibrosis was seen both in the cortical and medullary region in kidney of T2 group at day 14 (Fig. 4) and T1 group at day 28 (Fig. 6). At day 28, huge necrotic area with caseous mass was seen in T2 group eliciting cheesy appearance.

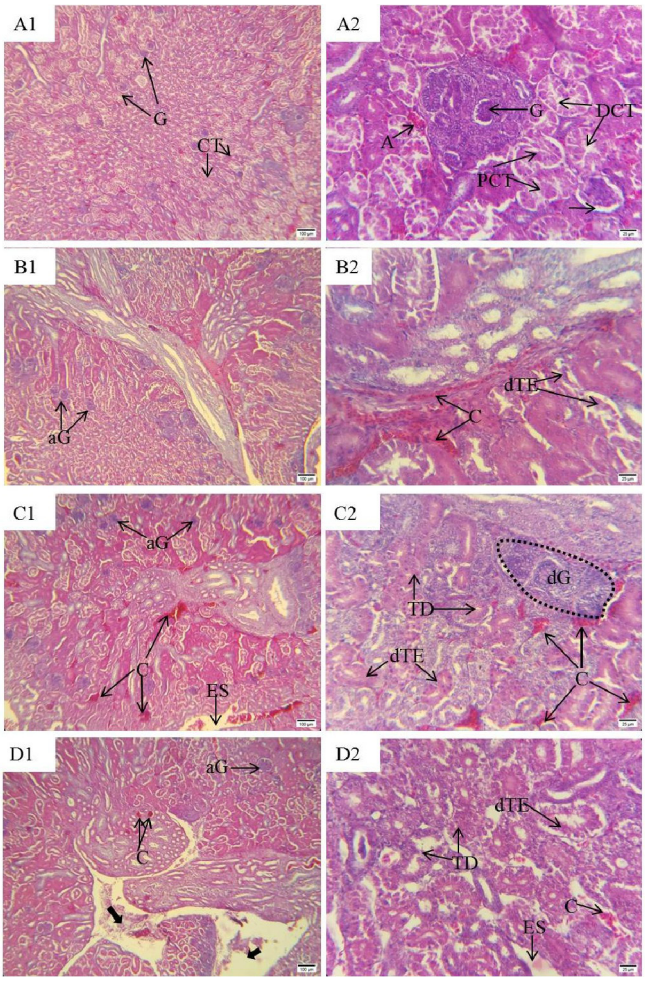

Fig. 5. Representative photomicrographs of transverse section (H& E stained) of kidney in the 21 day old broiler of control (A1, A2), treatment group-1 (B1, B2), treatment group-2 (C1, C2) and treatment group-3 (D1, D2) at 100x and 400x magnification. BC- Bowman capsule, G- Glomerulus, aG- Atrophied glomerulus, dG- Distorted glomerulus, US- Urinary space, CT- Convoluted tubules, PCT- Proximal convoluted tubule, DCT- Distal convoluted tubule, TD- Distorted and degenerated tubules, dTE- Degenerated tubular epithelium, C- Congestion, ES- Empty space, Thick arrows- Loss of tubules, A- Artery.

3.4. Histomorphometric alterations

The histomorphometric or biometric study on diameter of kidney glomeruli revealed a gradual numerical decrease. At day 7, both the longest and shortest diameters of glomerulus were numerically decreased from the control but significant decrease was found in T2 and T3 groups. This decrease was seen more in higher dose group compared to the lower dose group but the differences were not statistically significant (Table 1). At day 14 and 21, all the treated groups showed a significant decrease of diameter as compared to the control. But no significant difference was observed in respect to the dose variation (Table 1). At day 28, the diameter was decreased significantly in all the treated groups. The diameter was also decreased from day 21 which suggests extensive atrophy of the glomeruli (Table 1).

4. Discussion

The present study investigated whether the supplementation of different doses of dietary DEX affects the serum HDL, LDL level and causes any alterations on the gross morphology (i.e. color, weight, size and shape) histomorphology and histomorphometry (i.e. diameter of glomeruli) of kidney. The kidney of the control groups at different days revealed general gross and histomorphological characteristics as described by Abood et al., 2014; Pervenetskaya and Fomenko, 2018. However, the present data showed that DEX causes dose dependent changes in serum HDL and LDL concentration. The DEX treated group also revealed significant alterations in both the gross and histological architectures of the kidney.

4.1. Biochemical alterations

Our study demonstrated that dietary DEX was associated with significant ($P \leq 0.05$) increase in HDL compared to the control at day 7, 14 and 28. This is obvious because a higher glucocorticoid dose decreases plasma cholesteryl ester transfer protein activity in patients, thereby contributing to higher HDL level (Buning et al., 2017). Schroeder et al (2015), found similar result following DEX treatment in rheumatoid arthritis patients. However, significant ($P \leq 0.05$) decrease of serum HDL was seen in the treated groups compared to the control at day 21. This finding is biologically plausible as inflammatory process is linked to decreased HDL (Park et al., 2002). HDL has anti-inflammatory effect (Navab et al., 2011) and decreased serum HDL may lead to infiltration of mononuclear cells in the kidney. Dietary DEX induced kidney damage which led to mild to severe inflammation. The decreased HDL level may have inflicted joint effects that led to severe fibrosis in kidney. In our study, we also found that dietary DEX increases serum LDL level. Higher serum LDL level is associated with poor survival rate due to their accumulation in the heart and artery which leads to massive hypertension (Zadeh et al., 2002).

4.2. Gross morphometric observations

In gross morphological study, the kidney of DEX treated broilers revealed congestion with dark reddish or blackish kidney. None of the previous research claims this type of gross change as per the knowledge of the authors. The shape of the kidney also showed deviation from the control. The length and width of the cranial lobe of kidney was also decreased significantly which suggests atrophy of the kidney lobes. The total weight of left kidney was also decreased significantly in the treated groups compared to the control which justifies the above mentioned findings. Though Ortiz et al. (2002), demonstrated unchanged kidney weight in response to prenatal DEX treatment in rat.

4.3. Histomorphological alterations

Degenerated, shrunken and atrophied glomeruli were seen in the renal cortical and medullary areas. The kidney tubules were also degenerated and distorted. All of these findings justifythe wide empty spaces created between the tubules and these findings are in accordance with the report of Salman and Hassooni, (2020). Though, Choi et al. (2013) described that DEX treatment attenuates the renal injury by regenerating the cellular structures. There were also venous and inter tubular congestion. This might be due to the hypertension and increased venous pressure induced by DEX as discussed earlier. The histomorphological examinations revealed the existance of inflammation and fibrosis. Previously Straub and Cutolo (2016) explained that glucocorticoids have anti-inflammatory activities, although dose and duration are the most important points for this issue. Since glucocorticoid resistance is a consequence of inflammation, adequate anti-inflammatory therapy is mandatory. Long-term low-dose glucocorticoid therapy in chronic inflammatory diseases causes changes of the systemic and local cortisol-to-cortisone shuttle (reactivation and degradation of cortisol). Also the damage to the tissue may trigger the imflammation and inflammation triggers to fibrosis, which also

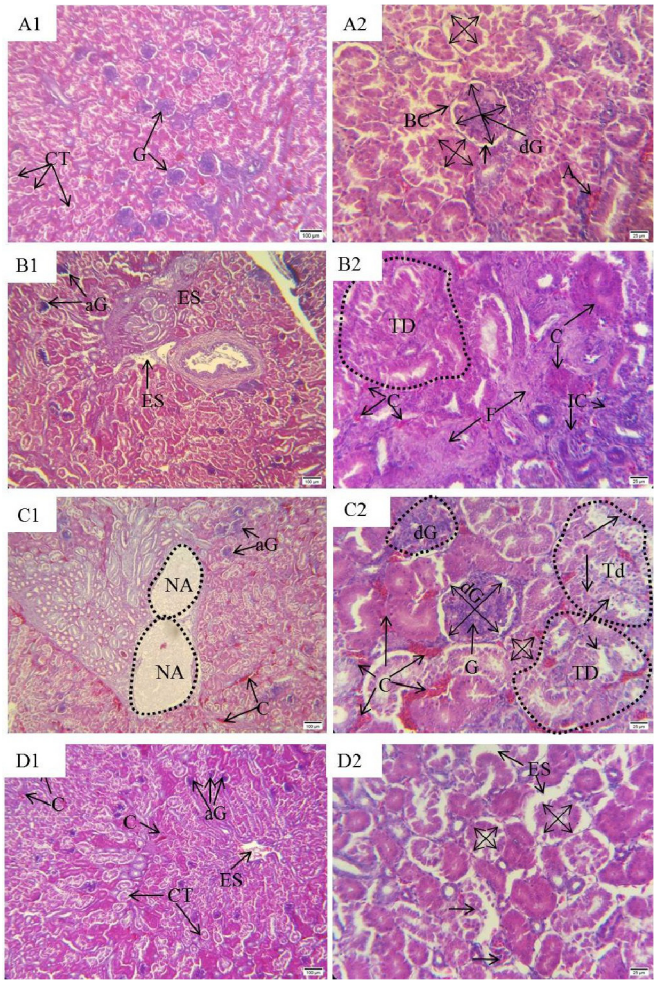

Fig. 6. Representative photomicrographs of transverse section (H& E stained) of kidney in the 28 day old broiler of control (A1, A2), treatment group-1 (B1, B2), treatment group-2 (C1, C2) and treatment group-3 (D1, D2) at 100x and 400x magnification. BC- Bowman capsule, G- Glomerulus, aG- Atrophied glomerulus, dG- Distorted glomerulus, US- Urinary space, CT- Convoluted tubules, PCT- Proximal convoluted tubule, DCT- Distal convoluted tubule, TD- Distorted and degenerated tubules, dTE- Degenerated tubular epithelium, PN- Pyknotic nuclei C- Congestion, ES- Empty space, A- Artery, IC- Inflammatory cells, F- Fibrosis, NA_ Necrotic area, Cross arrows- Diameter.

support the statement of Mack (2018) as in his review paper he mentioned that tissue damage and inflammation are important triggers for regeneration and fibrosis. At day 14, there was amelioration of the alterations mentioned at day 7. The changes were also extensive in higher dose group. There was extensive fibrosis in T2 group which is in agreement with Rafiq et al. (2011). These findings clearly indicate that DEX causes both age and dose dependent alterations in kidney histomorphology. At day 21, progressive and widespread histomorphological alterations were seen in all the treated groups. But there was remarkable reduction of inflammation. This might be due to the significant rise in serum HDL from day 14 as we have discussed the anti-inflammatory effect of HDL earlier. At day 28, significantly increased number of atrophied glomerulus was seen along with collapse of tubular structure. These findings are in agreement with Franco-Colín et al. (2006), Salman and Hassooni (2020). There was also extensive inflammation and fibrosis despite the serum HDL significantly increased from day 21. This suggests widespread tissue damage which triggered the inflammatory process and fibrosis. The huge necrotic area within the medullary

Table 1
Histomorphometric data on glomeruli of Control (C) and DEX treated (T1, T2, T3) broilers. LDG- Longest diameter of glomerulus, SDG- Shortest diameter of glomerulus. Values with asterisks (*) superscripts are significantly different from control ($P < 0.05$).

Groups	Day 7			Day 14			Day 21			Day 28		
	LDG (μm)	SDG (μm)	Atrophy (%)	LDG (μm)	SDG (μm)	Atrophy (%)	LDG (μm)	SDG (μm)	Atrophy (%)	LDG (μm)	SDG (μm)	Atrophy (%)
C	52.92 ± 4.24	44.64 ± 2.23	–	63.00 ± 2.41	63.72 ± 2.39	–	69.48 ± 1.73	56.52 ± 4.16	–	77.76 ± 7.05	68.04 ± 5.1	–
T1	43.56 ± 3.96	37.8 ± 4.83	20.4	49.68 ± 2.39*	33.84 ± 1.92*	31.6	41.04 ± 3.71*	33.48 ± 2.45*	38.04	36 ± 2.85*	29.88 ± 1.35*	45.8
T2	39.6 ± 3.77*	36.7 ± 5.04	24.5	45.36 ± 1.84*	38.16 ± 1.54*	42.2	44.28 ± 3.93*	35.28 ± 4.21*	45.7	36 ± 3.27*	30.24 ± 2.23*	57.9
T3	36.04 ± 1.84*	34.56 ± 2.5*	26.5	43.92 ± 2.39*	39.16 ± 2.89*	45.8	43.92 ± 2.88*	33.84 ± 2.44*	47.2	38.16 ± 2.44*	33.84 ± 2.51*	73.1

area also supports this statement. There were also numerous congested areas. This is may be due to the compound effects caused by glucocorticoid induced hypertension and increased arteriovenous pressure due to LDL accumulation narrowing the lumen of the vessels (Zadeh et al., 2002).

4.4. Histomorphometric alterations

In histomorphometric study, the kidney glomeruli revealed both dose and age dependent decrease of diameter. This clearly indicates the decrease of glomerular area which mismatches the findings of Ortiz et al. (2002), who described non-significant increase of glomerular area in response to prenatal DEX treatment. Looking as an overview at the obtained data from present study, it is evident that DEX treatment affects the gross and histological morphology and morphometry of kidney. The deleterious effects of DEX on kidney glomeruli and tubules may lead to reduced glomerular filtration rate and ultimately renal failure with associated cardiovascular problems like hypertension, venous congestion and heart failure.

5. Conclusion

Alterations in biochemical parameters, gross anatomy, histomorphology and histomorphometry of kidney of broiler due to different doses of dietary DEX were studied. In biochemical analyses, serum HDL level significantly decreased in all treated groups and serum LDL level decreased in T1 group at day 21 of the experiment. Gross study revealed dark congested kidney with reduced weight and size compared to the control. In histomorphological observation, various degree of congestion, inflammation and fibrosis were observed in the DEX treated groups. Degenerated, atrophied glomeruli and tubules were found distorted with degenerated tubular epithelia. Percentage of atrophied glomerulus was significantly increased as high as 73.1% at day 28 in T3 group. Wide necrotic area was found in T2 group at day 28. Biometric study showed significant decrease of glomerular diameter which also fortifies the findings of histological observation. All the data obtained in this study clearly indicates that dietary DEX changes serum HDL and LDL level as well as alters general gross and histomorphological characteristics of kidney. These alterations may lead to renal failure and associated abnormalities like hypertension, heart failure and ultimately fatality. However, further study is recommended to investigate the effects of dietary DEX on glomerular filtration rate, and its association with cardiovascular system. Moreover, the expression pattern of GC receptors in DEX treated broiler kidneys needed to be studied in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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