Research Article

Ultrasonographic and Histoarchitectural Evaluation of Liver and Kidneys in Black Bengal Goats While Drinking Saline Water

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Abstract | Natural water sources are becoming contaminated due to the salinization of surface and groundwater. This investigation aimed to examine the effects of water salinity on the ultrasonographic and histological appearance of the liver and kidneys. Twelve goats were divided into three groups. In group 1, all goats were given tap water as a control (TW) for eight weeks, whereas Group 2 goats received tap water with 0.5% salt (MSW), and Group 3 goats received tap water with 1.0% salt (HSW). Blood samples were taken from each animal weekly. Ultrasonography was conducted at the start and end of the experiment. Histopathological examination of liver and kidney samples was performed. Serum levels of ALT, AST, urea, uric acid, and creatinine in all groups did not differ significantly (P>0.05). At the end of the experiment, ultrasonographic examination of the liver surface showed an anechoic appearance in group 3. Histopathology of the liver in group 3 showed mild hepatocellular degeneration, dilatation of blood vessels, bile duct proliferation, and infiltration of inflammatory cells within the hepatocytes. Ultrasonographic examination of the left and right kidneys in group 3 revealed an anechoic appearance with a few hypoechogenic dots. The histopathological appearance of the kidneys in group 2 showed tubular necrosis with accumulation of water within the tubule and vacuole formation. In group 3, the histopathological appearance of the kidneys showed tubular dearrangement, necrosis (cellulitis), and severe hemorrhage. It is concluded that water salinity affects the function and appearance of the kidneys and liver in Black Bengal goats.

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Introduction

Goats are widely farmed in the dominant subsistence livestock farming system of Bangladesh. The size of the goat population in the country is 27.1 million, which appears to be the second largest in number, after poultry (DLS, 2024). The production potentials of goats are reliant on good health and nutrition management. Freshwater availability is a crucial input for maintaining animal health and achieving the desired farm productivity. Unfortunately, natural water sources in the coastal areas of Bangladesh are getting increasingly contaminated with salinity. It is anticipated that growing salt pollution of natural water sources may hamper livestock production in some regions of Bangladesh, which is also relevant in many other parts of the globe.

Animals that consume too much salt develop hypertension (Reynolds *et al*., 2013), elevated heart and breathing rates, muscular spasms, neurological conditions, and water retention (Kii and Dryden, 2005). Increased water salinity diminishes extracellular and interstitial fluids, blood and plasma volume, and urea concentrations in sheep (Assad and El-Sherif, 2002; Meintjes and Engelbrecht, 2004), while it can also decrease milk production in cows (Arjomandfar *et al*., 2010).

In animals and humans, the liver and kidneys are vital organs for maintaining water balance, electrolyte homeostasis, sodium conservation (Stevens *et al*., 2006), excreting metabolic waste products (Stevens *et al*., 2005), and detoxifying endogenous and exogenous compounds (Sallie *et al*., 1991). Any alterations in liver and kidney functions can be diagnosed by histological assessments and by performing many hemato-biochemical tests (Imran *et al*., 2011). Alternatively, non-invasive methods like ultrasonograms could also be used in assessing liver and kidney performance (Afshar *et al*., 2008). Ultrasound diagnostic methods can provide crucial clinical information on liver and kidney morphology without hampering the productivity of farm animals (Streeter and Step, 2007) or violating the animal welfare issues (Tharwat *et al*., 2024). Portable ultrasound tools can be utilized conveniently to diagnose farm animal pregnancy and internal disease conditions, even in farming conditions (Lazaridis and Kiossis, 2018).

In the context of increasing water salinity in many parts of the earth, it is important to see if goats can adapt to salty drinking water without suffering negative health consequences. Several researchers have investigated the effect of water salinity on human health in Bangladesh so far (Khan *et al*., 2011; Talukder *et al*., 2016). Our previous discoveries during the experiments on the Boer goats under European climatic conditions also revealed some interesting findings on goats' physiological responses to drinking water with different salinity concentrations (Runa *et al*., 2019a, b). The focus of earlier investigations was mostly on the effect of higher drinking water salinity on the farm animal production performance and physiological responses in arid and desert environments (Ghanem *et al*., 2018). On the other hand, until now, nothing is known about how water salinity affects the histological and ultrasonographical appearance of the liver and kidney in Black Bengal goats.

To our knowledge, no information exists on the production, health, or appearance of goats' livers and kidneys following consistent consumption of saline water in salinity-affected areas of Bangladesh, where animals are obligated to drink water with high salinity. Hence, we decided to use ultrasonography and histopathological techniques to assess the effect of drinking saline water on the liver and kidneys of Black Bengal goats.

Materials and Methods

During the experiment, the ethical research regulations approved by Bangladesh Agricultural University (ref. no.: AWEEC/BAU/2022 (26) were maintained duly.

Animals and management

The experiment was conducted on twelve Black Bengal goats purchased from local markets and kept at the Animal Research Farm, Department of Surgery and Obstetrics (DSO), Bangladesh Agricultural University, Mymensingh, Bangladesh. The goats aged between one and two years had an average body weight (BW) of 10.91 ± 1.71 kg. The experimental period was June-September 2021. Before the experiment, all goats were clinically examined to ensure their health status. They were then dewormed by oral administration of Fenbendazole and subcutaneous administration of Ivermectin, manufactured and marketed by Techno Drugs Ltd, Bangladesh. Subsequently, all goats were immunized to protect from tetanus infection by using Tetanus Toxoid (TT. Vax™, Popular Pharmaceuticals Ltd., Bangladesh) and allowed to feed and drink *ad libitum* during the experimental period.

For acclimatization purposes, the experimental goats were moved to the experimental pens two weeks before commencing the trials. A comfortable environment was created inside the pens by providing sufficient straw for bedding. Regular cleaning of the pens and proper ventilation were also ensured. One water bucket (diameter 28 cm, 10 l capacity), one feed trough (diameter 53 cm), and a separate bucket with the mineral supplement were provided for each pen.

Experimental design

Throughout the study, the goats were allocated into three subgroups (n= 4). In group 1, all goats were allowed to drink tap water (TW), which was considered as control. Moderate saline water (MSW: tap water with 0.5% salt) and high saline water (HSW: tap water with 1.0% salt) were provided to all goats and allowed to drink freely in groups 2 and 3, respectively.

Clinical examination

The condition of the nasal mucous membrane, ruminal movement, respiratory and pulse rates, and body temperature of goats were measured as part of routine clinical examinations to ensure they were healthy.

Blood sampling and serum biochemical analysis

Blood samples were collected from the jugular vein of the experimental goats. Samples were collected at the beginning of the trial and afterward once per week throughout the experimental period. For this purpose, a 19-gauge needle connected to a 10 ml disposable syringe was used. The collected blood was transferred to a labelled serum collection tube immediately after the collection. Then the tubes were carried to the hematology laboratory for biochemical analyses. The blood samples were centrifuged at room temperature for 15 minutes at 3000 rpm using a Tabletop Centrifuge (DSC-200-A2, Digisystem Laboratory Instruments Inc., Taiwan). After being pipetted into labeled Eppendorf tubes, the serum samples from the supernatant were kept at -20°C until further examination.

The CBS-30 Electrolyte analyzer and BS-5010 Chemistry analyzer were used to measure the blood electrolytes and other biochemical parameters, respectively, at the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were determined by the UV enzymatic Kinetic method using Cromatest (Linear Chemicals, Barcelona, Spain). Urea and uric acid were measured by the enzymatic colorimetric Endpoint method using Cromatest (Linear Chemicals, Barcelona, Spain). Creatinine was measured by the Kinetic colorimetric method using Cromatest (Linear Chemicals, Barcelona, Spain).

Ultrasonographic examination

Ultrasonography was performed using a portable ultrasound machine (MyLabFive Vet) with a 5 MHz curved linear probe, utilizing a standardized scanning process at the beginning of the investigation and after the saline water treatment was completed. The hair over the dorsal and ventral flank regions was cut, alcohol-washed, and coated using ultrasonographic gel to enhance the image quality. All ultrasonographic evaluations were performed in the standing and lateral recumbent positions. The scanning protocol started from the right kidney, directly before the 13th rib, and in the ventral region of the transverse process of the lumbar vertebrae. The liver was scanned between the 12th and 7th intercostal gaps on the right side of the belly. The size and location of the liver, along with the portal and hepatic veins diameters, were measured in each intercostal area. The pre-trial ultrasonographic findings were used as the baseline for changes in the liver and kidneys caused by drinking water salinity.

Histopathological examination

One animal from each group was humanely sacrificed at the end of the trial. The esophagus and rectum were clamped after the visceral cavity was opened to prevent the leaking of gastrointestinal contents and contamination of adjacent intestinal regions. The liver and kidney sites were standardized and exposed with sterile equipment as soon as the gastrointestinal tract was removed from the visceral cavity. Kidney and liver samples were collected (about 2 cm pieces of each) and placed into 10% neutral buffered formalin. They were then cleaned, dehydrated using ethyl alcohol in increasing grades, clarified using methyl benzoate, and embedded in paraffin wax. Before viewing under a microscope, several paraffin slices (3-5 microns thick) were cut and stained with hematoxylin and eosin.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 22 software. The data were compared using a one-way analysis of variance (ANOVA). *P-value* was adjusted by comparing all pairs using the Tukey HSD test. The values are presented as the mean ± standard error (SE). All differences were considered significant when $P<0.05$.

Results

Changes in liver functions

In the experimental goats (Table 1), compared to group 1 (100.45±9.51 and 23.20±2.89) and group 2 (111.68±7.69 and 21.97±5.26), both the AST and ALT were comparatively higher in group 3 (113.66±9.56 and 24.60±3.69). However, the differences were statistically insignificant (P>0.05).

Changes in kidney function

The creatinine, urea, and uric acid were relatively higher in group 2 than in groups 1 and 3 (Table 1). But the changes were statistically insignificant (P>0.05).

Changes in blood electrolytes

There were also no significant differences (P>0.05) in different blood electrolytes (potassium, sodium, and chloride) in groups 1, 2, and 3 (Table 2).

Table 1: *Changes of liver and kindey enzymes among different groups in Black Bengal goats (Mean ± SEM).*

Parameters	Group 1	Group 2	Group 3	P value
AST (U/L)	100.45±9.51	111.68±7.69	113.66±9.56	0.536
ALT (U/L)	23.20±2.89	21.97±5.26	24.60±3.69	0.901
Creatinine (mg/dL)	0.43±0.03	0.58±0.08	0.49±0.06	0.242
Urea (U/L)	48.11±3.0	53.27±3.08	53.06±2.17	0.341
Uric acid (mg/dL)	1.12±0.14	1.70±0.58	1.11±0.15	0.411

Table 2: *Different blood electrolytes among groups 1, 2, and group 3 (Mean ± SEM).*

Parameters	Group 1	Group 2	Group 3	P value	Reference range
Potassium (mmol/l)	5.09±0.38	5.01±0.26	5.76±0.58	0.409	3.4-6.1[1]
Sodium (mmol/l)	142.48±4.46	140.59±2.46	144.96±4.32	0.725	135-156[1]
Chloride (mmol/l)	109.27±3.68	108.29±1.92	109.55±3.56	0.957	98-110[1]

[1]*Jackson and Cockcroft (2002).*

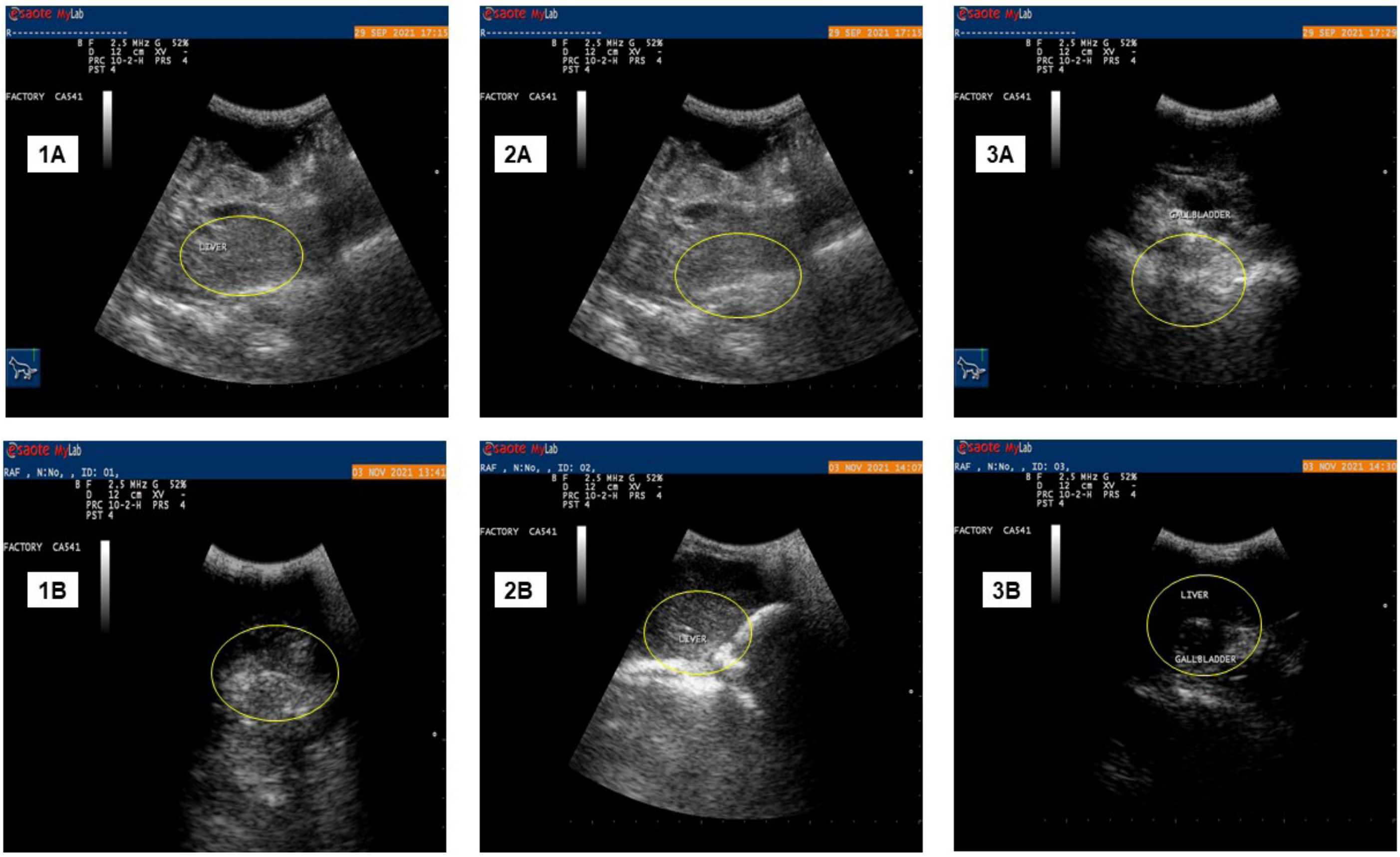

Figure 1: *Ultrasonographic examination of the liver in groups 1, 2, and 3 (1A, 2A, and 3A) at the onset of the experiment and (1B, 2B, and 3B) at the end of the experiment, respectively.*

Ultrasonographic examination of the liver

At the onset of the experiment, all goats in groups 1, 2, and 3 had normal echogenicity and no hyperechogenic dots in their liver (Figure 1; 1A, 2A, 3A). At the end of the experiment, the liver surface showed an anechoic appearance (black), normally resulting from the fluid-structure in group 3, where the goats consumed higher concentrations of saline water compared to groups 1 and 2 (Figure 1; 1B, 2B, 3B).

Ultrasonographic examination of the kidneys

At the beginning of the experiment, all goats in the TW, MSW, and HSW groups had normal echogenicity in both left and right kidneys (Figure 2). At the end of the experiment, goats of the MSW (2) and HSW groups (3) showed an anechoic appearance (black) in the left kidney and an anechoic with few hypoechogenic dots (white) in the right kidney (Figure 3), which is normally resulting from the fluid texture of the organ.

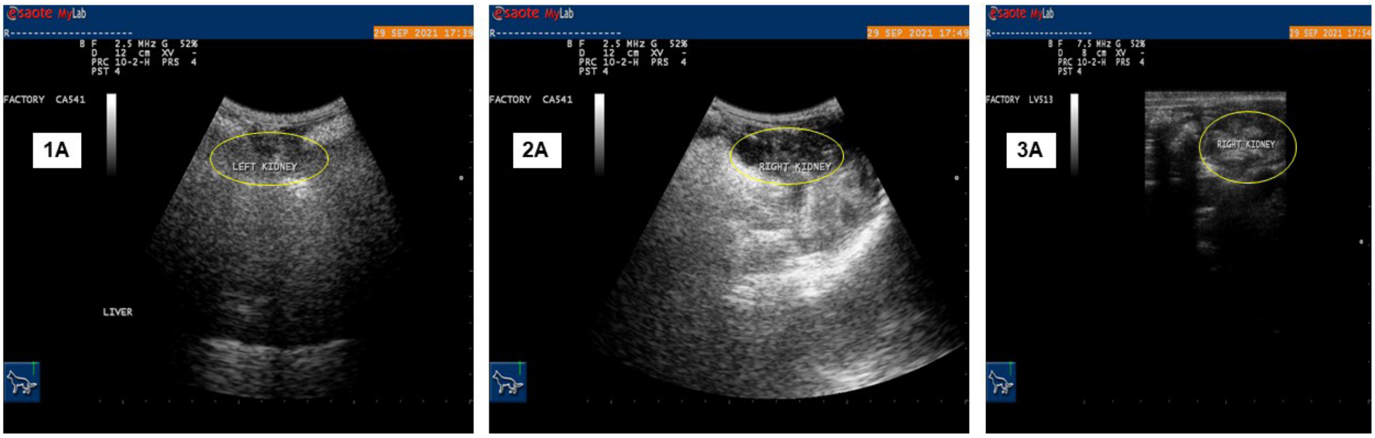

Figure 2: *Ultrasonographic examination of both kidneys in all groups of goats at the onset of the experiment. 1A, 2A, and 3A represent the figures of group 1, 2, and 3, respectively.*

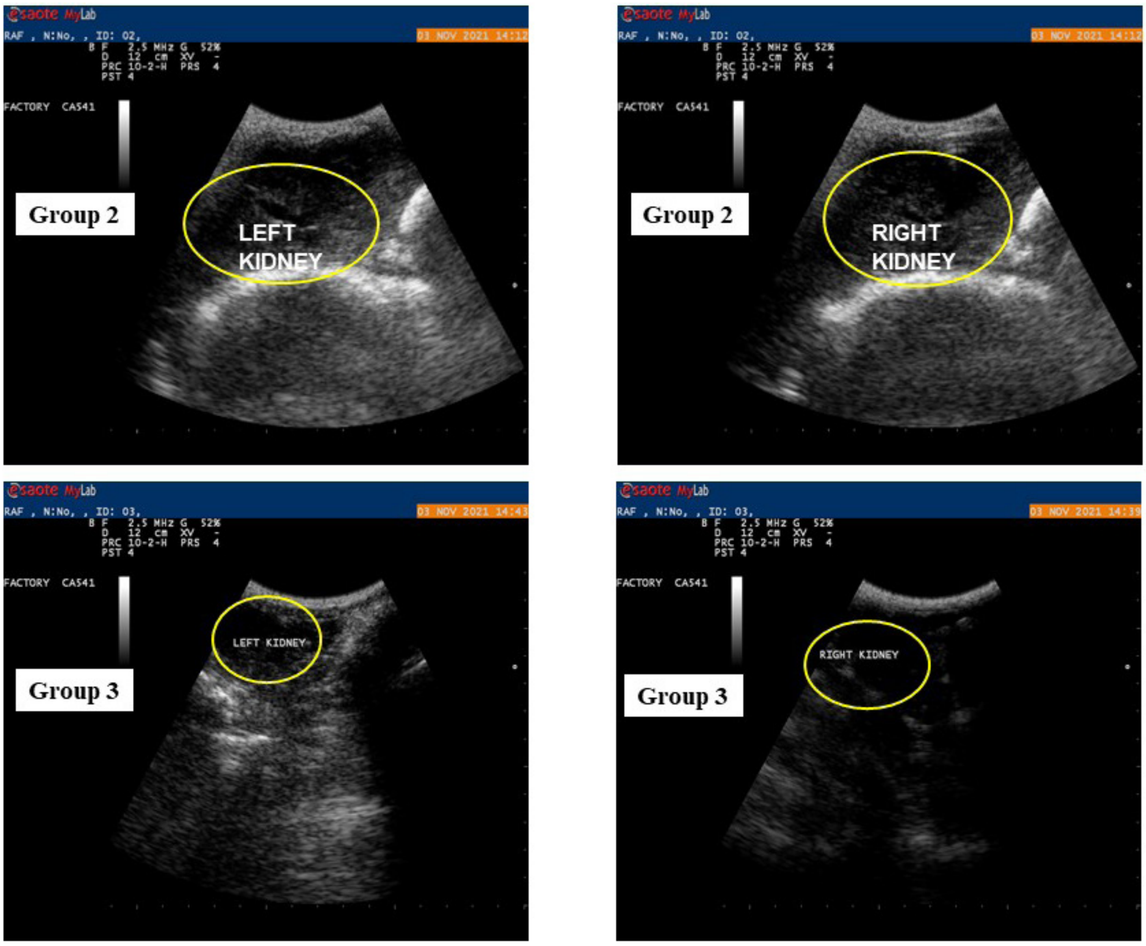

Figure 3: *Ultrasonographic examination of both kidneys in groups 2 and 3 at the end of the experiment.*

Histopathological examination of the liver

Histopathological changes of the liver in different groups are presented in Figure 4. The results show that at the end of the study, the liver of the goats in the control group (group 1, supplied with normal tap water) had a normal hepatocellular structure, and goats supplied with moderate saline water (MSW) in group 2 showed focal aggregation of inflammatory cells. Histopathology of the liver in goats supplied with high saline water (HSW) in group 3 showed mild hepatocellular degeneration, dilatation of blood vessels, bile duct proliferation, and infiltration of inflammatory cells within the hepatocytes. It was evident that goats supplied with different levels of saline drinking water had various levels of effect of the saline drinking water at the cellular level, without changing the gross appearances and structure of the liver.

Histopathological examination of the kidney

At the end of the study, the control (Group 1) group exhibited normal histopathological glomerular and tubular structures. The histopathological appearance of the kidneys in group 2 showed tubular necrosis with accumulation of water within the tubule and vacuole formation. In group 3, the histopathological appearance of the kidneys showed tubular dearrangement, water accumulation in the tubule, vacuole formation, and necrosis (Figure 5). Severe hemorrhage was also visible (Figure 6).

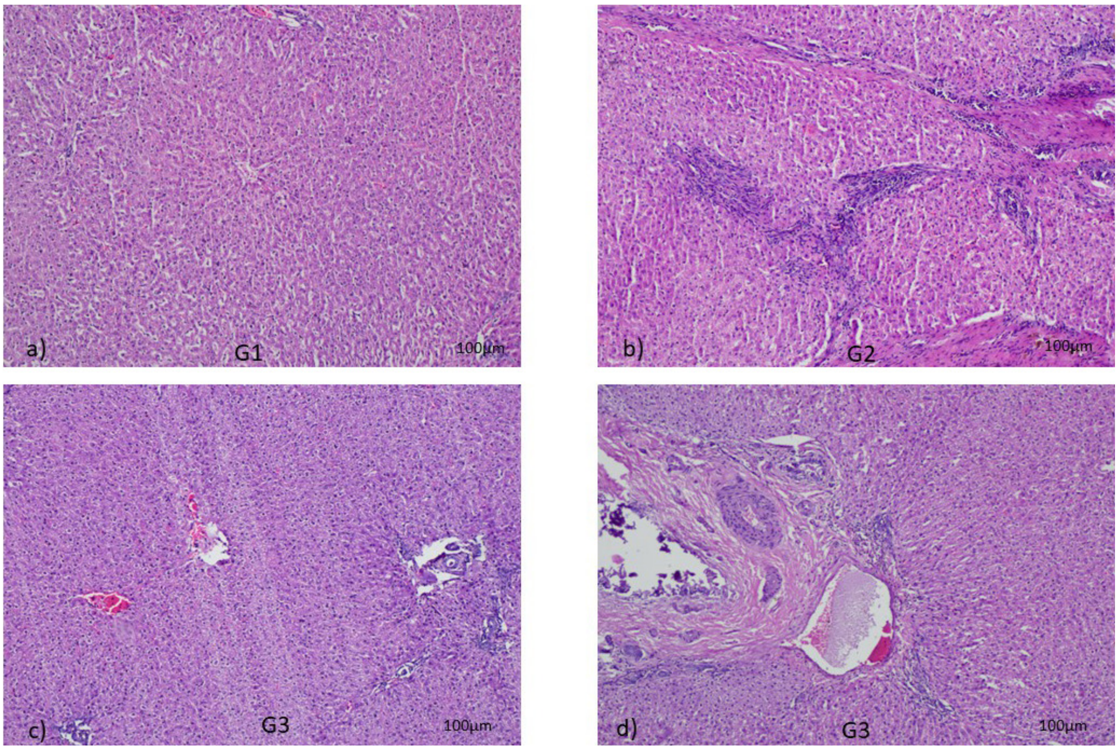

Figure 4: *Histopathology of liver (a) control (G1) appeared normal hepatocellular structure. (b) G2 showed focal aggregation of inflammatory cells. (c) G3 showed mild hepatocellular degeneration. (d) G3 showed dilatation of blood vessels, bile duct proliferation, and infiltration of inflammatory cells within the hepatocytes.*

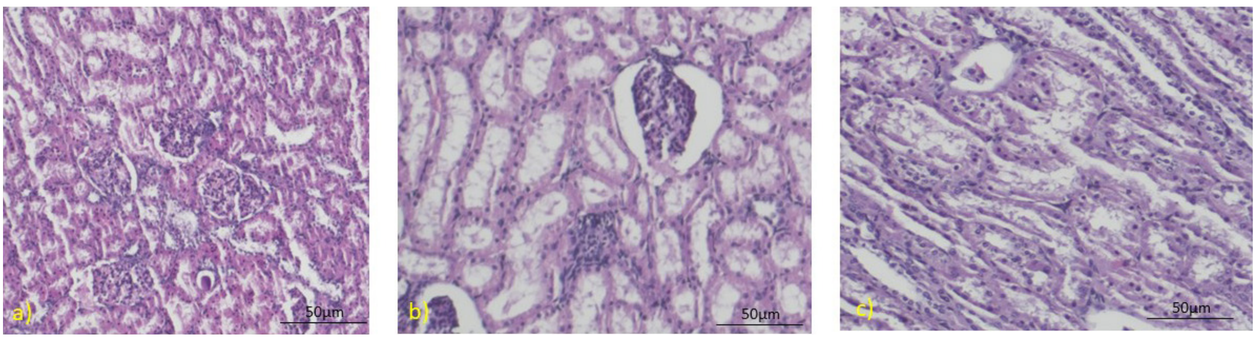

Figure 5: *Tubular change (A) control (Group 1) appeared normal glomerular and tubular structures. (B) Group 2 showed tubular necrosis with an accumulation of water within the tubule, vacuole formation. C) G3 showed tubular dearrangement, necrosis (cellulitis).*

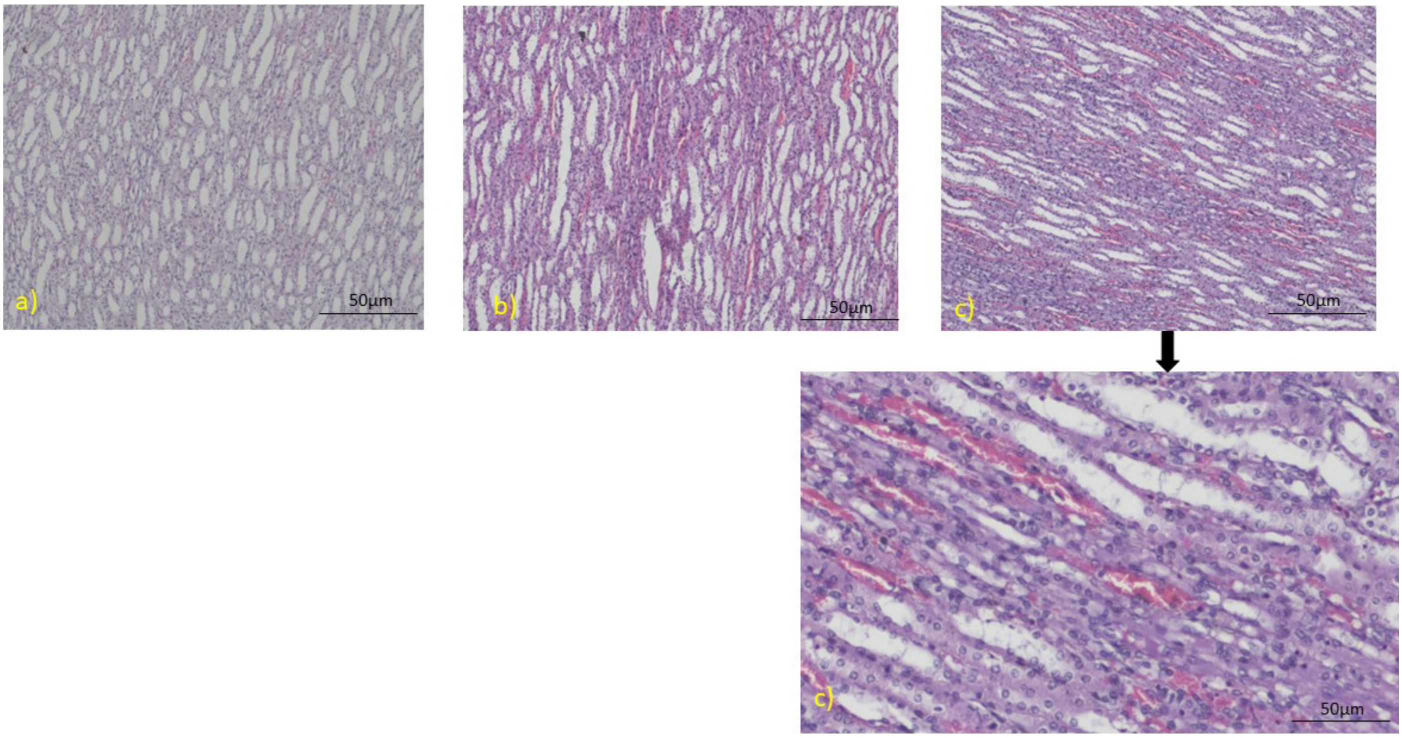

Figure 6: *Normal tubular structures of the kidney (a) Moderate to severe tubular hemorrhage (b+c)*

Discussion

In this study, we compared the effects of drinking saline water on the liver and kidneys of Black Bengal goats using ultrasonography and histological findings. The findings are important in the context of vital physiological roles of the liver and kidneys in maintaining the body's homeostasis and controlling metabolic processes (Stevens *et al*., 2005). According to Hussein and Elrashidy (2014), liver function tests in ruminants are not particularly useful for identifying liver disorders. Hepatic function tests cannot distinguish between various conditions that typically cause focal changes in the liver and metabolic disorders induced by diffuse liver alterations (Ettinger and Feldman, 2009). Compared to different invasive liver and kidney function tests, ultrasonography is a safe, accurate, quick, and non-invasive way to monitor liver and kidney problems in small ruminants.

An elevated AST and ALT value may indicate liver damage and liver cirrhosis (Gowda *et al*., 2009). Sheep supplied with different concentrations of saline drinking water showed a remarkable elevation of serum ALT, AST, urea, and creatinine with clinical symptoms of impaired liver and renal functions (Ghanem *et al*., 2018). Goats ingesting highly salinized water had noticeably elevated AST and ALT levels (Runa *et al*., 2022). These results are consistent with our results.

Goats ingesting saline water for a longer duration showed increased plasma K+ level (Runa *et al*. 2020). However, the results of our experiment (Table 1) show that there was no significant variation in serum sodium, potassium, and chlorine levels. This might happen due to the ingestion of saline water for a shorter duration. The findings on plasma mineral levels are also consistent with the results found in a similar study by Zoidis and Hadjigeorgiou (2018).

The ultrasonographic appearance of the liver and kidney of a goat has been thoroughly described by Scott (2017). The ultrasonographic method can be utilized in identifying fascioliasis, fatty liver, glomerulonephritis, hydronephrosis, urolithiasis, and other abdominal disease conditions in small ruminants. In the current study, the kidneys and liver of saline-treated and healthy goats were examined using ultrasonography in a standing position. The ultrasonographic examination of the goats supplied with only tap water (group 1) revealed liver fibrosis caused by fascioliasis. At the end of the trial, the liver surface in group 3, where the goats drank saline water at a higher concentration (1.0%), exhibited an anechoic appearance. Similar increases in echogenicity were observed after a cystic echinococcosis infection in sheep exhibiting clinical signs of hemorrhage and necrosis (Hussein and Elrashidy, 2014).

The histo-pathological features of the liver of the

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goats supplied only with tap water (group 1) showed a typical hepatocellular structure, group 2 displayed localized aggregation of inflammatory cells, and group 3 displayed modest hepatic deterioration, blood vessel enlargement, bile duct growth, and inflammatory cell infiltration into the hepatocytes. These hepato-cellular changes were in agreement with a previous study by Afshar *et al*. (2008).

Measuring the size of the kidneys provides important information to diagnose hydronephrosis (Attia-Ismail *et al*., 2008). The echogenic spots on the kidneys indicate that crystals were formed inside the kidneys, which is consistent with the findings of Ghanem *et al.* (2018). The blood was biochemically examined, and the goats in the HSW group had higher levels of urea and uric acid. Ghanem *et al*. (2018) speculate that the kidneys' appearance of echogenic dots could be due to an increase in uric acid crystals that formed because of the urine's lower pH. The male urinary system structure (long and narrow urethra with sigmoid flexure and urethral process) predisposes them to crystal deposition more than the female urinary system structure (short urethra, absence of sigmoid flexure and urethral process). The statement, however, did not corroborate our findings because the present study only included female goats.

The histology results in this study corroborated the ultrasonographic data and confirmed the changes in liver and kidney morphology in the goats treated with different concentrations of saline water. Significant histological abnormalities were observed in the right and left kidneys of the MSW and HSW groups at the study's completion. There were no changes in the TW control group. The TW group's glomerular and tubular architecture seemed normal. Consuming 0.5–1.0% saline water damages kidney tissue severely, resulting in tubular necrosis, tubule rearrangement with water buildup inside, and development of vacuoles in groups 2 and 3.

Conclusions

Drinking saline water causes significant structural damage to the kidneys and livers of Black Bengal goats, characterized by tissue hemorrhage and cellular degeneration. Advanced imaging reveals these internal changes through distinct anechoic patterns, signalling severe fluid imbalances and inflammation. Ultimately, high salt concentrations in drinking water pose a direct threat to livestock health by disrupting vital organ functions.

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Novelty Statement

Previous studies have examined the physiological responses and productivity of farm animals. This study provides a comprehensive understanding of how consistent saline water intake affects the liver and kidney functions of the Black Bengal goat, especially in Bangladesh's salinity-affected regions. The result contributes to new insights supporting the use of ultrasonography and histopathology in assessing the impact of consuming saline water.

Authors Contribution

RAR: Designed and supervised the study, data analysis, edited, provided critical evaluation of this manuscript and acquired fund for the research. MAR: Performed this experiment, data collection, acquisition, and drafted the manuscript. MMI: revised the manuscript and provided critical suggestions on this manuscript. MMR: Data collection and acquisition. MSS: Reviewed the manuscript and arrangement of data. NSJ: Provided critical suggestions and reviewed the manuscript.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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