Effect of Yogurt on Fluoride Induced Toxicity in Rabbits

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Abstract

Fluoride (F) is one of the major chemical substances that damage the renal tissue, resulting in an increase in the excretion of sodium (Na+) and chloride (Cl-), and enhancing creatinine (Cr), uric acid (UA), blood urea nitrogen (BUN), and potassium (K+) levels. Results revealed that on the 16th day in groups B, D, E, and G, Cr values were significantly (P<0.05) increased. On the 31st day in group F, the Cr value was significantly (P<0.05) decreased, whereas in groups B, D, E, and G, Cr values were significantly increased (P<0.05). On the 16th and 31st day, in yogurt-treated groups (C and F), UA values were significantly (P<0.05) decreased, whereas in groups B, D, E, and G, UA values were significantly (P<0.05) increased. On the 16th and 31st day, in groups B, D, E, and G, BUN levels were significantly (P<0.05) increased. On the 16th and 31st day, in groups B and E, Na+ levels were significantly (P<0.05) decreased, whereas in groups C, D, F, and G, Na+ levels were significantly (P<0.05) increased. Likewise, on the 16th and 31st day, in groups B, D, E, and G, K+ levels were significantly (P<0.05) increased. On the 16th day, in groups B and E, Cl- levels were significantly (P<0.05) decreased, while in groups F and G, Cl- levels were significantly (P<0.05) increased. On the 31st day, in groups B and E, Cl- levels were significantly (P<0.05) decreased, while in groups C, D, F, and G, Cl- levels were significantly (P<0.05) increased. It has been concluded that increased levels of Cr, UA, BUN, K+, and reduced levels of Na+ and Cl- were observed in all fluoride-treated groups. Yogurt-treated groups showed decreased levels of

Cr, UA, BUN, and K+, and increased levels of Na+ and Cl- in experimental rabbits.

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Introduction

Fluoride is extensively used and broadly distributed throughout the world in numerous forms. The presence of fluoride in the environment could vary depending on its chemical nature. The elemental fluorine (F) never occurs in nature in a free state due to its chemically active properties. It is a component of minerals found in rocks and soil and is created when it reacts with a variety of other elements (Barbier et al., 2010). In the atmosphere, fluoride compounds exist in various forms such as hydrogen fluoride, carbon tetrafluoride, and sodium fluoride (Mattsson and Paulus, 2020). In different countries, more than 260 million people suffer from fluorosis due to the improper use of fluoride, among them China is one of the major countries with most fluorosis cases (Herath et al., 2018). Fluoride entry in the human body via respiration, fluoride-containing products such as fluoride gel, dental products, mouthwash, toothpaste, and fluoride supplements (tablets, chewing gums, etc.) (Singh et al., 2020). The absorption of fluoride takes place through intestinal mucosa and then inhibits metabolic pathway (Zhou et al., 2020). Reportedly, serum creatinine level increased after giving 20 parts per million sodium fluoride in rats (Al Salhen and Mahmoud, 2016). The amount of urinary N-acetyl glycosaminidase activity, serum creatinine (Cr), uric acid (UA), and blood urea nitrogen (BUN) levels as well kidney lactate dehydrogenase (LDH) was elevated in the 12mg/kg, 24mg/kg, and 48mg/kg groups. Fluoride treatment lowers renal function as seen by the activity of renal Na+/K+-ATPase and ACP (acid phosphate) decreasing (Xiong et al., 2007). Yogurt is one of the most widely consumed fermented dairy products in the world, with a high level of public acceptance due to its health advantages (Weerathilake et al., 2014). *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subspecies *bulgaricus* are the most active bacterial strains in yogurt. Lactic acid bacteria (LAB) aid in the absorption of nutrients, vitamin production, and the prevention of unwanted microflora in the gut (Brodziak and Król, 2016). A harmful uremic toxin known as indoxyl sulfate accumulates in the plasma of those people suffering from chronic kidney diseases (CKD) (Niwa, 2010). This substance damages the cellular structure of renal tubular cells, glomerular mesangial cells, and vascular smooth muscle cell (Ng et al., 2014). In vitro studies reported that *Streptococcus thermophilus* reduces indoxyl sulfate. Combinations of *Streptococcus thermophilus* have shown great effectiveness in CKD, by decreasing the buildup of circulating uremic toxins (Pisano et al., 2018). Some probiotic bacteria consume urea, uric acid, creatinine, and another toxin as nutrition for growth. These harmful waste buildup in the circulation because of overworked and damaged kidney (Ranganathan, 2015).

Objectives

The objectives of this study were to determine fluoride-induced toxicity in the kidney and to assess the protective effect of yogurt against fluoride toxicity in the kidney of experimental animals.

Materials and Methods

Experimental animals and adaptation

Twenty-eight adult male and female rabbits (1kg B.W.) were bought locally from the market. The rabbits were reared in cages at the animal house, Sindh Agriculture University, Tando Jam. Before starting experiments, all animals were dewormed and kept under hygienic conditions for 2 weeks as an acclimatization period.

Experimental design

Animals were randomly divided into seven experimental groups (4 rabbits/group). Group-A (Control), Group-B (F 50mg/rabbit), Group-C (yogurt 15g/rabbit), Group-D (F 50mg + yogurt 15g/rabbit), Group-E (F 100mg/rabbit), Group-F (yogurt 30g/rabbit), Group-G (F 100mg + yogurt 30g/rabbit). A standard feeding regimen was adopted, and water was given ad libitum to them. Animals in all treatment groups received the prescribed treatment once daily orally in 500ml drinking water for 30 days.

Assessment of renal function

On the 16^{th} and 31^{st} day, a two ml blood sample was collected from the jugular vein in plain vacutainer tubes (red top). Serum was separated through centrifugation. After centrifugation, the serum was kept at -4°C. Thereafter, the following parameters were examined, i.e., serum uric acid, blood urea nitrogen, serum creatinine, and electrolytes (sodium, potassium, chloride). These parameters were determined by a spectrophotometer analyzer using biosystem BTS-350 kits.

Statical analysis

The data was analyzed statistically and tabulated using computer software Student Edition of Statistics (SXW), Version 8.1 (Copyright 2005, Analytical Software, USA).

Results and Discussions

Fluoride is present in the environment, and it comes from natural sources such as rocks and soils, as well as from industrial activities. It is toxic if the concentration is high. Drinking water from deep wells may have excessive quantities of fluoride in some places (Li et al., 2015). One of the most essential organs for removing fluoride from the body is the kidney. Approximately 60% of the daily fluoride taken by healthy people is eliminated through urine under normal physiological conditions. As a result, the kidney is one of the soft tissues that is most exposed to fluoride concentrations (Dharmaratne, 2019).

Creatinine

Results reveal the serum creatinine concentration in control and treatment groups of rabbits. Results show that on the 16th day, in groups B, D, E, and G, serum creatinine values were significantly increased ($P < 0.05$). Whereas, on the 31st day in group-F, serum creatinine values were significantly decreased ($P < 0.05$). Whereas, in groups B, D, E, and G, serum creatinine value ($P < 0.05$) was significantly increased compared to control (Figure 1). The value of serum creatinine in rabbits increases when the dose and time duration are increased. Yogurt containing beneficial bacteria reduced the concentration of creatinine. Similarly, this investigation agreed with (Xiong et al., 2007), who observed the concentration of serum creatinine (Cr) increased in the 12, 24, and 48 mg/kg groups, resulting in fluoride reducing or impairing renal function in mice. (Al-safei and Al-Mashhadane, 2021) recorded that sodium fluoride at 20 mg/kg in rabbits increased creatinine concentration. (Yang et al., 2020) observed that probiotics in the gastrointestinal tract metabolized creatinine and decreased its level. This rise was a result of chronic kidney disease. Because these substances pass through a concentration gradient from the bloodstream to the gastrointestinal tract, they would aid in lowering the blood level of creatinine. Probiotics, including *Lactobacillus paracasei*, *S. salivarius, thermophilus*, and *Lactobacillus plantarum*, reduced the level of creatinine in plasma.

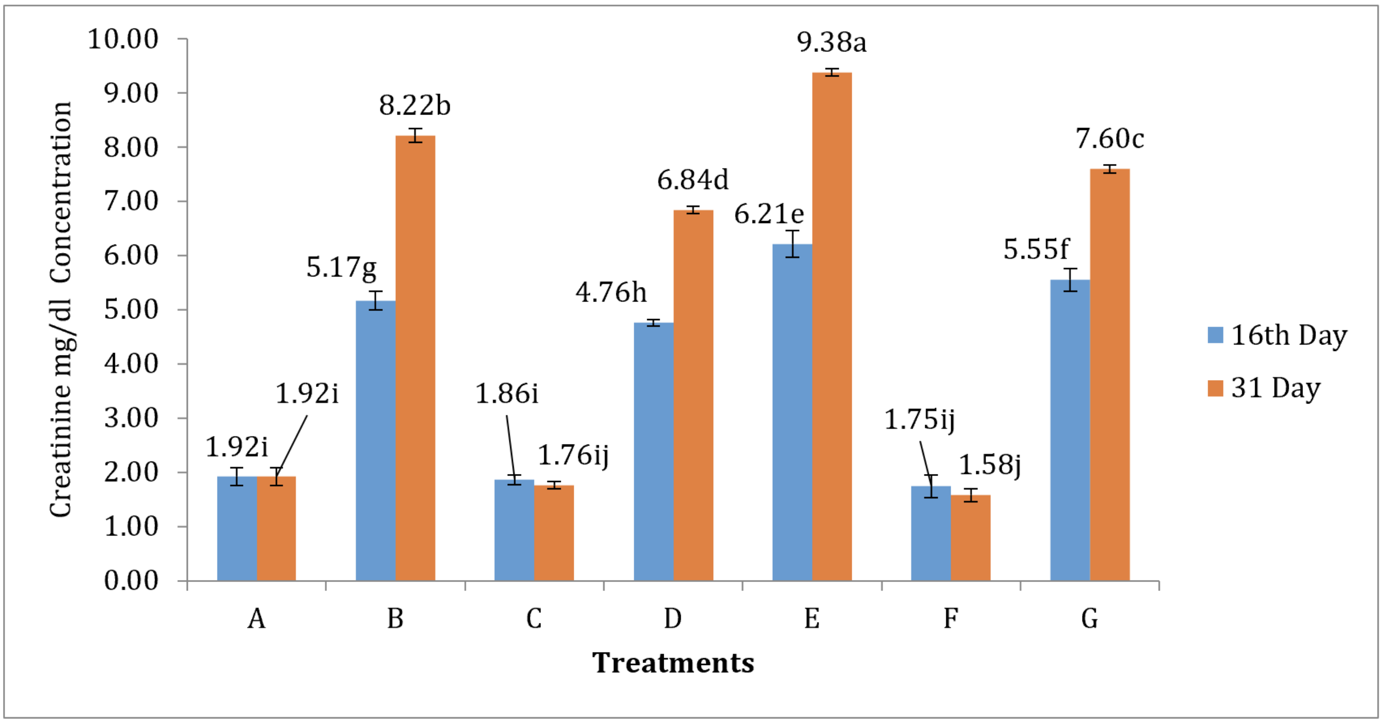

Figure 1. Serum creatinine concentration (mg/dL) in fluoride-induced toxicity and yogurt-supplemented amelioration in rabbits.

Uric acid

Data shown in (Figure 2) the serum uric acid concentration in control and treatment groups of rabbits. Results indicated that serum uric acid on the 16th day, in yogurt-treated groups i.e., groups C and F, serum uric acid levels were significantly decreased ($P < 0.05$). Whereas, in fluoride-treated groups i.e., groups B, D, E, and G, serum uric acid levels were significantly increased ($P < 0.05$). However, on the 31st day, statistically, a similar trend was noticed as indicated on the 16th day i.e., in groups C and F, serum uric acid levels were significantly decreased ($P < 0.05$), whereas, in groups B, D, E, and G, serum uric acid levels were significantly increased ($P < 0.05$) as compared to control. (Xiong et al., 2007) recorded that 12, 24, and 48 mg/kg fluoride in mice increased the concentration of uric acid in serum by increasing the dose of sodium fluoride. In the current study, yogurt containing beneficial bacteria reduced serum uric acid concentration. The present findings are consistent with (Choi et al., 2005) that there is a substantial inverse connection between dairy intake and serum uric acid level. Milk protein (casein and lactalbumin) has been demonstrated to lower blood uric acid levels in healthy people due to the uricosuric impact of these proteins. (Yamanaka et al., 2019) found that *lactobacillus gasseri* lowered serum uric acid levels in individuals with hyperuricemia and gout, which is characterized by a reduction in uric acid excretion or an increase in uric acid buildup due to aberrant purine metabolism. Reportedly, (Hamada et al., 2022) observed that *lactobacillus gasseri*-containing yogurt (85g) twice daily reduced the serum uric acid levels in patients suffering from marginal hyperuricemia.

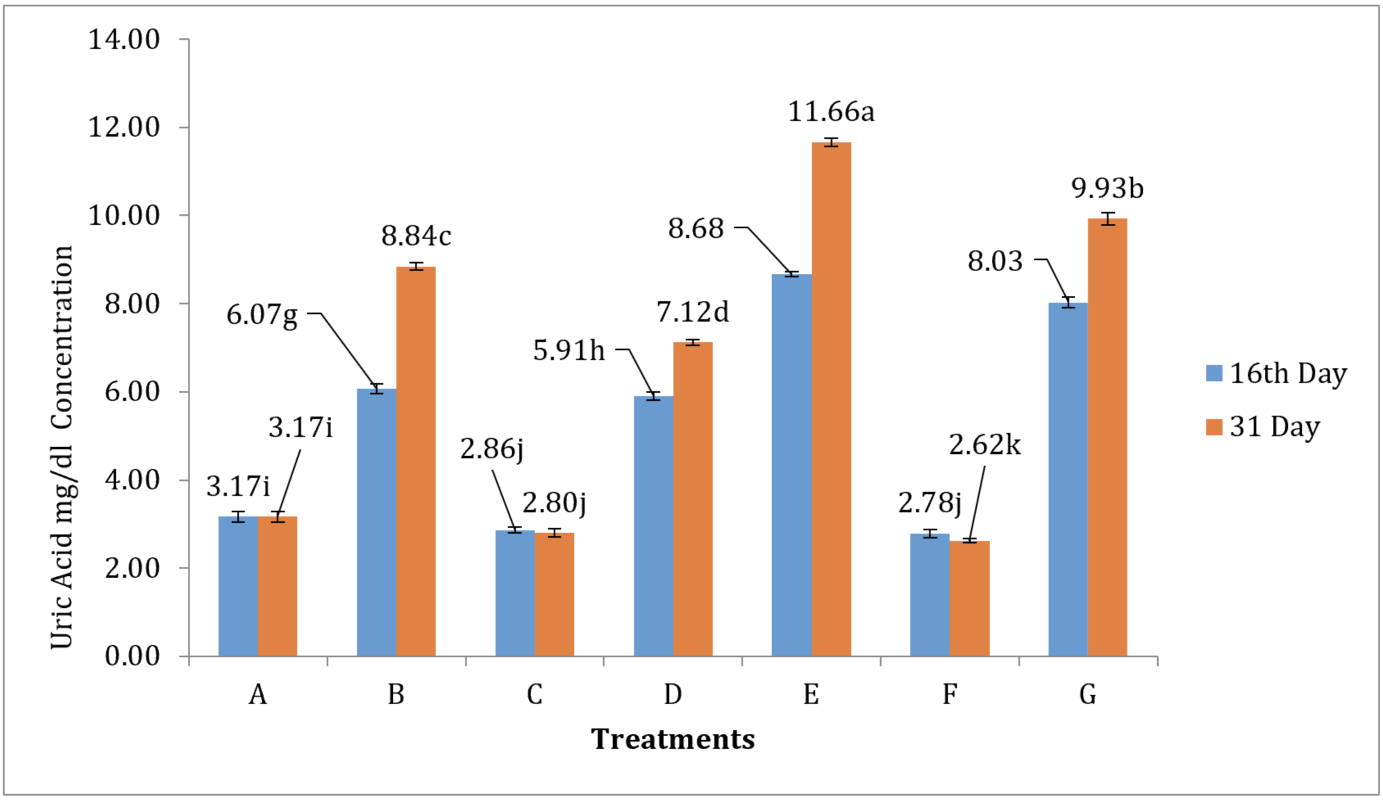

Figure 2. Serum uric acid concentration (mg/dL) in fluoride-induced toxicity and yogurt-supplemented amelioration in rabbits.

Blood urea nitrogen (BUN)

Serum blood urea nitrogen concentration in control and treated groups of rabbits shown in (Figure 3). Results indicated that on the 16th day, in groups B, D, E, and G blood urea nitrogen levels were significantly increased ($P < 0.05$). On the 31st day, mean blood urea nitrogen values similarly increased as on the 16th day i.e., groups B, D, E, and G blood urea nitrogen levels were significantly increased ($P < 0.05$) in comparison to control. The present study showed agreement with previous studies that the piglets fed fluoride 100 and 250 mg/kg for 50 days with a base diet showed an increased level of BUN (Zhan et al., 2006). Furthermore, in agreement, the concentration of BUN level increased when mice were treated with fluoride 12, 24, and 48 mg/kg, resulting in a decline in the function of the kidney (Xiong et al., 2007). It has also been reported that the short-term administration of probiotics reduced the serum blood urea nitrogen concentration and reduced the renal chances of renal disease by improving the renal function. Microorganisms from the lactobacillus and Bifidobacterium have been shown to reduce the concentration of blood urea nitrogen (Fagundes et al., 2018).

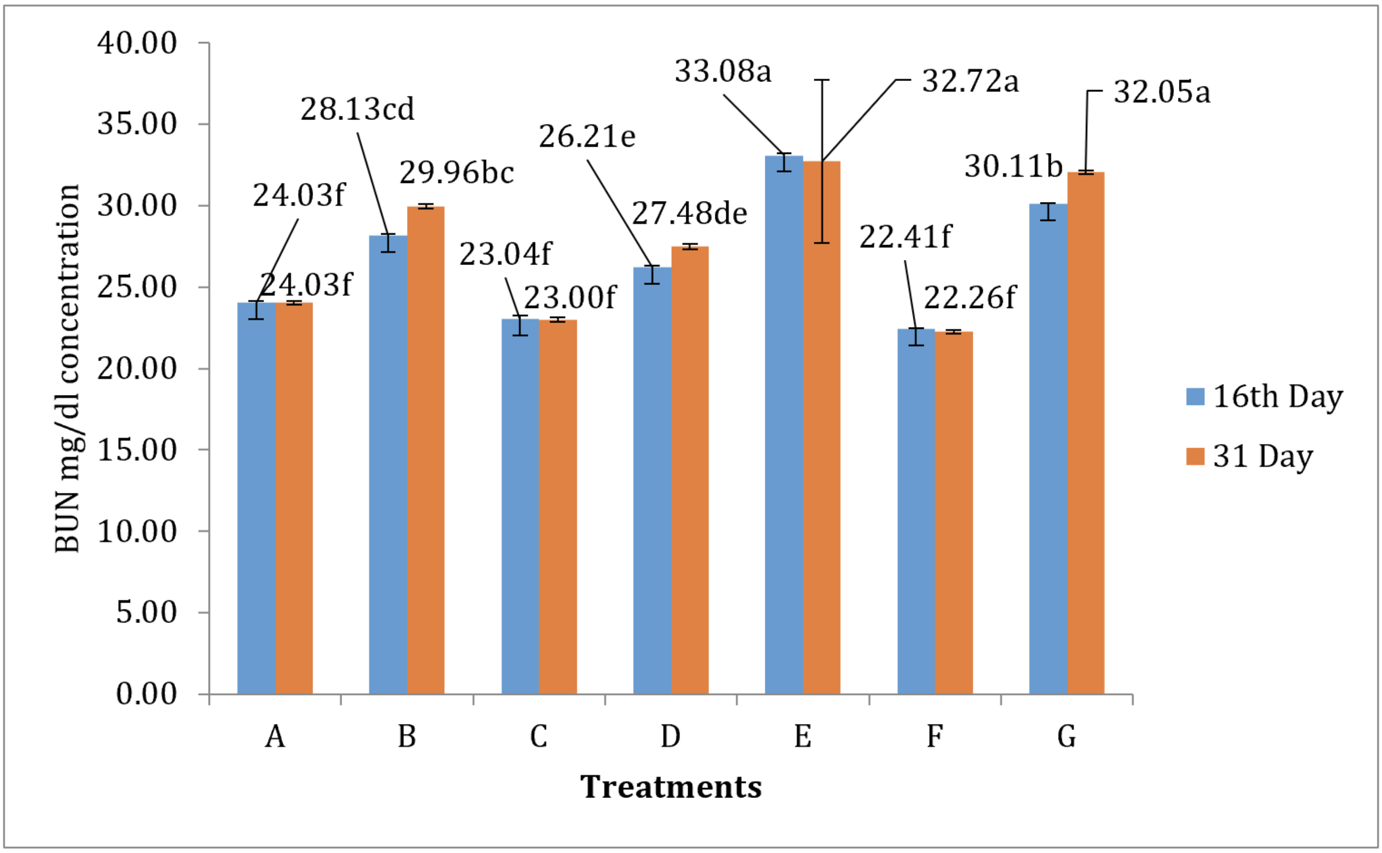

Figure 3. Serum blood urea nitrogen concentration (mg/dL) in fluoride-induced toxicity and yogurt-supplemented protection in rabbits.

Serum sodium

Data shown in (Figure 4) reveals the serum Na+ concentration in control and treated groups of rabbits. Results indicated that, on the 16th day, in groups B and E, Na+ levels were significantly decreased (P < 0.05). Whereas, in groups C, D, F, and G, Na+ levels were significantly increased (P < 0.05). On day 31st, in groups B & E, Na+ levels were significantly decreased (P < 0.05). Whereas, in groups C, D, F, and G, Na+ levels were significantly increased (P < 0.05) following treatment as compared to control. In agreement with the current results, it has been reported that piglets fed for 50 days with a base diet containing 100 and 250 mg/kg, show decreased serum Na+ levels. Ion may be affected via tubular reabsorption and osmotic disequilibrium between the luminal and medullary interstitial fluid (Agrawal et al., 2008). In agreement, (Barai et al., 2018) observed that the level of Na+ increased when treated with yogurt 50 ml, 100 ml, and 150 ml/kg B.W. (Girard et al., 2005) observed that the ingestion of castor oil considerably reduced net Na+ absorption from -196 ± 39 µm, in basal condition to 401 ± 100 µmole in experimental condition. In a dose-dependent manner, pre-treatment with *S. boulardii* at concentrations ranging from 1.2-12.0 x 1010 CFU/kg reduced the sodium secretory action of castor oil. The net sodium secretion was lowered to 89 ± 124 and 32 ± 86 µmoles at the two highest doses, respectively.

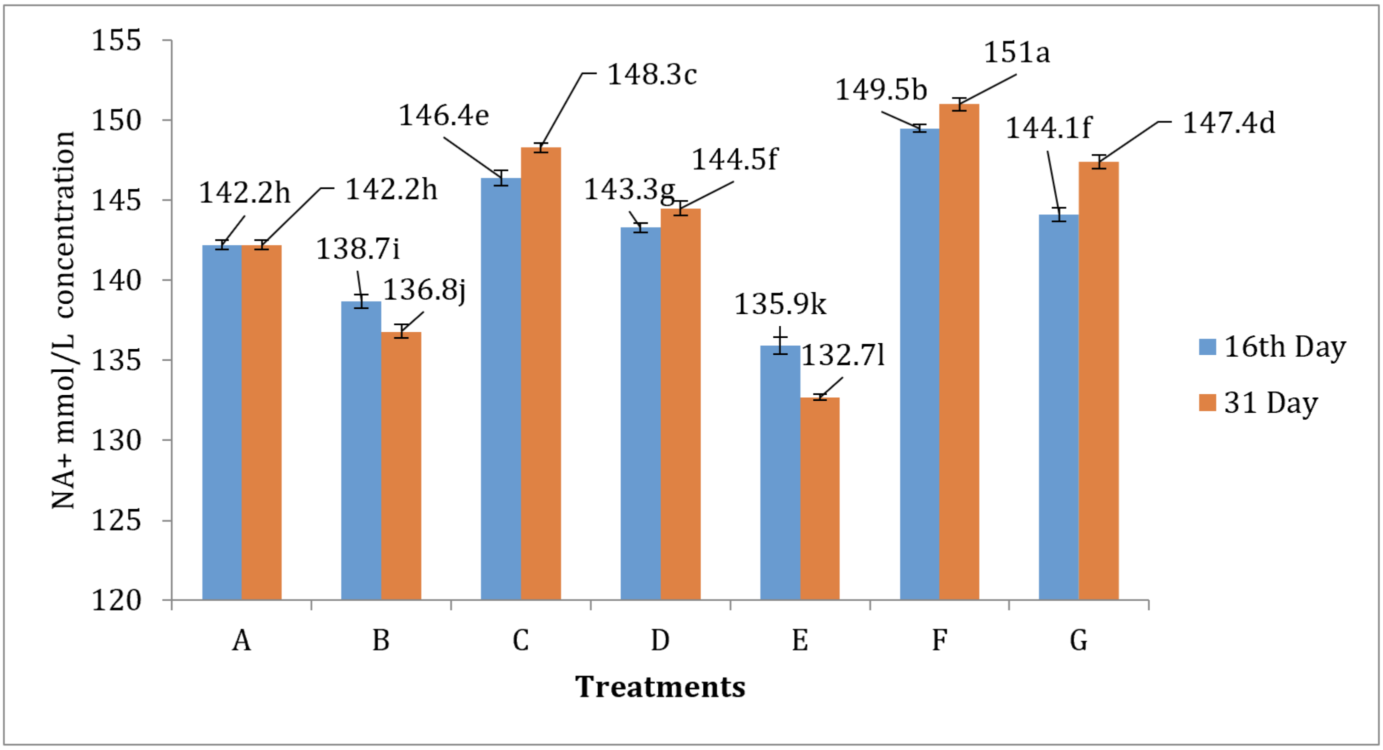

Figure 4. Serum Na+ concentration (mmol/L) in fluoride-induced toxicity and yogurt-supplemented betterment in rabbits.

Serum potassium

Data shown in (Figure 5) reveals the serum K+ concentration in control and treated groups of rabbits. Results indicated on 16th day, in treated groups-B, D, E and G, mean serum potassium values level was significantly increased (P˂0.05). Additionally, on 31st day, similar increasing trend was noticed i.e., in groups-B, D, E and G mean serum potassium values level was significantly increased (P˂0.05) after treatment in comparison to control. Reportedly, in agreement (Dalamaga *et al.*, 2008) recorded that increase fluoride consumption in human and other vertebrate induced electrolyte imbalance resulting increased level of potassium in serum. In another study, (Emejulu *et al.*, 2016) detected that the Piglet fed for 50 days with a base diet containing 100 and 250mg/kg, increased level of K+ in serum. The rise in K+ concentration generated by Na-F may suggest that membrane channel have been damaged and renal function has been affected. This study is contrast with the (Barai *et al.*, 2018) that 50mg/kg yogurt in mice reduced the level of potassium in serum that induced after oral administration of loperamide (3mg/kg). The secretory action of castor oil was inhibited by pre-treatment with S. boulardii at doses ranging from 1.2-12.0 x 1010CFU /kg. the yeast did not restore the net potassium content observed in the basal condition at the two largest doses, but it did significantly lower the castor oil induced net potassium secretion, with the net potassium secretion 0f 65.3 ± 43.6 and 51.5 ± 10.5 µmoles respectively (Girard *et al.*, 2005).

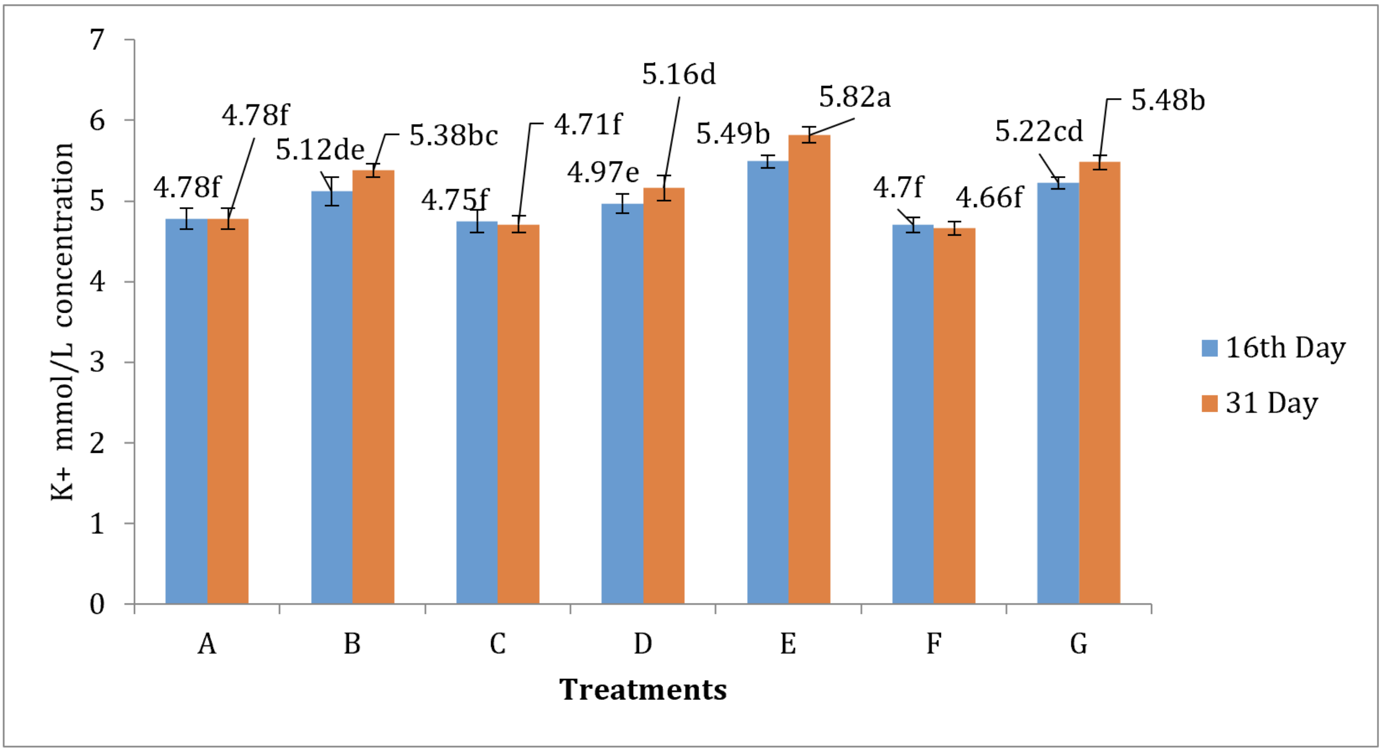

Figure 5. Serum K+ concentration (mmol/L) in fluoride-induced toxicity and yogurt-supplemented betterment in rabbits.

Serum chloride

Data shown in (Figure 6) reveal the serum Cl- concentration in control and treated groups of rabbits. Results indicated that on the 16th day in groups B and E, serum chloride levels ($P < 0.05$) decreased. Meanwhile, in groups F and G, serum chloride levels were significantly increased ($P < 0.05$). On the 31st day in groups B and E, serum chloride levels were significantly decreased ($P < 0.05$), while in groups C, D, F, and G, serum chloride levels were significantly increased ($P < 0.05$) following treatment as compared to control. The current study showed agreement with Lehnhardt and Kemper (2011) that the level of chloride reduced after the administration of sodium fluoride. Administration of fluoride decreased Cl- ion concentration in experimental animals. It has been observed that the level of Cl- elevated when treated with 50ml yogurt/kg body weight. Lactobacillus therapy reduced Cl- intestinal epithelial secretion in humans induced by enteropathogen (Girard et al., 2005). The use of castor oil significantly reduced net chloride absorption. Pretreatment with *S. boulardii* at doses ranging from 1.2-12.0 x 1010 CFU/kg reduced castor oil secretion. At the highest dose, yeast turned the net chloride secretion generated by castor oil into net chloride absorption (Al-Daihan and Bhat, 2019).

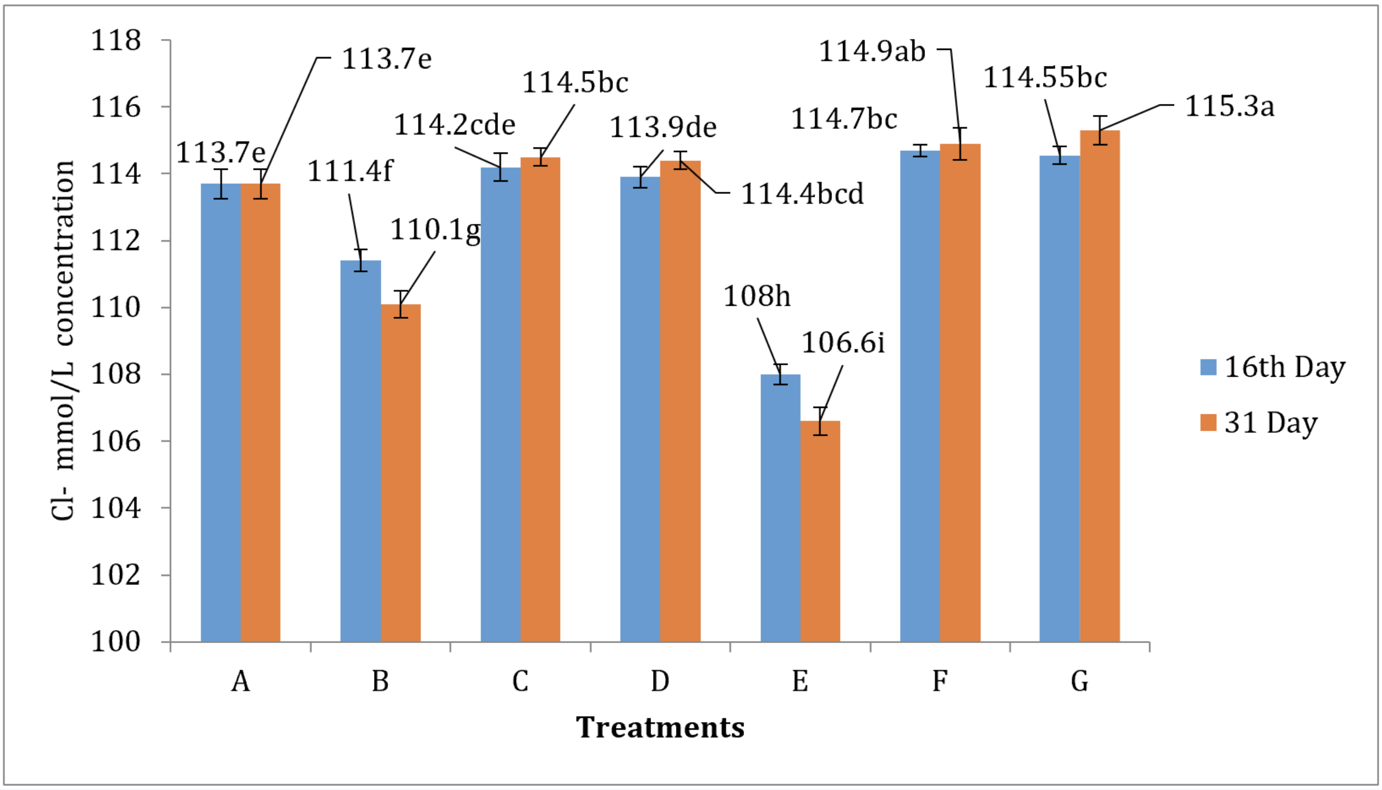

Figure 6. Serum Cl- concentration (mmol/L) in fluoride-induced toxicity and yogurt-supplemented amelioration in rabbits.

Conclusions

Based on the results of the present study, it was concluded that fluoride caused impaired renal function, which is evident in fluoride-treated groups with increased blood urea nitrogen, uric acid, creatinine, and K^+ levels in a dose-dependent manner. Yogurt supplementation decreased the elevated renal profile caused by fluoride intake in rabbits. Fluoride treatment decreased Cl^- and Na^+ levels, while yogurt supplementation inhibited it.

Authors' Contribution

Sallam, A., **S. Bughio**, and **R. Buriro** designed the project. **M.B. Arain** and **S. Bughio** wrote the manuscript. **M.A. Jamali** analyzed the data. **Sallam, A.**, and **Z. Lanjar** performed the experiment.

Conflict of Interest

The authors have no conflict of interest.

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