Research Article

Hepato-Nephrotoxic Effects of Induced Fluorosis in Rabbits and Broilers

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Abstract | Fluorosis in humans, animals and birds is often caused by drinking of fluoride contaminated groundwater. The study was designed to evaluate nephro-hepatotoxic effects of fluorosis on liver and kidneys in rabbits and broilers. Sixteen rabbits and sixteen broilers of four weeks age were divided into four subgroups each were given 0mg (control),50 mg, 100 mg and 200 mg Sodium Fluoride /liter in water daily for 18 days. The clinical signs and mortality were noted. Blood was collected at days 0, 5, 10, 15 and 18 of experiment, for evaluation of liver and kidney functions. Dose and time dependent significant ($p<0.05$) increase in serum Alanine aminotransferase (GPT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (γGT), uric acid and creatinine levels and significant ($p<0.05$) decrease of serum calcium levels were noted in all treatment groups of both species as compared to control. In rabbits and broilers, necropsy findings included mild inflammation and discoloration of liver along with nephritis. While in broilers, toxic lesions were observed on mucus membranes of duodenum and proventriculus along with nephritis. Histological lesions observed in livers of both rabbits and broilers included dilation of central vein and sinusoids and fatty degeneration of hepatocytes. Kidney tissues of both rabbits and broilers revealed marked shrinkage of glomeruli with widened bowman's spaces along with inflammatory cellular infiltration. It is concluded that high dose (200mg/l) of Sodium Fluoride causes liver and kidney dysfunction in both species along with lesions in digestive system of broilers.

Keywords | Fluorosis, Rabbits, Broilers, Liver, Kidney

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INTRODUCTION

Fluoride is a micronutrient naturally found in ground water, soil and plants in variable concentrations but is also sometimes added in small amounts in drinking water to maintain desired level (Takizawa, 2008). In the last few decades, due to water shortages the use of poor-quality groundwater having high fluoride contamination has become a serious threat to public health, wildlife, livestock and poultry. The World Health Organization (WHO) recommended Fluoride level in drinking water is 1.5 mg/l. Li et al. (2021), in their studies showed that more than recommended level causes health problems in animals. Fluoride contaminated groundwater has been reported in various areas of Pakistan and India with concentration ranging from 0.93 to 49.3 mg/L (Brahman et al., 2013; Farooqi et al., 2007; Kisku & Sahu, 2020; Rasool et al., 2018; Shah & Danishwar, 2003) .

Fluoride is good for dental and bone but excess amount of

(>1.5 mg/L) in long term causes dental and skeletal fluorosis; renal and neuronal disorders along with myopathy (Ayoob & Gupta, 2006). Humans, animals and birds are severely affected in fluoride contaminated areas. The fluoride concentration of up to 1.5 ppm in water causes chronic fluorosis in animals (Ranjan & Ranjan, 2015). Several studies have been conducted on fluorosis in birds, cattle, rabbits and sheep while outbreaks of fluorosis in pigs have also been reported in china (Kazi et al., 2018; Park et al., 2021; Tao et al., 2006).Excess fluoride intake in drinking water results decrease in milk production of cows and an increase in calving interval (Shupe et al., 1972).

In Pakistan, the groundwater quality is poor in many areas due to high fluoride levels. Most affected areas are Indus deltaic plain and Thar Desert in Sindh province (Rafique, et al., 2008; Rafique et al., 2015). As a result, people and animals are suffering from fluorosis in these areas (Farooqi et al., 2007). Keeping in view the above facts; the study was designed to evaluate the hepato-nephrotoxicity produced by induced fluorosis in mammalian (rabbit) and avian (chicken) models.

MATERIALS AND METHODS

Animal management and experimental protocol

The animals and birds were kept at the Animal House of Department of Veterinary Parasitology, Sindh Agriculture University Tandojam. A total of 24 rabbits (~ 1.5 kg BW) and twenty-four broilers (04 weeks age) were purchased from local market. An approval for the study was granted by the ethics committee, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam. Rabbits and broilers were divided into four subgroups, each having six animals, viz; A1, A2, A3, A4 and B1, B2, B3, B4. The animals in group A1 and B1 were kept as control and given normal water. The Groups A2-B2, A3-B3 and A4-B4 were given 50, 100 and 200 mg Sodium Fluoride/litter in drinking water respectively for 18 days.

Serum Biochemistry

The 3ml blood was collected on days 0, 5, 10, 15 and 18 of experiment. For evaluation of various biochemical parameters relating to liver and kidney functions, serum levels of Alanine aminotransferase (GPT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (γGT), Creatinine, Uric acid along with calcium were analyzed spectrophotometrically by using commercially available kits (HUMAN Diagnostic Co., Wiesbaden, Germany) through kinetic method as recommended by IFCC (International Federation of Clinical Chemistry) (Ceriotti et al., 2008).

Recording morbidity and mortality

All animals and birds were observed daily for development of clinical findings, morbidity and mortality.

Gross and Histopathological Examination

For studying lesions on various visceral organs necropsies were performed on dead while surviving broilers and rabbits were euthanized and dissected. For histopathological examination, the kidney and liver samples were cut into small pieces through sharp scalpel blade and were preserved in 10% formalin. Tissue samples were dehydrated in ethanol, cleared in Xylene, infiltrated / embedded in hard paraffin wax, sectioned and stained with Hematoxylin and Eosin stains. The histological lesions were recorded at 10 and 40X.

Statistical Analysis

Data was analyzed using two-way analysis of variance (ANOVA) to observe the difference between control group and experimental groups at different time intervals. Statistical differences among different treatment groups were determined by Duncan's Multiple Range test (DMR) at 5% level of significance (Akdogan et al., 2004).

RESULTS

Serum biochemical profile

Serum biochemical profile of rabbits and broilers treated with various concentrations of Sodium fluoride in drinking water and showing serum Alanine aminotransferase (GPT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (γGT), Serum creatinine, uric acid and calcium levels are shown in Table 1-3. Serum levels of Alanine aminotransferase (GPT), Alkaline phosphatase (ALP), Gamma glutamyl transferase (γGT),in all treatment groups of both rabbits and broilers significantly ($p<0.05$) increased in time and dose dependent manner as compared to control (Table 1). Serum creatinine and uric acid levels were significantly higher ($p<0.05$), in all treatment groups of both rabbits and broilers, as compared to control group (Table 2). The increases were dose dependent and time dependent. Moreover, time and dose dependent significant ($p<0.05$) decrease was seen in serum calcium levels in the groups treated with Sodium Fluoride as compared to control group (Table 3).

Clinical findings

There were no clinical signs observed in Rabbits during experiment, while in broiler chickens, paralysis of legs in few birds, decreased feed intake, increased water intake and intermittent diarrhea were observed in treatment groups (B2, B3, and B4) with signs being more pronounced with higher dose. There were only three deaths, one in each treatment group of broilers.

Table 1: Serum ALP, GPT and γGT levels of rabbits and broiler chicken given different concentrations of Sodium Fluoride in drinking water.

Test	Group	Day 0	Day 5	Day 10	Day 15	Day 18
ALP (IU/L)	A1 (0)	70.67 ± 0.0^{aA}	70.91 ± 0.01^{dA}	70.9 ± 0.02^{dA}	70.9 ± 0.04^{dA}	70.9 ± 0.03^{dA}
	A2(50)	70.8 ± 0.03^{aD}	72.5 ± 0.14^{cCD}	73.3 ± 0.05^{cC}	78.6 ± 0.14^{cB}	83.3 ± 0.15^{cA}
	A3 (100)	70.9 ± 0.0^{aE}	77.51 ± 0.23^{bD}	80.5 ± 0.07^{bC}	83.3 ± 0.29^{bB}	86.3 ± 0.23^{bA}
	A4 (200)	70.9 ± 0.0^{aE}	79.45 ± 0.06^{aD}	86.6 ± 0.13^{aC}	90.5 ± 0.12^{aB}	94.24 ± 0.84^{aA}
	B1 (0)	76.2 ± 0.08^{aA}	76.10 ± 0.02^{dA}	76.2 ± 0.03^{dA}	76.1 ± 0.07^{dA}	76.2 ± 0.06^{dA}
	B2(50)	75.4 ± 0.54^{abE}	77.4 ± 0.04^{cD}	79.6 ± 0.14^{cC}	82.38 ± 0.04^{cB}	85.05 ± 0.15^{cA}
	B3 (100)	74.5 ± 1.03^{bE}	78.2 ± 0.08^{bD}	80.3 ± 0.12^{bC}	84.5 ± 0.15^{bB}	88.17 ± 0.17^{bA}
	B4 (200)	75.4 ± 0.18^{abE}	83.3 ± 0.1^{aD}	85.1 ± 0.03^{aC}	88.62 ± 0.19^{aB}	92.42 ± 0.15^{aA}
GPT (IU/L)	A1 (0)	25.8 ± 0.01^{aA}	25.8 ± 0.01^{dA}	25.8 ± 0.01^{dA}	25.8 ± 0.01^{dA}	25.8 ± 0.01^{dA}
	A2(50)	25.7 ± 0.03^{aE}	28.3 ± 0.02^{cD}	30.8 ± 0.02^{cC}	33.8 ± 0.06^{cB}	35.2 ± 0.07^{cA}
	A3 (100)	25.6 ± 0.09^{aE}	31.7 ± 0.01^{bD}	34.3 ± 0.13^{bC}	36.62 ± 0.05^{bB}	38.6 ± 0.06^{bA}
	A4 (200)	25.6 ± 0.14^{aE}	35.6 ± 0.05^{aD}	37.4 ± 0.13^{aC}	42.33 ± 0.08^{aB}	45.58 ± 0.12^{aA}
	B1 (0)	37.5 ± 0.13^{aA}	37.6 ± 0.11^{dA}	37.6 ± 0.14^{dA}	37.7 ± 0.06^{dA}	37.7 ± 0.01^{dA}
	B2(50)	37.4 ± 0.16^{aE}	39.1 ± 0.02^{cD}	41.7 ± 0.77^{cC}	43.2 ± 0.11^{cB}	46.7 ± 0.08^{cA}
	B3 (100)	37.8 ± 0.08^{aE}	42.5 ± 0.05^{bD}	44.4 ± 0.16^{bC}	46.6 ± 0.12^{bB}	48.5 ± 0.15^{bA}
	B4 (200)	37.6 ± 0.07^{aE}	45.7 ± 0.07^{aD}	47.3 ± 0.08^{aC}	49.3 ± 0.2^{aB}	49.3 ± 0.24^{aA}
γGT (IU/L)	A1 (0)	6.2 ± 0.02^{aA}	6.1 ± 0.01^{dA}	6.2 ± 0.02^{dA}	6.2 ± 0.14^{dA}	6.3 ± 0.1^{dA}
	A2(50)	6.2 ± 0.05^{aE}	10.3 ± 0.16^{cD}	13.8 ± 0.29^{cC}	16.5 ± 0.08^{cB}	18.4 ± 0.1^{cA}
	A3 (100)	6.1 ± 0.05^{aE}	15.3 ± 0.05^{bD}	18.3 ± 0.05^{bC}	19.6 ± 0.14^{bB}	21.7 ± 0.13^{bA}
	A4 (200)	6.1 ± 0.03^{aE}	20.1 ± 0.04^{aD}	20.7 ± 0.01^{aC}	21.2 ± 1.33^{aB}	24.3 ± 0.95^{aA}
	B1 (0)	17.5 ± 0.12^{aA}	17.5 ± 0.12^{dA}	17.5 ± 0.13^{dA}	17.5 ± 0.13^{dA}	17.5 ± 0.13^{dA}
	B2(50)	17.3 ± 0.09^{aE}	18.5 ± 0.16^{cD}	21.6 ± 0.17^{cC}	24.5 ± 0.06^{cB}	26.5 ± 0.05^{cA}
	B3 (100)	17.4 ± 0.2^{aE}	20.4 ± 0.02^{bD}	24.3 ± 0.16^{bC}	28.6 ± 0.12^{bB}	31.1 ± 0.33^{bA}
	B4 (200)	17.6 ± 0.07^{aE}	45.7 ± 0.07^{aD}	47.3 ± 0.08^{aC}	49.3 ± 0.2^{aB}	49.3 ± 0.24^{aA}

Means±SD with different lowercase superscripts, for each species, show significant differences among the treatments for each time point (comparison per column), whereas different capital letters show significant differences among the time points for each treatment (comparison per line) ($p<0.05$).

Table 2: Serum creatinine and uric acid levels of rabbits and broiler chicken given different concentrations of Sodium Fluoride in drinking water.

Test	Group	Day 0	Day 5	Day 10	Day 15	Day 18
Creatinine (mg/dl)	A1 (0)	1.9 ± 0.05^{aA}	1.9 ± 0.11^{dA}	1.9 ± 0.52^{dA}	1.9 ± 0.17^{dA}	1.9 ± 0.31^{dA}
	A2(50)	1.9 ± 0.0^{aD}	3.3 ± 0.1^{cC}	4.4 ± 0.17^{cCB}	5.4 ± 0.24^{cB}	7.6 ± 0.13^{cA}
	A3 (100)	1.9 ± 0.0^{aD}	4.8 ± 0.02^{bC}	5.4 ± 0.1^{bCB}	6.6 ± 0.16^{bB}	8.9 ± 0.02^{bA}
	A4 (200)	1.9 ± 0.0^{aE}	8.6 ± 0.1^{aD}	10.2 ± 0.08^{aC}	13.9 ± 0.09^{aB}	17.4 ± 0.42^{aA}
	B1 (0)	1.9 ± 0.02^{aA}	1.9 ± 0.04^{dA}	1.9 ± 0.34^{dA}	1.9 ± 0.13^{dA}	1.9 ± 0.27^{dA}
	B2(50)	1.8 ± 0.04^{aE}	2.8 ± 0.03^{cD}	3.3 ± 0.11^{cC}	3.6 ± 0.12^{cB}	5.2 ± 0.15^{cA}
	B3 (100)	1.7 ± 0.04^{aE}	4.5 ± 0.09^{bD}	5.9 ± 0.28^{bC}	8.4 ± 0.14^{bB}	9.5 ± 0.09^{bA}
	B4 (200)	1.8 ± 0.08^{aE}	5.4 ± 0.08^{aD}	7.2 ± 1.12^{aC}	9.2 ± 0.19^{aB}	14.7 ± 1.54^{aA}
Uric Acid (mg/dl)	A1 (0)	1.6 ± 0.02^{aA}	1.6 ± 0.04^{dA}	1.6 ± 0.01^{dA}	1.6 ± 0.01^{dA}	1.6 ± 0.16^{dA}
	A2(50)	1.6 ± 0.04^{aE}	3.5 ± 0.25^{cD}	4.6 ± 0.04^{cC}	6.3 ± 0.15^{cB}	7.6 ± 0.1^{cA}
	A3 (100)	1.6 ± 0.01^{aE}	4.1 ± 0.04^{bD}	7.1 ± 0.22^{bC}	8.4 ± 0.15^{bB}	10.3 ± 0.25^{bA}
	A4 (200)	1.7 ± 0.06^{aE}	9.4 ± 0.09^{aD}	14.5 ± 0.18^{aC}	18.4 ± 0.19^{aB}	19.7 ± 0.04^{aA}
	B1 (0)	12.1 ± 0.05^{aA}	12.2 ± 0.13^{dA}	12.1 ± 0.1^{dA}	12.1 ± 0.03^{dA}	11.9 ± 0.18^{dA}
	B2(50)	12.1 ± 0.08^{aE}	14.1 ± 0.22^{cD}	23.3 ± 1.15^{cC}	28.1 ± 0.64^{cB}	29.9 ± 0.49^{cA}

	B3 (100)	12.1±0.04^{aE}	16.7±0.10^{bD}	26.7±0.1^{bC}	36.4±2.16^{bB}	35.1±0.57^{bA}
	B4 (200)	12.1±0.05^{aE}	18.9±0.33^{aD}	39.1±2.53^{aC}	58.5±2.4^{aB}	68.1±4.57^{aA}

Means±SD with different lowercase superscripts, for each species, show significant differences among the treatments for each time point (comparison per column), whereas different capital letters show significant differences among the time points for each treatment (comparison per line) ($p<0.05$).

Table 3: Serum calcium levels of rabbits and broiler chicken given different concentrations of Sodium Fluoride in drinking water.

Test	Group	Day 0	Day 5	Day 10	Day 15	Day 18
Calcium (mg/dl)	A1 (0)	21.4±0.03^{aA}	21.4±0.33^{aA}	21.4±0.46^{aA}	21.4±0.07^{aA}	21.4±0.61^{aA}
	A2(50)	21.5±0.01^{aA}	21.2±0.05^{aA}	20.4±0.1^{abB}	19.2±0.04^{bC}	18.8±0.01^{bD}
	A3 (100)	21.5±0.01^{aA}	20±0.11^{abB}	19.4±0.09^{bC}	18.6±0.06^{cbD}	16.4±0.17^{cE}
	A4 (200)	21.5±0.01^{aA}	19.8±0.02^{bB}	16.3±0.12^{cC}	15.61±0.02^{cD}	11.4±0.14^{dE}
	B1 (0)	13.4±0.07^{aA}	13.5±0.01^{aA}	13.3±0.18^{aA}	13.3±0.07^{aA}	13.5±0.01^{aA}
	B2(50)	13.6±0.06^{aA}	12.4±0.01^{bB}	10.5±0.06^{bC}	8.5±0.03^{bD}	6.9±0.22^{bE}
	B3 (100)	13.6±0.07^{aA}	8.6±0.03^{cB}	8.3±0.04^{cC}	6.7±0.06^{cD}	5.5±0.09^{cE}
	B4 (200)	13.6±0.06^{aA}	6.5±0.05^{dB}	5.5±0.12^{dC}	4.3±0.06^{dD}	3.5±0.18^{dE}

Means±SD with different lowercase superscripts, for each species, show significant differences among the treatments for each time point (comparison per column), whereas different capital letters show significant differences among the time points for each treatment (comparison per line) ($p<0.05$).

Gross and Histopathological Examination

In rabbits, postmortem lesions observed in kidney (Figure 1) included enlarged and inflamed kidneys, while in liver (Figure 2) there were inflammation and discoloration. The most common postmortem lesions observed in broilers were the toxic lesions on mucus membranes of proventriculus, swollen and congested spleen and enlarged gallbladder. The kidneys and liver were discolored and enlarged.

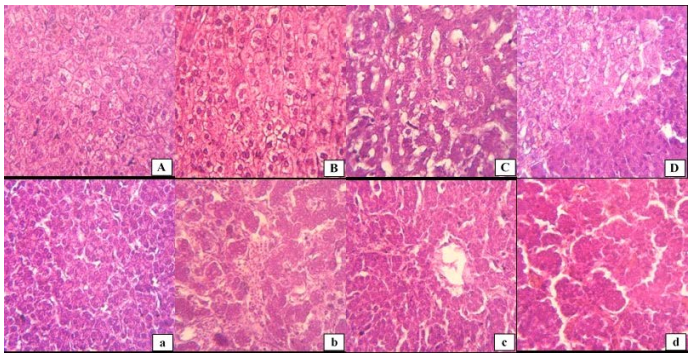

Figure 1: Photomicrographs of the kidneys of rabbits (Uppercase letters) and broilers (Lowercase letters) respectively, intoxicated with Sodium Fluoride. Sections from control group (A, a), groups given 50 mg/L (B, b), 100 mg/L (C, c) and 200 mg/L of NaF (D, d) in drinking water. (H&E) 400X.

The kidney tissues, in both species had mild inflammatory cellular infiltration, hyperemia in intertubular space, shrinkage of glomeruli with widened bowman's spaces (Figure 1). Sections of liver tissue of rabbit showed dilation of central vein and sinusoids and fatty degeneration of hepatocytes (Figure 2). On other hand, group of broilers showed hepatocytes surrounding the central vein were observed to possess darkly staining condensed nuclei and necrosis of the hepatocytes around the peri-portal and central vein area (Figure 2).

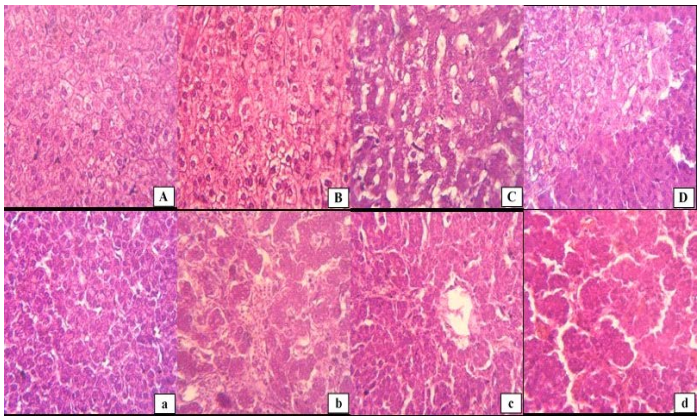

Figure 2: Photomicrographs of the Liver of rabbits (Uppercase letters) and broilers (Lowercase letters) respectively, intoxicated with Sodium Fluoride. Sections from control group (A, a), groups given 50 mg/L (B, b), 100 mg/L (C, c) and 200 mg/L of NaF (D, d) in drinking water. (H&E) 200X.

DISCUSSION

The fluorosis affects multiple organ systems of body including teeth, bones, gastrointestinal tract, nervous and muscular tissues. Among various modes of injury, reducing blood calcium levels, involvement of free radicals, increased generation of oxygen radicals and enhanced lipid peroxidation have been shown to play an important role in fluorosis

(Faccini & Teotia, 1974; Guo et al., 2003; Rzeuski, 1998). Most nutrients and toxins upon absorption, first pass through and are metabolized by liver and are excreted by kidneys. Therefore, these two organs are commonly affected in most toxicosis. While subtle damage is only reflected in changes of serum biochemical parameters, more severe damage is seen in on gross and microscopic levels. The present study was, therefore, designed to evaluate the effect of oral administration of high dose Sodium Fluoride in drinking water on liver and kidney structure and function.

In current study, both species belonging to two different taxonomic classes, showed structural and functional disturbance of liver and kidneys. The clinical signs of fluorosis and mortalities occurred only chicken, indicating greater susceptibility to fluoride toxicity. One probable reason could be the much higher growth and metabolic rate along with greater feed and water requirement in chickens.

Liver plays a major role in detoxification of various compounds. Serum ALP, γGT, GPT levels are important markers of liver function. In present study, increased serum levels of ALP, γGT and GPT show that liver is significantly affected in fluorosis. Similar results showed in rats and rabbits (Akinrinde et al., 2021; Al-safei & Al-Mashhadane, 2021). It is in line with various other studies (Guo et al., 2003). Similarly, Faccini and Teotia (1974) have also reported the high plasma alkaline phosphatase in the patients affected with fluorosis. Livers in both rabbits and broiler chicken tissues of rabbit showed dilation of central vein and sinusoids and fatty degeneration of hepatocytes while in broiler showed hepatocytes surrounding the central vein having degenerative changes and necrosis in some areas (Figure 2). Similar lesions observed in liver of rats, mouse and ducks (Akinrinde et al., 2021; Chen et al., 2019; Ouyang et al., 2021). Shashi et al. (2001) have also reported degenerative changes in liver of rabbits but changes were much more severe along with necrosis as duration of fluoride administration was 15 weeks. Similar findings have also been reported in fluoride exposed mice and rats (Akdogan et al., 2004; Akinrinde et al., 2021; Basha & Rao, 2014; Chinoy et al., 1993).

The kidney is the major route for removal of fluoride from the body, and consequently, this organ is sensitive to damage because of excessive fluoride exposure. In our study, serum levels of uric acid and creatinine in both rabbits and broilers increased with dose as well time duration. This study is also in agreement with Al-safei & Al-Mashhadane, (2021) and Dembińska-Kieć et al. (2017) who reported that in fluorosis produced significant increase in the concentration of creatinine in the blood due to impaired renal function. Similarly, Appleton (1995) also reported that high doses of sodium fluoride injection into rats resulted in increases in the concentration of urea, and creatinine in the plasma. In rat, kidney is exposed to concentrations of fluoride about five times higher than in other organs, as the tissue/plasma ratio for the kidney is approximately 5 to 1. In current study, mild inflammatory cellular infiltration, hyperemia in intertubular space, shrinkage of glomeruli with widened bowman's spaces were observed. Other researchers have found more or less severe gross and histopathological changes in kidneys as fluoride doses, routes of administration, duration of treatment and species vary. Basha and Rao (2014) found necrosis in glomerulus, degenerative changes in Bowman's capsule and alterations in glomerulus's tubular region in fluoride-treated albino mice. Similarly, Shashi et al. (2002) also reported same investigation necrotic and degenerative changes in kidney on rabbit however duration of treatment was fifteen weeks. Xu et al. (2006) reported similar the hypertrophy and hyperplasia in the renal tubules and damage kidney structure in fluoride-treated rats.

In the present study, rabbits and broilers were tested for serum calcium levels. The serum concentration of calcium in both rabbits and broilers decreased with fluoride dose as well time duration of treatment. Similar decrease has also been reported by various other researchers as well (Chandra, 1997; Madan et al., 2009; Tao et al., 2006; Wang et al., 1992). Similar observations were also reported by Faccini and Teotia (1974) who reported that increased urinary fluoride, a low urinary calcium excretion along with increased Ca and F in bone in the patients affected with fluorosis. Similarly, Appelton (1995) reported calcium decreased in blood due to renal insufficiency and cause in hypocalcemia in fluoride- treated rats. The decrease in serum calcium is related to a decrease of intestinal absorption of calcium by fluorine (Tao et al., 2006).

CONCLUSION

It is concluded that, there are dose and time dependent hepato-nephrotoxic effects of fluorosis in the rabbits and broilers as is reflected by gross and histopathological changes along with significant increase in serum ALP, γGT, GPT, creatinine, uric acid levels and decrease in calcium concentration.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

NOVELTY STATEMENT

Livestock and poultry farming industries in many parts of Pakistan have difficulties due to high fluoride content of groundwater. In the manuscript we have used a mammalian (rabbit) and avian (chicken) model to investigate the hepato-nephropathic effects of induced fluorosis. We report that dose-dependent subacute fluoride toxicity affects both function as well structure of liver and kidneys of rabbits and chicken.

AUTHORS CONTRIBUTIONS

Barirah Rehman Talpur conducted all experiments as part of MPhil research. Dr. Zaheer Ahmed Nizamani designed experiments and analyzed the data. Dr. Mansoor Tariq, Dr. Imdad Hussain Leghari and Aisha Rehman helped in data collection. Dr. Shahnawaz Kumbhar assisted in histopathological evaluation of slides.

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