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Original Research Article

Unraveling the Toxic Effects of Sodium Fluoride on Kidneys of Male Japanese Quails (*Coturnix japonica*)

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Abstract

Pesticides are commonly used in agriculture soils to distroy various pathogens. During the late 1950s and early 1960s, the media in Western Europe and North America extensively covered the issue of birds on agricultural land being poisoned by aldrin, dieldrin, and heptachlor, which were commonly used as seed dressings. To highlight this issue, an experiment was performed to examine the effects of sodium fluoride on Japanes quail growth, biochemical and histology of kidney. In the present work, forty-eight sexually male Japanese quail, weighing 105gm, were used, divided in 4 equal groups as A, B, C & D having 12 quails in every group. Quails were acclimatized for two weeks. After acclimatization experimental treatments were applied to their respective groups. Duration of experiment was 30 days. Sodium fluoride was given to quails orally with the help of gavage on daily basis. Quails in group C handled with 3.75mg/kg dose and group D handled with 5.0mg/kg dose showed significant changes in physical, biochemical and renal parameters as compared to control group. These treatments also cause significant reduction in final weight of birds. Birds in groups B with 2.5 mg/kg dose showed non-significant results. On 3.75mg/kg exposure, the highest accretion of fluoride occurred in the kidney. Sodium fluoride exposure significantly increased the kidney injury and other renal biomarkers. The histopathological variations such as necrosis of parenchymal cells, congestion and hemorrhage varied in dose dependent manner of sodium fluoride.
Keywords: Pesticides, Sodium Fluoride, Japanese quails, Histopathological alterations.

1- INTRODUCTION

The Japanese quail is regarded as a distinct species from the common quail. The Japanese quail is believed to have originated from the domestication of common quails in China and was introduced to Japan during the 11th or 12th century. Originally bred as domestic songbirds, the Japanese quail gained popularity in the 20th century for their meat and egg production purposes [1]. Upon reaching six to seven weeks old, they attain sexual maturity and start laying eggs. They exhibit a high rate of clutch, producing up to 280 eggs. Quail meat is not only delicious but also contains low fat content, aiding in the physical and cognitive development of youngsters. Quail eggs, as per nutritional standards, are superior to chicken eggs, as they contain a lower percentage of cholesterol. Both quail meat and eggs are beneficial for pregnant and nursing women [2]. Nevertheless, the production of Japanese quail encounters certain obstacles, including insufficient research on farm and environmental management practices [3]. Due to their petite physique, quick sexual maturation, prolific breeding, capacity to produce 3 to 4 generations annually, and relatively simple colony upkeep, the Japanese quail (Coturnix japonica) has gained significance as a laboratory animal [4].

Pesticide refers to any chemical substance utilized to eliminate certain types of plant or animal life, encompassing weed killers (herbicides), fungicides, insecticides, acaricides, and rodenticides [5]. During the late 1950s and early 1960s, the media in Western Europe and North America extensively covered the issue of birds on agricultural land being poisoned by aldrin, dieldrin, and heptachlor, which were commonly used as seed dressings. As a result, manufacturers, regulatory authorities, and pesticide users have since focused on the toxicity of pesticides to birds. This topic can generally be categorized into two areas: the impact of pesticides on captive birds in farms, zoological gardens, and wildlife parks; and the effects of pesticides on wild birds in the natural environment and on agricultural land [6].

Fluoride is found in plants and groundwater naturally [7]. Exposure to fluoride can harm not only bone-related organs but also non-bone organs, including those affecting the nerves and urinary system. The kidneys are particularly vulnerable to fluoride exposure, with fluoride-induced kidney damage often resulting in inhibition of intracellular enzymes and destruction of cell membrane structure, especially in the epithelial cells [8]. Past research indicates a possible correlation between environmental or occupational exposure to fluoride and renal damage [9]. Exposure to fluoride, whether it is acute or chronic, can lead to damage in different organs and tissues, including the enamel, skeletal tissues, brain and kidney [10, 11].

NaF, which can be found in soil, water, and nearly all plant structures, is a colorless crystalline solid or white powder [12]. Experimental models have clearly shown the renal toxicity of fluoride, particularly in the form of NaF, at high exposure levels in industry-related settings. However, the impact of low doses of fluoride on renal toxicity is still not well understood. It is suggested that fluoride alone may not be responsible for the observed renal toxicity at low doses, and mixtures with other salts may have a significant role to play. High fluoride levels may lead to an increased sodium/calcium ratio, which may be linked to certain renal complications [9].

The kidney plays a crucial role in eliminating fluoride from the body, making it vulnerable to damage in the event of excessive fluoride exposure [7]. An injection of 0.25 mmol NaF/kg or more led to kidney damage, as evidenced by increased urine GGT, diuresis, and phosphaturia. Histological analysis revealed that high doses of stannous fluoride and sodium pentafluoro stannite caused lesions in the kidney proximal tubules [13]. Renal toxicity resulting from NaF exposure has been demonstrated to cause caspase-mediated apoptosis and DNA damage in rats' kidneys. One of the ways NaF toxicity operates is by inducing tubular dysfunction, leading to diluted urine, impaired protein reabsorption, and increased urinary excretion of calcium and phosphate [14]. Elevated levels of NaF led to a rise in plasma concentrations of urea and creatinine [7]. Numerous studies have demonstrated a strong link between fluoride consumption and both kidney damage and cardiac injury.

2- MATERIALS AND METHOD

2.1 Study site

The research was conducted at govt employee's society near Baghdad campus Islamia University of Bahawalpur, which is located in the south Punjab region of Pakistan. The present study was managed in the laboratory of Department of Pathology the Islamia University of Bahawalpur conducted to study the morphological and nuclear changes in male Japanese quails exposed to Sodium fluoride.

2.2 Experimental Design

In the present work, 48 sexually mature male Japanese quail (*coturnix japonica*), weighing 105gm, were used. All birds had been placed in cages in an environmentally controlled room. Feed and water were supplied on daily basis. Male Japanese Quails were acclimatized for 15 days. After acclimatization quails were divided in 4 groups named as B, C, D and A as a control. Duration of experiment was 30 days.

Figure-1: Experimental Birds (Japanese quails)

2.3 NaF Treatments

Sodium fluoride was given to birds in groups (B, C and D) on each day basis for 30 days. Sodium fluoride was given to quails orally with the assist of crop tubes on every day basis for 30 days. The birds had been observed every day. Then, sampling was achieved on day 10, 20 and 30 days. During first trial 1cc of Sodium fluoride was given to birds but the mortality rate was increased and then trial 2nd and 3rd 0.5cc were given to the birds. Following treatments of sodium fluoride were used in this study:

1. Controlled (A group)
2. 2.5 ml NaF per Kg body weight (B group)
3. 3.75 ml NaF per Kg body weight (C group)
4. 5 ml NaF per Kg body weight (D group)

2.4 Physical parameter

Various physical parameter such as Frequency of crowing, Body weight and organ weight, different Clinical signs such as (Foam production), feed intake, Morphology and different behavioral signs are observed throughout the trail. Body weight was measured on weekly basis. The relative organ weight was measured on day 10, 20 and 30. The volume of testes was also measured.

2.5 Behavioral parameters:

All the male Japanese quail were examined daily in the afternoon for evidence of Morphological and clinical toxicity. Morphological and Clinical indications of all groups were observed and recorded on a daily basis. In all of the groups' behavioral changes were monitored and recorded. The response to stimuli, feathers, skin and crowing behavior of male Japanese quail, Different parameters of behavioral alterations included, the alertness of the birds, feathers loss, skin damage and weight loss (mounting on the pen mates) were observed as different markers of behavioral changes.

2.6 Biochemical parameters

Sampling was done on day 10, 20 and 30. In this sampling for microscopic examination 4 birds from every group were randomly picked and slaughtered by cutting their jugular vein. After slaughtering blood is collected in EDTA tubes for blood analysis and in centrifuge tube for centrifugation. At days 10, 20 and 30, around 3-4ml of blood was collected from every quail in each group. The weight of specimens and inner organs had been measured with the help of analytical weight balance and sample the sample organs were freeze by wrapping in foil paper in freezer for further procedure like histopathology.

2.7 Histopathological and Ultrastructural Assessment

Kidney specimens were fixed in 10% formalin for 24 h, following embedded in paraffin, and cut into 5 µm-thick slides, and they were prepared for hematoxylin and eosin (HE) staining. The slides were then sent to Rawalpindi for histopathology.

3- RESULTS

During the research, the Japanese quails that were kept in separate groups had a variety of clinical signs and behavioral changes due to exposure to different concentrations of Sodium Fluoride.

3.1 Weight Loss

Figure-2 shows the initial weight, final weight and weight loss of Japanese quails with respect to various sodium fluoride levels. Figure-2 shows that Japanese quails that were kept in group B didn't show any weight loss as they were exposed to lower level of NaF. While group C and group D quails showed significant weight loss. Group C (3.75 ml NaF per Kg body weight) showed 5% weight loss and group D (5 ml NaF per Kg body weight) showed 8% weight loss.

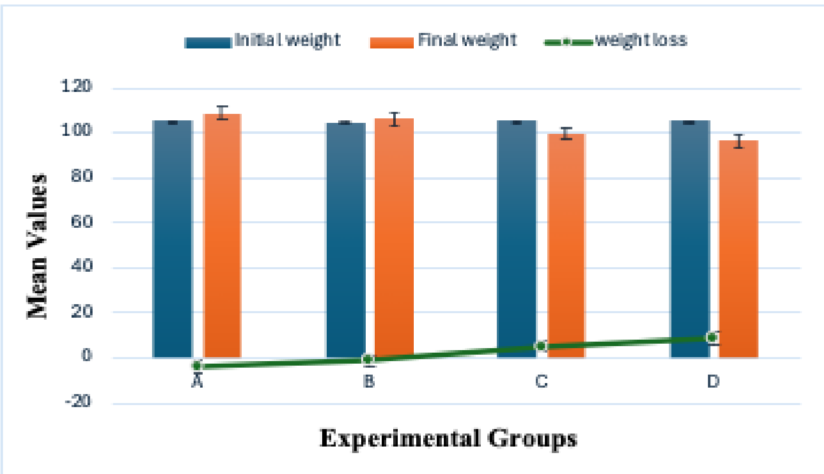

Figure-2: Graphical Representation of Initial Weight, Final Weight and Weight Loss of Japanese quails of Group A, B, C and D

3.2 Clinical signs

Dullness, depression and anorexia were also observed in Japanese quails that were given higher doses of Sodium Fluoride. The quails of control group A were not showing any clinical sign because they were not treated with Sodium Fluoride. The quails of group D were treated with higher doses of NaF. So, they showed severe clinical signs as compared to that of group A. The Japanese quails that were kept in group C and group B were given increasing dose of NaF so they showed significant change in clinical signs as compared to control group A. Dullness, depression, anorexia was recorded in the group C and D.

3.3 Alertness

Because they had not been exposed to NaF so all of the quails in control group A were highly energetic and alert. Striking similarities was recorded in the behavior of group B as they were treated with lower dose of NaF. The Quails of group C showed higher depression but that of group D showed severe dullness and sluggishness.

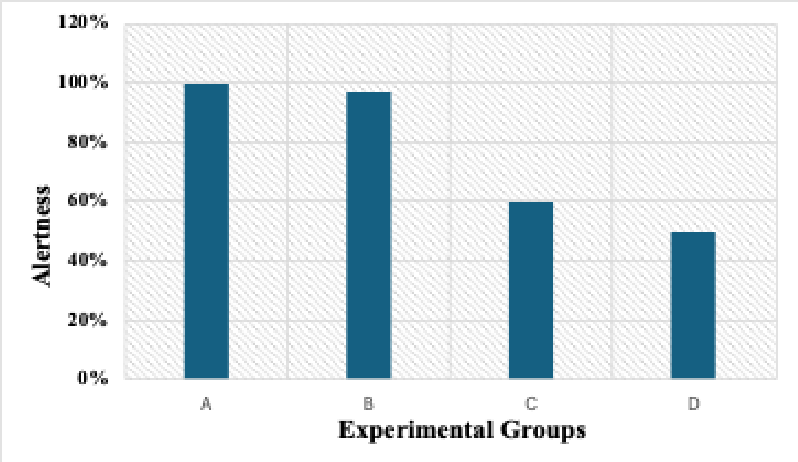

Figure-3: Graphical Representation of Initial Alertness of Japanese quails of Group A, B, C and D

3.4 Feed intake

Low feed intake was observed in quails with higher doses of NaF as compared to normal Japanese quail. Normal feed intake was recorded in control group A. The quails of group B were treated with lower dose of NaF so they did not show significant changes in feed intake, group C and D had a lower feed consumption.

3.5 Kidney

Grossly the consistency, color, structure, shape and texture of kidney of quails were normal in control group. Group B was exposed to lower dose of NaF so quails of group B showed similar results to control group. Group C and D were exposed to higher dose of NaF so they showed a prominent change and difference in color, structure, shape and texture of kidney

3.6 Absolute Organ Weight

Grossly the absolute weight of the kidney of quail in group A (control group) have been normal but a notable decrease in the Absolute weight of tissues of quail in group C and D as they were treated with high dose of NaF and the weight of the kidney was increased.

Table-1: Absolute Weight of Kidney of Japanese quails of Group A, B, C and D

Absolute weight of kidney				
Dosage	**A (Control group)**	**B**	**C**	**D**
2.5 ml/kg	0.765±0.107	0.757±0.412	0.757±0.483	0.786±0.638
3.75 ml/kg	0.748±0.103	0.790±1.054	0.708±0.216	0.710±0.452
5.0ml/kg	0.774±0.041	0.706±0.356	0.797±0.510	0.763±0.340

Table-2: Severity of different histopathological changes in various tissues of Japanese Quails (Coturnix japonica) exposed to various concentration of Sodium Fluoride after Ten Days

Histopathological lesions	**Groups/Treatments**			
	A (0.0mg/kg)	**B (2.5ml/Kg)**	**C (3.75ml/Kg)**	**D (5.0ml/Kg)**
Kidney (mg/kg)				
Congestion	-	+	+	++
Increased Bowman's space	-	+	++	++
Ceroid formation	-	+	+++	+++++
Edema	-	++	++	+++++
Necrosis of tubular cells	-	+	++	++++
Melan macrophage aggregates	-	++	++	++++
Nuclear hypertrophy	-	++	+++	++++
Deterioration of glomerulus	-	+	++	+++
Degeneration and obliteration of renal tubule	-	++	+++	+++++
Thyroidization	-	++	+++	++++

Table-3: Severity of different histopathological changes in various tissues of Japanese Quails (Coturnix japonica) exposed to various concentration of Sodium Fluoride after Twenty Days

Histopathological lesions	Groups/Treatments			
	A (0.0mg/kg)	B (2.5ml/Kg)	C (3.75ml/kg)	D (5.0ml/Kg)
Kidney (mg/kg)				
Congestion	-	++	+++	+++
Increased Bowman's space	-	++	+++	+++
Ceroid formation	-	++	+++	+++
Edema	-	++	+++	++++
Necrosis of tubular cells	-	++	+++	+++++
Melan macrophage aggregates	-	+++	+++	++++
Nuclear hypertrophy	-	+++	++++	++++
Deterioration of glomerulus	-	++	+++	++++
Degeneration and obliteration of renal tubule	-	+++	++++	+++++
Thyroidization	-	+++	++++	++++

Table-4: Severity of different histopathological changes in various tissues of Japanese Quails (Coturnix japonica) exposed to various concentration of Sodium Fluoride after Thirty Days

Histopathological lesions	Groups/Treatments			
	A (0.0mg/kg)	B (2.5ml/Kg)	C (3.75ml//Kg)	D (5.0ml/Kg)
Kidney (mg/kg)				
Congestion	-	+++	++++	+++++
Increased Bowman's space	-	+++	++++	++++
Ceroid formation	-	+++	+++	+++
Edema	-	+++	++++	+++++
Necrosis of tubular cells	-	+++	+++	++++
Melan macrophage aggregates	-	++++	++++	+++++
Nuclear hypertrophy	-	+++	+++	++++
Deterioration of glomerulus	-	+	+++	+++++
Degeneration and obliteration of renal tubule	-	++	+++	++++
Thyroidization	-	+++	++++	+++++

The observation of histopathological lesions was recorded normal in the control group A as they were not exposed to NaF. The group B was exposed to lower dose of NaF so the Quails that kept in group B showed non-significant results. Group C and D were exposed to higher dose of NaF so they showed a remarkable increase in degeneration, thyroidization, nuclear hypertrophy and deterioration of glomerulus. These results shows that the effect of sodium fluoride on kidney is dose dependent. With the increasing dose the degeneration and obliteration of renal tubules, thyroidization and edema become more and more severe. Similarly, more severe necrosis and nuclear hypertrophy is noticeable in high dose groups as compared to those which were given low dose.

4- DISCUSSIONS

Santoyo-Sanchez [9] found that low concentrations of sodium fluoride can cause renal damages, if exposure is prolonged. He also found that NaF exposed population kidneys also face deficiency of phosphate and calcium. Our findings were also in accordance with these results [15] and other researchers found that kidney tissues of soft animals accumulate more fluoride concentrations as compared to other tissues. Kidneys are responsible for removal of excessive fluoride from the body, that's why, higher concentrations of fluoride accumulate in kidney tubules. The highest fluoride concentrations were always found in kidney and the lowest in serum, similar effects are visible in our results. Necrosis, vacuoler degeneration and granular degeneration were obvious in renal tubular epithelial cells in the Fluoride group. In this group renal tubules walls were also thickened.

Yu [16] described fluorosis as a disease that causes nonspecific damage to multiple organs, resulting from chronic excessive fluoride intake through food and water, with the extent of damage increasing over time. According to [17], a significant reduction in GSH levels in the kidney and other tissues was observed in groups treated with NaF [9] found that exposure to fluoride significantly increased Jv, indicating a notable diuretic effect. Despite this, the GFR remained stable, suggesting that fluoride did not impact glomerular function. Additionally, free-water clearance was notably reduced, implying increased renal water reabsorption in the presence of fluoride. The total protein concentration decreased, likely due to the increase in Jv and the overall dilution of the urine. However, the fractional excretion and excretion of calcium significantly rose, indicating decreased calcium reabsorption, which points to impaired renal tubular function [11]. Previous studies have shown a relationship between fluoride exposure and oxidative stress in renal tissue.

Luo [18] observed that the NaF-treated groups exhibited degeneration and necrosis of tubular cells, infiltration of inflammatory cells, swelling of the glomeruli, and the presence of renal tubular hyaline casts. The histopathological damage caused by fluoride was noted to be dependent on both the dose and duration of exposure. Tubular cells exhibited granular and vacuolar degeneration, with necrotic tubular cells, hyaline casts in the renal tubules, and infiltration by inflammatory cells being observed. Similarly [19], reported that the tubular epithelium often desquamated into the lumen of the tubules in NaF-treated groups, which showed significant histomorphological alterations. These included severe degeneration and congestion of glomerular tufts and interstitial blood vessels, along with extensive inter-tubular hemorrhages. Additionally, large lymphocytic aggregates were observed in the inter-tubular areas, accompanied by tubular degeneration and necrosis. The glomerular tufts were severely atrophied, with disruption of Bowman's capsule in several areas.

In the present study we report the earliest effects of increasing-dose fluoride administration (similar to the levels reached in environmentally exposed populations) on renal function and the renal handling. These findings highlight the role of water tubular management and explain the impacts on glomerulus, which has been previously described in similar models. Both beneficial and deleterious effects have been attributed to fluoride administration. Water fluoridation programs, topical applications and therapeutic administration have been and will most likely continue to be seriously questioned. Our results confirm that the administration of high dose of sodium fluoride will cause severe damage to renal tubules, glomerulus, nephrons, congestion, Melanin macrophage aggregates and renal membrane leading to renal dysfunctioning.

5- CONCLUSIONS

The present study finds that NaF in excess of 2.0 mg/kg can induce the renal histological lesions, and inflammatory responses. The results of present experiment found out that sodium fluoride has significant effects on the physiology and functioning of kidneys by causing histopathological changes in glomerulus, tubules and Bowman's capsule. The results revealed that sodium fluoride has acute toxicity in birds at low concentrations and cause severe damage on chronic exposure. Therefore, it is a matter of concern for populations living in continual fluoride exposure. Awareness must be created by arranging seminars and policies should be made to limit fluoride use on fields.

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