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Mitigation of Fluoride Toxicity in Broiler Chicks using Fruit Pod Extracts of *Moringa oleifera* and *Prosopis cineraria*

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ABSTRACT

Purpose: Fluoride toxicity (fluorosis) caused by high levels of fluoride in drinking water is a global issue affecting both humans and animals. Although poultry birds are less susceptible than other animals, fluorosis still compromises growth and production. While several plant-based products have demonstrated ameliorative effects in domestic animals, their use in mitigating fluoride toxicity in chicks remains unexplored. This study aims to evaluate the potential of aqueous extracts of *Moringa oleifea* (Drumstick tree) and *Prosopis cineraria* (Khejri) fruit pods in mitigating fluoride toxicity in broiler chicks.

Methods: Sixty broiler chicks were randomly assigned to four groups (15 birds each). Gr I (control) received normal drinking water, while Gr II to IV were provided with water containing 400 ppm sodium fluoride to induce toxicity. In addition, Gr III received 100 mg of *M. oleifera* fruit pod extract per 100 g body weight per day, and Gr IV received the same dosage of *P. cineraria* extract. The treatment continued for 40 days, and plasma fluoride concentrations, bone fluoride levels, histopathology, and blood biochemistry were assessed.

Results: Plasma fluoride concentrations were significantly lower in Gr III and IV compared to Group II. On day 40, bone fluoride levels in Gr III and IV were also reduced relative to Gr II. Cortical thinning and bending of both proximal and distal ends of tibia were prominent in Gr II but were less prominent in Gr III and IV. Histopathology of the tibia, liver, and kidneys showed significant degenerative changes in Gr II, which were either less severe or absent in Gr I, III, and IV. Blood biochemical markers indicated liver and kidney damage in fluoride-intoxicated chicks, but these effects were mitigated in the extract-treated groups.

Conclusion: Aqueous extracts of *M. oleifera* and *P. cineraria* fruit pods exhibit ameliorative potential against fluoride toxicity in broiler chicks. These extracts may offer a promising approach for mitigating environmental toxicants in poultry production.

Key-words: Amelioration, Chicks, Fluoride toxicity, *Moringa oleifera*, *Prosopis cineraria*

INTRODUCTION

Excessive fluoride intake has been reported to cause toxic effects on bones and soft tissues across almost all animal species, though the degree of susceptibility varies[1]. Among livestock, cattle are the most sensitive, followed by sheep, horses, pigs, rabbits, rats, guinea pigs, and poultry[2]. Despite their relatively high tolerance to fluoride toxicity, poultry experience compromised growth, reduced production potential, and altered blood biochemical parameters when exposed to excessive fluoride intake[3-6]. Under natural conditions, chronic fluoride intoxication, or fluorosis, in domestic animals and birds is primarily linked to high fluoride concentrations in drinking water[7,8]. However, most studies on chicks have focused on fluoride exposure through feed, with only a few investigations examining drinking water as the source of fluoride intake.

Various ameliorative agents have been tested for mitigating fluoride toxicity in different animal species, with varying degrees of success. Among these, aluminum[9], selenium[10], and boron[11] have shown potential to reduce fluoride toxicity. However, these compounds present certain limitations, as they can be toxic themselves, particularly with prolonged use. There is also growing global concern over drug residues in poultry products, which pose a threat to public health and contribute to the rise of antimicrobial resistance. As a result, the poultry industry is increasingly seeking safer alternatives to conventional drugs.

Plant-based products with known therapeutic potential are emerging as a promising solution, given their availability and low cost[12]. Extracts from plants such as *Emblica officinalis*, *Mangifera indica*, *Limonia acidissima*, *Averrhoa carambola*, *Moringa oleifera*, and *Tamarindus indica* have demonstrated efficacy in mitigating fluoride toxicity in laboratory animals and livestock due to their richness in phytochemicals[13]. The pods of *Moringa oleifera* (Drumstick tree) and *Prosopis cineraria* (Khejri) are particularly rich in nutrients and antioxidants, and are consumed as food in several countries worldwide[14,15]. These plants have also been used in traditional medicine for the treatment of various human ailments[14,15]. However, their potential application in mitigating fluoride toxicity in chicks has not yet been explored. Thus, the present study aims to evaluate the effects of *M. oleifera* and *P. cineraria* fruit pod extract supplementation in fluoride-intoxicated chicks.

MATERIAL AND METHODS

M. oleifera and P. cineraria fruit pods

Fruit pods of *M. oleifera* and *P. cineraria* were collected from various locations in Bikaner, Rajasthan, India. Scientists from the ICAR-Central Institute for Arid Horticulture (CIAH), Bikaner authenticated the plant materials. The voucher specimens were prepared in duplicate, one was kept for in-house record, while other was submitted to ICAR-CIAH, Bikaner, Rajasthan, India. After collection, the fruit pods were thoroughly washed with distilled water and air-dried in the shade at room temperature for 15 days. The dried pods were then homogenized into a fine powder. The powdered material was stored in glass-stoppered bottles in a cool, dry place, protected from direct sunlight and moisture until extracts were prepared. Proximate analysis of the powdered fruit pods was carried out following standard methods[16].

Preparation of aqueous extracts

Powdered fruit pods of *M. oleifera* and *P. cineraria* (50 g each) were mixed with distilled water (500 ml) in conical flasks and left to stand overnight at room temperature. The mixture was shaken vigorously several times during this period. The following day, the mixture was filtered through Whatman No. 1 filter paper. The resulting filtrate was evaporated to dryness in a hot air oven at 37°C to obtain a semisolid material, and stored at -20°C until use. Before oral administration to chicks, the extract was diluted to the desired concentration with distilled water. The extractability of the plant extracts was calculated using the following formula:

$$\% \text{ Extractability} = \frac{\text{Weight of extract obtained (gm)} \quad \text{X } 100}{\text{Powder taken (gm)}}$$

Experimental protocol

Sixty healthy day-old broiler chicks (weighing 38-42 g) were procured and housed under an electric brooder at the Poultry Farm, College of Veterinary and Animal Sciences, Bikaner, for a 5-day acclimatization period. Thereafter, the chicks were randomly divided into four equal groups (Gr. I to Gr. IV). Gr. I chicks served as negative control and received normal tap water. Gr. II to IV chicks received tap water added with 400 ppm sodium fluoride. Gr. II served as positive control and received no plant extract treatment, while Gr. III and Gr. IV chicks individually received 100 mg fruit extracts / 100 gm body weight/ day of plant *M.*

oleifera and *P. cineraria*, respectively for 40 days. Blood samples were collected from the wing vein on days 0, 20, and 40. Clinical monitoring was conducted throughout the experimental period, and body weights were recorded on each sampling day. On day 40, the chicks were sacrificed, and samples of the liver, kidney, and long bone (tibia) were collected for further analysis.

The experimental chicks were fed a standard broiler diet (starter mash during weeks 0-3 and finisher mash from week 4 onward), formulated according to BIS[17] standards by the Department of Animal Nutrition, College of Veterinary and Animal Sciences, Bikaner. The chicks were maintained on a deep litter system with free access to feed and drinking water.

Fluoride estimation

Plasma fluoride concentration was measured following the method described by Cernik[18] using a digital ion analyzer (Orion Star A214 Benchtop pH/ISE Meter, Thermo Scientific, USA) equipped with a fluoride-specific electrode (Combination Fluoride Electrode, Orion Research Model, Thermo Scientific, USA). Quality control was ensured through repeated standard solutions and slope determination[19]. The electrode operation (slope) was checked, and the instrument was calibrated before fluoride estimation. Fluoride concentrations in blood plasma and water samples were measured after mixing the samples with Total Ionic Strength Adjustment Buffer (TISAB) II[20].

Fluoride concentration in bone samples was estimated by first ashing the tibia bone in a muffle furnace. The ash was then dissolved in 2 ml of 0.25 M HCl, to which 8 ml of TISAB II buffer was added. The fluoride concentration in the resulting mixture was determined using the digital ion analyzer equipped with the fluoride-specific electrode[21].

Fluoride concentration in plant materials and poultry feed was estimated following AOAC methods[22]. The plant material and poultry feed samples were dried at 80°C and ground into a fine powder. The powdered sample was dissolved in 10% HCL, and the pH of the solution was adjusted to 5.2 using a sodium acetate solution (45% W/V). The final volume was adjusted to 100 ml, and the fluoride concentration in the solution was measured using the ion-selective electrode after mixing with TISAB II.

Radiographic examination

Dorso-ventral radiographs of the long bones (tibia) were taken on day 40, before sacrifice. The operating parameters for the X-ray machine were set at 58 kV and 2.5 mA[23]. The radiographs were examined for bone density, diaphyseal width of the tibia, and any abnormal changes in the bone or articular surfaces.

Histopathology

Small pieces of liver and kidney tissue, approximately 2-5 mm thick, were cut and fixed in 10% formal saline. After 24-48 hours of fixation, the tissues were processed using standard techniques to obtain paraffin-embedded sections of 4-5 µm thickness. These sections were then stained with routine Hematoxylin and Eosin for histopathological examination[24]. Tibia bone samples were decalcified using Gooding and Stewart's fluid before sectioning and staining with Hematoxylin and Eosin[25].

Blood biochemistry

The activities of alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT), as well as the concentrations of creatinine and urea nitrogen in blood plasma, were estimated following standard methods using kits supplied by AGD Biomedicals (P) Ltd., Mumbai, India.

Statistical analysis

The data obtained were subjected to statistical analysis by adopting appropriate methods using SPSS, version 10.0, statistical software[26].

RESULTS & DISCUSSION

Proximate composition and fluoride estimation in plant materials, broiler feed and drinking water

The proximate composition of the dried fruit pod powders of *M. oleifera* and *P. cineraria*, as well as broiler starter and finisher feeds, is presented in Table 1. The dry matter content and crude protein levels were higher in *M. oleifera* compared to *P. cineraria*, while crude fibre and ash content were greater in *P. cineraria*. *M. oleifera* fruit pods are excellent sources of protein, calcium, iron, vitamin A, ascorbic acid, and antioxidant compounds, including carotenoids, flavonoids, vitamin E, and phenols[27]. A study reported the nutritional value of *M. oleifera* pods (per 100 g) as follows: calories 25 KCal, protein 2.5 g, fat 0.1 g, carbohydrates 3.7 g, fibre 4.8 g, vitamin C 120 mg, calcium 30 mg, phosphorus 110 mg, potassium 259 mg, copper 3.1 mg, and iron 5.3 mg[28]. The mature seeds of *M. oleifera* contain 332.4 g crude protein, 414.0 g crude fat, 212.2 g carbohydrates, and 44.4 g ash per kg of dry matter, and are rich in cysteine, methionine, oleic, palmitic, and behenic acids[28].

The pods of *P. cineraria* are well-known for their nutritional and nutraceutical value, have been consumed by desert dwellers since ancient times[29]. They contain 8.55% moisture, 5.00% crude fibre, 2.30% crude fat, 28.42% total protein, 51.01% total carbohydrate, and 9.71% ash[29]. Furthermore, the fruit pods of *P. cineraria* are rich in phosphorus, magnesium, zinc, selenium, calcium, and iron. One notable property of dried fruit pods is their ability to retain nutritional content even after processing[30].

The broiler starter and finisher feeds used in this study contained sufficient nutrients to meet the nutritional requirements of the chicks. The crude protein content in the broiler starter ration was 22.3%, while the broiler finisher ration contained 21.05%. According to BIS specifications, the crude protein and crude fibre levels for broiler starter feed should be 23% and 6%, respectively, while for broiler finisher feed, they should be 20% and 6%, respectively. Previous studies have reported crude protein contents of 23.69% and 21.20%, crude fibre contents of 4.87% and 6.14%, and ash contents of 7.86% and 7.88% in broiler starter and finisher feeds, respectively[31].

In this study, the fluoride concentrations (mean ± S.E.) in broiler starter and finisher feeds were found to be 4.7 ± 0.23 mg/kg and 4.5 ± 0.61 mg/kg, respectively, which are within safe levels[3]. The tap water contained 0.90 ± 0.59 ppm fluoride, which is below the safe limit of 1.50 ppm[19].

Table 1. Proximate composition of *M. oleifera* and *P. cineraria* fruit pods, broiler starter and finisher feeds.

S. No.	Proximate principles (%)	*M. oleifera* fruit pods	*P. cineraria* fruit pods	Broiler starter feed	Broiler finisher feed
1.	Moisture	06.43	08.55	08.10	08.50
2.	Dry Matter	93.57	91.40	91.90	91.50
3.	Crude Protein	43.26	18.25	22.30	21.05
4.	Ether Extract	21.36	10.60	03.75	04.35
5.	Crude Fibre	12.08	20.93	05.00	04.90
6.	Total Ash	04.45	05.34	03.00	03.50
7.	Nitrogen Free Extract	12.42	36.28	57.85	57.70

Aqueous plant extracts

The aqueous extracts of *M. oleifera* pods and *P. cineraria* obtained in this study were semisolid in consistency and dark brown in color, with extractability percentages of 21% and 18.3%, respectively. One previous study reported an extractability percentage of 30% for aqueous seed extracts of *M. oleifera*, also exhibiting a semi-solid consistency[32]. In contrast, another study reported an extractability percentage of 8.3% for methanolic extracts of dried *P. cineraria* pods[33]. Additionally, an extractability percentage of 13.6% was observed for aqueous extracts of *P. cineraria* pods in a different study[15].The variation in extractability may be attributed to differences in the maturity stage of the fruit pods or individual plant effects, as the chemical composition of *P. cineraria* fruit pods can vary among individual trees and is influenced by various environmental factors[30].

Change in body weight

Changes in body weight (BW) of chicks on different observation days are presented in Table 2. On day 20, BW of Gr II, III, and IV chicks were significantly ($p<0.05$) lower compared to Gr I (control). On day 40, the mean BW was highest in Gr I, followed by Gr IV and Gr III; while it was lowest in Gr II. When comparing the percent increase in mean body weight of chicks from day 0 to day 40, the values were 1711.59%, 1673.36%, 1690.29%, and 1691.84% for Groups I, II, III, and IV, respectively. These results indicate that excess fluoride exposure resulted in a decreased growth rate, while concurrent oral administration of the different plant extracts had some positive effects. However, no other abnormal symptoms could be recorded in chicks receiving excess fluoride during the experimental period.

Table 2. Changes in body weight (gm) of birds on different observation period.

Day of observation	*Group I*	Group II	Group III	Group IV
Day 0	121.33 ± 0.033^{aA}	114.66 ± 0.088^{aA}	117.83 ± 0.033^{aA}	119.16 ± 0.067^{aA}
Day 20	840.50 ± 0.251^{bB}	804.16 ± 0.775^{aB}	811.16 ± 0.723^{aB}	809.18 ± 0.454^{aB}
Day 40	2198.00 ± 0.731^{cC}	2033.33 ± 0.285^{aC}	2109.50 ± 0.413^{abC}	2135.16 ± 0.914^{bC}

Values bearing different superscript in small letters in a row and capital letters in a column differ significantly ($p \leq 0.05$).

Fluoride concentration in blood plasma and long bone

Fluoride concentrations in blood plasma and long bones are illustrated in Figures 1 and 2, respectively. On day 40, there was a significant increase ($p<0.05$) in mean plasma fluoride concentration in birds treated with sodium fluoride alone compared to the control group. In contrast, birds receiving aqueous extracts of *M. oleifera* and *P. cineraria* showed statistically significant lower plasma fluoride concentrations compared to Gr II.

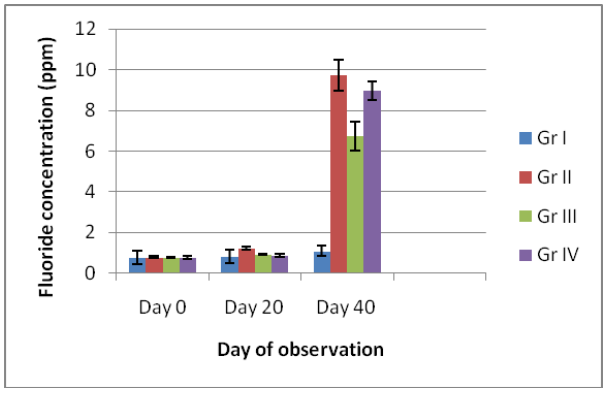

Figure 1. Changes in plasma fluoride concentration in birds.

The mean fluoride concentrations (ppm) in the tibia for Gr I, II, III, and IV were 375.9, 1259.2, 1201.2, and 1232.6 ppm, respectively. These results indicate that bone fluoride concentrations increased in all groups exposed to excess fluoride, with the most substantial increase observed in Group II. Notably, birds receiving plant extracts exhibited lower bone fluoride concentrations compared to the positive control.

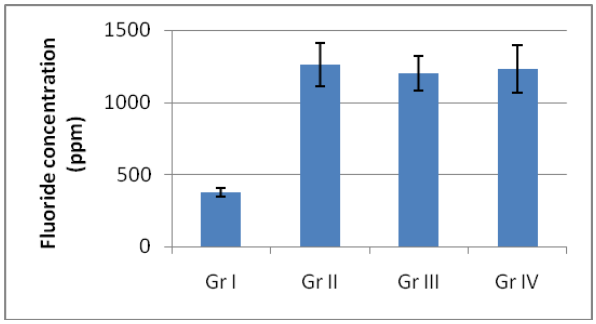

Figure 2. Bone fluoride concentration on day 40 in birds.

Radiographs of tibia

Radiographic changes in bones are considered very good indicators of fluorosis in humans[34]. However, there are no reports available on the use of radiographs for diagnosing fluoride toxicity in chicks. In the present study, the radiographic appearance of tibia was almost normal in control birds on day 40. Normal leg bones of birds appear symmetrical on both sides, without distortion, enlargement, or fractures[23]. In poultry, leg deformities are commonly identified through radiographic findings such as inward or outward projection of the tibial-metatarsal joint, distortion of long bones, overlapping/ absence of tibial articular cartilage, and joint effusions[23].

In fluoride-intoxicated Gr II birds, radiographs revealed bone bending, cortical thinning at the proximal end, and narrowing of the medullary cavity (Figure 3). These changes were less pronounced in broiler chicks co-treated with *M. oleifera* and *P. cineraria* fruit pod extracts. The cortical thickness in Gr III was comparable to that of the control group, indicating the beneficial effects of *Moringa* fruit extract supplementation. However, cortical thinning was still observed at the proximal tibia in both Gr II and Gr IV, though milder in Gr IV. The articular surfaces and medullary cavities of the tibia appeared normal across all groups, and the tibio-tarsal-metatarsal joint space was unaffected on day 40. The bending of the tibia in Gr II birds suggests a loss of tensile strength due to fluoride intoxication, a phenomenon not observed in control or plant extract co-treatment groups.

In a study conducted on rabbits exposed to 200 ppm or more of fluoride in drinking water for 90 days, radiographic findings revealed a widening of the metaphysis, thinning of the cortical region, and osteopenic changes like osteoporosis and osteomalacia[35]. Similar findings were reported in Wistar albino rats, with significant thinning of the epiphyseal growth plate and bone trabeculae in the femoral bone under fluoride intoxication[36]. In humans, large variations have been reported in the radiographic appearance of bones due to chronic fluoride toxicity. The majority of reports suggest an increase in bone density, blurring or haziness of trabeculae, compact bone thickening, periosteal bone formation, and ossification of the attachments[34], while others describe a decrease in bone density, osteopenia, and osteoporosis[37,38]. Some reports suggest smaller doses of fluoride promote osteogenesis, while larger doses result in increased bone resorption and defective matrix formation[39]. High fluoride exposure is also associated with reduced collagen synthesis, further supporting this hypothesis[40]. Osteoclasts exhibit greater sensitivity to changes in fluoride concentration compared to osteocytes and osteoblasts. Fluoride exposure exacerbates the apoptosis of osteocytes and osteoclasts, particularly when induced by parathyroid hormone administration[41].

In poultry, the effects of excess fluoride on bone mineralization, radiographic appearance, and long bone strength have shown variability. One study

noted a nearly 40% increase in tibia strength following exposure to 200 ppm fluoride in drinking water for eight weeks[42]. In another study on White Leghorn chickens, supplementation with drinking water containing 16.8 nmol/L fluoride *ad libitum* for 4 weeks led to enhanced bone maturation and secondary mineralization in cortical bone[4].

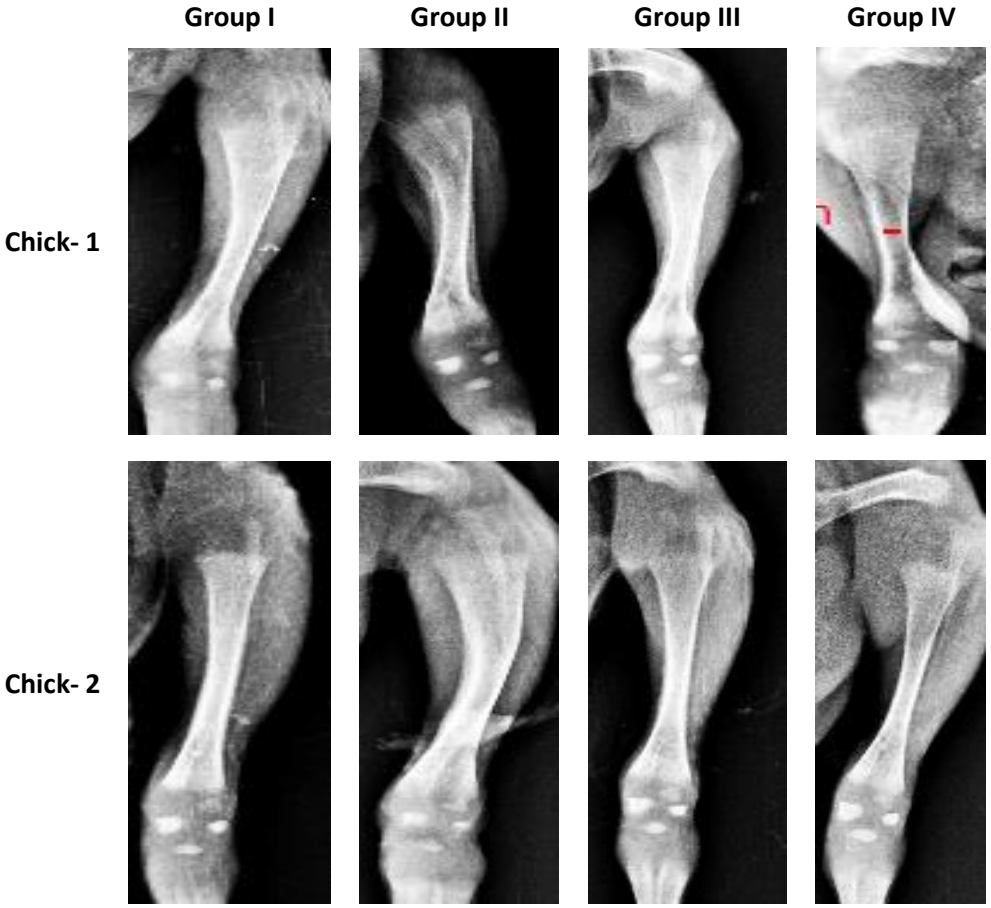

Figure 3. Radiographs of the tibia of two representative birds on day 40 of the experiment.

Histopathology and blood biochemistry

The tibia of control birds exhibited normal tissue architecture, whereas a marked reduction in the bony matrix was observed in fluoride-intoxicated birds (Figures 4a and b). However, in both *Moringa* and *Prosopis* co-treated birds (Figures 4c and d), the bony tissues showed an almost normal matrix, indicating the significant ameliorative effects of these plant extracts. This study suggests that excessive fluoride intake induces pronounced alterations in bone tissue architecture. In fluoride-intoxicated rabbits, notable structural changes were recorded in the long bones and costo-chondral junction (CCJ), including disorganized chondrocyte arrangement, nodular protrusions in the metaphysis, hemorrhages, growth plate discontinuation, and reduced bone marrow elements[38]. Similar histopathological changes in the long bones of fluorotic red deer have also been documented[43]. However, to the best of our knowledge, no prior studies have reported on the micro-architectural changes in bone tissues of birds subjected to excessive dietary fluoride, making the results of the present study novel.

In hepatic tissues, control birds displayed normal architecture (Figure 5a), while mild to moderate congestion was evident in both control and plant extract co-treated birds. Mild degenerative changes were observed in the liver cells of Gr II birds (Figure 5b), highlighting the hepatotoxic potential of excess fluoride. Interestingly, the absence of degenerative changes in the plant extract-supplemented groups (Figures 5c and d) suggests a hepatoprotective role of *Moringa* and *Prosopis* extracts. Given the liver's active role in metabolism, it is particularly vulnerable to fluoride toxicity[44]. Previous studies have reported compression of sinusoids and swelling of hepatocytes, along with coarse cytoplasmic granularity in fluoride-intoxicated rabbits[45]. Similarly, in rats, fluoride exposure has been linked to hepatocellular necrosis, degenerative changes, hyperplasia, vacuolization in hepatocytes, and centrilobular necrosis[46]. In fluoride-intoxicated chicks,

significant hepatic alterations include inflammation, portal vein congestion, and sinusoidal dilation, while kidney lesions often feature glomerular segmentation, dilation of Bowman's space, renal tubule epithelial necrosis, and hemorrhages has been reported in another study[47]. Additionally, dilation of the central vein and sinusoids, along with fatty degeneration of hepatocytes, has been observed in rabbits and broilers exposed to 200 mg sodium fluoride per litre in drinking water[6].

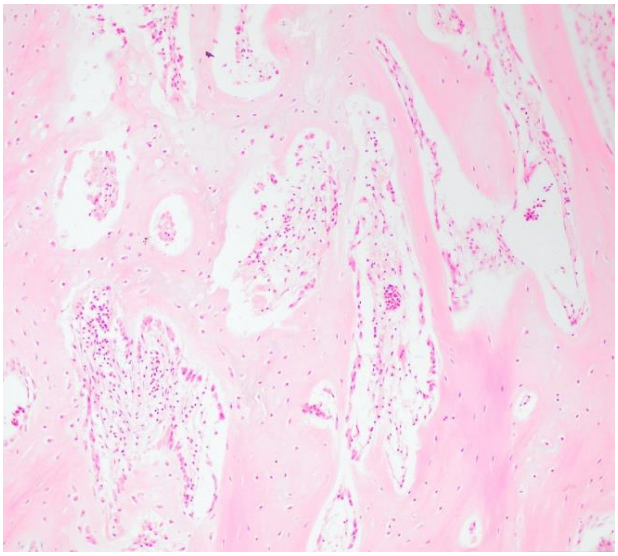

Figure 4a. Normal architecture of tibia in control birds (Group I). H and E stain, X100.

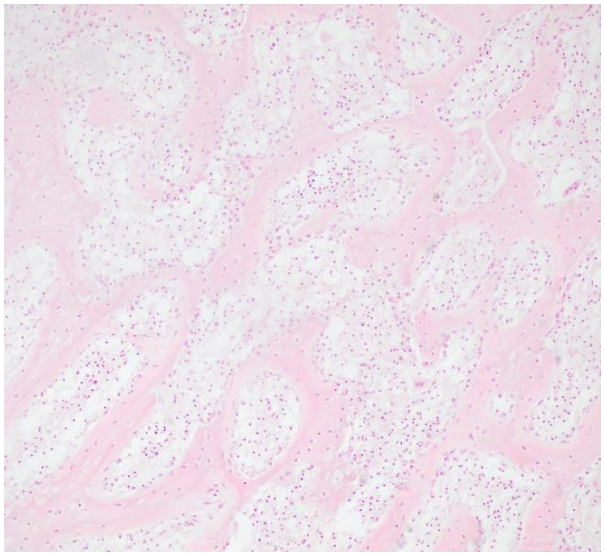

Figure 4b. Reduction in bony matrix of tibia in fluoride intoxicated chick (Group II). H and E stain, X100.

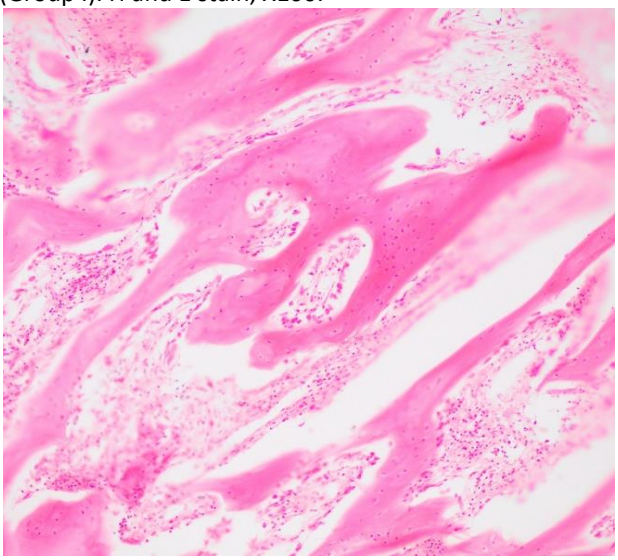

Figure 4c. Tibia in Moringa co-treated birds showing almost normal bony matrix (Group III). H and E stain, X100.

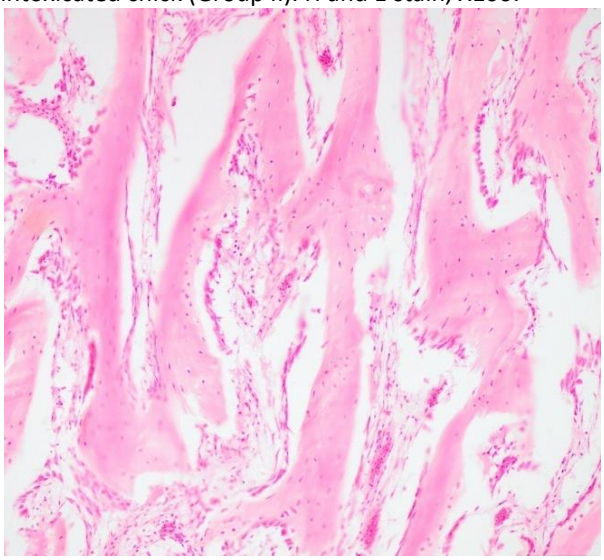

Figure 4d. Tibia in Prosopis co-treated birds showing almost normal bony matrix (Group IV). H and E stain, X100.

Alterations in the plasma levels of alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST) in fluoride-intoxicated birds across different observation days indicated that the liver was likely stressed in detoxifying excess fluoride (Table 3). These enzyme fluctuations were less pronounced in the Moringa and Prosopis extract co-treated birds, suggesting the protective effects of these plant extracts. Consistent with the present study, a significant ($p<0.05$) increase in serum AST, ALT, and ALP activities was observed on day 42 in birds receiving diets containing 800 and 1200 mg fluoride/kg[5]. Elevated levels of these liver marker enzymes have also been reported in fluorosis cases in children[48], rats[49], pigs[50], rabbits[45], and chicks[6]. However, the hepatotoxic effects of fluoride remain a topic of debate, as some studies suggest that excessive fluoride intake has no significant impact on serum ALT and AST activities in humans[51].In another study, male albino mice that received oral sodium fluoride at a dosage of 10

mg/kg/day for 30 days exhibited ballooning degeneration of hepatocytes, necrosis, and mononuclear cell infiltration in hepatic lobules[52]. These changes were accompanied by elevated levels of liver marker enzymes in fluoride-intoxicated mice. Similarly, in rats treated with sodium fluoride, hepatic changes included focal hepatocyte necrosis and hepatocyte drop-off[53].

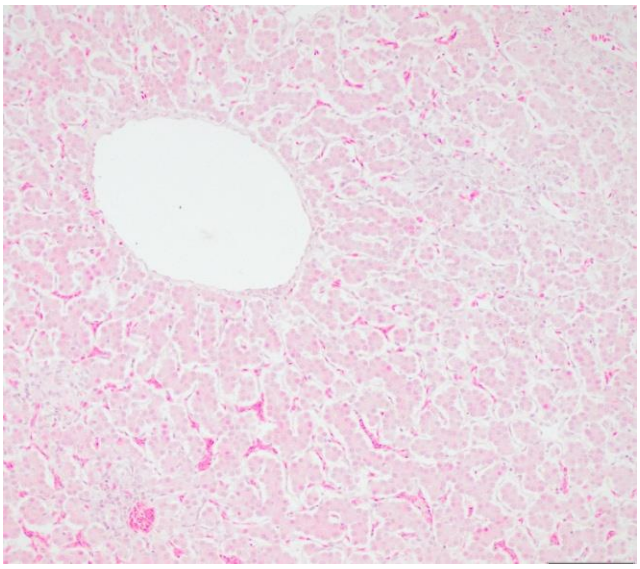

Figure 5a. Liver in a control chick showing normal tissue architecture with mild congestion. H and E stain, X100.

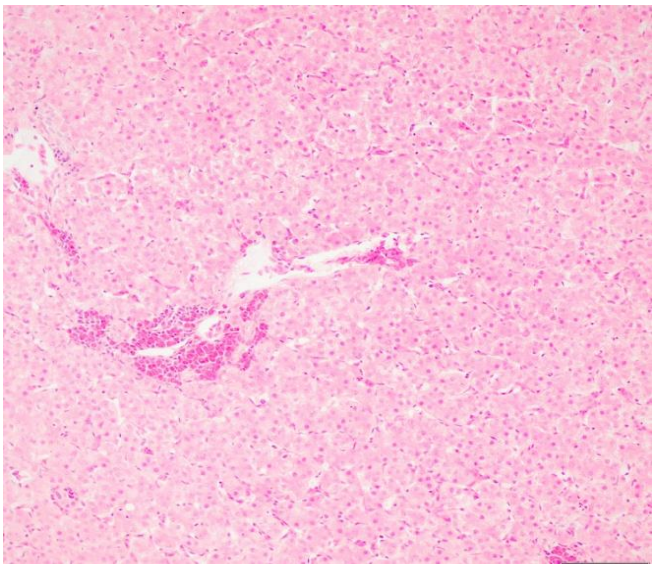

Figure 5b. Hepatic tissues in a fluoride intoxicated chick showing congestion and mild degeneration of hepatocytes (Group II). H and E stain, X100.

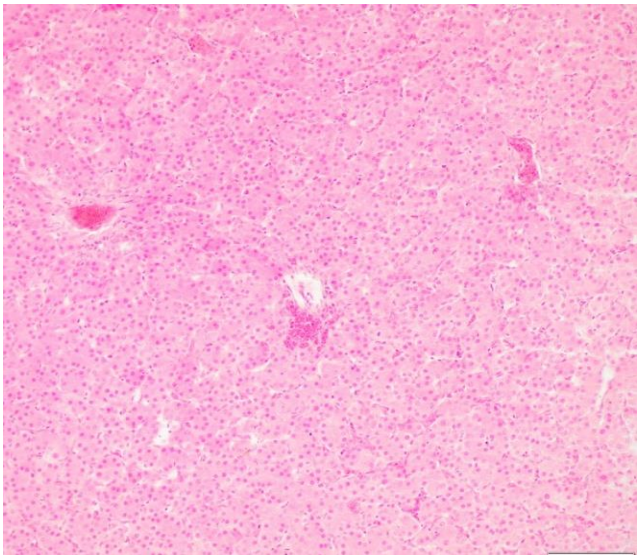

Figure5c. Hepatic tissues in a fluoride intoxicated and Moringa extract supplemented chick showing mild congestion. HandE stain, X100.

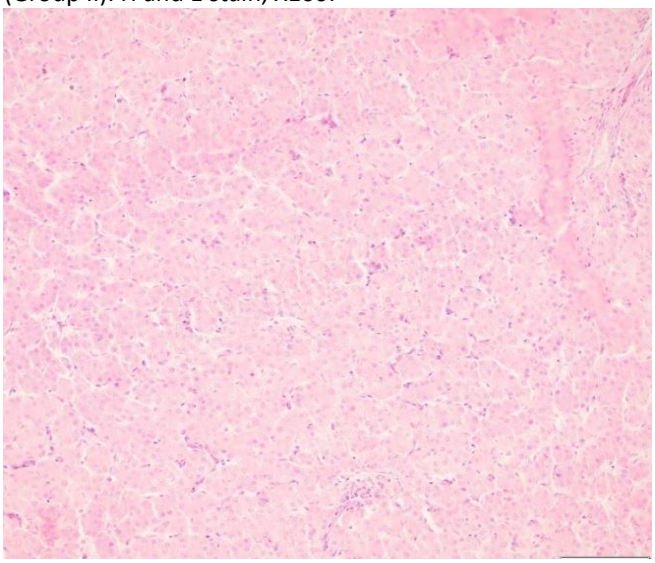

Figure 5d. Hepatic tissues in a fluoride intoxicated and Prosopis extract supplemented chick showing moderate congestion. HandE stain, X100.

In control birds (Figure 6a), kidney tissues exhibited normal architecture, while in birds exposed to excess fluoride alone, mild congestion and degeneration of the tubular epithelium were observed (Figure 6b). In the Moringa and Prosopis extract co-treated groups, only mild congestion was evident, with no major degenerative changes (Figures 6c and d). The histological findings, including congestion and degeneration of the renal tubular epithelium in Gr II birds, suggest the nephrotoxic effects of fluoride in chicks. This hypothesis is further supported by changes in plasma creatinine and urea nitrogen levels in experimental birds (Table 3). Similar observations have been made in fluoride-intoxicated rabbits[45] and rats[53].A recent study reported significant shrinkage of glomeruli, widening of Bowman's space, and inflammatory cellular infiltration in the kidneys of broiler chicks exposed to 200 mg sodium fluoride per

liter of drinking water for 18 days[6]. Since urine is the major route for fluoride elimination, the kidneys play a crucial role in protecting against fluoride toxicity[5,54]. In healthy individuals, approximately 50% of ingested fluoride is excreted through the kidneys, making this organ more vulnerable to fluoride toxicity than most other soft tissues[55]. Fluoride exposure inhibits various enzyme systems in the kidneys[56], including decreasing Na^+-K^+-ATPase activities[57].Increases in serum urea nitrogen and creatinine levels are well-documented in cases of fluoride toxicity in both humans and animals[48,58]. Fluoride's toxic effects in rabbits have been characterized by tubular vacuolization and necrosis, glomerular hypertrophy and atrophy, interstitial edema, and nephritis[59]. Serum or plasma creatinine concentration is a key biomarker of kidney function in humans, domestic animals, and birds[5,60]. Consistent with the present findings, elevated plasma creatinine levels have been observed in poultry fed with 800 mg fluoride/kg or more in their feed[5].

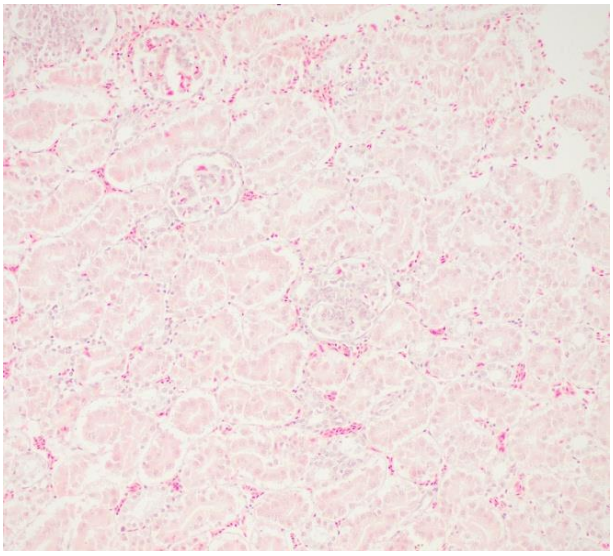

Figure 6a.Kidney in a control bird showing normal tissue architecture. H and E stain, X100.

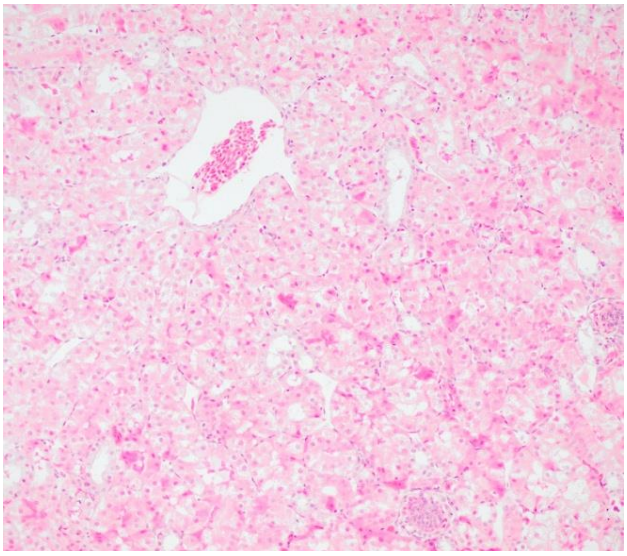

Figure 6b. Kidney in a fluoride intoxicated bird showing congestion and degeneration in tubular epithelium. H and E stain, X100.

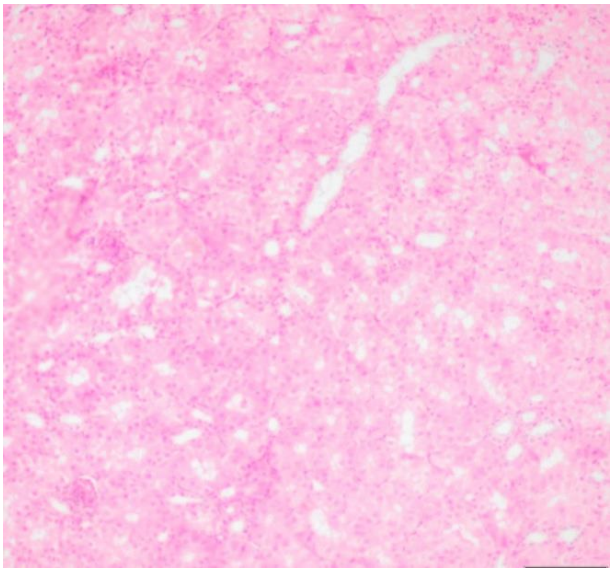

Figure 6c. Kidney in a fluoride intoxicated and Moringa extract co-treated chick showing mild congestion. H and E stain, X100.

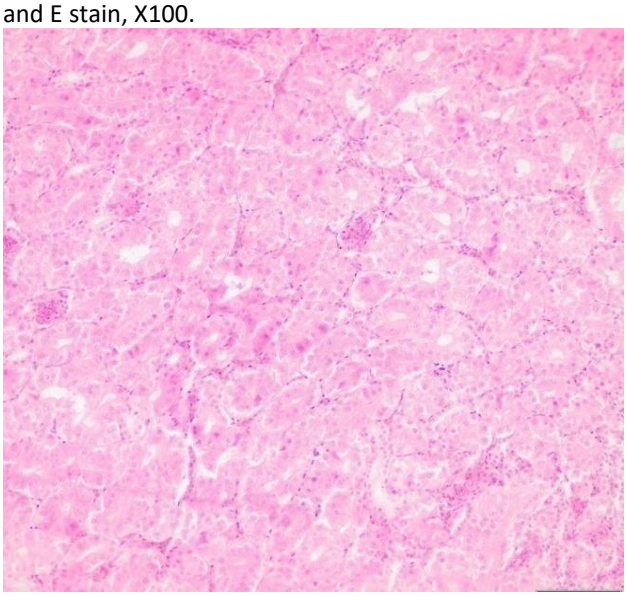

Figure 6d. Kidney in fluoride intoxicated and Prosopis fruit extract supplemented chick showing mild congestion. H and E stain, X100.

Table 3. Changes in ALP, AST, ALT, Creatinine and Urea in blood plasma of birds.

Parameter	*Group*	Day 0	Day 20	Day 40
ALP (U/L)	I	705.33±10.47^{aA}	773.30±08.71^{aA}	0963.33±73.62^{aB}
	II	702.33±11.54^{aA}	990.66±41.70^{cB}	1264.33±90.81^{cC}
	III	708.50±10.07^{aA}	916.20±173.39^{bB}	1074.00±62.62^{bC}
	IV	707.83±11.69^{aA}	945.70±73.62^{bB}	1113.50±107.41^{bC}
SGOT(AST) (U/L)	I	146.08±0.639^{aA}	147.227±0.716^{aA}	151.642±1.432^{aB}
	II	147.08±0.611^{aA}	167.027±0.910^{cB}	177.672±1.210^{cC}
	III	146.61±0.872^{aA}	154.933±2.461^{bB}	159.008±1.040^{bC}
	IV	146.20±0.765^{aA}	157.113±1.726^{bB}	163.622±1.347^{bC}
SGPT(ALT) (U/L)	I	12.497±0.015^{aA}	13.270±0.072^{aA}	15.885±0.042^{aB}
	II	12.654±0.015^{aA}	16.577±0.217^{cB}	23.727±0.699^{cC}
	III	12.418±0.015^{aA}	15.680±0.106^{bB}	19.972±0.203^{bC}
	IV	12.873±0.015^{aA}	16.057±0.072^{bB}	20.487±0.267^{bC}
Creatinine (mg/dl)	I	0.427±0.012^{aA}	0.513±0.023^{aA}	0.612±0.022^{aA}
	II	0.423±0.067^{aA}	0.553±0.041^{cA}	0.772±0.061^{cB}
	III	0.419±0.065^{aA}	0.529±0.012^{bA}	0.663±0.043^{bB}
	IV	0.421±0.017^{aA}	0.530±0.020^{bA}	0.692±0.052^{bB}
Urea Nitrogen (mg/dl)	I	0.340±0.055^{aA}	0.423±0.105^{aB}	0.437±0.162^{aB}
	II	0.346±0.067^{aA}	0.457±0.301^{aB}	0.603±0.571^{cC}
	III	0.345±0.035^{aA}	0.440±0.093^{aB}	0.565±0.189^{bC}
	IV	0.340±0.044^{aA}	0.428±0.098^{aB}	0.585±0.445^{bC}

Values bearing different superscript in small letters in a column and capital letters in a row differ significantly ($p \leq 0.05$).

Functional properties of plant extracts

A comprehensive evaluation of changes in body weight, blood biochemistry, plasma, and bone fluoride levels, as well as histopathological alterations in bone, liver, and kidney tissues, suggests that supplementation with aqueous extracts of *M. oleifera* and *P. cineraria* fruit pods confers beneficial effects in ameliorating fluoride toxicity in chicks. *M. oleifera* seeds have been shown to mitigate fluoride toxicity in experimentally fluorotic rats[61]. Similarly, a study reported that the aqueous extract of *M. oleifera* seeds at a dose of 50 mg/kg body weight for 90 days provided significant protection against fluoride toxicity in rabbits exposed to fluoridated drinking water[32].

M. oleifera, also known as horseradish, drumstick tree, ben oil tree, kelor, marango, moonga, saijhan, and sahjna, originates from the Indian subcontinent and has since spread to tropical and subtropical regions worldwide[62]. The plant contains a diverse range of phyto-compounds, including pterygospermin, isothiocyanates, glucosinolates, alkaloids, glycosides, sterols, polyphenols, and other bioactive compounds[63,64], making it highly valued for its nutritional and medicinal properties. Various parts of the plant-roots, bark, leaves, flowers, fruits, and seeds-have been utilized in traditional medicine for their health benefits[65]. Specifically, *M. oleifera* pods are rich in essential nutrients like potassium, calcium, phosphorus, iron, vitamins A and D, essential amino acids, and antioxidants such as beta-carotene, vitamin C, and flavonoids[66,67]. These attributes lend the plant its well-documented roles in antiulcer, diuretic, anti-inflammatory, and wound-healing therapies[68,69]. Research has also demonstrated its potent antioxidant properties in various animal models[70-72], making it a potential candidate for protecting against fluoride-induced toxicity in birds. The beneficial effects of aqueous *M. oleifera* extracts observed in this study may be attributed to its nutritional and functional properties, which helped in mitigating the toxic effects of excess fluoride on bone and soft tissues.

P. cineraria, commonly known as the Khejri or Ghaf tree, is integral to the Indian desert ecosystem, renowned for its cultural, economic, and ecological significance, especially due to its remarkable tolerance to harsh climates[73,74]. This plant has long been used in traditional medicine to treat ailments such as asthma, leprosy, leukoderma, tumors, dysentery, bronchitis, and piles. It exhibits diverse biological activities, including anticancer, bactericidal, fungicidal, antiviral, and anthelmintic properties[75]. Additionally, its role in removing heavy metals like lead and cadmium from wastewater has been explored in various studies[76-79].

The efficacy of *P. cineraria* in mitigating fluoride toxicity, particularly in arid and semi-arid regions, is attributed to its unique phytochemical composition[80]. The bioactive compounds present in its leaves, pods, and bark- such as tannins, flavonoids,

alkaloids, saponins, and antioxidants- contribute to its protective effects against fluoride-induced toxicity[81]. Its antioxidant properties play a crucial role in reducing oxidative stress and scavenging free radicals generated by excessive fluoride exposure[82]. Furthermore, it enhances calcium absorption, which helps counteract the adverse effects of fluoride on bone health[83]. To maximize the potential benefits of *P. cineraria* in mitigating toxicities, various formulations can be derived from its leaves, fruit pods, or bark.

CONCLUSIONS

The results of the present study indicate that excess fluoride intake in chicks leads to a decrease in growth rate and alterations in bone, liver, and kidney tissues. Fluoride concentrations in blood plasma and long bones increase, accompanied by changes in blood biochemical parameters. Supplementation with aqueous extracts of *M. oleifera* and *P. cineraria* fruit pods has beneficial effects by restoring growth rates and preventing toxic insult to bone and soft tissues. Therefore, these two plant extracts exhibit functional food properties and may have potential applications in the poultry industry for promoting health and production while minimizing the adverse effects of environmental pollutants and toxicants.

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CONFLICT OF INTERESTS

None

ETHICS STATEMENT

The experimental protocol was reviewed and approved by The Institutional Animal Ethics Committee, CVAS, Bikaner, Rajasthan, India (CVAS/IAEC/2023-24/26; dated 17.07.2023).

AUTHORS CONTRIBUTIONS

DK and AA contributed to the study's conception, design, and experimentation. RR performed the laboratory analysis and wrote the first draft. SSC conducted the statistical analysis and edited the manuscript. KG carried out the histopathology. AKB and PB performed the radiography and its interpretation.

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