Clinicopathological Study On Fluoride Toxicosis and Aluminum Sulfate-Ration -Supplement In Chickens

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ABSTRACT

The present study was performed to investigate the clinicopathological changes associated with sodium fluoride toxicity and aluminum sulphate ration supplement in Balady chickens. One hundred and fifty, one day old Balady chickens were divided into six equal groups. Gp.1 was the control. Diet of gps. 2&3 was supplemented with 800 and 1600 ppm sodium fluoride respectively , the ration of gp. 4 was supplemented with 640 ppm aluminum sulphate . The ration of gp. 5 was supplemented with 800 ppm sodium fluoride and 640 ppm aluminum sulphate ,while that of gp. 6 was supplemented with 1600 ppm sodium fluoride together with 1280 ppm aluminum sulphate.

Clinically the chickens showed anorexia, poor growth with yellowish-brown diarrhea and toe deformation which led to stiffness and lameness with high mortality. Gps. 2-6, showed macrocytic hypochromic anemia with decreased thrombocytic count and increased clotting time.

Biochemical analysis, revealed hepatic damage (increased activity of liver enzymes ALT, AST and ALP with decreased total protein, albumin and globulin levels) and disturbed renal function (increased uric acid and creatinine, decreased calcium, magnesium and non significant change in inorganic phosphorus). Pathological results confirmed the biochemical results which revealed toxic effect of sodium fluoride and aluminum sulphate on the liver and kidneys.

INTRODUCTION

Fluoride is a naturally occurring element. Fluoride compounds are constituents of minerals in rocks and soil. Water passes over rocks and dissolves the fluoride compounds that are present, creating fluoride ions. The result is that small amounts of soluble fluoride ions are present in all water sources, including the oceans. Fluoride is present to some extent in all foods and beverages *(1,2).*

Aluminum is the most abundant metal in the earth's crust, and the third most abundant element therein, after oxygen and silicon. It makes up to about 8% by weight of the earth's solid surface. Aluminum is too reactive chemically to occur in nature as a free metal. The chief source of aluminum is bauxite ore *(3).*

Aluminum compounds inhibit the toxic effects of fluoride by forming less soluble complexes with fluoride and they decrease the absorbability of fluoride *(4)* or neutralize the hydrogen fluoride produced in the stomach *(5).*

The present study was performed to investigate the signs, clinicopathological changes (hematological and biochemical) and lesions associated with sodium fluoride and aluminum sulphate toxicity in Balady chickens. Moreover the evaluation of aluminum sulphate, supplement to ration was considered.

MATERIAL AND METHODS

I-Chickens

One hundred and fifty (one day old) Balady chickens were kept under standard hygienic conditions and on a commercial well balanced ration. All chickens were vaccinated against ND by Hitchner B1 via eye drop at the age of 7 days old and against Gumboro disease via drinking water when 15 days old *(6).*

II- Chemicals

Sodium fluoride (NaF) was obtained from Avondala laboratories (supplies and services) limited. Banbury, Oxon, England. Assay: 97%.

Aluminum sulphate $Al_2(So_4)_3$ was obtained from Laboratory chemicals, Alpha group:(98%).

III- Experimental design

The birds were divided into 6 equal groups and treated from the one day old till the age of 7 weeks.

Gp.1 was given normal ration(control).Gp. 2 was given ration containing 800 ppm sodium fluoride. Gp.3 was given ration containing 1600 ppm sodium fluoride. Gp. 4 was given ration containing 640 ppm aluminum sulphate. Gp.5 was given ration containing 800 ppm of sodium fluoride and 640ppm aluminum sulphate. Gp. 6 was given ration containing 1600 ppm of sodium fluoride and 1280 ppm aluminum sulphate

Five chickens from each group were weighed weekly. The feed consumption, body weight and body gain for each group was calculated weekly. Mortality rate was recorded in all groups.

Blood Samples

Two blood samples were collected from wing vein of five chickens in each group three times at the end of the 3rd , 5th and 7th weeks of age. The first blood sample was collected in a clean EDTA –containing tube and used for hematological examinations. The second blood sample was collected without anticoagulant for separation of serum samples for the different biochemical assays *(9)*.

Tissue Specimens

Liver and kidneys were collected from five chickens from each group(1-6) after sacrification at the end of 3rd , 5th and 7th weeks from the experiment for pathological examination.

Hematological studies

Erythrocytic count was performed using the improved Neubaur hemocytometer with Natt and Herrick's solution as diluting fluid *(10)*. The hemoglobin was estimated by cyanmethemoglobin method (after centrifugation). Packed cell volume(PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined. Thrombocytic count and clotting time were determined *(11)*.

Clinicobiochemical analysis

The serum activities of ALT and AST were determined *(12)*,besides the activity of ALP was estimated *(13)*. The serum total protein *(14)*, albumin *(15)*, globulin *(16)*, uric acid *(17)*, creatinine *(18)*, calcium *(19)*, inorganic phosphorus *(20)* and magnesium *(21)* levels were determined.

Histopathological examination

Specimens from the liver and kidneys were collected from gps.1-6, fixed in 10 % neutral buffered formalin. Five micron thick paraffin section were prepared , stained with hematoxylin and eosin (H&E) and examined microscopically *(22)*.

Statistical Analysis

The obtained data were statistically analyzed, using F- test *(23)*.

RESULTS AND DISCUSSION

Chickens of gps. 2&3 showed anorexia, decreased growth rate, small in size **(fig.1)**, depression, ruffled feathers with yellowish-brown diarrhea and toe deformation **(fig.2)** and led to stiffness and lameness. These signs were mild in chickens which received aluminum sulphate gp.4 and also treated gps. 5&6 . The mortality rate was 12% in gp. 2 , 20% in gp.3 , 28% in gp.4 and 8% in gps. 5&6 . These results may be due to fluoride inhibition of growth by restricting feed consumption *(24)*. Reduction in growth rate in fluoride toxicity resulted in anemia *(5)*. The stomach is very sensitive to fluorides. Fluoride toxicity in the intestine, inhibits of sodium absorption and hence the transport of fluids through the intestinal wall, leading to diarrhea *(25)*. Increase in mineral content by fluoride toxicity and increasing tibia magnesium indicate impaired bone mobilization *(26)*. Fluoride leads to deposition of calcium salts in tissues and results in bone deformation *(27)*. Fluoride toxicity causes enlarged joints which become sore and stiff, leading to lameness *(28)*. A positive relationship exists between mortality and dietary fluoride as fluoride interferes with birds resistance to common diseases *(29)*.

The clinical signs, observed in chickens of gp. 4, may be due to immunosuppressive effect of aluminum sulphate (as toxic chemical), which is considered among bird-stressors *(30)*.

All groups showed a reduction in feed consumption, body weight and body gain throughout the experimental periods (3rd , 5th and 7th weeks) (Tables 1-3). Such reduction was obvious in gps. 2&3 throughout the experimental periods. The gain observed decrease in body weight may be due to the decreased feed intake and its duration. The decreased feed intake could be due to gastric and intestinal irritation produced by sodium fluoride *(29)*. Moreover the decreased growth performance in gp. 4 may be due to decreased feed consumption which resulted in decreased gain body weight *(31)*. On the other hand, aluminum did not affect body weight of broiler chicks (one-week old) fed on 400 and 800 ppm of aluminum, for 6 weeks *(7)*. The difference may be due to different breeds.

The hemogram in the present study Table 4, reveals highly significant decrease in red blood cell-count, hemoglobin concentration and packed cell volume in all groups producing macrocytic hypochromic anemia, throughout the experimental periods (3rd , 5th and 7th weeks). These results in gps. 2&3 may be due to the hemolytic effect of fluoride *(32)*. Hemolytic anemia may result from toxic chemicals. Also, toxins depress erythropoiesis *(9)*. Decreased RBCs count, Hb and PCV in chronic fluorosis in farm animals due to the lower serum iron and hemolytic effect of fluoride was reported *(33)*.On the other hand, aplastic anemia in cattle *(34)* and megaloblastic anemia in human resulted from administration of 5-fluorouracil as fluoride source *(35)*. These variation may be due to the difference in species.

The results in gp.4 may be due to aluminum intoxication which causes resistance to erythropoietin by interference with heme synthesis and accumulation of protoporphyrin leading to anemia *(36,37)*. Aluminum produces peroxidative changes in the erythrocyte membrane, leading to hemolysis. Therefore, the depressed erythrocytic count in animals intoxicated with aluminum may be the consequence of both the hemolytic action of aluminum and the shortened survival time of erythrocytes.

Chickens of gps. 5 &6 , showed similar results. Aluminum supplement did not improve the on hematological changes caused by fluoride toxicity *(38)*.

Thrombocytic count, in the present study, was highly significantly decreased in gps. 2-6 throughout the experimental periods (3rd, 5th and 7th weeks). 5-fluorouracil (fluoride source) is a pyrimidine analog that inhibits thymidylate synthetase, an enzyme necessary for the formation of thymidylic acid,which resulted in thrombocytopenia in chickens *(11)*.

The clotting time, was prolonged in gps. 2,3 &6 after 3rd and 5th weeks and in gp. 3 after 7th week. Prolonged whole blood clotting time is seen in a variety of coagulopathies in domestic animals including severe liver disease, thrombocytopenia and the presence of circulating anticoagulants (sodium fluoride) *(9)*. Our results get along with the use of sodium fluoride as an anticoagulant.

Table 5 shows that the liver enzyme, (ALT and AST) activities were highly significantly increased throughout the experimental periods in all groups. AST has a wide distribution in the body's tissues and although it is not liver specific, it is possibly the single most useful enzyme for indicating liver disease. The increased activities of ALT and AST could be due to liver damage (degenerative changes) caused by fluoride intoxication. The increased serum activity of ALT is directly related to the degree of hepatic damage in hepatocytes and AST is usually associated with cell necrosis of different tissues, either muscular or hepatic. Increased AST activity occurs with hepatopathy by toxic chemicals (fluoride and/or aluminum) *(9, 39,40)*. The histopathological changes in the liver of gps. 2-6 confirmed this result. The liver revealed vacuolar and hydropic degeneration, focal coagulative necrosis with granular basophilic material and portal fibrosis in addition to lymphocytic infiltration **(figs.3-6)**.

The ALP activity was highly significantly increased throughout the experimental periods in gps. 2-6 . The fluorine acts on periostium which is the main source of ALP enzyme and the hyperactivity of the periostium is

considered the main cause of increased ALP activity *(41)*. ALP is widely distributed in the body and is found in high concentration in bone (osteoblast). In the chickens the intestinal isoenzyme of alkaline phosphatase makes the largest contribution to plasma alkaline phosphatase activity and is affected by intestinal disturbances and inappetence *(9,39)*. Fluoride has an intense, dose dependent osteogenic action and osteofluorosis is associated with increased bone ALP activity *(42)*. Alkaline phosphatase is non-organ-specific enzyme which is found mostly in the duodenum and kidneys. ALP enzyme is considered to be good indicator of osteoblastic activity, its level is also increased in growing birds and in some cases of liver disease*(40)*. The increased in ALP activity is probably related to the abnormal formation of bone*(5)*.

The proteinogram, in the present study, showed highly significant decrease in gps. 2-6 throughout the experimental periods, with hypoalbuminemia and hypoglobulinemia in gps. 2-6 . The histopathological changes in the liver in gps. 2-6 confirmed the current result. The serum protein concentration of avian blood is lower than that of mammals. Albumin is the largest individual protein fraction in avian plasma. Hypoproteinemia can occur with chronic renal or hepatic disease,malnutrition and malabsorption *(9,39)*. The majority serum proteins are produced by the liver. A reduction in the total serum protein indicates of the severity and progression of hepatopathy. Other possible causes include any disease associated with anemia and malnutrition. The major protein produced by the liver and forms most of plasma protein is albumin so that a hypoalbuminemia is usually responsible for a drop in total serum protein. Hypoglobulinemia, associated with reduction in total protein indicates liver failure and malnutrition *(40)*. The decreased total protein could be attributed to the inhibitory effect of fluoride on protein synthesis*(43, 44)*.

Table 6 shows hyperuricemia (gps. 3, 5&6) throughout the experimental periods. Uric acid is the primary catabolic product of protein, non protein nitrogen and purines in birds. The avian kidney excretes uric acid primarily by tubular excretion. Hyperuricemia, in birds, occurs with starvation, massive tissue destruction and renal disease*(9,39)*. Levels of uric acid vary with age and species. Birds excrete 60-80% of their nitrogenous waste as uric acid (synthesized in the liver but excreted through the kidney). Hyperuricemia, in birds, occurs with decreased feed intake(due to catabolism of the body's tissues as liver and renal damage) and in toxicosis of the kidneys (fluoride and/or aluminum) *(40)*.

Creatinine, showed non-significant change in gps. 2-6 at the end of the 3rd week of age. A highly significant increase in gp. 2 at the end of the 5th week and was observed in gps. 2-6 at the end of the 7th week of age. These results showed that creatinine level was time dependent. Creatinine is not a major non protein nitrogen component of avian blood. Serum creatinine has a questionable value in the evaluation of renal function in birds. Increased serum creatinine in birds indicates renal damage*(9,39)*.

The changes in uric acid and creatinine was confirmed by our histopathological results as grossly, the kidneys were enlarged and congested. Microscopically,cloudy swelling, hydropic degeneration and coagulative necrosis were encountered in the renal tubular epithelium. Basophilic granular casts were noticed inside the lumens of some renal tubules**(figs.7-12).**

Hypocalcemia was found in gps. 2, 3&6 at the end of the 3rd and the 5th weeks of age, and in gps. 2-6 at the end of the 7th week of age, it was time dependent. The decreased blood calcium may be due to kidney failure to reabsorb calcium from glomerular filtrate and diarrhea *(45)*. Fluoride causes hypocalcemia by removing blood and tissue-calcium through precipitation *(46,47)*. Hypocalcemia is associated with renal failure and hypoalbuminemia, which can result in a depressed-production of protein-bound calcium and eventually hypocalcemia *(40)*. The decreased blood calcium values could be related to the inhibition of calcium absorption from intestine due to CaF formation *(38)*.

Table 1. Feed consumption/gm/chicken(mean values) for gps. 1-6, throughout the experimental periods.

Periods(week) / Groups	1st	2nd	3rd	4th	5th	6th	7th
1	115	122	257	282	318	400	436
2	102	108	150	193	254	334	370
3	102	107	147	178	237	300	364
4	110	115	182	204	303	385	400
5	100	102	157	221	306	366	391
6	102	103	152	209	292	340	380

(1) Control gp., (2) NaF 800 ppm, (3) NaF 1600ppm, (4) $Al_2(SO_4)_3$ 640ppm,
(5) NaF+ $Al_2(SO_4)_3$800+640ppm and (6) NaF+ $Al_2(SO_4)_3$1600+1280ppm.

Table 2. Body weight/ gm/chicken(mean values ± SE) for gps. 1-6, throughout the experimental periods.

Periods(week) / Groups	1st	2nd	3rd	4th	5th	6th	7th
1	47.00 ±0.95	98.43 ±4.18	160.90 ±3.80	220.50 ±7.92	278.70^{a} ±17.33	341.92^{a} ±15.73	393.02^{a} ±18.37
2	45.69 ±0.17	87.46 ±0.57	147.40 ±9.01	196.40 ±9.30	230.52^{b} ±14.70	257.78^{bc} ±19.38	280.90^{b} ±32.70
3	45.85 ±0.12	92.60 ±1.96	150.60 ±5.92	195.54 ±9.03	226.38^{b} ±9.80	238.38^{c} ±15.91	272.16^{b} ±17.96
4	47.14 ±0.19	97.41 ±0.98	158.34 ±9.61	200.44 ±5.06	247.38^{ab} ±14.12	290.28^{b} ±12.45	329.98^{b} ±16.05
5	44.81 ±0.91	96.11 ±1.85	154.48 ±9.72	198.98 ±9.51	239.60^{ab} ±12.41	270.28^{bc} ±12.82	296.14^{b} ±22.08
6	45.81 ±0.98	91.78 ±2.40	149.10 ±6.97	191.54 ±18.59	226.64^{b} ±17.81	259.48^{bc} ±22.06	291.60^{b} ±17.44

Means at the same column with different letters are significantly different

Table 3. Body gain/ gm/chicken (mean values ± SE) for gps. 1-6 , throughout the experimental periods.

Periods (week) / Groups	2nd	3rd	4th	5th	6th	7th
1	51.43^{a} ±3.31	62.47^{a} ±2.48	59.60^{a} ±4.39	58.20^{a} ±10.62	53.22^{a} ±6.72	51.10^{a} ±10.44
2	41.77^{b} ±0.56	59.94^{ab} ±8.89	49.00^{b} ±5.84	34.12^{c} ±8.92	27.26^{c} ±5.18	23.12^{c} ±14.05
3	46.75^{ab} ±1.84	58.00^{b} ±4.11	44.94^{bc} ±3.27	30.84^{c} ±4.25	25.78^{c} ±9.23	20.00^{c} ±3.73
4	50.26^{a} ±0.82	60.93^{ab} ±8.63	42.10^{c} ±4.93	46.94^{b} ±11.50	42.90^{b} ±10.50	39.70^{b} ±6.14
5	51.29^{a} ±1.02	58.37^{b} ±7.91	44.50^{c} ±5.22	40.62^{bc} ±4.30	30.68^{c} ±4.60	25.86^{c} ±12.98
6	45.97^{ab} ±1.47	57.32^{b} ±4.80	42.44^{c} ±13.00	35.10^{c} ±5.83	32.84^{c} ±11.19	32.16^{bc} ±11.11

(1) Control gp., (2) NaF 800 ppm, (3) NaF 1600ppm, (4) $Al_2(SO_4)_3$ 640ppm,
(5) NaF+ $Al_2(SO_4)_3$800+640ppm and (6) NaF+ $Al_2(SO_4)_3$1600+1280ppm.
Means at the same column with different letters are significantly different.

Table 4.Hematological changes (mean values± SE) of gps. 1-6 ,at different experimental periods.

Parameter / Group &Periods		RBCs $\times 10^6$ µl	Hb gm %	PCV %	MCV fl	MCHC %	Thrombocyte $\times 10^3$ µl	Clotting time (seconds)
3 weeks post administration	1	3.04±0.11^{a}	13.62±0.29^{a}	36.20±0.66^{a}	119.29±3.14^{d}	37.71±1.34^{a}	44.00±0.71^{a}	50.20±6.30^{c}
	2	1.84±0.14^{bc}	9.72±0.37^{b}	27.60±0.75^{b}	152.79±10.48^{bc}	35.30±1.53^{b}	36.00±0.84^{c}	198.80±15.00^{a}
	3	1.35±0.14^{c}	9.20±0.88^{b}	28.00±1.41^{b}	213.17±16.11^{a}	33.34±4.27^{c}	34.80±0.73^{c}	209.40±12.99^{a}
	4	1.73±0.16^{bc}	9.68±0.18^{b}	26.80±1.02^{b}	158.91±12.61^{b}	35.23±0.80^{b}	38.80±1.16^{b}	75.20±4.58^{c}
	5	2.16±0.31^{b}	9.46±0.39^{b}	27.40±0.87^{b}	136.50±17.85^{c}	34.52±0.83^{bc}	39.00±0.45^{b}	88.6o±19.77^{c}
	6	1.90±0.17^{bc}	9.36±0.31^{b}	25.60±1.17^{b}	141.26±18.13^{c}	35.64±1.95^{b}	40.00±0.71^{b}	145.60±13.32^{b}
5 weeks post administration	1	3.11±0.14^{a}	13.70±0.22^{a}	37.60±4.46^{a}	123.6±19.889^{c}	38.03±3.46^{a}	40.40±1.03^{a}	70.60±14.36^{c}
	2	1.70±0.28^{b}	8.88±0.36^{cd}	27.80±1.88^{b}	197.22±52.69^{a}	32.22±1.08^{d}	32.00±0.84^{cd}	166.80±35.67^{ab}
	3	1.66±0.29^{b}	8.12±0.31^{d}	25.40±2.36^{b}	166.45±23.84^{ab}	33.36±3.80^{cd}	30.00±0.55^{d}	191.20±29.81^{a}
	4	1.80±0.12^{b}	9.28±0.41^{c}	27.00±1.00^{b}	153.68±13.59^{b}	34.78±2.83^{bc}	37.60±0.93^{b}	126.40±9.61^{bc}
	5	1.77±0.35^{b}	9.76±0.23^{bc}	27.60±1.43^{b}	185.21±40.03^{a}	35.66±1.66^{b}	34.00±0.71^{c}	113.40±28.82^{c}
	6	1.80±0.32^{b}	10.48±0.23^{b}	28.80±0.58^{b}	184.76±38.92^{a}	35.48±1.41^{b}	33.60±1.33^{c}	177.80±31.21^{ab}
7 weeks post administration	1	3.02±0.18^{a}	13.74±0.30^{a}	36.80±0.58^{a}	121.40±6.40^{d}	37.38±1.07^{a}	41.20±0.58^{a}	35.23±15.54^{b}
	2	1.84±0.11^{b}	8.76±0.40^{b}	25.00±1.14^{b}	136.65±4.74^{c}	34.23±1.94^{b}	35.20±0.66^{b}	133.40±27.25^{ab}
	3	1.75±0.21^{b}	7.76±0.17^{b}	25.60±1.47^{b}	156.36±23.00^{b}	29.73±1.62^{c}	31.20±0.58^{c}	154.80±19.57^{a}
	4	1.18±0.23^{c}	8.72±0.28^{b}	26.60±1.08^{b}	260.21±53.53^{a}	32.43±1.68^{bc}	36.40±1.21^{b}	128.00±16.80^{ab}
	5	1.75±0.17^{b}	8.40±0.43^{b}	26.80±0.97^{b}	159.01±16.43^{b}	31.48±1.83^{c}	34.80±0.58^{b}	96.00±13.18^{b}
	6	1.90±0.16^{b}	7.68±0.58^{b}	25.00±0.84^{b}	136.58±9.96^{c}	30.69±1.97^{c}	36.80±1.16^{b}	98.00±17.10^{b}

(1) Control gp., (2) NaF 800 ppm, (3) NaF 1600ppm, (4) $Al_2(SO_4)_3$ 640ppm, (5) NaF+ $Al_2(SO_4)_3$ 800+640ppm and (6) NaF+ $Al_2(SO_4)_3$1600+1280ppm.

Means at the same column at the same period with different letters are significantly different.

Table 5. Biochemical parameters (mean values± SE) of gps. 1-6,at different experimental periods.

Groups& period \ Parameter		ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)
3 weeks post administration	1	7.80±0.66^{b}	72.00±2.76^{b}	173.20±15.83^{d}	3.66±0.29^{a}	1.53±0.18^{a}	2.11±0.17^{a}
	2	26.06±3.79^{a}	102.40±3.59^{a}	687.62±63.73^{ab}	2.10±0.25^{b}	0.98±0.15^{b}	1.12±0.19^{b}
	3	28.60±4.19^{a}	104.80±2.96^{a}	753.77±34.63^{a}	2.02±0.13^{b}	1.01±0.11^{b}	1.01±0.18^{b}
	4	25.80±3.67^{a}	100.20±3.05^{a}	477.77±9.04^{c}	2.13±0.11^{b}	1.14±0.09^{ab}	0.98±0.10^{b}
	5	24.00±3.11^{a}	101.80±2.97^{a}	542.44±46.94^{c}	2.13±0.32^{b}	1.12±0.11^{ab}	1.01±0.31^{b}
	6	25.20±4.21^{a}	101.20±3.59^{a}	566.66±66.55^{bc}	2.36±0.27^{b}	1.38±0.12^{ab}	0.98±0.21^{b}
5 weeks post administration	1	9.40±1.08^{b}	74.80±5.25^{b}	164.57±23.77^{d}	3.65±0.43^{a}	1.77±0.21^{a}	1.84±0.41^{a}
	2	30.40±7.43^{a}	106.80±5.44^{a}	726.69±73.25^{ab}	2.19±0.16^{b}	1.08±0.03^{b}	1.11±0.15^{b}
	3	30.60±4.82^{a}	107.80±3.74^{a}	784.99±65.46^{a}	2.34±0.19^{b}	1.11±0.21^{b}	1.22±0.27^{b}
	4	29.60±4.36^{a}	101.80±3.25^{a}	581.77±17.20^{c}	2.30±0.07^{b}	1.22±0.17^{b}	1.08±0.23^{b}
	5	23.80±4.00^{a}	103.40±3.63^{a}	601.78±46.94^{b}	2.44±0.05^{b}	1.09±0.11^{b}	1.35±0.15^{b}
	6	26.60±3.31^{a}	105.20±4.54^{a}	679.91±51.86^{ab}	2.19±0.07^{b}	1.29±0.21^{ab}	0.90±0.27^{b}
7 weeks post administration	1	8.00±0.55^{b}	75.00±4.60^{b}	208.43±23.77^{b}	3.70±0.20^{a}	1.92±0.10^{a}	1.79±0.27^{a}
	2	28.60±4.03^{a}	107.40±2.01^{a}	719.26±59.57^{a}	1.98±0.12^{c}	0.92±0.03^{b}	1.06±0.08^{bc}
	3	30.80±3.40^{a}	108.60±3.61^{a}	723.76±61.04^{a}	1.96±0.07^{c}	1.09±0.17^{b}	0.88±0.16^{c}
	4	28.00±3.33^{a}	103.20±3.54^{a}	560.27±60.55^{a}	2.35±0.16^{bc}	1.08±0.08^{b}	1.27±0.21^{ab}
	5	24.80±2.74^{a}	104.80±3.76^{a}	665.87±46.94^{a}	2.43±0.15^{b}	1.00±0.09^{b}	1.43±0.16^{ab}
	6	27.40±4.07^{a}	107.40±2.01^{a}	685.87±67.79^{a}	2.21±0.11^{bc}	1.08±0.21^{b}	1.12±0.14^{bc}

(1) Control gp., (2) NaF 800 ppm, (3) NaF 1600ppm, (4) $Al_2(SO_4)_3$ 640ppm, (5) NaF+ $Al_2(SO_4)_3$ 800+640ppm and (6) NaF+ $Al_2(SO_4)_3$1600+1280ppm.

Means at the same column at the same period with different letters are significantly different.

Table 6 . Renal function tests (mean values± SE) of gps. 1-6,at different experimental periods.

Groups &periods \ Parameter		Uric acid (mg/dl)	Creatinine (mg/dl)	Calcium (mg/dl)	Inorganic phosphorus (mg/dl)	Magnesium (mg/dl)
3 weeks post administration	1	5.42±0.57^{c}	1.55±0.14	10.70±0.97^{a}	4.64±0.22	3.22±0.22^{a}
	2	7.05±0.86^{bc}	1.70±0.14	7.17±1.27^{b}	4.73±0.24	2.33±0.14^{b}
	3	13.37±2.03^{a}	1.46±0.13	6.58±0.94^{b}	4.85±0.10	2.32±0.14^{b}
	4	7.58±0.76^{bc}	1.52±0.04	9.00±0.47^{ab}	4.92±0.17	2.32±0.32^{b}
	5	7.31±0.92^{bc}	1.46±0.13	8.82±1.03^{ab}	4.87±0.30	2.42±0.13^{b}
	6	9.88±0.90^{b}	1.45±0.19	7.88±0.52^{b}	4.86±0.17	2.40±0.14^{b}
5 weeks post administration	1	4.67±0.65^{bc}	1.64±0.08^{b}	10.11±0.97^{a}	4.15±0.33	3.42±0.14^{a}
	2	7.13±0.83^{b}	2.60±0.24^{a}	6.17±0.56^{b}	3.77±0.23	1.38±0.11^{b}
	3	9.86±1.28^{a}	1.80±0.23^{b}	6.15±0.99^{b}	4.17±0.13	1.35±0.09^{b}
	4	3.77±0.52^{c}	1.64±0.17^{b}	8.92±1.07^{ab}	4.25±0.18	1.38±0.04^{b}
	5	7.40±0.69^{a}	1.45±0.16^{b}	8.65±0.88^{ab}	3.69±0.20	1.65±0.16^{b}
	6	8.97±0.99^{a}	2.01±0.34^{ab}	7.37±0.95^{b}	4.04±0.28	1.44±0.17^{b}
7 weeks post administration	1	4.26±0.08^{c}	1.38±0.28^{b}	11.06±0.80^{a}	4.57±0.34	3.75±0.24^{a}
	2	6.59±0.58^{bc}	2.50±0.19^{a}	7.14±0.61^{c}	3.06±0.58	1.62±0.19^{b}
	3	9.98±0.94^{a}	2.75±0.17^{a}	6.03±0.70^{c}	3.68±0.35	1.58±0.24^{b}
	4	6.39±0.52^{bc}	2.41±0.16^{a}	7.86±0.62^{bc}	3.46±0.46	1.58±0.20^{b}
	5	8.45±1.12^{ab}	2.50±0.32^{a}	7.72±0.59^{bc}	3.65±0.16	1.64±0.12^{b}
	6	9.26±1.14^{a}	2.53±0.15^{a}	7.97±0.62^{bc}	3.63±0.36	1.84±0.12^{b}

(1) Control gp., (2) NaF 800 ppm, (3) NaF 1600ppm, (4) $Al_2(SO_4)_3$ 640ppm, (5) NaF+ $Al_2(SO_4)_3$ 800+640ppm and (6) NaF+ $Al_2(SO_4)_3$1600+1280ppm.

Means at the same column at the same period with different letters are significantly different.

Fig. 1. Gp.(2), slow gain in body weight when compared with the control

Fig.2. Gp.(3), toe deformation, depression and ruffled feathers

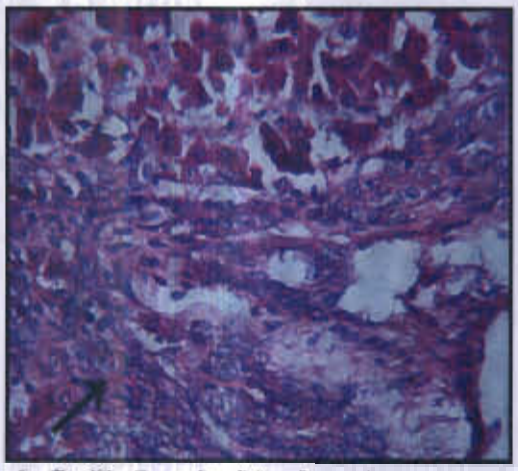

Fig.3. Gp.(2), 7 weeks PA, photomicrograph of the liver showing portal fibrosis (arrow) (HE x 1200).

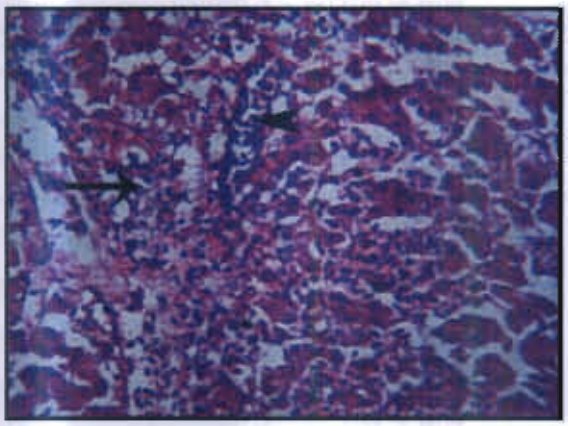

Fig. 4. Gp.(3) ,3 weeks PA, photomicrograph of the liver showing focal coagulative necrosis (arrow) with granular basophilic cytoplasm (arrowhead)(HE X300).

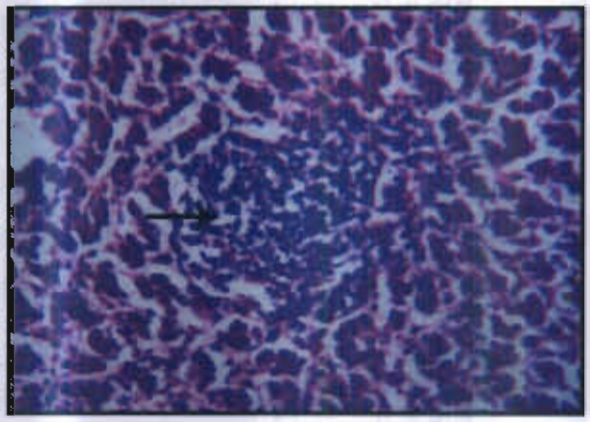

Fig. 5. Gp.(4), 3 weeks PA, photomicrograph of the liver showing few lymphocytic infiltrations in the portal area (arrow)(HE x 300).

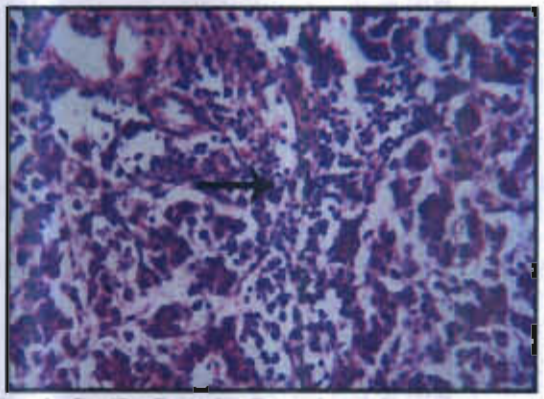

Fig. 6. Gp.(5), 7 weeks PA, photomicrograph of the liver showing mild portal fibrosis and lymphocytic infiltration (arrow) (HE x 300).

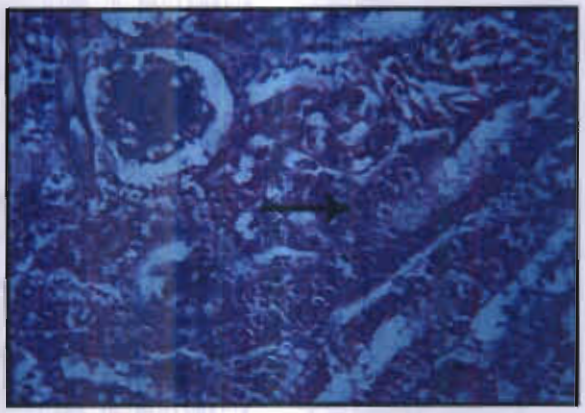

Fig. 7. Gp.(2), 3 weeks PA , photomicrograph of the kidney showing cloudy swelling and hydropic degeneration in the renal tubular epithelium (arrow) (HE x 1200).

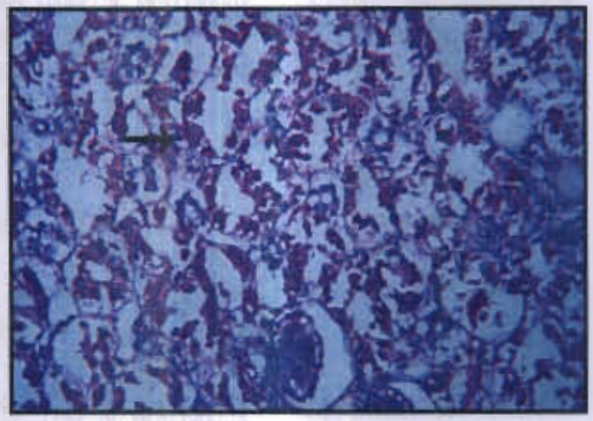

Fig. 8. Gp.(3), 5 weeks PA , photomicrograph of the kidney showing tuhular coagulative necrosis (arrow) (HE x 300).

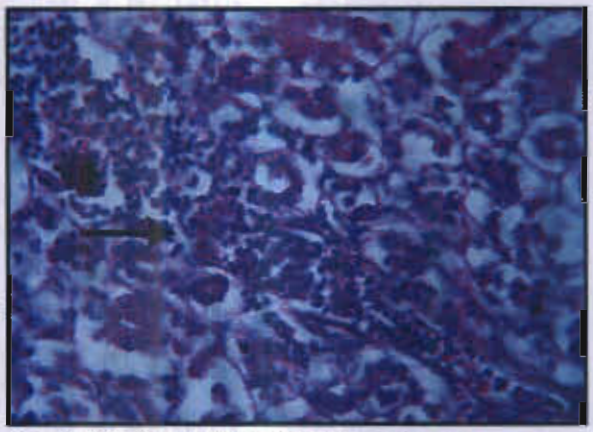

Fig. 9. Gp.(4), 3 weeks PA, photomicrograph of the kidney showing focal mononuclear cell infiltration (arrow) (HE x 300).

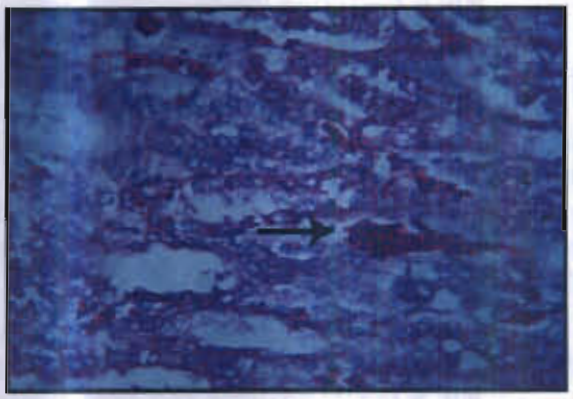

Fig. 10. Gp.(5), 3 weeks PA, photomicrograph of the kidney showing congestion (arrow) (HE x 300).

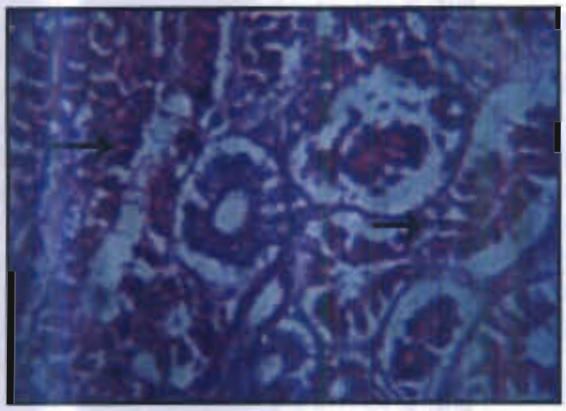

Fig. 11. Gp.(6), 5 weeks PA, photomicrograph of the kidney showing coagulative necrosis represented by pyknosis (arrow) (HEx 1200).

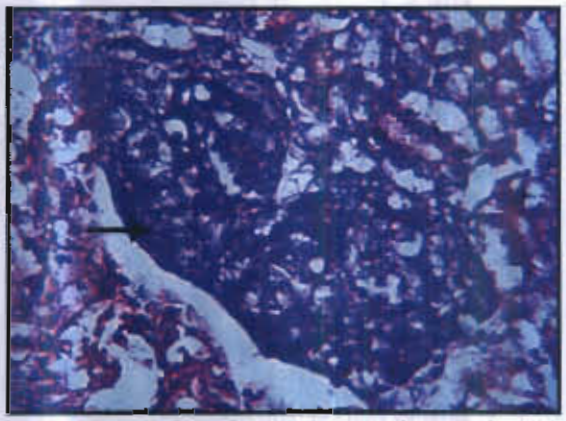

Fig. 12. Gp.(6), 7 weeks PA, photomicrograph of the kidney showing regenerated renal tuhules represented by hasophilic epithelium (arrow) (HE x 300).

Inorganic phosphorus showed non significant change in gps. 2-6 at the end of the 3rd , 5th and 7th weeks of age. This result may be due to elevated serum phosphorus associated with renal disease to compensate the low serum phosphorus induced by impaired intestinal absorption by aluminum. Moreover anorexia contributed to hypophosphatemia *(9,38,40)*.

Magnesium, was highly significantly decreased in gps. 2-6 throughout the experimental periods. Fluoride can pass easily through cell membranes by nonionic diffusion binding magnesium and making it unavailable for various biological functions *(48)*.

Macroscopically, the liver and kidneys were enlargemed, congested and hemorrhagic. Microscopically, the liver revealed vacuolar and hydropic degeneration, focal coagulative necrosis with granular basophilic cytoplasm and portal fibrosis **(figs.3-6).** Kidneys, revealed severe toxic tubular nephrosis represented by cloudy swelling, hydropic degeneration and coagulative necrosis in the renal tubular epithelium. Glomerular hypercellularity, besides hyaline and cellular casts were seen in the renal tubules throughout the experimental periods*(7,27,47,49, 50)*.**(figs.7-12).**

It could be concluded that sodium flouride in Balady chickens caused macrocytic hypochromic anemia and thrombocytopenia with prolonged clotting time besides liver and kidney damage. Moreover, aluminum sulphate - supplement to the ration of gps. 5&6 induced a very mild effect of on signs, mortality rate and histopathological changes. It did not improve the hematological or biochemical parameters.

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الملخص العربى

دراسات باثولوجية إكلينيكية على تأثير التسمم بالفلوريد فى الدواجن

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*رئيس بحوث بمعهد بحوث صحة الحيوان بالزقازيق
ماجستير العلوم الطبية البيطرية*

أجريت هذه الدراسة لمعرفة تأثير فلوريد الصوديوم و كبريتات الألومنيوم على الصحة العامة و التغيرات فى الدم و التغيرات الكيميائية و التغيرات الباثولوجية فى الكتاكيت البلدى. بالإضافة إلى تقييم اضافة كبريتا ت الألومنيوم للعليقة كملطف للتسمم بفلوريد الصوديوم وقد إستخدم لهذه الدراسة عدد مائة و خمسين كتكوت بلدى (عمر يوم). و قد قسمت الكتاكيت إلى ست مجاميع متساوية. المجموعة الأولى: تركت بدون معاملة كمجموعة ضابطة، المجموعة(٢&٣): تم اضافة ٨٠٠ و١٦٠٠ جزء من المليون من فلوريد الصوديوم للعليقة ، المجموعة (٤): تم اضافة ٦٤٠ جزء من المليون من كبريتات الألومنيوم للعليقة والمجموعة(٥&٦): تم اضافة ٨٠٠ جزء من المليون (فلوريد الصوديوم)+٦٤٠ جزء من المليون(كبريتات الألومنيوم) و١٦٠٠ جزء من المليون(فلوريد الصوديوم)+١٢٨٠ جزء من المليون(كبريتات الألومنيوم) لعليقتها بالترتيب.

لقد أظهرت المجاميع(٢-٦) فقدان للشهية مع نقص فى النمو مع وجود إسهال بنى مصفرو لقد ظهرت تشوهات فى الأصابع أدت إلى جمود فى الحركة و ظهور العرج مع إرتفاع النفوق. لوحظ وجود نقص فى إستهلاك العليقة وتاخر زيادة وزن الجسم في المجاميع(٢-٦) حيث أظهرت أنيميا ذات الحجم الكبيرلكرات الدم الحمراء مع نقص فى عدد الصفائح الدموية و زيادة مدة التجلط.

وقد أظهرت النتائج البيوكيميائية وجود تليفات بالكبد (زيادة فى إنزيمات الكبد الألانين أمينوترانسفيريز والاسبرتيت أمينوترانسفيريز و الفوسفاتيز القلوى مع نقص البروتين الكلي والجلوبيولين) مع وجود اضطراب في وظائف الكليتين (زيادة فى حمض البوليك وقيمة الكرياتينين مع نقص فى الكالسيوم والماغنسيوم مع عدم وجود تغيير معنوى فى الفوسفور). التغيرات الباثولوجية تتماشي مع النتائج الكيميائية والتى تشير إلى التأثير السمى لفلوريد الصوديوم وكبريتات الألومنيوم.

نستخلص من هذا البحث أن:

١- فلوريد الصوديوم في الكتاكيت البلدي أدى إلى أنيميا التحلل الدموي مع نقص الصفائح الدموية وزيادة مدة التجلط بالإضافة الى تأثيره الضار على الكبد والكليتين .

٢- تقييم كبريتات الألمونيوم كملطف للتسمم بالفلوريد (مجاميع ٥ و٦) أظهر تأثير خفيفا جدا على الأعراض الإكلينيكية ومعدل النفوق والتغيرات الباثولوجية ولم يظهر تأثيرا على التغيرات في صورة وكيمياء الدم .