

## Effect of boric acid on sodium fluoride toxicity in chicks

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### Abstract

The aim of this study to explore the therapeutic effect of boric acid on the neurobehavioral (motor activity) level, and histopathologic changes in the brain, liver and kidneys against fluorosis. In this study rose chicks have been used and determined medium lethal sodium fluoride dose at 346.5 mg/kg orally. The chicks divided into four random groups each one consists of 10 chicks. The first group considered to be a control group, the second received 20 mg/kg of sodium fluoride, the third group received 10 mg/kg of boric acid and the fourth received 20 mg/kg of sodium fluoride and boric acid at the same previous dosages. After two weeks of daily treatment, neurobehavioral measures were taken, the use of boric acid has a major effect to improve the neurobehavioral measurement and develop complications of ALT, AST, creatinine, Ca, MDA. The results indicate that boric acid may be a therapeutic agent against the fluoride toxicity of the brain, liver and kidney. This result support by histopathological changes which represented by inflammation, congestion of portal vein and dilation of sinusoids in the liver and vacuolation, vasogenic edema and gliosis in the brain and Kidney of showed segmentation of glomeruli, dilation of Bowman's space, necrosis of epithelial cells renal tubules and hemorrhage of NaF group, while the liver of the NaF with boric acid group showed an improvement the results of histopathological examination of the liver, brain and kidneys compared to the NaF group alone. The results revealed that boric acid has a preventing effects against fluoride after two weeks of treatment with boric acid.

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### Introduction

Fluoride is an essential substance in the production of fluoridated dental preparations and in the drinking water (1). Drinking water, fruit, dental products, pesticides and rodenticides are the major sources of fluoride toxicity. Excessive exposure to fluorides, especially in the tooth and skeleton, leads to many health problems (2). Fluorine's electronegativity means that it is negatively charged and appears to pass through ion channels via cell membranes (3). Excessive ingestion of fluorine contributes to toxicity, which poses a major health threat with skeleton and dental defects. The major cause of fluorosis is contamination of organic and inorganic waste by drinking water (4). Since

fluorine is ionic in drinking water, it is rapidly absorbed through the intestinal epithelia and, after accumulation in various organs, interferes with metabolic processes (3). Studies in rats have shown that fluoride in drinking water can damage and build soft tissue (5). In addition, it reduces the amount of antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD) and peroxidase glutathione, by generating fluorine-inducing free radicals. Furthermore, increased lipid peroxidation is a major oxidative stress biomarker. Studies in rats find that exposure to fluoride in rat tissue improves lipid peroxidation as ROS production (6).

Fluoride exposure was also shown to cause chromosome defects and damage to the DNA with

genotoxic effects (7). The median lethal dose is 2,95 g/kg of boric acid in chick (8,9). Boric acid is an ingredient present as a trace element in animal bodies. It's a typical boron shape. It is water soluble and can be used in biochemical processes (10). Monobasic molecules of boric acid contain hydroxyl structures and release protons in a reaction (3). The integrity of the cell membranes and redox metabolism can play a significant role, even with a high association of boric acid to certain important molecules that are associated with biochemical and physiological processes, such as dinucleotide nicotinamide adenine, dinucleotide (11).

Boric acid is used in many fields of industry and agriculture. It moves through the digestive system easily when swallowed into the bloodstream (4). Latest findings indicate that the boric acid can be used to treat some types of cancer in animals (12). In previous studies, boric acid was identified as having protective effects from inflammatory and oxidative damage in rat, in hormone metabolism, membrane signaling and different processes of enzymes, Boric acid function as an antioxidant (13).

The aim of this study was to investigate therapeutic impacts of boric acid on neurobehavioral and motor toxicity and the histopathological impacts of brain, liver and kidney and to correlate all of this with enzyme- and mineral-level changes in chicken's bodies due to an increase in the prevalence of fluorosis clinics. The neuro-motor behavior of the chicks, liver enzyme and creatinine functions, phosphors and calcium, and MDA and their relationship with evident pathologic changes in the brain, liver and kidney were examined in order to confirm our hypothesis.

## **Materials and methods**

### **Animals**

In this study the chicks were species of rose meat, brought from one day and grew up in animal house halls of the College of Veterinary Medicine. The experiment started at age 7 days and has been supplied with concentrated feed provided by a local company in order to provide fodder.

### **Chemicals**

Sodium fluoride in powder from BDH limited pool England was used in this study and boric acid in powder form Scharlou company Spain, dilute formalin was used for sample preservation, Kits for measuring enzymes and minerals from Biolabo, Biomerieux, France company.

### **Experimental design**

The median lethal dose of NaF in chickens was calculated according to the Dixon method by up and down method (14). The chicks were divided into 4 main groups each one consists of 10 chicks. The first group was considered a control group was not given anything. the second group received a dose of 20 mg/kg body weight of

sodium fluoride and the third group received a dose of 10 mg/kg body weight of boric acid, while the fourth group received a dose of 20 mg/kg sodium fluoride and boric acid at 10 mg/kg. The treatment was for a while for 5 days/week for 2 weeks of treatment. The volume of admiration was 5-10 ml/kg.

Neurobehavioral and tonic immobility response tests were taken. Then the blood was collected from the animals by cutting their jugular veins to extract the serum for biochemical studies. The animals slaughtered in order to collect organs and store them in formalin before histopathology was performed and the pathological changes were study.

### **Median lethal dose LD<sub>50</sub>**

Calculated according to Dixon up and down method (14).

### **Open felid motor activity test**

A purpose-built wooden box measuring 90 x 60 x 30 cm, with a divided floor into 24 equal squares and a square side length of 15 cm, was used for this test. Each chick is subjected to the test alone. The counting of the number of squares for the chick crosses for 3 minutes. After 2 weeks of treatment at the end of this test, animals were subjected to the tonic immobility response test (15).

### **Tonic immobility response test**

This test is conducted by calmly putting each chick on the side of the body, then placing the right hand palm on the animal for 15 seconds to calm down, then quietly pulling the hand and measuring the time of the chick still without moving (the time of inactivity) before the chick starts to resist and move, an animal that fails to calm down is tried again for 5 attempts with an interval between attempts. The overall rest is 300 seconds (16).

### **Malondialdehyde measurement**

Malondialdehyde was measured according to the Buege and Aust method (17).

### **Histopathic examination**

Necropsy was performed at the end of the experiment to study microscopic pathological changes in the Brain, kidneys and liver. The organs were quickly removed and washed with tap water.

They transferred to special containers to store the samples in a 10% neutral buffered formalin solution for three days, then washed in tap water Later, the specimen crossed in alcohol, xylene, enameled in the form of patterns, microtome slides of up to 5 microns with a thickness of 4-5  $\mu$ m were cut off from each block and then stained with hematoxylin and eosin.

Light microscope was used for study, and the affected areas were photographed (18).

**Statistical analysis**

The ANOVA test was used to analyze parametric data (one way), the spss software was used, then the results were subjected to the LSD test. As for the non-parametric data, the PAST program was used to submit it to the Mann Whitney test.

**Results**

The median lethal dose of fluoride in chicks was 346.5 mg/kg (Table 1). The results after 2 weeks of acute fluoride treatment indicated a significant decreased in the number of squares crossed by chicks in the group treated with fluoride alone in open field test, as well as an increase in the calm time in the tonic immobility response test compared to the control group and other groups (Table 2). A significant increase in enzymes AST, CREATININ, ALT and decrease in Ca in the group treated with fluoride alone was found in the results of the biochemical tests compared to the control and other groups, a significant increase in the ALT level was observed when the fluoride-treated group was compared with the rest of the groups (Table 3).

In the group treated with fluoride and boric acid, calcium recorded a significant difference compared to the group treated with fluoride alone. These findings indicate that the group treated with fluoride and boric acid improved compared to the group treated with fluoride alone. MDA showed a significant increase in the sodium fluoride-only

treatment group compared to the rest of the groups (Table 3).

The histological changes in the liver, brain and kidneys showed the presence of differentiated histopathological changes after two weeks, which represented by congestion of sinusoids and portal vein in liver of chicks in boric acid group (Figure 1).

Photomicrograph of chick's brain of boric acid group shows normal architecture of brain tissue representing by neurons, glial cells, and blood vessels (Figure 2).

Photomicrograph of chick's kidney of boric acid group shows normal architecture of renal tissue representing by glomeruli, proximal renal (Figure 3).

Table 1: The median lethal dose of sodium fluoride in chicks

Variables	Result
LD <sub>50</sub>	346.5 mg/kg, orally
The range of the doses	400-325 mg/kg, orally
Initial dose	400 mg/kg, orally
Last Dose	325 mg/kg, orally
Number of chicks	6 (XX0XX0) *
Increase or decrease in dose	25 mg/kg, orally
Signs of toxicity	Fatigue, feather erections, lack of movement and paralyzes other signs

\* X means death and O refer to the survival of chicks.

Table 2: Tonic immobility response and Motor activity (Number of squares) after 2 weeks of treatment with sodium fluoride and boric acid alone, or both of them in chicks

Groups and doses	Tonic immobility response time	Motor activity
Control	60.30±18	5±1
Sodium Fluoride 20 mg/kg	* 300±60	*2 ± 1
Boric Acid 10 mg/kg	<sup>a</sup> 70.44 ± 27	<sup>a</sup> 4±1
Sodium Fluoride 20mg/kg and Boric Acid 10 mg/kg	<sup>a</sup> 75 ± 14	<sup>a</sup> 4 ± 1

Each group consists of 8 animals, mean ± stander error represent data. \* significant difference from the control group, P<0.05. a represented a significant difference from the group of sodium fluoride.

Table 3: Concentration of enzymes in the serum after 2 weeks of Treatment with sodium fluoride and boric acid alone or together in chicks

Treatment and doses	ALT (Unit/l)	AST (Unit/l)	Crea (mg/100ml)	P (mg/100ml )	Ca (mg/100ml)	MDA (Nanomol/L)
Control	11± 0.4	152±32	0.07 ±0.01	6.95 ±3	10.9 ±3	11±3
Sodium Fluoride 20 mg/kg	13 ± 2*	164±13*	0.1±0.03*cb	6.57 ±2	8.92 ±2*bc	13.5±2*bc
Boric Acid 10 mg/kg	10.7 ± 2 <sup>a</sup>	157±28	0.06± 0.08	6.61 ± 1	10.8± 4	11.6±3
Sodium Fluoride20 mg/kg and Boric Acid 10 mg/kg	11.5± 2 <sup>ab</sup>	149±22 <sup>ab</sup>	0.07± 0.01 <sup>a</sup>	7± 1	9.3± 2	12±2

Each group consists of 6 animals. Data represent as mean ± SE. \*significant difference from the control group, P<0.05. a represented a significant difference from the group of sodium fluoride. b significant difference from the group of boric acid. c significant difference from sodium fluoride and boric acid group.

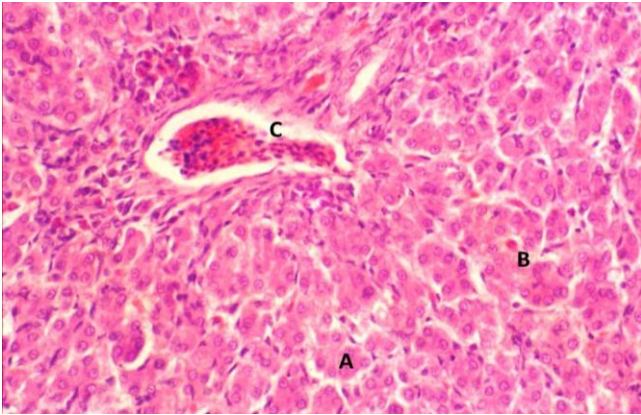


Figure 1: photomicrograph of chicken liver of boric acid group (for 2 weeks) shows normal architecture of hepatic tissue representing by hepatocytes (A), congestion of sinusoids (B) and portal vein (C). H&E stain, 400X.

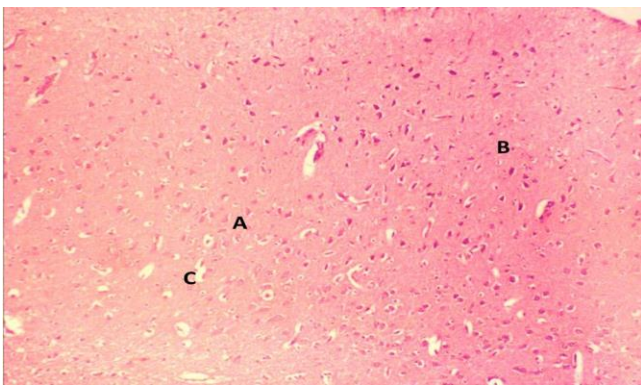


Figure 2: photomicrograph of chicken brain of boric acid group (for 2 weeks) shows normal architecture of brain tissue representing by neurons (A), glial cells (B), and blood vessels (C). H&E stain, 100X.

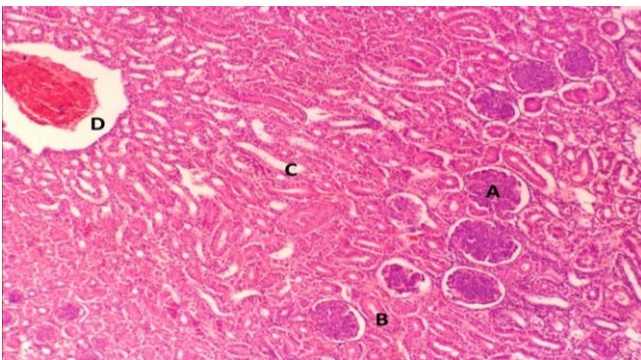


Figure 3: photomicrograph of chicken kidney of boric acid group (for 2 weeks) shows normal architecture of renal tissue representing by glomeruli (A), proximal renal tubules (B), distal renal tubules (C) and congestion of renal vein (D). H&E stain, 100X.

While in NaF group showed focal infiltration of inflammatory cells, congestion of portal vein and dilation of sinusoids (Figure 4-6). In the brain of NaF group shows vacuolation, vasogenic edema, gliosis and congestion of blood vessels (Figure 7 and 8) more than lesion (Figure 9) shows recent thrombus (erythrocytes, platelets and fibrin).

Kidney of NaF group shows segmentation of glomeruli, dilation of Bowman's space, necrosis of epithelial cells lining renal tubules and hemorrhage (Figure 10).

Atrophy of glomeruli, cell swelling and necrosis of epithelial cells lining renal tubules, focal infiltration of inflammatory cells and congestion of renal vein (Figure 11 and 12), while in the groups of mixture of NaF and boric acid shows mild cell swelling of hepatocytes, congestion of central vein and portal vein (Figure 13 and 14).

In the NaF with boric acid group shows normal architecture of brain tissue except vasogenic edema (Figure 15). Kidney of NaF with boric acid group appears mild vacuolar degeneration of epithelial cells lining renal tubules, congestion of blood vessels, sinusoids (Figure 16).

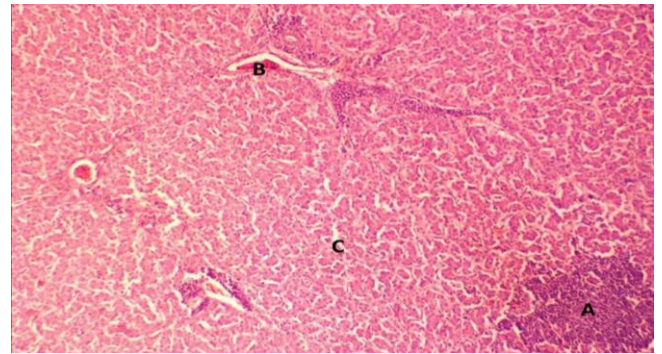


Figure 4: photomicrograph of chicken liver of NaF group (for 2 weeks) shows focal infiltration of inflammatory cells (A), congestion of portal vein (B) and dilation of sinusoids (C). H&E stain, 100X.

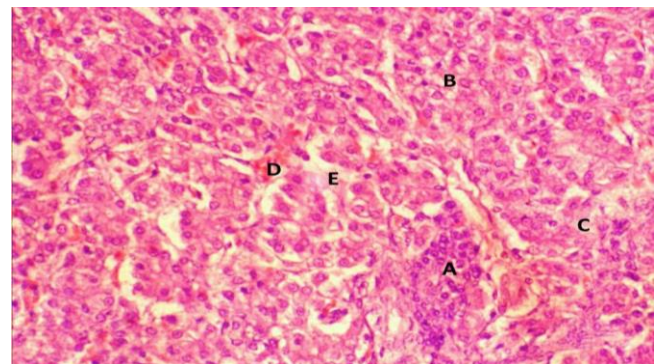


Figure 5: photomicrograph of chicken liver of NaF group shows focal infiltration of inflammatory cells (A), vacuolar degeneration (B) necrosis of hepatocytes (C), congestion of sinusoids (D) dilation of sinusoids (E). H&E stain, 400X.

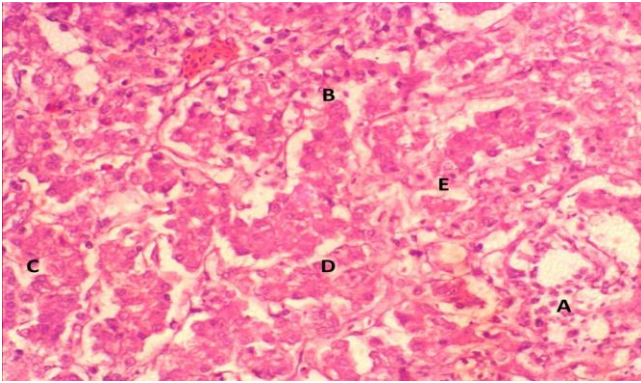


Figure 6: photomicrograph of chicken liver of NaF group shows infiltration of inflammatory cells (A), vacuolar degeneration (B) and necrosis of hepatocytes (C), congestion of sinusoids (D) and dilation of sinusoids (E). H&E stain, 400X.

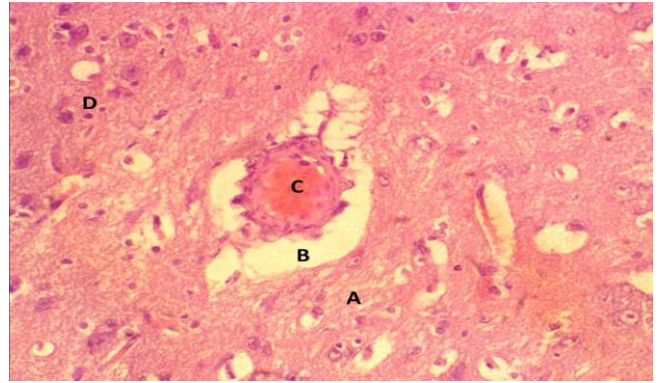


Figure 9: photomicrograph of chicken brain of NaF group (for 2 weeks) shows presence of vacuolation in cytoplasm of neurons (A), vasogenic edema (B), recent thrombus (erythrocytes, platelets and fibrin) (C) and gliosis (D). H&E stain, 400X.

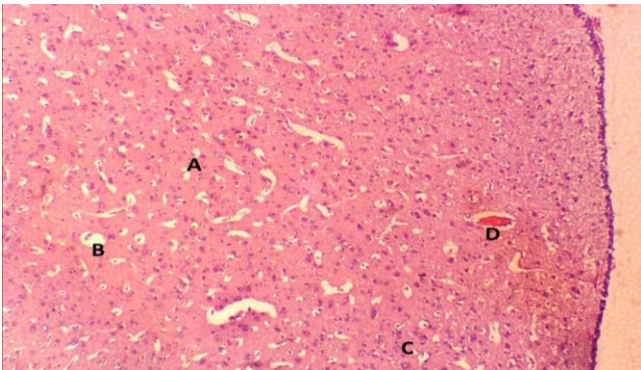


Figure 7: photomicrograph of chicken brain of NaF group (for 2 weeks) shows vacuolation (A), vasogenic edema (B), gliosis (C) and congestion of blood vessels (D). H&E stain, 100X.

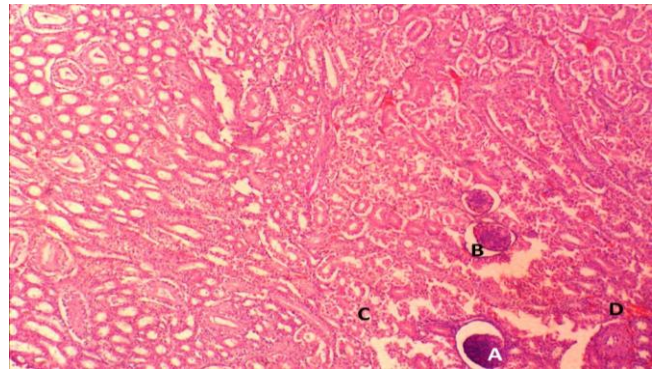


Figure 10: photomicrograph of chicken kidney of NaF group shows segmentation of glomeruli (A), dilation of Bowman's space (B) necrosis of epithelial cells lining renal tubules (C) and hemorrhage (D). H&E stain, 100X.

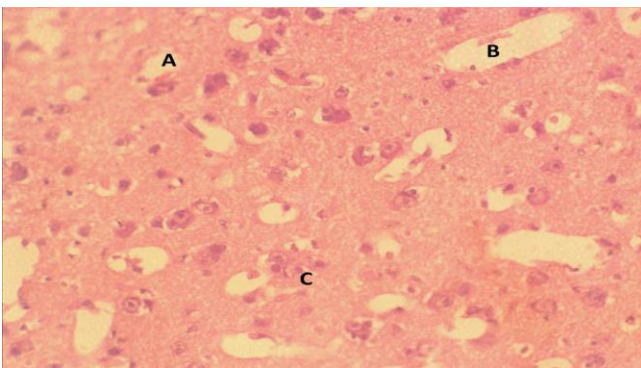


Figure 8: photomicrograph of chicken brain of NaF group (for 2 weeks) shows presence of vacuolation in cytoplasm of neurons (A), vasogenic edema (B) and gliosis (C). H&E stain, 400X.

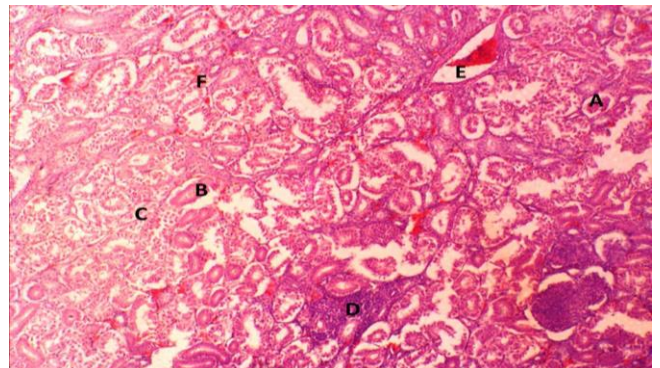


Figure 11: photomicrograph of kidney of NaF group shows atrophy of glomeruli (A), cell swelling (B) necrosis of epithelial cells (C), focal infiltration of inflammatory cells (D) congestion (E) and hemorrhage (F). H&E stain, 100X.

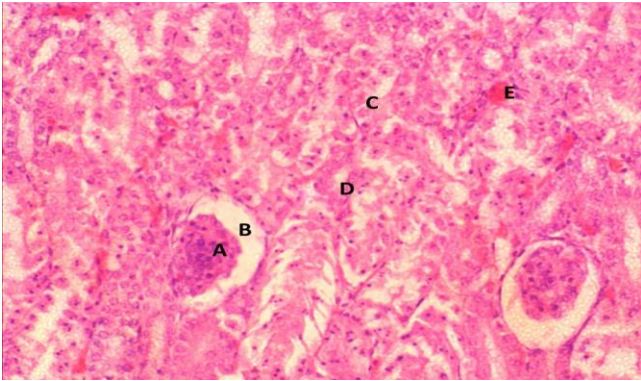


Figure 12: photomicrograph of chicken kidney of NaF group shows atrophy of glomeruli (A), dilation of Bowman's space (B), cell swelling (C) and coagulative necrosis of epithelial cells lining renal tubules (D) and congestion of blood vessel (E). H&E stain, 400X.

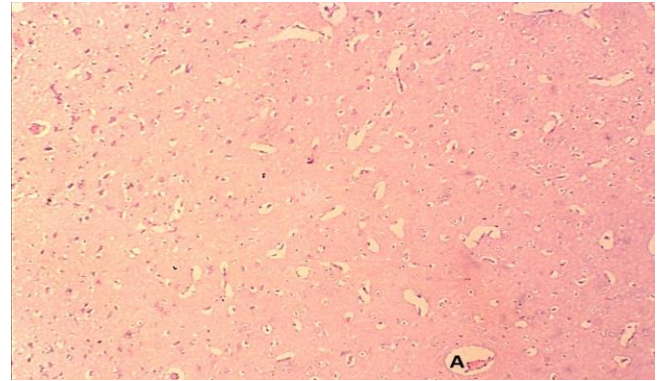


Figure 15: photomicrograph of chicken brain of NaF with boric acid group (for 2 weeks) shows presence the normal architecture of brain tissue and cells all over the section, with one exception is presence vasogenic edema (A). H&E stain, 100X.

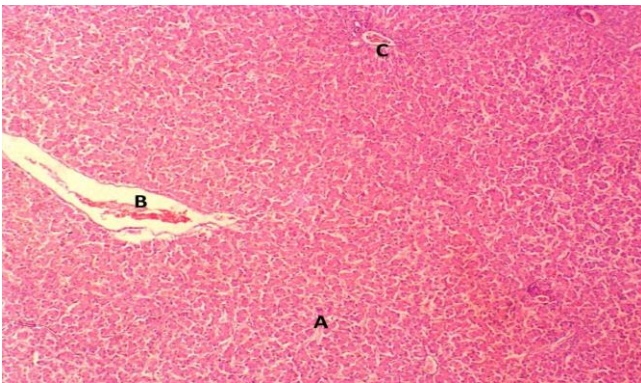


Figure 13: photomicrograph of chicken liver of NaF with boric acid group (for 2 weeks) shows mild cell swelling of hepatocytes (A), congestion of central vein (B) and portal vein (C). H&E stain, 100X.

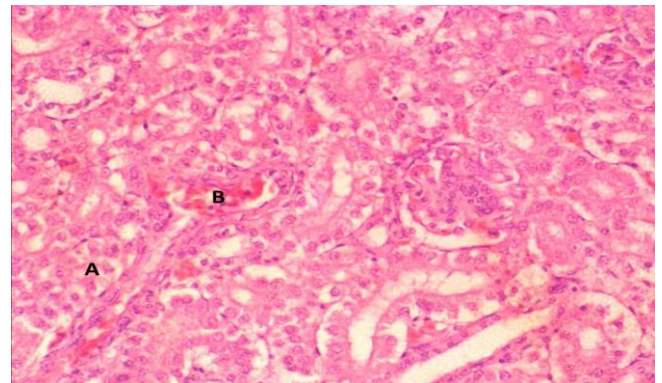


Figure 16: photomicrograph of chicken kidney of NaF with boric acid group shows mild vacuolar degeneration of epithelial cells lining renal tubules (A) congestion of blood vessels sinusoids (B). H&E stain, 400X.

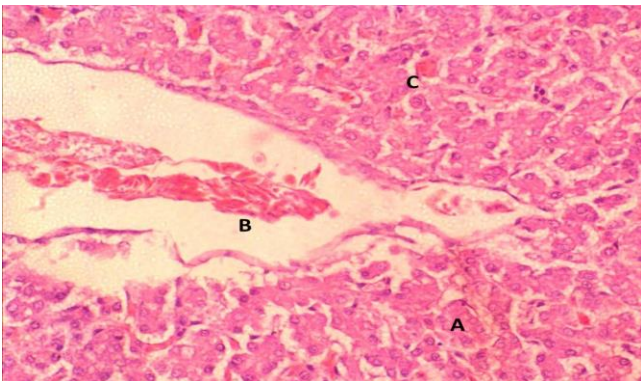


Figure 14: photomicrograph of chicken liver of NaF with boric acid group (for 2 weeks) shows mild cell swelling of hepatocytes (A), congestion of central vein (B) and sinusoids (C). H&E stain, 100X.

## Discussion

The group treated with mixture of fluoride with boric acid showed an improvement in the animal clam time in the tonic immobility test relative to the animals treated with fluoride alone. Whereas prolonged duration of inactivity (clam) has been reported in chicks of sodium fluoride group this may be due to animal's feelings of fear and anxiety (opposite of clam), or to the effects on serotonin and other neurotransmitters (19).

Synapses that play a significant role in the transmission of nerve signals are strongly linked to neurotoxicity due to oxidative stress (20). Since fluorine has the property of crossing the blood-brain barrier (7), nerve cells are highly susceptible to fluoride damage and neuronal fat and protein oxidation due to their high mitochondrial content, especially the presynaptic nerve cell and this result

confirms that boric acid in cells may be an antioxidant agent (6,21).

As it was found that fluoride exposure in rabbit increased levels of MDA in synapses by raising oxidative stress (22), our findings are compatible with other research. In one study, treatment with boric acid was also found to affect synaptosomes in the brain of rat, as it led to a significant improvement in antioxidant enzyme levels (23).

For their effect on the elimination of free radicals. In other words, by helping the antioxidant defense mechanism, boric acid helps protect cells. In the group treated with fluoride alone the movement of animals was also greatly affected by inhibiting the movement and a decreased number of squares crossed by the chick within the open field, and this may be due to inhibiting the activity of Na/K ATPase in the brain tissue, which is reflected in the open field neurobehavioral and motor activity (24).

The sodium-potassium pump is a protein imbedded in the neural membrane that plays an important role in the maintenance of the cells' electrochemical membrane potential, it is also involved in the provision inside and outside the cells of electrolyte balances (25).

Fluoride binds to and prevents the function of ion channel proteins in cell membranes (26), which causes the membrane translucency to deteriorate and this explains was found in this research.

An improvement in the neuromotor activity of the animals was recorded in the groups treated with fluoride with boric acid, as well as a marked improvement in the level of the ALT and AST enzymes. This improvement may be attributable to the fact that boron interacts with fluoride and forms a fluoride-boron complex that is not absorbable, and this complex is excreted outside the body via the kidney (27).

That result was supported by histopathological changes in liver and kidney, where improvement was observed with the pathological changes in the liver and kidneys of the group treated with mixture of NaF with boric acid. In previous studies, fluoride in rats was the cause of oxidative stress, damage to DNA, activation of apoptotic pathways, and changes in the cell cycle (6,13).

The development of cellular degeneration and the resulting degeneration due to tissue damage and necrosis in the liver or heart or possible which muscle damage due to the use of a drug or toxic chemical (28) is a possible mechanism for raising the concentration of ALT and AST enzymes in the serum. There are variations in enzyme levels that may be due to individual differences, as the reaction of birds to medications differs and varies depending on the stress to which the animal is subjected and the sensitivity of the animal. The liver is a primary foreign body detoxification site, and the toxic agent is readily altered by metabolism. Because of its capacity to remove and concentrate toxic substances from highly

specialized cells, the kidney also is a special target organ for toxic foreign substances (29).

Therefore, one of the target organ for the toxicity of sodium fluoride that has been linked with high levels of creatinine, ALT and AST enzymes in fluoride-treated animals, as well as with the toxic reactions of certain organs that have appeared in the form of pathological changes in the liver and brain organs, whereas fluoride is primarily involved in the production of reactive oxygen and derivatives of nitrogen.

The results showed a decrease in serum calcium levels only in the fluoride group compared to the rest of the groups, and this corresponds to many studies that attributed to the fact that fluoride interferes with the metabolism of calcium (30,31).

In the group treated with fluoride with boric acid, there is a substantial improvement in the level of liver function enzymes and creatinine, as boric acid acts to preserve the integrity of the cell membrane, maintain its function and minimize DNA damage because of its similar affinity with other metabolites in the cells of the body of the animal by acting on the balance of oxidation and reduction (32,33).

## **Conclusion**

Boric acid, by behavioral studies assisted via histopathological brain examination, has a preventive effect on fluoride toxicity at the level of open field motor activity and nervous behavior of chicks, and has also help to minimize fluoride toxicity in the liver and kidneys. Evaluating the function of the enzymes ALT, AST, creatinine, Ca and MDA with histopathological modifications in the liver and kidney sections has shown this.

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## **Conflict of interest**

There is no conflict of interest.

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## تأثير حامض البوريك في السمية المحدثة بفلوريد الصوديوم في أفراخ الدجاج

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### الخلاصة

الهدف من هذه الدراسة هو الكشف عن التأثير العلاجي لحامض البوريك على مستوى السلوك العصبي (الفعالية الحركية) والتغيرات المرضية النسجية في الكبد والدماغ والكلية ضد التسمم المحدث بالفلوريد. استخدم في هذه الدراسة أفراخ دجاج نوع روز وأوجدت الجرعة المميّنة الوسطية لفلورايد الصوديوم وكانت ٣٤٦,٥ ملغم/كغم. قسمت الأفراخ إلى أربعة مجاميع عشوائية كل مجموعة مكونة من ١٠ أفراخ، اعتبرت المجموعة الأولى مجموعة سيطرة وأعطيت المجموعة

المرضية المتمثلة بوجود الالتهاب واحتقان الوريد البابي وتوسع الجيبانيات في الكبد والتفجي والوذمة حول الأوعية الدموية وتجمع الخلايا الدبقية في الدماغ ولوحظ في الكلية وجود نقص في الكبيبات وتوسع في محفظة بومان وتنخر الخلايا الظهارية المبطنة للنبيبات الكلوية في المجموعة المعاملة بفلوريد الصوديوم. في حين لوحظ في المجموعة المعاملة بفلوريد الصوديوم مع حامض البوريك وجود تحسن في التغيرات المرضية النسجية في الكبد والدماغ والكلية مقارنة مع المجموعة المعاملة بفلوريد لصوديوم لوحده. بينت النتائج أن حامض البوريك له تأثير وقائي ضد السمية المحدثة بفلوريد الصوديوم بعد أسبوعين من العلاج بحامض البوريك.

الثانية فلوريد الصوديوم بجرعة ٢٠ ملغم/كغم وأعطيت المجموعة الثالثة حامض البوريك بجرعة ١٠ ملغم/كغم وأعطيت المجموعة الرابعة كلا من فلوريد الصوديوم بجرعة ٢٠ ملغم/كغم وحامض البوريك بنفس الجرعة السابقة. أجريت الاختبارات السلوكية العصبية بعد أسبوعين من المعالجة اليومية ولوحظ في مجموعة فلوريد الصوديوم مع حامض البوريك خاصة وجود تحسن في القياسات السلوكية العصبية وتحسن المضاعفات التي حدثت في الأنزيمات ناقلة أمين الالانين وناقلة أمين الاسبارتيت والكرياتنين والكالسيوم المالوندايديهايد. أشارت النتائج أن حامض البوريك قد يكون له تأثير مفيد ضد التسمم بالمحدث بفلوريد الصوديوم وتأثيره على الدماغ والأعصاب والكبد والكلية. تم دعم هذه النتائج بدراسة التغيرات

