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Effect of folic acid on fluoride induced morphological alterations in the liver of albino wistar rats

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ABSTRACT

Objective: To observe the histomorphological changes in liver of Albino wistar rats induced by Fluoride and effect of Folic acid.

Methodology: This quasi experimental study was conducted at Anatomy department & postgraduate Laboratory of Isra University Hyderabad Sindh from April 2018 to September 2018. Total forty healthy rats 8-12 weeks old and weight 150-250gm were selected. Animals were divided in 4 groups in equal numbers. Animals of Group A, were given normal diet, Group B, received distilled water mixed with Fluoride (10.0mg/kg) and group C received distilled water mixed with Fluoride (10mg/kg) along with folic acid supplement (2.5mg/kg). Group D, animals received Fluoride (10mg/kg) for four weeks initially and then folic acid (2.5mg/kg) mixed distilled water was given for additional four weeks. After completion of experiment, the rats were sacrificed and hepatic tissues were processed to prepare paraffin blocks. 4-6 micrometer sections were obtained for slides and stained with hematoxylin and eosin to observe under light microscope. All the data was recorded in proforma.

Results: Liver weight was insignificant among all study groups. On histological examinations, fibrotic changes were significantly higher among all experimental groups as compare to control group whereas folic acid consumption reduced it. Necrotic changes, hepatic inflammatory changes, sinusoidal dilatation and congested portal veins were found higher among animals of experimental group B as compared to group C and group D, while no changes found in control group.

Conclusion: Fluoride exposure that impair liver architecture, is potently supported by the portal inflammation, necrosis, and histological alterations. Folic acid is the best supplement to prevent and revert the hepatic histological alterations caused by fluoride.

Key Words: Fluoride, Folic acid, Liver.

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Introduction

Folic acid (pteroylmonoglutamate), a vital water-soluble vitamin, is antioxidant to prevent human body from oxidative damage and has anti-inflammatory effects.[1] Folic acid is an strong regulator of oxidative stress because it has a free radical, scavenging property and protects cells against oxidative injury.[2] Many studies indicate folic-acid supplementation's potential health advantages containing reduction in neural-tube defect's risk, cardiovascular, hematological, kidney diseases and also improves memory in cognitive deficits.[3] Supplements of folic acid also prevent hepatocellular damage from many other toxic substances e.g. arsenic, carbon tetrachloride and improve liver functions by inflammatory stress blocking and normalizing metabolic activities within the liver. Moreover, oxidative stress and anti-inflammatory effect of folic acid (FA) in experimental animals results in better outcome.[4]

Fluoride belongs to halogen family and is a most reactive and electronegative than other elements and is a vital industrial and natural ecological pollutant which subsists together with the further element(s) as fluoride compound(s) that are components of minerals within soil & rocks.[5] Fluoride interacts with the human body and environment via food, water, tea, edible marine oil, detergents, industrial exposure, drugs, cosmetics, fertilizers, tooth pastes etc.[6] Fluoride has impacts on teeth and skeletal tissues at lower intensities during drinking water. Too much interaction to fluoride within drinking-water, or in amalgamation with interaction to Fluoride resources, can result in several adverse outcomes, which may vary from crippling-skeletal-fluorosis to mild-dental-fluorosis with rise in period & level of exposure.[7] Fluoride can cause many histological hepatic and renal changes and is responsible for oxidative stress that induce disturbances in liver and kidney functions.[8] In liver, fluoride exposure shows micro-necrotic-foci within hepatocytes, vacuolar-degenerations, hepatocellular-hypertrophy and sinusoidal dilatation with distended central vein bounded via deep-blue-erythrocytes in the mice exposed to fluoride.[9] Fluoride pollution within groundwater is well-known to be a serious challenge globally. The WHO's suggested tolerance limit for fluoride within drinking-water is 1.5 mg/L. Recent reports show that the lethal effects of excessive fluoride cause a provocation of inflammatory reaction(s), protein synthesis inhibition and oxidative stress, the progression of the cell cycle, and structural damages. Most of these cellular incidents eventually result in cell- death. It induced apoptosis that was exhibited within cells from various tissues and organs of body.[10-12]

Since 2012, around 4.35 hindered million people globally received fluoridated water at suggested levels (i.e., around 5.40% of the worldwide populace).[13] Around two hundred fourteen million of them are residing in the U.S. The World Dental Federation, the WHO, and the FDI, advocated that water fluoridation is effective and safe.[14] The Centers for Disease Control and Prevention reported that water fluoridation comes in the top ten pronounced public health accomplishments of the year 2000 in the U.S. Regardless of this, its application is debated as a public health measure. Even though the mechanism by which fluoride provokes these toxic effects are incompletely revealed, growing evidence exhibits that fluoride can provoke the generation of reactive oxygen species (ROS) and disrupt the normal hepatic antioxidant systems, demonstrating that oxidative stress contributes significantly in fluoride-provoked hepatotoxicity. Though, the comprehensive molecular mechanism causing oxidative stress provoked by fluoride yet remains largely mysterious.[15] An essential nutrient, Folic acid, is essential for the replication of DNA and as a substrate for various enzymatic reactions contributing to the synthesis of amino acid and metabolism of vitamin.[16] In Pakistan 40% diseases are waterborne and fluoride is considered as one of the serious contaminants in drinking water.[17] Keeping in view the above reports, the way to deal with fluoride intoxication could be the use of antioxidants which could prevent damage caused by free radicals. No such studies have been found in the literature on the preventive effect of folic acid liver damage induced by fluoride. Therefore this study is an attempt to observe morphological changes in liver due to fluoride and protective effect if any of folic acid supplementation in Albino wistar rats.

Methodology

This was a quasi-experimental study was conducted after ethical approval Isra University Hospital Hyderabad. The animal protocols were followed at Animal House Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. The study was conducted from April 2018 to September 2018. All the healthy adult albino rats with average weight of 150-250 gm and without any gross abnormality were selected. Offspring's, moribund rats and pregnant female rats were excluded.

Total 40 rats (n=40) were grouped into four categories (each of 10 rates from either group): -

Group A, Control (n= 10) Rats were provided with normal food accompanied by distilled water ad libitum for 4 weeks.

Group B, Experimental (n=10) were given Fluoride (10mg/kg-bw/day) orally in distilled water with standard diet for four weeks.

Group C, Experimental (n=10) were given Fluoride (10mg/kg-bw/day) + folic acid supplementation (2.5mg/kg-bw) orally in distilled water with normal diet for 4 weeks.

Group D, Experimental (n=10) were given Fluoride (10mg/kg-bw/day) for 4 weeks then stopped and followed by FA (2.5mg/kg-bw/day) for another 4 weeks orally in distilled water with normal diet.

All animals were acclimatized for 6 days before start of experimental work. The animals were kept in plastic cages and were equipped with stainless steel feed containers and plastic drinkers with stainless steel nozzles. They were allowed free access to standard chow diet and water before and after experiments. Saw dust were used as beddings and were changed daily. The animals were housed under a

hygienic and well-ventilated environment at room temperature 26°C and 12 hours light/dark cycle. The weights of all group animals were measured and noted prior to the start of the experiment and prior to sacrifice. After completion of experiment, the weights of all animals were measured. The rats were sacrificed by cervical dislocation. Liver were removed by dissection and after washing with normal saline, the gross morphological changes were recorded and the liver were fixed in 10% formalin. The tissues were processed to prepare paraffin blocks. 4-6 micrometer sections were obtained for slides and stained with hematoxylin and eosin to observe under light microscope for histological assessment. All the data was recorded in the proforma. Data was analyzed by using SPSS (Statistical packages for social sciences) version 22.0. Chi-square test and t-test were applied and a p-value ≤0.05 was taken as significant.

Results

The mean liver weight of control group was 5.90±1.14gm, group B 5.44±0.97 gm, group C 5.40±0.80 gm and mean liver weight of group D was 6.03±0.85 gm. Though there was no significant difference in mean liver weight among study groups (P-value=0.462). Table I.

Table I: Mean comparison of liver weight (gm) among study groups (n=40)

Groups	Liver weight		p-value
A vs B	5.90±1.14	5.44±0.97	0.462
A vs C	5.90±1.14	5.40±0.80	
A vs D	5.90±1.14	6.03±0.85	

Fibrotic changes were significantly higher among all experimental groups as compare to control group, p-value 0.013. Necrotic changes were found higher among animals of experimental groups, and in only one animal of control group, p-value 0.013–Histomorphological changes in different groups of rats is presented in Table II.

Hepatic inflammatory changes, sinusoidal dilatation and congestion is due to inflammatory changes present in intralobular area, was found only among animals of experimental groups, while no animal was noted with sinusoidal dilatation in control group. Various histological features are shown in Figure 1,2 and 3.

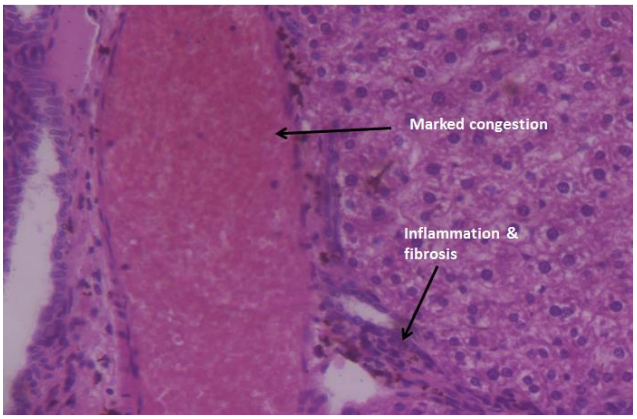

Figure1. Histological section of liver of experimental group B rat with areas of lymphocytic infiltration, marked congestion and fibrosis. (H&E)X400

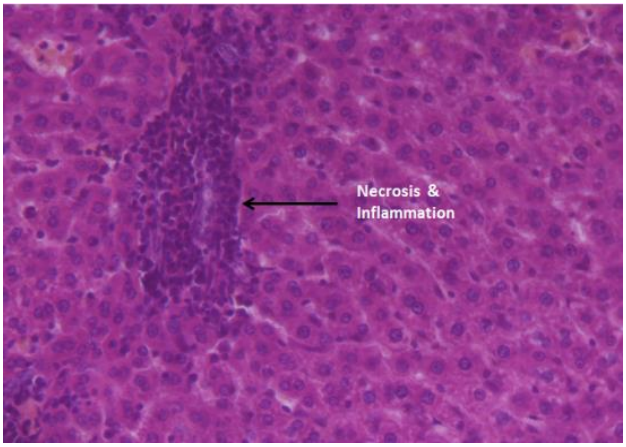

Figure 2. Histological section of liver of experimental group B rat with areas of necrosis and inflammation. (H&E)X400

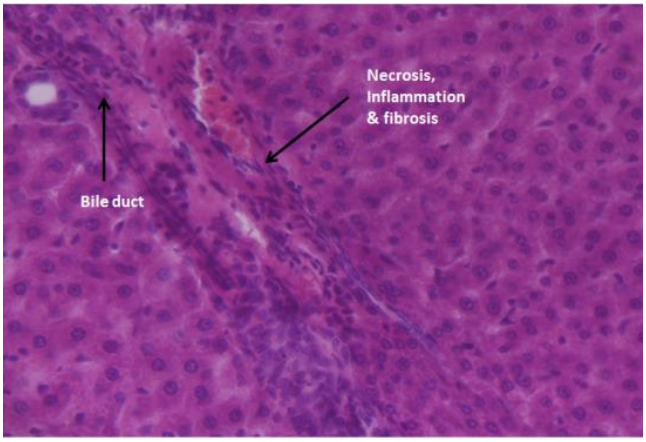

Figure 3. Photomicrograph showing histological section of liver of group D rat with marked reduction in inflammation, necrosis and fibrosis. (H&E)X400

Table II: Comparison of histomorphological changes in different groups of rats. (N=40)

Morphological changes	Groups					p-value
	Group A	Group B	Group C	Group D	Total	
Fibrosis	1	8	4	3	16	0.013
Necrosis	1	8	4	3	16	0.013
Inflammatory cell infiltration	1	9	5	5	20	0.005
Sinusoidal dilatations	0	7	6	5	18	0.008
Congested portal vein	0	6	4	0	10	0.034

Discussion

Excessive fluoride is significantly associated with fibrosis in liver p-value 0.013 in this study. These findings are consistent with D Mukhopadhyay et al who had observed similar histopathological changes in the liver of zebra fish.[18] In the present study, necrotic changes in liver were observed mostly in experimental group B as compared to group C and D which showed additional consumption of folic acid minimize histological alterations in liver which is induced by fluoride. Ghosh J et al[19] reported that fluoride exposure caused death of hepatic cells mostly through necrotic pathway which is supported by DNA fragmentation and cytometric flow analyses. Consistently with the present study, Luo Q et al[20] also observed the necrosis and degeneration of the tubular cells, glomeruli swelling in the kidney of experimental animals which were receiving sodium fluoride orally at the different doses for 42 days. A study conducted by Da Silva Pereira HA et al[21] inconsistently observed that high fluoride levels did not exhibit variations in the hepatic cellular structures as hepatocytes, sinusoids and portal canal showing typical morphology.

Present study revealed that infiltrative inflammatory changes in liver were higher in experimental group B as compared to group C & D. These results suggested that fluoride significantly associated with infiltrative changes in liver and folic acid equally reduced and prevent it, Perera T et al[22] observed infiltrative and focal necrotic changes after 15 days of fluoride administration.

Sinusoidal dilatations and congested portal vein of liver were mostly marked in experimental group B as compare to control group while in experimental group D no rat was found with congested portal vein. The results significantly showed that fluoride is linked to sinusoidal dilatations and congested portal vein, however among group C and D hepatic injury are minimum due to use of folic acid along with fluoride or after its wash out period. Similarly, in the study of Song GH et al[13] reported that prolonged and excessive fluoride consumption causes dilatation of central vein of the hepatic sinusoids and lobule. These outcomes showed that long-term intake of fluoride resulted in severe hepatic impairment among rats which modulate liver cell apoptosis. Moreover, some authors like Thangapandiyan et al has also reported that these histopathological changes in fluoride treated hepatic tissues might be cause of the accumulation of the free radicals through fluoride ions.[14]

According to the findings of present study, histological alteration may be decreased by folic acid administration and it can be said that the folic acid is the protector element among those underwent consumption of fluoride. Ebtihal a et al reported that it is one of the powerful antioxidants because has a free radical scavenging property. It enhances the cell survival rate and inhibits apoptosis. Folic acid as a cheap, safe, and well-tolerated supplement has a beneficial role in various health disorders.[23] Stanhewicz AE at el found an inverse association amid folic acid supplementation intake and cardiovascular health. This association has grown interested in many clinical studies which recommended that supplementation of folic acid could overturn endothelial dysfunction among CVD patients.[24]

No studies have been found regarding the association of liver histological alteration and folic acid. While other vitamins are used by the researchers as antioxidant effects to cure the liver histological alteration induced by fluoride as Abdel-Wahab et al found possible defensive effects of thymoquinone to reduce the oxidative stress and toxicity of sodium fluoride in livers of the rats, and found significant cure effects of thymoquinone.[25] Chinoy et al.[26] also reported that hepatic function can recover from intoxication induced by fluoride and aluminum by modifying effects of vitamins E (tocopherol) and vitamin C (ascorbic acid). As oxidative stress of fluoride describes astate of uncontrolled overproduction of free radicals beyond a threshold for proper antioxidant neutralization resulting in impairment to macromolecules such as DNA, lipids, and proteins. However, folic acid, like other natural antioxidants, is limited by poor stability, short half-life in vivo, low bioavailability, and is easily degraded by proteolytic or gastrointestinal enzymes. When folic acid reacts with oxidizing free radicals, this hydroxyl group can contribute significantly in inhibiting the oxidation effect

and take part in the biosynthesis of DNA/RNA in addition to interconversion of amino acids. It inhibits apoptosis and reduces MDA levels and oxidative stress markers. Thus, supplementing with antioxidants has been considered to have a vital contribution in decreasing the level of fatigue resulting from free radical and oxidative stress.[27]

Conclusion

Fluoride exposure impairs liver architecture specially necrosis, portal inflammation, fibrosis, sinusoidal dilatations and congested portal vein.

Folic acid is the best supplement to prevent and revert the hepatic histological alterations caused by fluoride. There are no significant effects of fluoride found on animal body weight and liver weight.

More research should be conducted on this topic with various doses and with different time of exposure in order to observe damage on other organs of the body.

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