PROTECTIVE ROLE OF FOLIC ACID ON HEPATIC MARKERS AND MORPHOLOGY COUNTER TO NEGATIVE EFFECTS OF FLUORIDE ON ALBINO WISTAR RATS

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ABSTRACT

Objective: To demonstrate the protective role of folic acid supplementation on hepatic markers counter to negative effects of fluoride in Albino wistar rats. **Methodology:** Quasi experimental study was conducted at the Isra University, Hyderabad from May-September 2019. Thirty healthy albino wistar rats with average weight of 150-250 gm. without any gross abnormality were selected by non-random purposive sampling. Unhealthy, female and pregnant rats were excluded. All rats were divided into three group i.e.Group-A (Control group),Group-B (Fluoride treated experimental group) and Group-C (Fluoride and folic acid treated experimental group).All animals were acclimatized for 1 week before start of 4 weeks experimental work. Liver function testsand serum bilirubins were tested using commercially available kits. Gross hepatic morphological details were recorded and changes in severity were observed using a graded scale. Data was analyzed by using SPSS ver. 22.0. **Results**: Significant difference in mean post experimental body weight was observed in all three groups. The relative liver weight was significantly raised in Group-B as compared with other experimental group ($p<0.05$). Statistically significant rise in hepatic serum markers was observed after fluoride administration in Group-B. Treatment with folic acid administration significantly reduced serum levels of hepatic markers ($p<0.05$). Fibrotic, Necrotic, hepatic inflammatory changes, sinusoidal dilatation and congestion is due to inflammatory changes present in intra-lobular area higher among group-B as compared to group-A. **Conclusion:** Folic acid supplement is highly effectivein preventing and reverting the hepatic serum markers levels and histological alterations caused by fluoride.

Key Words: Fluoride, Folic acid, Hepatic markers.

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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 avital 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 ground water is ell-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.Mostofthesecellularincidentseventually 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 world wide 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 accomplishment softhe 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 influoride-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 synthesisof 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 demonstrate the protective role of folic acid supplementation on hepatic markers and morphology counter to negative effects of fluoride in Albino wistar rats.

METHODOLOGY

This was a quasi-experimental study was conducted from May 2019 to September 2019 at the Isra University, Hyderabadafter gaining approval from the ethical review board of University. The animal protocols were followed at Animal House Department of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. ThirtyAlbino Wistar rats were picked using the standard poweranalysis sample size formula for animal studies.[18,19] Healthy adult albino rats with average weight of 150-250 gm and without any gross abnormality were selected by non-random purposive sampling. Rats not meeting the weight inclusion criteria, moribund rats and pregnant female rats were excluded.

Total 30 rats (n=30) were grouped into four categories (each of 10 rates from either group):

GroupA,Control(n=10)Ratswereprovidedwithnormal food accompanied by distilled water ad libitum for 4 weeks.Group B, Experimental (n=10) were given Fluoride (10mg/kg-bw/day) [20, 21] 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) [21, 22] orally in distilled water with normal diet for 4 weeks.

All animals were acclimatized for 1 week 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 was used as bedding which was changed daily.The animals were house dundera 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 before the commencement as well as after the completion of the experiment. The rats were sacrificed by cervical dislocation.Blood was collected by cardiac puncture. 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. The changes in severity were observed using a graded scale adopted from a previous study.[23] The grading scale consists of rankings according to the tissue damage; (0) none, (I) mild, (II) moderate and (III) severe.

Liver function tests (LFT) and serum bilirubin were tested using commercially available kits. All the data was recorded in the proforma. Data was analyzed by using SPSS (Statistical packages for social sciences) version22.0. Descriptive data was expressed as Mean±SD while statistical significance level was evaluated using one-way ANOVA. Post hoc Tukey's test was used to compare the findings between the groups. Significance level was set at $p \leq 0.05$.

RESULTS

Significant difference in mean post experimental body weight was observed in all three groups i.e. in group A there was a rise in body weight, while in experimental groups B and C significant decline in mean body weight was observed. However, in group C the weight loss was not as much as seen in group B. There was a statistically significant difference ($p<0.05$) between the experimental groups (Figure 1). The relative liver weight was significantly raised in Group B as compared with other experimental groups ($p<0.05$). (Table. 1)

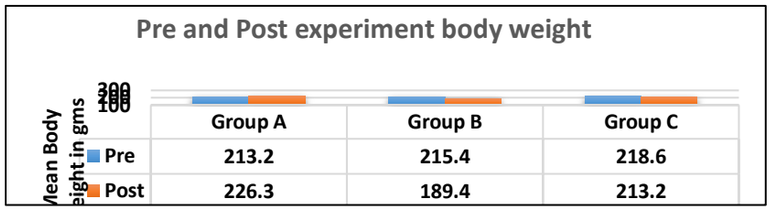

Figure 1. Distribution of pre and post experimental body weights among control and experimental groups.

Table 1. Distribution of body weight and relative liver weight among animal groups		
Groups	Body-weight (gm)	Relative liver weight/ 100 gm of body-weight (g)
Group A	226.3±13.14	3.21±0.23
Group B	189.4±26.11[a,c]	7.14±0.95[a,c]
Group C	213.2±5.87[a, b]	3.88±0.41[a,b]

[a]p value < 0.05 as compared with Group A, [b]p value < 0.05 as compared with Group B, [c] p value <0.05 as compared Group C

A statistically significant rise in serum markers of hepatic function (LFT) was observed after Fluoride administration in Group B. Treatment with Folic acid administration significantly reduced serum levels of LFTs ($p<0.05$). (Table II)

Table II: Difference in hepatic markers among all animal groups					
Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)
Group A	43.21±8.63	45.81±7.4	81.6±15.12	0.26±0.07	0.19±0.03
Group B	210±17.73[a]	165±12.03[a]	261±6.73[a]	1.39±0.08[a]	1.12±0.06[a]
Group C	72.11±9.63[a,b]	71.56±9.02[a,b]	120.3±6.04[a,b]	0.51±0.08[a,b]	0.28±0.03[a,b]

[a]p value < 0.05 as compared with Group A, [b]p value < 0.05 as compared with Group B, [c] p value <0.05 as compared Group C

On histological examination, Fibrotic changes were significantly higher among experimental groups as compared to control group. Necroticchanges, hepatic inflammatory changes, sinusoidal dilatation and congestion is due to inflammatory changes present in intra-lobular area, were found markedly higher among animals of group B. Histomorphological changes in different groups of rats is presented in TableIII.

Table III: Grading wise comparison of histopathological changes in hepatic tissues of rats					
	Fibrosis	Necrosis	Inflammatory cell infiltration	Sinusoidal dilatations	Congested portal vein
Group A	0	0	0	0	0
Group B	***	***	***	***	***
Group C	*	**	**	*	**

Grading score follows: none (0), mild (*), moderate (**) and severe (***)

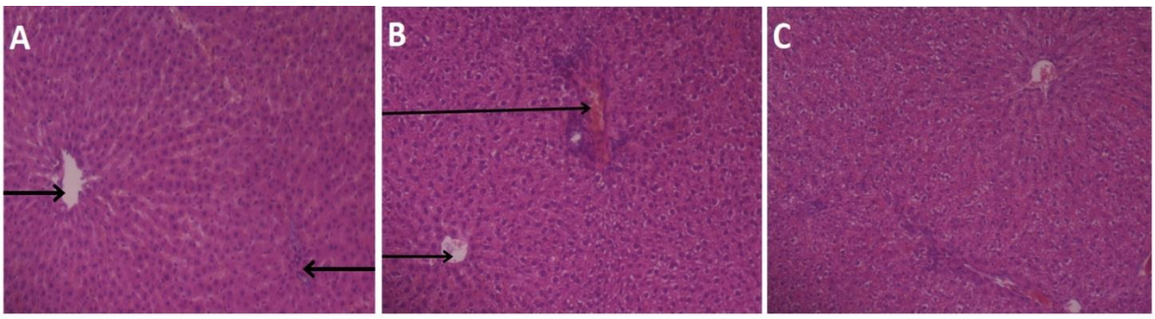

Figure2. Photomicrograph showinghistological section of liver of control and experimental rats. (H&E) X 400.
(A) – Control group rat with normal hepatic histological architecture without any infiltration.
(B) – Experimental group B rat with areas of lymphocytic infiltration, marked congestion and fibrosis.
(C) – Experimental group C rat with marked reduction in inflammation, necrosis and fibrosis.

DISCUSSION

Excessive fluoride is significantly associated with fibrosis in liver in this study. These findings are consistent with D Mukhopadhyay et al who had observed similar histopathological changes in the liver of zebra fish.[24] In the present study, necrotic changes in liver were observed mostly in experimental group B as compared to group C, which showed additional consumption of folic acid minimize histological alterations in liver which is induced by fluoride Ghosh J et al[25] 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[26] 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[27] 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. These results suggested that fluoride significantly associated with infiltrative changes in liver and folic acid equally reduced and prevent it, Perera T et al[28] observed infiltrative and focal necrotic changes after 15 days of fluoride administration.

Similarly, in the study of Song GH et al[7] 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, which is consistent with the current study. Moreover, Than gapandiyan 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 fluorideions.[14]

According to the findings of present study, histological alteration as well as levels of serum hepatic markers 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. Woo et al reported similar findings where folic acid supplementation was associated with marked reduction in hepatic injury as reflected by serum markers of hepatic function, as well as normalization of the hepatic histological architecture, which is consistent with the present study.[29] Folic acid as a cheap, safe, and well-tolerated supplement has a beneficial role in various health disorders.[30]

As oxidative stress of fluoride describes a state of uncontrolled over production of free radicals beyond a threshold for proper antioxidant neutralization resulting in impairment to macromolecules such as DNA, lipids, andproteins. 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.[21] 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 inter conversion of aminoacids. 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 oxidativestress.[31]

CONCLUSION

Folic acid supplement is highly effective in preventing and reverting the hepatic serum markers levels and histological alterations caused by fluoride

ETHICS APPROVAL: The ERC gave ethical review approval

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin

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