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Effect of Combined Exposure to Chronic High Fat Diet and Arsenic on Kidney Function in Male Mice

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A B S T R A C T

A number of risk factors could potentially affect the process of arsenic-induced kidney diseases. Arsenic is a common environmental and occupational contaminant dispersed world-wide, which can have an influence on developing chronic kidney disease (CKD) by several mechanisms. In recent years, alteration in the lifestyle as well as food habits among the people led to an increased use of food comprising a high-fat level. This important susceptible factor can have an effect on toxicity induced by arsenic. The present study was designed to evaluate the chronic exposure high fat diet (HFD) on arsenic-induced oxidative stress in kidney tissue of mice. Mice were randomly divided into 6 different groups (n=12). A low fat diet (LFD) control, LFD + arsenic 25 ppm, LFD + arsenic 50 ppm, HFD control, HFD + arsenic 25 ppm, HFD +arsenic 50 ppm. At the end of experiments, body weight and kidney weight to body weight ratio, biochemical parameters, oxidative stress markers, and kidney histological were evaluated. Our study showed that HFD increased arsenic-induced kidney damage through oxidative stress in mice. These investigations could be important for clinical research to protect against arsenic-induced kidney toxicity.

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Introduction

Chronic kidney disease (CKD) remains the leading cause of mortality worldwide [1]. Epidemiological studies were done in different countries and it is recognized that environmental factors are associated with this disease. Arsenic is a heavy metalloid that exposure to it has been recognized as a risk factor for cardiovascular disease, hypertension, peripheral artery disease, diabetes mellitus as well as renal disease [2, 3]. WHO has estimated that about 140 million people are exposed to high concentrations of arsenic via consumption of contaminated drinking water in areas such as Bangladesh, India, Taiwan, Xinjiang, Inner Mongolia, and Shanxi of China [4]. It is well known that kidney is the main organ involved in the excretion of arsenic and a primary target for the toxic effects of it, which its disorder is characterized by clinical symptoms and biochemical alterations [5]. Among several mechanisms, oxidative stress is a relatively common cause of arsenic toxicity in the kidney [6]. Oxidative stress damage caused by arsenic plays a vital role for biochemical alteration [7].

Obesity is a major risk for chronic diseases, including type-2 diabetes, cardiovascular disease, hypertension, and some types of cancer [8, 9]. In the last decades, changes in food habits in developing countries have led to increasing the quantity of lipids in their diet [10]. Diet rich in cholesterol facilitate the growth and progression of injury because cholesterol accumulation in the kidneys can lead to generation of reactive oxygen species (ROS), lipid peroxidation, and finally oxidative damage [11]. HFD significantly increased BUN, creatinine, urinary urea, urinary albumin excretion, and kidney weight which are measured as the marker of protein leakage, an indicator of renal damage [12]. According to these findings, enhancement of lipid levels, nutrient uptake, and subsequently increase in the electron flow in the mitochondrial respiratory chain lead to an augment reactive ROS generation, increased oxidative stress, kidney malfunctioning, and kidney damage [13].

There is no evidence that shows the effects of chronic exposure HFD on arsenic toxicity in kidney. The present study has conducted to indicate the relationship between chronic consumption of HFD and chronic arsenic toxicity of kidney and also has investigated the mechanism of oxidative stress.

Materials and Methods

Chemicals

Sodium arsenite (99% pure), thiobarbituric acid, trichloroacetic acid, reduced glutathione (GSH) was purchased from Sigma- Aldrich (St. Louis, USA). LFD (11% of all calorie supply from fat) and HFD (57% of all calorie supply from fat) obtained from Javaneh Khorasan lab. Iran. 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and 1,1,3,3-tetramethoxypropane was obtained from Merck (Darmstadt, Germany).

Animal's preparation

Male NMRI mice (30-35 g)(n=72) were obtained from the animal facility of Ahvaz Jundishapur University of Medical Science (AJUMS), which is fully accredited by AJUMS animal care guidelines with an ethics committee grantee No. IR.AJUMS.REC.1395.405. The NMRI mice model is widely used for studying metabolic disease, toxicity, and infection. Mice were divided into six groups (n=12): LFD (control group), LFD + arsenic 25ppm, LFD + arsenic 50ppm, HFD (control group), HFD + arsenic 25ppm and, HFD + arsenic 50ppm. Control groups received deionized drinking water. Water containing arsenite was freshly prepared every three days to minimize its oxidation. The monitoring of water and food consumption and body weight has been done every week in all experimental groups [14].

Animal sacrifice and collection of blood and tissue samples

After 20 weeks of experimenting, the overnight fasting animals were anesthetized by ketamine and xylasine. Blood was carefully collected by cardiac puncture in heparin tubes. Plasma

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samples were separated immediately and used for the various biochemical analyses. The kidneys were also removed and used separately to prepare 10% (w/v) homogenate in 0.1 M Tris–HCl buffer (pH 7.4) and centrifuged at 7,000 g for 10 minutes at 4° C. The supernatant fraction was used for determination of antioxidant factor assays. Additionally, the remainder of kidneys was fixed in 10% formalin solution and then stained with hematoxylin and eosin (H&E) [15, 16].

Measurement of body weight and kidney weight to body weight ratio

Body weights of mice in all the groups were measured and recorded every week and kidney weight of every animal was measured after sacrificing animal. Kidney weight to body weight ratio was also calculated.

Biochemical parameters of kidney function

Urea (Lot. No., 95006), creatinine (Lot. No., 99004), total protein (Lot. No., 95002), and albumin(Lot .No., 95003) concentrations were quantified using commercial kits(Pars Azmoon,Tehran, Iran) and were assessed by Automatic Analyzer(Alpha Classic-AT plus, Isfahan, Iran).

Oxidative stress markers

Determination of Malondialdehyde (MDA) level

The tissue MDA levels were determined by the method of Draper and Hadley based on the reaction of MDA with thiobarbituric acid (TBA) at 95° C. The sample was mixed with 2.5 vol of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation. Then the supernatant was used to react with 0.67% TBA in a boiling water-bath for 15 minutes. After cooling, the absorbance was read 532 nm. The results were expressed as nmol/g tissue [17].

Determination of GSH

GSH contents were measured in the kidney homogenate applying the Ellman's reagent. Briefly, TCA (20%) along with EDTA (1 mM) was added to the tissue homogenate (500 µl). mixture (200 µl) was added to DTNB (1.8 ml, 0.1 mM) and then was incubated for 20 min at room temperature. The absorbance was read at 412 nm. Results were reported as mol/g tissue. All data were expressed in µmol /g tissue using a GSH standard curve prepared by applying an authentic GSH standard [18].

Determination of ROS level in kidney tissues

The level of ROS in kidney tissue was measured by using 2′, 7′-dichlorofluorescindiacetate (DCFDA) that converted into highly fluorescent DCF by cellular peroxides. In each test, 2mL homogenate tissue was mixed with 40 ml of 1.25mM DCFDA in methanol for ROS estimation. All samples were incubated for 15 minutes in a water bath at 37° C. Fluorescence was calculated using a fluorimeter, at 488nm excitation and 525 nm emission wavelength [19].

Statistical analysis

Data were presented as means ± SE for different experiments. All the results were analyzed using Graph Pad Prism (version 5.04). Statistical significance was determined using the one-way analysis of variance with the Tukey post hoc test. Statistical significance was set at $p < 0.05$.

Results

The effects of diet and arsenic exposure on water, arsenic, food, and calorie intakes

Water consumption in the HFD control group was less compared to LFD control group ($p<0.001$). Also water intake decreased in arsenic exposure groups compared to their control group. The average daily intake of arsenic was estimated

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using the daily water intake. The results showed a significant increase of arsenic administration in arsenic 50ppm exposure groups compared to arsenic 25ppm exposure groups ($p<0.05$). In general, mice in LFD group consumed more food than mice in HFD group, while mice in HFD and arsenic 50ppm group consumed less food than LFD control group($p<0.01$). Calorie intakes were estimated using food intake data. There was no difference between calorie intake in LFD groups, but exposure to arsenic showed a significant decrease in calorie intake in the HFD groups ($p<0.05$) (Table1).

Table 1. The effects of diet and arsenic exposure on average daily food intake, water drink, arsenic, and calorie intake.

Groups Variables	food intake(g/day)	water drink(mL/day)	As intake(µg/day)	Calorie intake(kcal/day
LFD Control	10.71±2.5	11.37±1.3	0	42.48±5.2
LFD+As 25ppm	10.18±3.3	7.34±0.7**	185.75±17.5	40.74± 9.6
LFD+As 50ppm	8.56±1.7	4.27±0.84***	213.5±24.7&	34.68±3.5
HFD Control	9.50±2.8	8.48±0.9***	0	57.3±5.4**
HFD+ 25 ppm	8.65±1.6#	6.18±1.6##	154.5±30.8	51.3±4.2&
HFD+ 50 ppm	7.73±1.7**	4.34±1.4###	217.4±36.7^	45.7±4.5#$

All data are given as Mean± SEM of different experiments (n = 12).*: Significantly different from LFD, #: Significantly different from HFD, &: Significantly different from LFD + As 25 ppm, $: Significantly different from LFD + As50 ppm, ^: Significantly different from HFD + As 25 ppm. *, #, &, ^ and $ p < 0.05, **and ## p< 0.01, * and ###p< 0.001.**

The effects of diet and arsenic exposure on body weight and kidney weight to body weight ratio

The body weight of mice was significantly heavier in the HFD control group compared to LFD control group ($p < 0.001$). In regard to the animals co-treated with arsenic 50ppm and HFD, body weight decreased compared to LFD control group ($p < 0.01$). Also in both HFD groups, body weight decreased compared to their control groups (Fig. 1 A).

Furthermore, kidney to body weight ratio in HFD control group was significantly less than in LFD control group as shown in Figure1B ($P < 0.001$). As well as in HFD and LFD groups treated with arsenic, the post-experiment kidney weight to body weight ratio decreased compared to their control groups (Fig. 1B).

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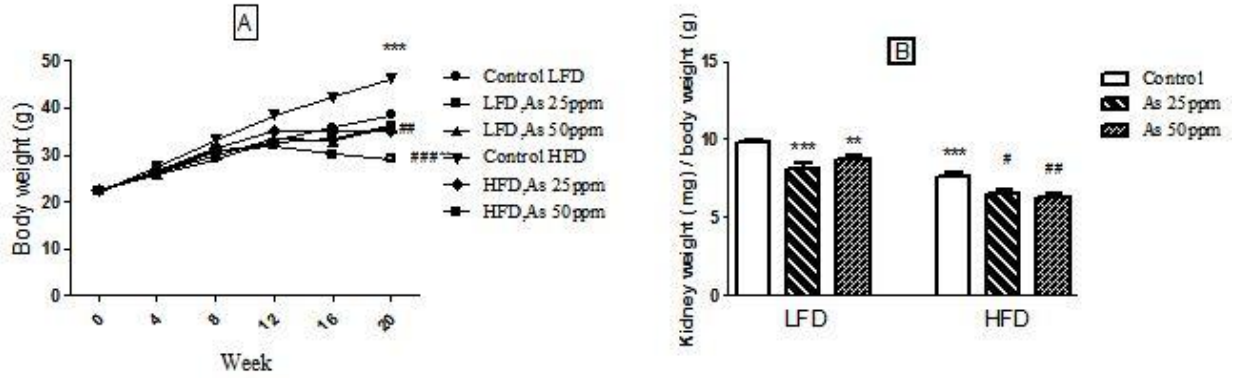

Fig. 1. Changes of body weight and kidney weight to body weight ratio in control LFD and HFD groups and As 25 and 50 ppm treated LFD and HFD groups. (A) Body weight. (B) Kidney weight to body weight ratio.(Mean ± SE; n = 12, for A-B). *: designate significant difference from LFD, #: designate significant difference from HFD, ** and ## p< 0.01, *** p< 0.001.

Blood Biochemical parameters

The arsenic did not affect plasma total protein, albumin, and urea levels in LFD groups, but significantly (P<0.05) decreased plasma total protein and albumin and increased urea in HFD groups. In addition, plasma creatinine levels increased in HFD control group in comparison with LFD control group. Also in Arsenic 50ppm and LFD and HFD co-treated groups, the plasma creatinine increased after the experiment but it had no effect on the group co-treated with arsenic 25ppm (Table2).

Table 2. The effects of Diet (high fat diet and low fat diet) and arsenic exposure (25 and 50 ppm) on Total protein (mg/dl), Albumin (mg/dl), Urea(mg/dl), and Creatinine(mg/dl) of plasma

Groups Variables	Low fat diet			High fat diet		
	Control	As 25ppm	As 50ppm	Control	As 25ppm	As 50ppm
Total protein(mg/dl)	7.21±0.8	5.60± 0.8	5.49±0.6	4.91±0.6*	4.48±0.5**	4.15±0.3**
Albumin(mg/dl)	3.82±0.4	3.43±0.3	2.50±0.4	2.36±0.1*	1.92 ±0.2**	1.80±0.09***
Urea (mg/dl)	39.71±1.8	68.25±7.4	79.95±8.6	99.02±4.4*	94.19±13.9*	120.3±15.8***
Creatinine(mg/dl)	0.21±0.02	0.39±0.06	0.56±0.05**	0.47±0.04*	0.53±0.07	0.77±0.04##

Each value was presented as means ± SEM (n = 12). *: Significantly different from LFD *, p < 0.05, **, ##p< 0.01, *** p< 0.001.

The effects of diet and arsenic exposure on kidney oxidative stress

As depicted in Fig.2A, the ROS level of kidney significantly increased in all HFD groups compared to LFD control group. Besides, arsenic 50ppm increased ROS level in LFD and HFD group compared to their control groups. GSH assessment results showed a decrease in all HFD groups and arsenic 50ppm and LFD co-treated group (p<0.05) compared to LFD control group (Fig.2B). The

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results of lipid peroxidation revealed that exposure to HFD and arsenic alone had no effect on MDA level of kidney tissue but it was significantly increased in arsenic and HFD co-treated groups compared to LFD and HFD control groups ($p<0.01$) (Fig. 2C).

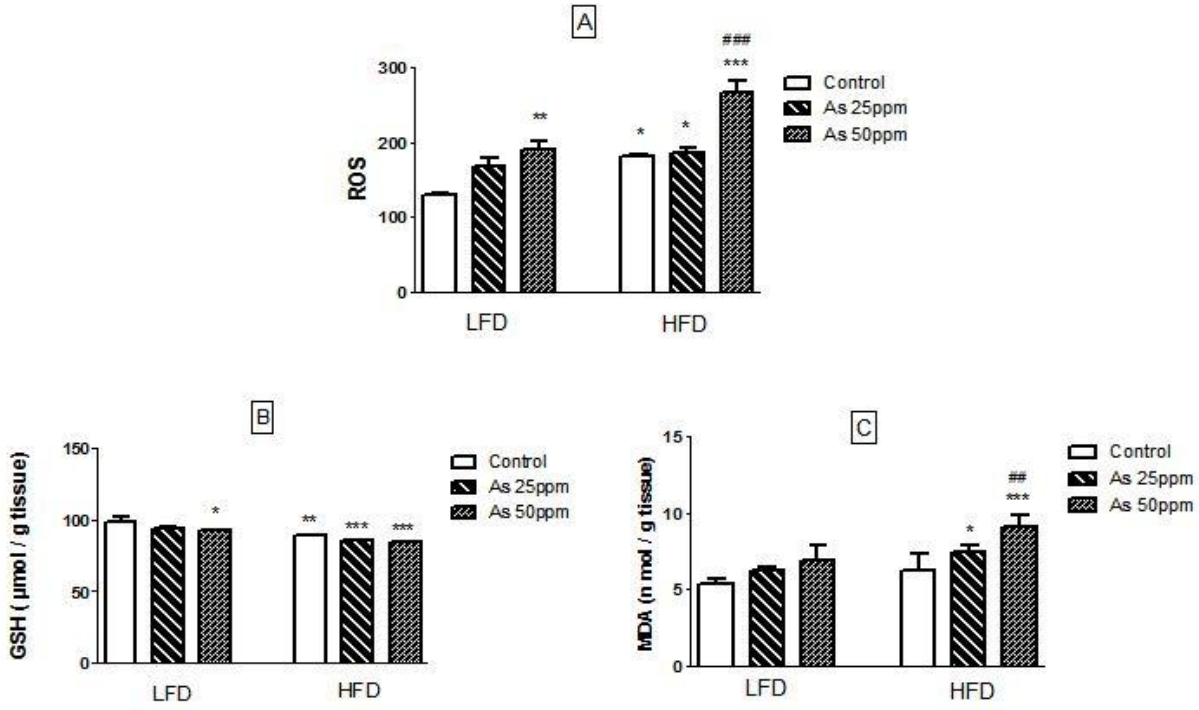

Fig. 2. Changes in ROS formation(A), GSH level (B) and Lipid peroxidation(MDA)(C) in kidney tissue in control LFD and HFD group and As 25 and 50 ppm treated LFD and HFD groups.(Mean ± SEM; n = 12, for A-C).*: designate significant difference from LFD, #: designate significant difference from HFD, *p<0.05, **p< 0.01, *** and ### p< 0.001

Histopathological Analysis

The examination of the HE-stained sections of the kidney (Figure 3) in the LFD control group showed the typical structure of kidney. But in the LFD group to which arsenic 50ppm was given($p<0.05$) and in all HFD groups, cellular swelling, brush border loss, and infiltration of inflammation cells significantly increased compared to LFD and HFD control groups, while the diameter of glomerules was significantly decreased (Table3).

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Table 3. Effect of high fat diet and As on cellular swelling(%), brush border loss(%), infiltration of inflammation cells and the diameter of glomerul(µm).

Histological criteria	groups					
	LF Control	LF25	LF50	HF control	HF25	HF50
Normal (%)	98.7±1.2	98.6 ± 1.7	89.4±8.2*	90.3±11.5*	78.4±9.3**#	65.7±10.1**#
Cellular swelling (%)	0.09±0.01	0.08±0.02	8.2±5.2*	6.1±2.6*	13.3±3.7*##	19.6±4.3**#
Brush border loss (%)	0.02 ± 0.1	1.1 ± 0.3*	3.8±2.8*	3.7±1.5*	9.5±2.1*#	13.8±1.9*#
Infiltration of inflammatory cells	0.01± 0.001	0.1± 0.03*	0.5±0.07*	0.7±0.04*	1.9±0.08*#	2.6±0.9*#
Glomerul diameter (µm)	237±6.3	207.7±2.9	198.8±5.4*	90.6±0.26**	186.2±3.1**	154.1±16.1***#

Each value was presented as means ± SEM (n = 12). *: Significantly different from LFD, #: Significantly different from HFD,* p < 0.05, **, ##p< 0.01, *** p< 0.001.

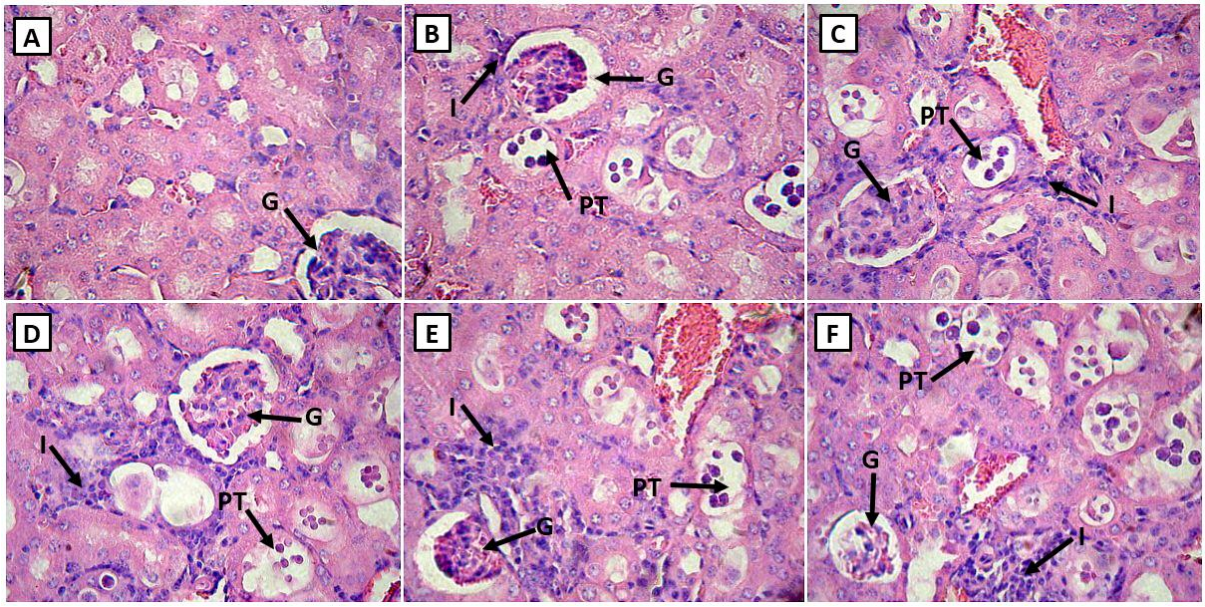

Fig. 3. Histological changes of mice kidney in various groups (A–F). H&E staining. A: control LFD; B: LFD+ As 25 ppm; C: LFD+ As 50 ppm; D: Control HFD; E: HFD + As 25 ppm; F: HFD+ As 50 ppm**.** I: Infiltration of inflammatory cells, G: glomeruli, PT: Proximal tubule, magnifications: ×400

Discussion

The present study has shown that HFD, obviously worsen arsenic induced kidney injury with regard to induction of oxidative stress. It has been reported in many studies that chronic exposure to inorganic arsenic in experimental animals leads to increased levels of urea and creatinine, decreased clearance and increased protein losses through urine, edema in renal cortex and interstitial field, swelling of tubule cells, necrosis in tubule

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epithelium, and also dilatation and hyperemia of glomerular [20]. However, people's exposure to environmental arsenic is not the only development factor leading to kidney diseases such as metabolic syndrome related glomerulopathy; lifestyle and diet are also playing a key role in this development. In the present study we found that HFD could enhance arsenic induced renal dysfunction as well as obesity, which are also the second major risk factors that lead to an increased incidence of chronic kidney disease. It may be associated with increased oxidative stress in the kidney.

Arsenic itself decreased body weight by interfering with multiple metabolic pathways [21]. In the present study we found that when the HFD is administered together with arsenic, body weight and kidney weight to body weight ratio gradually reduce. In addition, arsenic decreased water intake in a dose-dependent manner, and the same effect was revealed in the HFD control group compared to LFD control group. Even though animals treated with arsenic 50ppm drank less water, our result showed that they received a significant amount of arsenic. Furthermore, concomitant arsenic and HFD administration caused food and calorie intake limitation.

It has been shown in various studies that arsenic and HFD through kidney injury, lead to an increase in urea and creatinine, and a reduction in total protein and albumin [22, 23]. Our findings indicated that HFD caused significant increase of plasma urea and creatinine that is an indicator for defective kidney function in HFD group. Reduced renal protein and albumin in these groups indicate enhanced catabolism of proteins that ultimately results in increased production of urea in these animals. But arsenic alone did not affect plasma total protein, albumin, and urea levels in LFD groups, while significantly decreased plasma total protein and albumin and increased urea in HFD groups. Also plasma creatinine increased in groups treated with arsenic 50ppm but didn't change in groups treated with arsenic 25ppm during the period of experiment. So the present results determine that arsenic combined with HFD led to more changes in these biomarkers in the interest of kidney damage.

Because of the injury, renal tissue acts as a source of ROS and these free radicals activate the process that will lead to a reduction in antioxidant defense mechanisms and finally increase oxidative stress [24, 25]. ROS themselves can reduce the activity of antioxidant enzymes such as GPX that use GSH as substrate. GSH represents one of the important biomarkers of oxidative stress which forms the first line of defense against free radicals.

In the present study, we demonstrated that arsenic in combination with HFD leads to an increase in the production of ROS and depletion of glutathione levels in kidney tissue. The membrane polyunsaturated fatty acids are extremely susceptible to free radical-induced oxidative damage. Also the interaction between free radicals and polyunsaturated fatty acids initiates the self-disseminating lipid peroxidation reactions which results in impaired membrane function and generation of MDA [26]. MDA is the final product of lipid peroxidation and an indicator used to evaluate the oxidation of membrane lipids [27]. In this study, it has been detected that MDA level didn't change in the groups receiving either HFD or arsenic, but it was significantly higher in the arsenic 50ppm and HFD co-treated groups. The further changes of these factors in the groups co-treated with arsenic and HFD represents development of oxidative stress.

The histological assessments of different kidney segments of experimental animals in this study showed that exposure to arsenic led to increased cellular swelling, brush border loss, and decreased diameter of glomerules. A study by SM Prabu has shown that arsenic leads to tubular necrosis, inflammatory cell infiltration, tubular degeneration, hemorrhage, swelling of tubules, and vacuolization [28]. In the present study, the combination of arsenic and HFD led to aggravate above-mentioned signs along with Infiltration of inflammation cells. This could be due to the accumulation of free radicals as a result of increased lipid peroxidation by free arsenic ions and also HFD in the renal tissue. The increased formation of lipid peroxides and associated ROS led to damage in kidney cells membrane and also other pathological changes in mice kidney.

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Conflict of interests

Authors certify that there is no actual or potential conflict of interest in relation to this article.

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