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Original Article

Chronic exposure to arsenic and high fat diet additively induced cardiotoxicity in male mice

Akram Ahangarpour[1], Leila Zeidooni[2,*], Azin Samimi[2], Soheila Alboghobeish[3], Laya Sadat Khorsandi[4], and Mitra Moradi[1]

[1]Health Research Institute, Diabetes Research Center, Department of Physiology, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, I.R. Iran.
[2]Department of Toxicology and Student Research Committee, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, I.R. Iran.
[3]Department of Pharmacology and Student Research Committee, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, I.R. Iran.
[4]Cell and Molecular Research Center, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, I.R. Iran.

Abstract

Diet is one of the important risk factors that could potentially affect arsenic-induced cardiotoxicity. The present study was undertaken to investigate the effect of high fat diet on arsenic-induced cardiotoxicity in mice. Mice were divided into six different groups (n = 12), two control groups received either low fat diet (LFD) or high fat diet (HFD) along with deionized drinking water and four test groups given LFD + 25 ppm arsenic, LFD + 50 ppm arsenic, HFD + 25 ppm arsenic, and HFD + 50 ppm arsenic in drinking water for 5 months. The body weight, heart weight to body weight ratio, cardiac biochemical markers, lipid profile, and histological examination of heart were evaluated. The results demonstrated that arsenic exposure led to a significant decrease in heart glutathione level, catalase enzyme activity, and a significant increase in reactive oxygen species (ROS), malondialdehyde levels, and biochemical enzymes. The administration of HFD resulted in above-mentioned changes as well as an alteration in lipid profile; however, arsenic exposure alone or along with HFD caused a reduction in lipid profile factors, except HDL level. Our results revealed that HFD increased arsenic-induced heart injury in the mice. This effect may be because of reduction in antioxidant activities and/or increase in oxidative stress and ROS in mice heart tissues. These findings could be important for clinical intervention to protect against or prevent arsenic-induced cardiotoxicity in humans.

Keywords: Heart; Arsenic; High fat diet; Chronic exposure; Cardiotoxicity

INTRODUCTION

Cardiovascular disease is both a familial and lifestyle disorder affecting millions of people in the world.

Despite rapid advances that have been made in the treatment of cardiac disease, it remains a leading cause of mortality in many countries (1,2).

There are many risk factors associated with cardiovascular diseases, including dyslipidemia, hypertension, cigarette smoking, diabetes, obesity, and physical inactivity (3). Arsenic toxicity is one of the risk factors associated with cardiovascular diseases that includes direct myocardial injury, cardiac arrhythmias, cardiomyopathy (2,4), ischemic heart disease (5), and blood pressure (6,7).

One of the mechanisms of arsenic toxicity is oxidative stress (8) and decrease in levels of antioxidants (8), decrease in levels of antioxidants, and increase in levels of oxidative products leading to widespread arsenic toxicity (9).

Moreover, many studies have reported that oxidative stress plays a key role in diverse types of cardiovascular diseases (4).

A number of studies have shown that nutrition is an important factor in arsenic-induced toxicity.

*Corresponding author: L. Zeidooni
Tel: +98-9374666363, Fax: +98-6133332036
Email: leilazeidooni@gmail.com

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HFD enhanced arsenic-induced hepato-fibrogenesis (10) and acute exposure to HFD clearly aggravated the accumulation of arsenic in liver tissues (11). However, there is no evidence to show the effects of chronic exposure to HFD on arsenic toxicity in the heart. Industrialization and economic growth of modern society have led to changes in lifestyle of the people. One such phenomenon is the increasing consumption of fast foods that contain high level of fat.

The present study was designed to determine if the chronic exposure to HFD exacerbates chronic arsenic-induced cardiotoxicity in mice.

MATERIALS AND METHODS

Chemicals

Sodium arsenite (99% pure), 2,7-dichlorofluoresceindiacetate (DCFDA), thiobarbituric acid (TBA), trichloroacetic acid (TCA), and glutathione (GSH) were purchased from Sigma-Aldrich (St Louis, Missouri, USA). 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was obtained from Merck (Darmstadt, Germany). Creatine phosphor kinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride (TG), cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) kits were obtained from (Pars Azmoon, Iran). NaCl, KCl, Na_2HPO4, K_2HPO_4, and other chemicals were of the highest grade commercially available.

Animals and treatments

Seventy-two male Naval Medical Research Institute (NMRI) mice weighing 30-35 g were obtained from the animal facilities of Ahvaz Jundishapur University of Medical Science (AJUMS). All animal experiments were approved by the Animal Research Ethics Committee of AJUMS (ethical approval ID: IR.AJUMS.REC.1395.636) and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals. Mice were housed (six per cage) in polycarbonate cages with temperature at 20 ± 4 °C and 10% humidity with a 12 h light/12 h dark cycle. Control mice were either fed LFD (11% fat) or HFD (58% fat) (purchased from Javaneh Khorasan Lab, Iran) for 5 months. Four test groups given LFD + 25 ppm arsenic, LFD + 50 ppm arsenic, HFD + 25 ppm arsenic, and HFD + 50 ppm arsenic in drinking water for 5 months. Some studies have shown that, the grain-based diet can contain between 19.5-28.6 ppb arsenic which could have compromised the study design.

To avoid this problem in the present study, we used only purified diets that did not contain any grain components. The level of arsenic in HFD was 10 ppb and in LFD was 15 ppb, which is very low compared to the examined concentration (25 and 50 ppm).

Previous studies suggest that mice are less susceptible to arsenic toxicity than human, partly because of a faster metabolism and clearance of arsenic. Therefore, it is necessary to use a higher exposure concentration of arsenic than the environmentally relevant concentrations in mouse experiment.

A recent study showed that 10-fold higher arsenic concentration in drinking water is needed (50 ppm) to get arsenic concentrations similar to those seen in humans exposed to arsenic. Therefore, in the present study mice were administered arsenic in diH_2O in doses of 25 or 50 ppm for 5 months (12-14).

Water containing arsenic was freshly prepared every 3 days to minimize oxidation. Water and food consumption and body weight was monitored every week in all six exposure groups (15).

Determination of the heart weight to the body weight

At the end of 5 months, mice were sacrificed and their hearts were quickly excised and weighed. Then the ratio of heart weight to body weight was calculated for each animal (1).

Preparation of heart homogenates

Heart tissue was homogenized with phosphate buffer (1 mM; pH 7.4) (10% w/v) using glass homogenizer. The homogenate was then centrifuged at 12,000 × *g* for 30 min at 4 °C. The supernatant was collected and used for the following experiments (1).

Assay of serum cardiac markers and lipid profiles

Twenty-four hours after the last experimental day, the overnight fasting animals were anesthetized by ketamine-zylazine and blood samples were directly collected by cardiac puncture and centrifuged at 3500 rpm for 20 min (1). Serum samples were kept at −70 °C until biochemical assessments were performed. Serum samples were used to measure total cholesterol, TG, HDL, LDL, AST, ALT, LDH, and CPK using standard assay kits (1,16).

Determination of catalase enzyme in heart tissue

Catalase enzyme activity was assayed according to the method used by Goth (17). Accordingly, 500 µL of 0.05 mmol tris-HCl, 1 mL H_2O_2, and 50 µL of sample were mixed and incubated for 10 min, and then reaction was stopped by the addition of 500 µL ammonium molybdate solution (4%). The absorbance was read at 410 nm and expressed as U/g tissue (17).

Determination of glutathione in heart tissue

Glutathione content was measured according to the method described by Thomas and Skrinska (18). Heart homogenates were incubated with 1 mL of 20% TCA and 1 mL of 1 mM ethylenediaminetetraacetic acid for 5 min, which was used as protein precipitant. The total homogenate was centrifuged at 12,000 × g for 30 min at 4 °C. Then, 200 µL of supernatant was mixed with 1.8 mL of 0.1 mM DTNB. The GSH reacts with DTNB and forms a yellow-colored complex. The absorbance was read at 412 nm and expressed as moles of GSH/g tissue (18).

Determination of thiobarbituric acid reactive substances in heart tissue

The extent of lipid peroxidation in terms of malondialdehyde (MDA) formation was measured. One mL of heart homogenate sample was briefly mixed with 1 mL TCA (20%) and 2 mL TBA (0.67%) and heated for 1 h in boiling water bath. After cooling, the mixture was centrifuged and absorbance of the supernatant was measured at 532 nm against suitable blank. The amount of thiobarbituric acid reactive substances (TBARS) was calculated by using a molar extinction coefficient of $\varepsilon = 1.56 \times 10^5$ /M/cm and expressed as mol/g tissue (18).

Determination of reactive oxygen species level in heart tissue

The level of reactive oxygen species (ROS) in the heart tissue was measured using DCFDA that gets converted into highly fluorescent dichlorofluorescein (DCF) by cellular peroxides. Heart homogenate (10% w/v) was prepared in phosphate buffer (1 mM; pH 7.4). For each ROS estimation test, 2 mL homogenate tissue was mixed with 40 mL of 1.25 mM DCFDA in methanol. All the samples were incubated for 15 min in a water bath at 37 °C. Fluorescence was calculated using a fluorimeter, at 488 nm excitation and 525 nm emission wavelength (19).

Histopathological analysis

The heart of mice were removed, fixed in 10% formalin solution, dehydrated in graded alcohol concentrations, and subsequently embedded in paraffin. Sections of 5 µm thickness were prepared and stained with hematoxylin and eosin (H&E) for the histopathological analysis of the heart.

Statistical analysis

Data was expressed as means ± SE for three different groups. All the results were analyzed using Graph Pad Prism (V. 5.04). Statistical significance was determined using the one-way analysis of variance with the Tukey's post hoc test. Statistical significance was set at $P < 0.05$.

RESULTS

Effect of high fat diet and arsenic on body weight

Fig. 1 shows body weight in the LFD and HFD fed control and arsenic-treated mice. While expected, the control mice fed with HFD for 20 weeks weighed more than the LFD control mice ($P < 0.001$). The HFD mice exposed to 25 and 50 ppm arsenic weighed less than the HFD controls mice ($P < 0.01$) and

($P < 0.001$), respectively. Furthermore, arsenic exposure in both the concentrations led to a small decrease in weight of mice that were fed LFD for 20 weeks; however, these differences were not statistically significant**.**

Effect of high fat diet and arsenic on heart weight to the body weight

Fig. 2 shows that there was no statistically significant difference in the control HFD group compared with the control LFD group with respect to average heart weight to the body weight ratio.

Similarly, there was no statistically significant difference between LFD arsenic 25 ppm and LFD arsenic 50 ppm fed mice compared with the LFD control mice. But the LFD arsenic 50 ppm fed mice showed statistically significant difference compared with LFD arsenic 25 ppm ($P < 0.001$).

Lastly, arsenic exposure significantly reduced the difference in heart weight to body weight ratio between HFD arsenic 50 ppm and LFD arsenic 50 ppm fed mice and significantly increased heart weight to body weight ratio in HFD fed mice ($P < 0.001$).

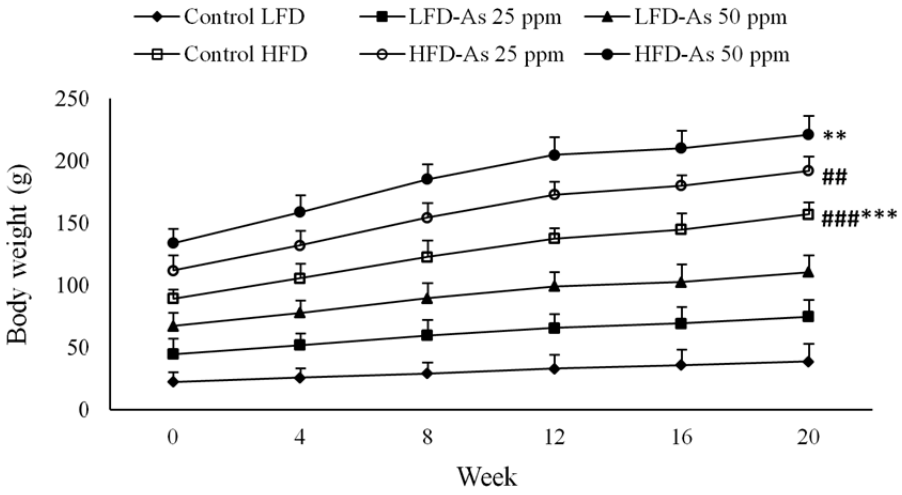

Fig. 1. Body weight in control LFD or HFD fed and arsenic 25 or 50 treated LFD or HFD mice. HFD, high fat diet; LFD, low fat diet; As 25, arsenic 25 ppm; As 50, arsenic 50 ppm. Values are mean ± SD; n = 12. $^{\#\#}$ $P < 0.01$, significantly different from HFD; $^{\#\#\#}$ $P < 0.001$, significantly different from HFD; ** $P < 0.01$, significantly different from LFD; *** $P < 0.001$, significantly different from LFD.

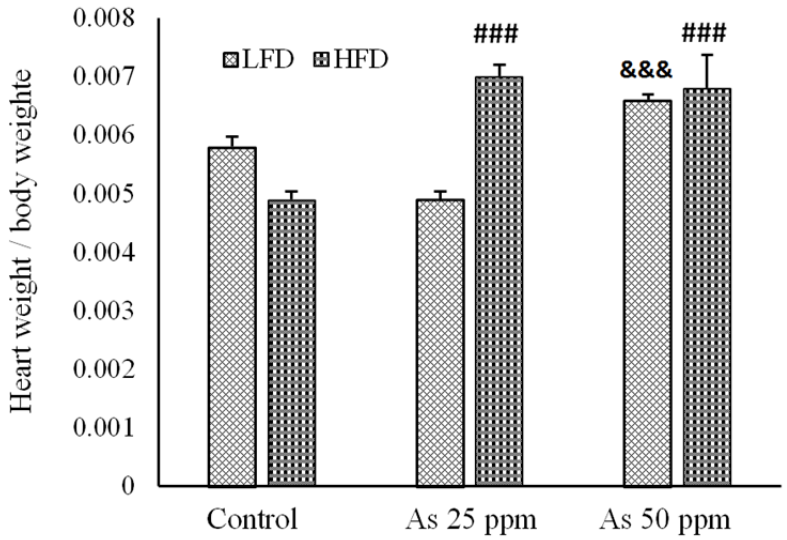

Fig. 2. Heart weight to the body weight ratios in control LFD or HFD fed and arsenic 25 or 50 treated LFD or HFD mice. HFD, high fat diet; LFD, low fat diet; As 25, arsenic 25 ppm; As 50, arsenic 50 ppm. Values are mean ± S.E.M; n = 12. $^{\#\#\#}$ $P < 0.001$, significantly different from HFD; $^{\&\&\&}$ $P < 0.001$, significantly different from LFD + As 25 ppm.

Effect of high fat diet and arsenic on lipid profile

Lipid profile of fasted mice was measured in all the treatment groups, and there was no statistical significant difference between the LFD groups. However, the results of the HFD control mice indicated a higher plasma levels of TG, very low density lipoproteins (VLDL) ($P < 0.05$), cholesterol, and LDL ($P < 0.01$) and a lower plasma levels of HDL ($P < 0.01$) compared to the LFD control group. Also, plasma TG and VLDL levels decreased in HFD arsenic 25 ppm ($P < 0.05$) and HFD arsenic 50 ppm ($P < 0.01$) groups, when compared to HFD control group. Expectedly, cholesterol ($P < 0.01$) and LDL ($P < 0.05$) increased in HFD arsenic 25 ppm group versus LFD arsenic 25 ppm group.

Concomitant administration of HFD and arsenic 50 ppm induced a similar effect on plasma cholesterol levels ($P < 0.05$) compared with LFD arsenic 50 ppm group. However, cholesterol and LDL levels showed a significant decrease in HFD arsenic 50 ppm when compared to the HFD control mice ($P < 0.05$) (Table 1).

Effect of high fat diet and arsenic on plasma levels of heart enzymes

The CPK level did not indicate statistically significant difference in the control HFD mice compared with the LFD control. Further, exposure to arsenic 50 ppm increased plasma CPK level in the LFD and HFD fed mice when compared to their respective control groups ($P < 0.05$) and ($P < 0.001$), respectively. Plasma LDH level was not statistically significant difference between the HFD and LFD control groups.

Arsenic 50 ppm and arsenic 25 ppm exposure increased LDH level in the LFD and HFD fed mice compared with their respective control groups ($P < 0.001$) and ($P < 0.01$), respectively.

Plasma AST level did not show statistically significant effect in the control HFD mice when compared with LFD control mice. However, the ALT level was significantly greater in the control HFD group, and the HFD arsenic 25 and HFD arsenic 50 ppm fed mice compared with LFD control ($P < 0.001$). Furthermore, plasma AST and ALT levels in the LFD plus arsenic 25 ppm and LFD plus arsenic 50 ppm groups was not statistically significantly different compared with LFD control. But, exposure to HFD arsenic 50 ppm increased plasma AST and ALT level when compared to HFD control group, ($P < 0.001$) and ($P < 0.05$), respectively (Table 2).

Effects of high fat diet and arsenic on heart tissue reactive oxygen species levels

Increased ROS formation is expressed as DCF fluorescence intensity unit.

While shown in Table 3, arsenic 50 ppm exposure in the HFD and LFD groups induced a significant increase of ROS in heart tissues, ($P < 0.001$) and ($P < 0.01$), respectively.

Exposure to arsenic 50 ppm in the HFD group also resulted in significantly greater ROS formation compared with HFD arsenic 25 ppm group ($P < 0.001$). However, ROS formation was not statistically significant between HFD and LFD control groups.

Table 1. Effect of high fat diet and arsenic on plasma lipid profiles.

Variables	Low fat diet			High fat diet		
	Control	As 25 ppm	As 50 ppm	Control	As 25 ppm	As 50 ppm
Triglyceride	91.5 ± 2.5	70.2 ± 4.9	70 ± 2.8	120.5 ± 10.8*	88.1 ± 3.9$^{\#}$	63.1 ± 1.9$^{\#}$
Cholesterol	92.4 ± 4.6	88.5 ± 7.3	100.5 ± 10.5	193 ± 14.2**	171.4 ± 11.9$^{\&\&}$	160.1 ± 19.4$^{\#\$}$
HDL	81.4 ± 7.2	78.5 ± 6.1	90 ± 5.1	113.8 ±12.2**	118.0 ± 9.5$^{\&\&}$	110.0 ± 7.2$^{\$\$}$
LDL	13.5 ± 5.9	14.6 ± 3.5	16.7 ± 3.9	27.9 ± 3.1**	23.9 ± 3.5$^{\&}$	20.7 ± 6.5$^{\#}$
VLDL	17.8 ± 0.9	14.1 ± 1.5	14.1±0.9	25.1 ± 2.8*	18.1 ± 0.5$^{\#}$	12.1 ± 0.2$^{\#}$

(HDL), high-density lipoprotein; (LDL), low-density lipoprotein; (VLDL), very low-density lipoprotein; As 25, arsenic 25 ppm; As 50, arsenic 50 ppm. *, significantly different from LFD; $^{\#}$, significantly different from HFD; $^{\&}$, significantly different from LFD + As 25 ppm; $^{\$}$, significantly different from LFD + As 50 ppm.*, $^{\#}$, $^{\&}$, and $^{\$}$, $P < 0.05$; **, $^{\#\#}$, $^{\&\&}$, and $^{\$\$}$, $P < 0.01$.

Table 2. Effect of high fat diet and arsenic on plasma heart markers.

Variables (U/L)	Low fat diet			High fat diet		
	Control	As 25 ppm	As 50 ppm	Control	As 25 ppm	As 50 ppm
CPK	346.5 ± 49.41	430 ± 29.4	509 ± 68.26*	449.25 ± 51.5	582.5 ± 71.9**	757.5 ± 81.5$^{\#\#\#***}$
LDH	2693.2 ± 485.4	3426 ± 202.2**	3856.6 ± 256.7***	3233 ± 283.4	3719.3 ± 369.5$^{\#***}$	3885.6 ± 210.1$^{\#\#***}$
AST	149 ± 25.97	143.2 ± 39.09	143.8 ± 34.8	164.5 ± 47.48	226.1 ± 30*	267.8 ± 22.29$^{\#\#}$***
ALT	41.2 ± 19.58	63 ± 12.7	44 ± 4.3	146.2 ± 5.4***	166.6 ± 15.6***	179 ± 19.1$^{\#}$***

(As), arsenic; (CPK), creatine phosphorkinase; (LDH), lactate dehydrogenase; (AST), aspartate transaminase; (ALT), alanine transaminase. *, significantly different from LFD (control); $^{\#}$, significantly different from HFD (control); $^{\$}$, significantly different from HFD + As 25 ppm. *, $^{\#}$, and $^{\$}$, $P < 0.05$; **, $^{\#\#}$, and $^{\$\$}$, $P < 0.01$; *** and $^{\#\#\#}$ $P < 0.001$.

Table 3. Effect of high fat diet and arsenic on antioxidant marker in heart tissue.

Variables	Low fat diet			High fat diet		
	Control	As 25 ppm	As 50 ppm	Control	As 25 ppm	As 50 ppm
MDA[a]	6.3 ± 0.4	8.2 ± 0.25	11.9 ± 1.05**	10.3 ± 10.4*	11.5 ± 0.43	14.56 ± 0.7$^{\#}$
GSH[b]	109 ± 4.5	87 ± 5.1**	75.2 ± 4.4***	62 ± 5.4***	52 ± 5.5	43 ± 5.5$^{\#\#}$
Catalase[c]	152.7 ± 1.9	115.7 ± 10.1***	55.6 ± 5.2$^{\&\&\&***}$	105 ± 5.6***	46.5 ± 3.6$^{\#\#\#}$	33.3 ± 3.97$^{\#\#\#}$
ROS[d]	160.2 ± 0.28	202.5 ± 13.67	221.3 ± 17.1**	192.5 ± 10.2	215.6 ± 12.8	296.8 ± 26.22$^{\$\$\$\#\#\#}$

(a), nmol/g tissue; (b), µg/g tissue; (c), U/gr tissue; (d), FIU/g tissue; (As), arsenic; (MDA), malondialdehydyde; (ROS), reactive oxygen species; (GSH), glutathione. *, significantly different from LFD (control); $^{\#}$, significantly different from HFD (control); $^{\$}$, significantly different from HFD + As 25 ppm; $^{\&}$, significantly different from LFD + As 25 ppm. *, $^{\#}$, $^{\&}$, and $^{\$}$, $P < 0.05$; **, $^{\#\#}$, $^{\&\&}$ and $^{\$\$}$, $P < 0.01$; ***, $^{\&\&\&}$, and $^{\#\#\#}$, $P < 0.001$.

Effect of high fat diet and arsenic on heart tissue glutathione levels

GSH assessment results showed a significant decrease in control HFD group compared to control LFD fed mice ($P < 0.001$).

Furthermore, both arsenic doses significantly decreased this antioxidant enzyme in the LFD groups compared with LFD control ($P < 0.01$) and ($P < 0.001$) respectively. Arsenic 50 ppm exposure in HFD fed mice significantly decreased GSH level compared to HFD control group ($P < 0.01$) (Table 3).

Effects of high fat diet and arsenic on heart tissue thiobarbituric acid reactive substances

The results of lipid peroxidation analysis revealed that heart tissue MDA levels were significantly different in the two control groups.

Also, arsenic 50 ppm exposure significantly increased MDA levels in the HFD ($P < 0.05$) and LFD ($P < 0.01$) fed mice when compared to their respective controls (Table 3).

Effects of high fat diet and arsenic on heart tissue catalase enzyme levels

The results of catalase enzyme activity in heart tissue showed that this antioxidant enzyme was significantly decreased in the control HFD group compared to the control LFD fed mice ($P < 0.001$). Furthermore, arsenic exposure at both doses significantly decreased this antioxidant enzyme in the LFD and HFD fed mice compared with their respective controls ($P < 0.001$) (Table 3).

Histopathological analysis

Fig. 3 illustrates the histopathological assessments of different cardiac segments of experimental animals. In the LFD control group, there were no fatty changes in mice cardiac tissue. But, in the LFD arsenic 25 ppm and LFD arsenic 50 ppm groups, increased fatty changes were observed in mice cardiac tissues.

In the control HFD group, the fatty changes were higher than in the control LFD fed mice. In the HFD arsenic 25 ppm and HFD arsenic 50 ppm groups, the fatty changes were more than their respective control groups.

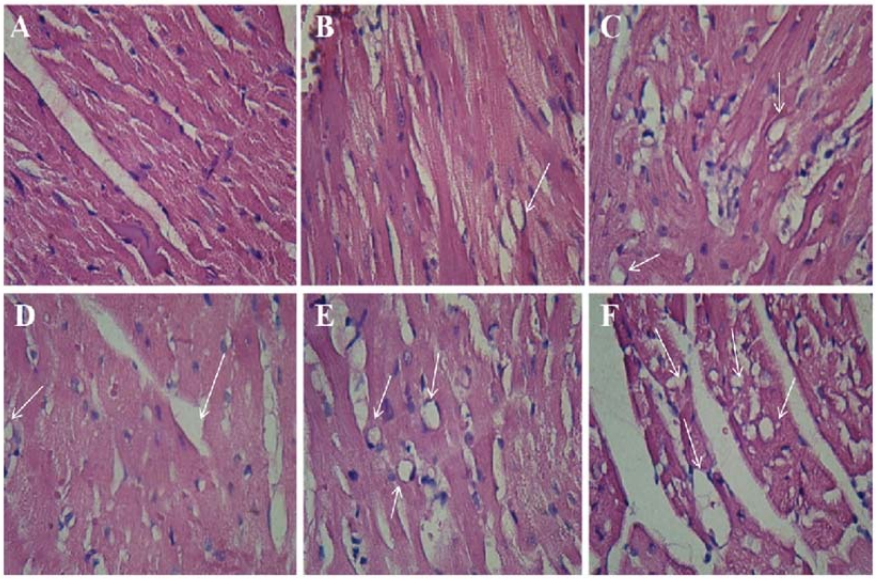

Fig. 3. Haematoxylin and eosin stained heart section of (A), LFD (control); (B), LFD arsenic 25 ppm; (C), LFD arsenic 50 ppm; (D), HFD (control); (E), HFD arsenic 25 ppm; (F), HFD arsenic 50 ppm at magnification of 400×. (HFD), high fat diet; (LFD), low fat diet.

DISCUSSION

The present study has shown that chronic exposure to HFD exacerbated chronic arsenic-induced cardiotoxicity in mice. Heart is one of the main target organs in arsenic toxicity (20) and previous studies have shown that arsenic toxicity led to cardiac oxidative stress by decreasing intracellular antioxidant concentration (1); in addition, chronic exposure to inorganic arsenic in experimental animals produced inflammation and oxidative damages in heart, including cardiac cell degradation (21). Additionally, it has been demonstrated that arsenic exposure can lead to cardiac and vascular diseases (22) including ischemia (23), QT prolongation, hypertension, atherosclerosis, impaired microcirculation (22,24), electrophysiological abnormalities (25), arrhythmias, and pericarditis (26). Arsenic alone can cause cardiotoxicity and there are other factors that can increase the cardiac toxicity of arsenic; for example dietary factors can enhance arsenic toxicity (11). A HFD has been demonstrated that enhances cardiac abnormalities including coronary resistance and myocardial fibrosis (27). One study indicated that chronic HFD can enhance arsenic-induced hepatofibrogenesis (10) and another study reported obesity as a key risk factor that can led to cardiac diseases (28). Thus, the present study was designed to determine that chronic exposure to HFD exacerbate chronic arsenic-induced cardiotoxicity in mice.

Consumption of HFD for 5 month led to increased body weight. But, when HFD was administered along with two doses of arsenic (25 and 50 ppm), body weight was reduced and heart weight to the body weight ratio increased and arsenic 50 ppm in combination with LFD also led to the increase in heart weight to the body weight ratios. Based on these results, we suggest that reduction of body weight and increase in heart weight to body weight ratio is because of arsenic and not HFD exposure. Also, we proposed that arsenic exposure led to hypertrophy of the heart tissue. These results are similar to Flora, *et al.* study that showed arsenic toxicity caused cardiac hypertrophy and reduction of body weight (29), and the study by Yan, *et al* showing that myocardium in arsenic-treated mice had cell swelling with volume of the three to four fold more than that of normal cells (30). Another study reported that arsenic caused reduction of body weight by interfering with diverse metabolic pathways (29).

HFD led to dyslipidemic changes such as increased serum levels of TG, total cholesterol, LDL, and VLDL. Moreover, increased production of ROS as well as reduced antioxidant defense mechanisms have been suggested to play a role in dyslipidemia induced by HFD consumption (31). Hence, in accordance with previous study, findings from the present data indicate that HFD induces hyperlipidemia mediated via oxidative stress and imbalances between ROS and antioxidant enzyme activities. Interestingly, arsenic administration caused a favorable reduction in lipid profile factors, except HDL levels. Previous study has shown that arsenic inhibited signal transduction mechanisms that are responsible for adipocytes differentiation which is responsible for fat (TG) accumulation in adipose tissues. Thus, the limited fat accumulation in HFD mice exposed to arsenic is because of the inhibition of adipocytes differentiation by arsenic (15).

Our study indicated that arsenic alone and in combination with HFD induced heart tissue injury through increasing oxidative stress as is evident from enhanced tissue levels of MDA and ROS, and decreased tissue content of GSH and catalase enzyme, which are well-known biomarkers of oxidative stress. Arsenic toxicity is due to its direct binding with –SH groups or indirectly through generation of ROS (32-34). Previous studies have suggested that one of the key sources of ROS production could be mitochondria (35). In this study, arsenic alone and in combination with HFD could increase ROS production in heart tissue.

In our study, depletion of glutathione level in heart tissue after arsenic exposure may be because of enhanced ROS production and oxidative stress that can result in increased consumption of glutathione by myocardium. Glutathione has been shown as an important biomarker of oxidative stress, which forms the first line of antioxidant defense against arsenic-induced damages. A reduction in tissue GSH level indicates oxidative damage (11) and catalase enzyme causes scavenging of superoxides and hydrogen peroxides (H_2O_2) that are produced by arsenic (11,36,37). Thus, we suggest that arsenic could impair the ability to detoxify H_2O_2 via catalase enzyme resulting in accumulation of H_2O_2 in heart tissues. Exposure to arsenic in combination with HFD reduced glutathione level and catalase enzyme in heart tissue indicating oxidative stress. Interaction of free radicals with poly-unsaturated fatty acids initiates the self-disseminating lipid peroxidation reactions (38) resulting in impaired membrane function and generation of MDA (39) which is a well-known oxidative stress biomarker

Previous studies have shown that arsenic can cause elevation of MDA levels in heart tissue (16). Our study also showed that arsenic exposure alone and along with HFD enhances the lipid peroxidation in heart tissue. Hence, combination of both factors leads to an oxidative stress as a result of ROS production and lipid peroxidation of membranes, thereby causing degradation of phospholipids and finally cellular deterioration in heart tissue.

The histological assessments of different cardiac segments in our study showed that exposure to arsenic alone and along with HFD led to fatty changes in heart tissue that became more severe when arsenic and HFD were consumed together. Of course, most changes observed in mice heart were fatty changes and ultra-structure study did not perform on the mice heart. Ultra-structure studies may be needed for more details and it is suggested to be assessed in future studies.

CONCLUSION

In conclusion, our study results reveal that chronic arsenic exposure in the drinking water caused heart injury and HFD significantly enhanced arsenic-induced heart injury in mice. This effect may be explained by reduced antioxidant activities and/or increased oxidative stress and ROS in mice heart tissue. These findings could be important for clinical intervention to protect against or prevent arsenic-induced cardiotoxicity in humans.

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