Letter to the Editor

Differential Alterations of Lipid Status and Lipid Metabolism, Induction of Oxidative Stress by Sodium Arsenate in Female Rat's Liver and Kidney*

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Three groups of female rats (*n*=6 per group) were exposed to 1 and 10 mg/L of sodium arsenate for 90 days. The antioxidant and peroxidation parameters in kidney and liver tissues were evaluated, also the fatty acid composition were determined. In liver the synthesis pathway of n-3 polyunsaturated fatty acids (PUFA) seemed to be more affected than the n-6 PUFA, while in kidney the n-6 pathway was more affected. The Δ5 'desaturase index' was decreased in kidney. Both in liver and kidney an increase in the percentage of *trans* PUFA was observed, also we noted a disturbance in antioxidant parameters as SOD, GPx, CAT, and GSH (*P*<0.05). While the protein carbonyl levels increased only in liver.

Arsenic is a natural element that exists in the environment in both organic and inorganic forms. Human exposure to arsenic, especially to the inorganic form, occurs in environmental and occupational settings. In spite the trivalent arsenicals (AsIII) are more cytotoxic and genotoxic than their pentavalent (AsV) counter parts, but the inorganic arsenate (iAsV) is usually the predominant form in water and soil[1].

Arsenic is a widespread environmental contaminant with mutagenic and carcinogenic effects. Despite these effects, arsenic is used as herbicides, rodenticides, insecticides, food conservative, and by product of used fossil fuel[2].

Human arsenic exposure is related to severe health problems such as skin cancer, diabetes, liver, kidney, and central nerve system (CNS) disorders[1].

It was reported that arsenic increased the generation of reactive oxygen species (ROS), depressed the functions of antioxidant defense system and led to oxidative damage[3].

Free radicals attack PUFA, and the composition of fatty acids (FA) membrane changes[4].

Several studies have shown a relationship between elevated tissue Pb and oxidative stress biomarkers and fatty acid composition[5]. Other metals, such as Cu and Cd can also affect FA profiles in plants and animals[5]. Moreover, in our previous studies[6-7] marked perturbations of antioxidant markers lipid peroxidation and changes in the FA composition in livers and kidneys of male rats exposed to sodium arsenate have been proved. We noted higher percentage of saturated fatty acids (SFA) and trans FA, but lower percentages of cis unsaturated fatty acids (cis UFA).

SFAs and trans fatty acids (TFAs) may have adverse effects on human health[8]. They are risk factors for cardiovascular diseases (CVD); they favor inflammation, insulin resistance, and obesity. These fatty acids also induce endothelial dysfunctions and an unfavorable blood lipid profile, including increased LDL-c and decreased HDL-c levels[8].

In this study we selected two organs: the liver as it is considered the major site for iAs metabolism and the kidneys as they are the major route of As excretion. The objective herein was to compare the effect of arsenate on oxidative stress biomarker and acid profile between liver and kidney of female rats.

Eighteen female adult Wistar rats (Central Pharmacy, Tunisia), weighing 130±8 g, were divided equally into 3 groups, and given drinking water containing 0, 1, and 10 mg/L of arsenic as sodium arsenate[6] (Na_2HAsO_4 $7H_2O$; Sigma, St.Louis, MO) for 90 d. The animals from each group were sacrificed at the end of the exposure. The liver and kidney tissues from each animal were isolated. The rules of the

doi: 10.3967/bes2015.115

*The present study was supported by the Tunisian Ministry of Higher Education and Scientific Research through the Research Laboratory of 'Nutrition-Functional Food and Vascular Health' LRNAFS LR12ES05, Faculty of Medicine, Monastir.

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Tunisian Society of the care and use of laboratory animals, was followed throughout the experimental duration and all the experiments were approved by the Animal Ethics Committee of the Faculty of Medicine, Monastir.

A part of liver and kidney samples was minced and homogenized (10%, w/v) for 30 s, in ice-cold 1.15% KCl 0.01 mol/L sodium, potassium phosphate buffer (pH 7.4). Then it was centrifuged (6000 g for 20 min at 4 °C). The supernatant fraction was used to evaluate the antioxidant parameters: gluthation peroxidation (GPx), catalase (CAT), superoxide dismutase (SOD) activities and gluthation level (GSH), the indicators of lipid peroxidation LPO[6]. The protein content was determined by using bovine serum albumin as a standard according to Bradford method[9]. We also determined the protein carbonyl (PC) level as described by Kharroubi et al.[6].

The second part of fresh tissue pieces collected from the liver and kidney was used to evaluate the fatty acid composition. The total lipids were extracted from the tissues following the method of Folch[6]. Fatty acids were analyzed as fatty acid methyl esters (FAMEs) by gas chromatography. FAMEs were identified by comparing their retention times with those of individual standards. Results were expressed as percentage of individual FAMEs from total FA[6].

The data were analyzed by using the statistical package for Social Sciences (SPSS) program, release 11.0 for windows (SPSS, Chicago, Il, USA). In each assay, the experimental data represent the mean of six independent assays ± standard mean deviations. Statistical significance of the difference in value of different treatment groups was calculated by Mann-Whitney test. The statistical significance was set at $P<0.05$.

In our experiment, arsenate intoxication induced pronounced changes in the liver and the kidney fatty acid profiles for the two exposed groups.

In the liver, the decreased level of cis UFA and the increased levels of trans unsaturated fatty acids (trans UFA) characterized the fatty acid composition of liver of female rats treated with sodium arsenate for 90 d (Table 1). The most important change in *cis* UFAs was the decrease in the percentage of docosahexaenoic acid (DHA, C22:6n-3) ($P<0.05$) and its precursor eicosapentaenoic acid (EPA, C20:5n-3) (0.21±0.03 *vs.* 0.3±0.1). So we can presume that the intoxication by sodium arsenate in liver affect the synthesis pathway of n-3 PUFA than the synthesis pathway of n-6 PUFA, since no significant variation was detected neither in arachidonic acid (AA, C20:4n-6) nor in its precursor dihomo-γ-linolenic acid (DGLA, C20:3n-6) percentages.

Table 1. Saturated, cis Unsaturated Fatty Acids and Trans Unsaturated Fatty Acids (per cent) in Liver of Female Rats Treated with 1 and 10 mg/L for 90 d

Fatty Acids	Content (%)		
	Control	1 mg/L	10 mg/L
C14:0	0.25±0.06	0.30±0.03	0.21±0.05
C15:0	0.12±0.01	0.13±0.01	0.12±0.02
C16:0	19.70±2.40	18.20±1.20	20.50±1.60
C18:0	18.00±1.20	19.00±0.90	19.00±1.60
C20:0	0.09±0.02	0.10±0.03	0.13±0.02
C24:0	0.84±0.12	0.70±0.07	0.76±0.05
ΣSFAs	39.00±1.60	38.6±1.00	40.6 0±2.00
C16:1	1.30±0.40	1.20±0.20	0.70±0.20*
C18:1 (isomers)	10.24±1.2	10.60±1.40	7.20±1.30*
C20:1	0.12±0.02	0.12±0.02	0.12±0.03
∑MUFA	11.70±1.30	11.90±1.00	8.40±1.50*
C18:2	10.60±1.40	10.90±1.40	10.50±0.80
C18:3 (isomers)	0.55±0.14	0.54±0.15	0.42±0.07
C20:3n-6	0.60±0.10	0.60±0.20	0.50±0.12
C20:4n-6	19.30±0.80	18.70±0.90	19.50±0.70
C20:5n-3	0.30±0.05	0.27±0.05	0.21±0.03*
C22:6n-3	8.49±1.01	8.18±0.40	4.90±0.50*
ΣPUFA	41.10±3.60	41.17±2.90	37.68±0.61
C16:1 trans	0.20±0.02	0.20±0.03	0. 18±0.03
C18:1-9 trans	0.10±0.03	0.10±0.04	0.11±0.01
C20:1 trans	0.10±0.03	0.09±0.01	0.10±0.01
∑MUFA trans	0.41±0.03	0.41±0.04	0.50±0.02
C18:2n (isomers) trans	0.31±0.03	0.32±0.04	0.40±0.02*
∑UFA trans	0.72±0.06	0.73±0.08	0.93±0.03*
PUF/SFA	1.09±0.3	1.07±0.20	0.90±0.30
C18:0/C16:0	0.90±0.30	1.04±0.40	0.92±0.20
C20:4/C20:3	32.30±8.20	36.10±9.60	39.00±5.30
C16:1/C16:0	0.06±0.02	0.06±0.30	0.03±0.01
C18:1/C18:0	0.57±0.20	0.54±0.20	0.40±0.10
C18:3/C18:2	0.02±0.01	0.02±0.005	0.01±0.01
SFA/PUFA	0.90±0.05	0.94±0.01	1.10±0.01*

Note. Statistical significance of the difference in value of different treatment groups was calculated by Mann-Whitney test. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA polyunsaturated fatty acids. *$P<0.05$ (as compared with respective controls).

The decrease in the DHA and EPA can explain the significant increase in the index of cell membrane viscosity (SFA/PUFA)(P<0.05)(Table 1). Moreover, we noted a negative correlation between the percentage of DHA and the ratio (SFA/PUFA) r=-0.8 and between EPA and the previous ratio r=-0.5. So we can presume that the depletion of both DHA and EPA is the result in peroxidation of PUFA, and especially omega 6 PUFA leading to the degradation of phospholipids and ultimately result in cellular deterioration. However, no significant increase in TBARS or CD was observed in livers of treated female rats (Table 2). Contrarily to the present results, arsenate intoxication induced in male rats significant increased in the level of MDA at both high and low dosages at 90 d of intoxication[6]. The trans UFA profile changes in treated female rats consists essentially on the *trans* PUFA [C18:2n (isomers) trans] at 10 mg/L (Table 1). In our previous study we proved that changes in the lipid status can lead to liver dysfunction and may exacerbate disruption in the balance of pro-oxidant/anti-oxidant and mainly trans FA may represent a direct source of oxidative stress for the organism[6]. In fact to the changes in the lipid status, in our present study, we noted significant alterations in the different parameters of oxidative stress (SOD, CAT, GPX, GSH) (Table 2). Relative to the controls, the SOD activity was markedly decreased by 38.6% at 10 mg/L of exposure for 90 d. The CAT activity was raised at both 1 mg/L and 10 mg/L exposures for 90 d compared with control (P<0.05). Our results also showed that the treatment with arsenate at 1 mg/L could lead to the increase of GPx activity compared with the control rats (P<0.05). However, for the group treated at 10 mg/L, the activity was markedly decreased compared with the control (P<0.05). A marked decrease of GSH level is noted after arsenate exposure at 1 mg/L and 10 mg/L for 90 d; usually compared with the respective control (P<0.05). Moreover, we noted negative correlations between the percentage of trans PUFA and the antioxidant parameters levels like SOD (r=-0.4), GPX (r=-0.2) and GSH (r=-0.78). Those correlations present a proof that the increase in the trans UFA and especially the trans PUFA leads to a disturbance in the balance of pro-oxidant/anti-oxidant by decreasing the efficiency of the antioxidant system.

In the kidney, the main changes that have been observed in treated rats were the increase in saturated fatty acid C24:0 and in unsaturated fatty acids omega 3: the DGLA (C20:3n-6)(Table 3). However, the next step in n-3 PUFA synthesis, the formation of arachidonic acid (AA, 20:4n-6), was not enhanced, in fact, there was a decrease in this fatty acid (Table 3). The decrease of percentage of AA, the accumulation of its precursor (C20:3n-6) and the decrease of the index desaturase Δ5 (C20:3/C20:4) (Table 3), indicate that arsenate may induce the decrease in the activity of Δ5 desaturase[7]. But we cannot rule out the eventuality that the decrease in AA may be related to the increase in oxidation of this highly unsaturated fatty acid. However, no increase in TBARS or in CD was observed in kidneys of treated rats. Those observations, confirm the last deduction, that arsenate induces a decrease in the activity of desaturase, mainly Δ5.

Table 2. Antioxidant Enzyme Activities, Lipid Peroxidation Parameters and Protein Carbonylation Level in Liver and Kidney of Female Rats Treated with 1 and 10 mg/L for 90 d

Items	Liver			Kidney		
	Control	1 mg/L	10 mg/L	Control	1 mg/L	10 mg/L
SOD (U/mg proteins)	1.14±0.10	1.17±0.20	0.70±0.01*	0.22±0.01	0.22±0.04	0.3 0±0.01*
CAT (μmol/mg proteins)	47.8±1.3	51.6±0.8*	58.5±3.1*	54.4±4.2	46.6±0.7*	21.5±2.2*
GPx (U/mg proteins)	4.6±0.1	5.6±0.7*	3.6±0.3*	12.3±1.4	18.1±7.3	23.9±1.6*
GSH (mg/g tissue)	14.6±1.5	12.0±1.5*	11.6±0.8*	1.2±0.1	1.8±0.3	2.3±0.1*
TBARS (nmol/mg proteins)	18.6±2.5	17.6±2	17.2±1.4	19.8±2.9	22.1±2	23.3±2.2
CD (μmol/mg proteins)	1.3±0.1	1.4±0.3	1.6±0.17	2.9±0.1	2.2±0.5	2.9±0.02
PC (μmol/mg proteins)	4.03±0.80	5.08±0.70*	6.00±0.90*	0.28±0.06	0.31±0.02	0.35±0.05

Note. Data are expressed as means±SD (n=10 rats per group). *P<0.05, as compared with controls. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH), thiobarbituric acid reacting substance (TBARS), conjugated dienes (CD) and protein carbonyl (PC) levels in the liver and kidney of rats treated by sodium arsenate at two increasing doses during two periods.

The *trans* UFA profile changes in tissues of animals exposed to metals have not received much attention in the literature and remain unexplored. Here, in rats intoxicated by arsenate we observed an increase in the percentage of trans UFA [both trans PUFA [C18:2n-6 (isomers) and MUFA (C16:1 and C18:1)](Table 3). Negative correlation was noted between the percentage of trans PUFA and the activity of the antioxidant parameter CAT (r=-0.8).

Table 2 shows the changes in antioxidant enzymes activities, lipid peroxidation level, the glutathione content in kidney. A 90 d exposure at 10 mg/L of arsenate caused the increase in some antioxidant parameters SOD, GPx, and GSH in comparison with the control group (P<0.05). Conversely, there was a significant depletion (P<0.05) of CAT activity after the exposure to 1 mg/L and 10 mg/L of sodium arsenate. While no significant alterations were seen in the kidney lipid peroxidation level measured as TBARS and conjugated dienes (CD).

The elevation in the level of some antioxidant parameters (GSH, SOD, and GPX in kidney tissue of female rats under arsenate treatment and in the level of CAT in the liver tissue) could be due to the adaptation of the antioxidant system to counteract to oxidative stress and a positive correlation is determined between the level of trans PUFA and GSH (r=0.9), GPX (r=0.9), and SOD (r=0.27) of the kidney and between the trans PUFA and CAT activity in the liver (r=0.8).

Those results give evidence that the trans FA may represent a direct source of oxidative stress by the alteration of the activity or level of antioxidant parameters (by decreasing or increasing), and by disturbing the balance of pro-oxidant/anti-oxidant.

The significant increase in the level of protein carbonyl (PC) (P<0.05, 10 mg/L) observed in the liver tissues reflected the oxidative damage to protein carbonyls kidney protein carbonyl level tented to increase in all the treated groups (P≥0.05)(Table 2). In our present study, we observed that the free radicals damage to proteins might be more important than the damage to lipids and especially in liver. This observation is consistent with previous study of Reznick and Parcker which deduced that in oxidative stress situations *in vivo*, the free radicals damage to proteins might often be more important than damage to lipids[10].

In conclusion, our results demonstrated that the changes of female's fatty acid profile induced after sodium arsenate exposure was very different between liver and kidney and may be they are depend on the type of tissue. In the liver the synthesis pathway of n-3 PUFA seemed to have been more affected by arsenate exposure, while the n-6 pathway seemed to have been more affected in kidney than the n-3 pathway by probably reducing the activity of desaturase Δ5. The increasing trans FA was associated with the disturbance of the antioxidant

Table 3. Saturated, cis Unsaturated Fatty Acids and Trans Unsaturated Fatty Acids (per cent) in Kidney of Female Rats Treated with 1 and 10 mg/L for 90 d

Fatty Acids	Content (%)		
	Control	1 mg/L	10 mg/L
C14:0	1.59±0.50	1.67±0.02	1.94±0.35
C15:0	0.42±0.07	0.55±0.30	0.58±0.20
C16:0	31.85±3.46	30.87±4.33	30.93±5.65
C18:0	32.30±9.10	32.72±7.80	32.72±7.14
C20:0	0.69±0.25	0.55±0.00	0.69±0.28
C24:0	0.63±0.13	0.66±0.01	1.13±0.28*
∑SFAs	67.50±13.50	67.63±12.5	67.1±13.90
C16:1	1.75±0.66	1.58±0.02	1.06±0.30
C18:1 (isomers)	16.03±5.5	14.97±4.23	12.84±3.4
C20:1	0.41±0.20	0.42±0.02	0.37±0.15
∑MUFA	17±6.36	17.79±4.27	14.57±3.90
C18:2	7.27±3.20	6.71±0.04	6.66±1.23
C18:3 (isomers)	0.21±0.10	0.22±0.07	0.20±0.12
C20:3n-6	0.30±0.03	0.50±0.00*	0.69±0.02*
C20:4n-6	5.19±0.71	2.88±0.02*	2.15±0.28*
C20:5n-3	4.57±0.65	4.87±1.57	3.29±0.54
C22:6n-3	0.36±0.10	0.35±0.10	0.33±0.12
∑PUFA	17.94±5.38	13.9±1.64	14.9±2.33
C16:1 trans	0.34±0.00	0.39±0.00	0.66±0.1*
C18:1 (isomers) trans	0.40±0.01	0.43±0.04	0.52±0.05*
C20:1 trans	0.05±0.01	0.06±0.01	0.07±0.01
∑MUFA trans	0.84±0.02	1.15±0.06	1.35±0.36*
C18:2n-6 (isomers) trans	0.45±0.07	0.52±0.02	1.31±0.03*
∑UFA trans	1.29±0.09	1.68±0.1	2.56±0.63*
PUF/SFA	0.26±0.10	0.2±0.07	0.26±0.1
C18:0/C16:0	1.01±0.05	1.02±0.04	0.77±0.03
C20:4/C20:3	19.5±2.55	5.7±0.14**	3.4±0.23**
C16:1/C16:0	0.05±0.02	0.05±0.01	0.03±0.01
C18:1/C18:0	0.46±0.02	0.47±0.01	0.58±0.02
C18:3/C18:2	0.01±0.00	0.02±0.00	0.01±0.00
MUFA/SFA	0.25±0.08	0.26±0.10	0.25±0.10
SFA/PUFA	1.93±0.50	2.13±1.00	1.94±0.80

Note. Statistical significance of the difference in value of different treatment groups was calculated by Mann-Whitney test. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. *P<0.05, **P<0.01 (as compared with respective controls).

enzymatic system and, therefore, with the increase of oxidative stress in rat's tissues (liver and kidney), and might serve as sensitive biomarkers of toxic metal exposure in rats.

We can also deduced that sodium arsenate intoxication might induce a protein peroxidation more than lipid peroxidation. Moreover we can conclude that liver is more sensitive to sodium arsenate induced oxidative stress than kidney.

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Received: April 21, 2015;

Accepted: October 15, 2015

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