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Effects of crude oil and its inorganic component (vanadium) on antioxidant enzymes in liver and kidneys of rats

Abstract

Antioxidant defense were studied in liver and kidney of male albino rats received an aqueous solution of ammonium metavanadate (AMV; 0.30 mg V/mL) or/and crude oil (CO; 0.75 ml/kg bw ip)) for 21-day period. AMV or/and CO treatments resulted in a significant ($P<0.001$) induction in aminotransferases (ALT and AST) activities in serum, as well as lipid peroxidation in liver and kidney. Concomitantly significant ($P<0.001$) decline in superoxide dismutase (SOD) activity and glutathione (GSH) content was obtained. In AMV and AMV-CO treatments, glutathione-S-transferase (GST) activity significantly ($P<0.001$) reduced in liver and induced in kidney while in CO treatment, GST activity significantly ($P<0.01$) induced in liver and was unchanged in kidney. Signs of toxicity were observed in all vanadium-treated animals as evidenced by some deaths, decreased weight gain. These data indicated that kidney is more vulnerable to the caused by the AMV or/and CO-induced oxidative stress than liver as well as that the oxidative stress at co-exposure to AMV and CO may be more markedly advanced than at separate exposure.

Keywords: *Crude oil, Ciprofloxacin, Cytochrome P-450 1A, Lipid peroxidation, Rat*

Introduction

Pollution by crude oil is a wide spread and a common problem, such pollution arises either accidentally or operationally wherever oil is produced, transported, stored, processed or used. It has been described as a complex mixture of over 6000 potentially different hydrocarbons and metals [1]. Vanadium metal is naturally occurring to varying degrees in virgin crude oil. Another source of vanadium could be blending of reside products into the crude system; the source of vanadium in crude oil is related to earth's mantle because hydrocarbons are primordial and stable at great depth within the earth. Devastating effects on the environment may result from massive incidental and/or intentional burning of crude oil-containing vanadium and it's spilling into the sea [2, 3].

Vanadium and other trace metals, unlike organic pollutants, are not biodegradable in the environment. Therefore, inorganic vanadium compounds redistributed by human activity tend to build up in the ecosystem to levels which may be toxic to living organisms. Vanadium toxicity is a true concern for industrial workers and military personnel exposed to its compounds on land and sea. In addition to vanadium exposure at the work place [4], the general population is also increasingly exposed to this metal [5]. A growing body of evidence indicates that transition metals act as catalysts in the oxidative deterioration of biological macromolecules, and therefore, the toxicities associated with these metals may be due at least in part to oxidative tissue damage [6]. In-depth studies in the past few decades have shown metals like vanadium has the ability to generate reactive radicals, which in turn may cause neurotoxicity, hepatotoxicity and nephrotoxicity in humans and animals [6, 7].

Heavy metal poisoning and chemical toxicity lead to the accumulation of toxins in our tissues and organs causing nutritional deficiencies, hormonal imbalances and the breakdown of the immune system, the central nervous system, and the organs of the body. This breakdown of bodily organs and systems will encourage numerous diseases and disorders (listed above) to take hold in the body. As the important roles of the liver in detoxification of

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toxins and the kidney in elimination of harmful toxins and metabolic wastes in the urine, the present study is thus aimed to investigate the oxidative status of both organs in rats simultaneously exposed to ammonium metavanadate and crude oil.

Materials and methods

The kits for aminotransferases (ALT and AST), glutathione (GSH), superoxide dismutase (SOD), glutathione-S-transferase (GST), were obtained from Cayman chemical, E. Ellsworth Road, Ann Arbor, USA. Ammonium metavanadate (NH_4VO_3) and all the other chemicals and reagents used were purchased from high commercial company from Almaty, Kazakhstan. Fresh crude oil was obtained from the oil field Biikzhal, western Kazakhstan.

6-month-old male albino rats in the weight range of 238-248 g were obtained from the Animal House, Faculty of biology and biotechnology - Almaty – Kazakhstan and acclimatized for at least 20 day before putting them on different treatments. During the experiment, the animals were housed in plastic cages and placed in a well-ventilated rat house (humidity was around 70 %, temperature was 21 ± 2 °C and 12 h light/dark cycle), and fed with commercial pellets (protein 21%, fat 6.78%, fiber 3.26%, salts and vitamins) and water *ad libitum*, All animals were housed according to the ethical rules in compliance with institutional guidelines.

The animals were divided into four groups and received daily: deionized water to drink—(Group I, control); water solutions of: ammonium metavanadate (AMV; 0.30 mg V/mL)—Group II; crude oil (CO; 0.75 ml/kg bw ip five days in week)—Group III and AMV-CO at the same doses as in group II and III for AMV and CO—Group IV for 3-weeks period. The doses for ammonium metavanadate and crude oil were chosen on the basis of other authors studies [8] and [9] respectively.

Blood samples were taken by puncturing the abdominal aorta of the animals after giving light ether anesthesia. The collected blood samples were kept at room temperature for 30 min and then were centrifuged at 2000 rpm for 10–15 min to separate the serum. Serum was used for the estimation of the liver marker enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Then the animals were sacrificed by exsanguination under light anesthesia. Liver and kidneys were removed immediately for the estimations of enzymes activities and perfused with normal saline (0.9%, w/v) in order to take care of red blood cell contamination.

Biochemical analysis

The content of malondialdehyde (MDA) was determined in the liver and kidney spectrophotometrically at wave length 532 nm according to the method of Burlakova et al. [10] and its content was expressed as (nmol/g).

The content of glutathione (GSH) was determined spectrophotometrically in the liver and kidney at wave length 405 nm according to the method of Baker et al. [11] and its content was expressed as (μM).

The activity of superoxide dismutase (SOD) was determined in the liver and kidney spectrophotometrically at wave length 450 nm according to the method of Marklund, [12] and its activity was expressed as (Unit/ml).

The activity of glutathione-S-transferase (GST) was determined in the liver and kidney spectrophotometrically at wave length 340 nm according to the method of Habig et al. [13] and its activity was expressed as (nmol/min/ml).

Statistical analysis

All data expressed as mean ± SE and statistical analysis was made using the Statistical Package for Social Sciences (SPSS 18.0 software and Microsoft Excel 2010). For tests, analysis of differences between groups consisted on a one-way analysis of variance (ANOVA) with repeated measures, followed by post-hoc comparisons (LSD test). Differences were considered statistically significant at $p< 0.05$ and marked as (†), highly significant at $p< 0.01$ and marked as (*), and very highly significant at $p< 0.001$ and marked as (**) [14].

Results

All the results of various treatment groups have been compared with their normal controls.

Animals on multiple dosing with AMV and AMV-CO suffered from conjunctivitis, congested facial vessels, loss of appetite, weight loss, dehydration, kyphosis, distress and emaciation,

owing to this the chances for the survival of these animals were reduced while animals on multiple dosing with crude oil alone did not show any visible signs of distress or intoxication, similarly noticeable changes were not observed in their behavior. The variations in the body weights of the animals subjected to different treatments are shown in Table 1. Body weights of AMV, CO and AMV-CO treated rats significantly ($p<0.001$) reduced by (35, 10.3 and 38.2%,) respectively compared to control. The fluid intake in AMV and AMV-CO treated rats significantly ($p<0.001$) decreased by (59.1 and 46.4 %) respectively while in CO-treated animals, the fluid intake changed insignificantly ($p>0.05$).

Changes in MDA, GSH contents, SOD and GST activities in the liver

The liver content of MDA significantly ($p<0.001$) induced as a result of AMV, CO and AMV CO treatments by (50, 22.86 and 87.14%) respectively whereas the content of GSH reduced significantly ($p<0.001$) in AMV and AMV CO treatment by (60.1 and 63.8%) respectively and was unchanged in CO treatment (table 2). The activity of SOD had significant ($p<0.01$, 0.001 and 0.001) inhibition as a result of AMV, CO and AMV CO treatment by (17.1, 28.9 and 46.1%) respectively. For the GST activity significantly ($p<0.001$) reduced in AMV and AMV CO treatment by (41.9 and 14%) respectively while in CO treatment, GST activity increased significantly ($p<0.01$) by (6.7%) Table 3.

Changes in MDA, GSH contents, SOD and GST activities in the Kidney

Exposure to AMV, CO and AMV-CO resulted in significant ($p<0.001$, 0.01 and 0.001) induction in MDA content by (64.9, 27 and 78.4 %) respectively (table 2) and concomitant significant ($p<0.001$) decrease in the GSH content and SOD activity, the decrease in GSH content was by (59.6, 35.3 and 60.9 %) respectively (table 2) while in the SOD activity was by (39.8, 32.5 and 47%) respectively (table 3). For the GST activity induced significantly ($p<0.001$) in AMV and AMV CO treated rats by (19.4 and 21.9%) respectively while in CO treated rats, GST activity was unchanged (table 3).

Table 1 - Changes in fluid and food intake and body weight gain in AMV or/and CO-treated rats.

Treatment	Initial weight (g)	Final weight (g/ 3 weeks)	Fluid intake (mL/kg b.w./24 h)	Food intake (g/kg b.w./24 h)
Control	245.00±2.65	264.00±3.56	110.00±2.04	94.00±2.74
AMV	238.00±2.48	171.50±3.28**	45.00±3.34**	34.50 ± 3.59**
CO	248.00±2.94	236.75±4.39**	112.00±2.20	82.00±3.24†
AMV CO	243.50±3.40	163.25±3.64**	59.00±2.68**	47.00±2.94**
	ANOVA			
F- ratio	2.102	174.063	174.683	80.259
p- value	0.153	0.000**	0.000**	0.000**

Values are significant in comparison with control mean ± SE; **CO** = crude oil; **AMV** = ammonium metavanadate. Significant, †$p<0.05$ and **$p<0.001$.Figures in parentheses indicate percent (%) values.

Changes in MDA, GSH contents, SOD and GST activities in the liver

The liver content of MDA significantly ($p<0.001$) induced as a result of AMV, CO and AMV CO treatments by (50, 22.86 and 87.14%) respectively whereas the content of GSH reduced significantly ($p<0.001$) in AMV and AMV CO treatment by (60.1 and 63.8%) respectively and was unchanged in CO treatment (table 2). The activity of SOD had significant ($p<0.01$, 0.001 and 0.001) inhibition as a result of AMV, CO and AMV CO treatment by (17.1, 28.9 and 46.1%) respectively. For the GST activity significantly ($p<0.001$) reduced in AMV and AMV CO treatment by (41.9 and 14%) respectively while in CO treatment, GST activity increased significantly ($p<0.01$) by (6.7%) Table 3.

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Changes in aminotransferases (ALT and AST) activities in the serum

The serum activities of ALT and AST were significantly ($p<0.001$) increased in AMV, AMV CO and CO treatment by (164.3, 142.9 and 44.6%) and (45.6, 50.9 and 50.9%) respectively Table 4.

Discussion

The purpose of this study was to investigate the effects of AMV or/and crude oil on lipid peroxidation and selected antioxidant parameter in liver and kidney of rats. In the present study a significant reduction was observed in the body weight of the AMV or/and CO treated groups. This reduction in weights might be due to low food consumption. A consistent reduction in body weight by vanadium has also been reported by [15]. Changes in body weight have often been used as indices of toxicity of chemicals [16].

Table 2 - Changes in MDA and GSH contents in liver and kidney of AMV or/and CO-treated rats.

		Liver			Kidney	
Treatment		MDA content (nmol/g)	GSH content (μM)		MDA content (nmol/g)	GSH content (μM)
Control		0.070±0.001	20.43±0.59		0.37±0.034	1.56±0.05
AMV		0.105±0.003**	8.16±0.40**		0.61±0.022**	0.63±0.06**
CO		0.086±0.003**	19.04±1.36		0.47±0.018*	1.01±0.05**
AMV CO		0.131±0.002**	7.39±0.25**		0.66±0.026**	0.61±0.03**
		ANOVA				
F- ratio		119.772	80.015		26.029	84.849
p- value		0.000**	0.000**		0.000**	0.000**

Values are significant in comparison with control mean ± SE; **CO** = crude oil; **AMV** = ammonium metavanadate. Significant, *p≤0.01 and **p<0.001; Figures in parentheses indicate percent (%) values.

Table 3 - Changes in SOD and GST activities in liver and kidney of AMV or/and CO-treated rats.

		Liver			Kidney	
Treatment		SOD activity (U/ml)	GST activity (nmol/min/ml)		SOD activity (U/ml)	GST activity (nmol/min/ml)
Control		0.076±0.004	246.70±4.74		0.083±0.001	103.78±2.99
AMV		0.063±0.002*	143.21±2.64**		0.050±0.002**	123.89±2.08**
CO		0.054±0.003**	263.13±2.70*		0.056±0.004**	107.11±3.10
AMV CO		0.041±0.003**	212.07±2.74**		0.044±0.002**	126.53±2.49**
		ANOVA				
F- ratio		24.021	255.175		49.967	18.319
p- value		0.000**	0.000**		0.000**	0.000**

Values are significant in comparison with control mean ± SE; **CO** = crude oil; **AMV** = ammonium metavanadate. Significant, *p≤0.01 and **p<0.001; Figures in parentheses indicate percent (%) values.

Table 4 - Changes in aminotransferases (ALT and AST) activities in serum of AMV or/and CO-treated rats.

Treatment	ALT (mMol/h.L)		AST (mMol/h.L)
Control	0.56±0.047		0.57±0.017
AMV	1.48±0.043**		0.83±0.007**
CO	0.81±0.039*		0.86±0.050**
AMV CO	1.36±0.048**		0.86±0.012**
	ANOVA		
F- ratio	98.473		27.053
p- value	0.000**		0.000**

Values are significant in comparison with control mean ± SE; P = petrol; AMV = ammonium metavanadate. Significant, *p≤0.01 and **p<0.001; Figures in parentheses indicate percent (%) values.

Administration of AMV or AMV-CO caused significant decrease in fluid intake. Decreased consumption of drinking water containing vanadium has been reported and discussed by [17]. Due to the reduced fluids intake, it is possible that the rats exposed to AMV or AMV-CO were affected by some degree of dehydration, which might to some extent contribute in the cause of animals death in this study and thus has to be taken into account under interpretation of the results.

The significance and biological implications of chemically induced oxidative stress have been reviewed extensively by Byczkowski and Channel [18]. Oxidative stress is a pathophysiological process in which intracellular balance between endogenous as well as exogenous pro-oxidants and antioxidants is shifted towards pro-oxidants, leaving cells unprotected from free radical attack. The results obtained in this study, allow us to state that ammonium metavanadate, at the concentration used (0.30 mg V/mL) taken up by the rats with drinking water for 3 weeks, may induce oxidative stress in kidneys and to lesser degree in liver through increased MDA production and, thereby, contribute to the enhancement of their susceptibility to oxidative injuries. Many authors reported that, vanadium administration enhance the lipid peroxidation in in vitro [19] and in vivo conditions [20]. Furthermore, the MDA-enhancing effect of crude oil at a dose of 0.75ml/kg b.w 5 days in a week for 21days that was supported by Khan, et al. [21] who also reported that exposure to petroleum-contaminated environment and the ingestion of petroleum-contaminated diet have been reported to stimulate the formation of lipid peroxidation products in animals. Lipid peroxidation that is a consequence of the activity of oxygen free radicals (e.g. superoxide anion, hydroxyl radical and alkylperoxyl radical) has been implicated as a mediator in oxidative stress in animals [22, 23]. The results reported here also provide evidence that the tested dose of crude oil consumed by the rats together with AMV at co-application induced lipid peroxidation was more markedly advanced than at separate exposure.

The changes in the enzymatic and non-enzymatic components of the antioxidant system in vanadium and crude oil-treated rats have also been intensively examined. The study of [24, 25] demonstrated that vanadium administration attenuate the antioxidant defence system. In order to evaluate if the defence mechanisms against vanadium toxicity occurred in the present study were sufficient to protect the cellular membrane against vanadium mediated oxidative damage, we analysed SOD, GST activities and GSH concentration in the liver and kidneys supernatant. Superoxide dismutase is considered as the first line of defense against deleterious effects of oxy radicals in the cell by catalyzing the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen [26]. The present study indicated decrease in the activity of superoxide dismutase in AMV or/and CO treated animals. Inhibition to the SOD activity of each exposure group in the present study indicated that plenty of superoxide anions (O_2^-) had been produced through the redox metabolism by AMV or/and CO in liver and kidney of rats. The antioxidant defense system could not produce strong enough SOD activity to remove these O_2^-. A decrease in SOD activity in liver and kidney of rats would diminish the ability of these organs to scavenge free radicals

GSTs belong to a group of multigene and multifunctional detoxification enzymes and an important condition affecting GST expression is known to be oxidative stress. GSTs are also essential components of the cellular antioxidant defence system, since they catalyze the conjugation of GSH to several dangerous compounds produced by lipid peroxidation [27]. the decreased GST activity in the liver after AMV or AMV-CO treatment explained by AMV or AMV-CO intoxication led to generation of ROS which can oxidize -SH groups of the enzyme leading to disulfide bond formation and, thereby, causing its inactivation [28]. The level of GST expression is considered to be an important factor to protect organs against the deleterious effect of toxicants. Chiapotto, et al., [29] reported inactivation of GST by different concentrations of acetaldehyde and the result of this study on the hepatic GST activity on rats received AMV and AMV-CO seems similar. The induction of GST activity in the kidney after AMV and AMV-CO and in the liver after CO treatment indicated the corresponding defense mechanism to these exogenous compounds was established to eliminate the increased oxidation products.

Glutathione is made by all the cells in the body and plays a major role in detoxifying the body of many toxic pollutants, including toxic metals and chemicals, in additionally it is the body's master antioxidant. Glutathione deficiency impairs the body's ability to get rid of toxins whether they are environmental or the body-products of cellular metabolism. So its inhibition in liver and kidneys of rats following AMV or AMV-CO treatment indicate that they slowly become toxic, storing away poisons in their tissues.

The activities of serum marker enzymes ALT and AST showed a significant elevation after AMV or/and CO treatment. Changes in these enzymes have been considered to be indicators of cell viability and changes in cell membrane permeability. Elevation of ALT activity appears to reflect hepatic disease and it is more specific for hepatic disease than AST because of the biological location of the enzymes. Though the activity of either enzyme particularly AST may be elevated also in extra hepatic tissue.

Conclusion

Based on the results of the present study it can be concluded that vanadium or crude oil, and especially co-exposure to both, can lead to lipid oxidation in the liver and kidney. The most important and new finding of the study is revealing that kidneys is more vulnerable to the caused by the ammonium metavanadate or/and crude oil-induced oxidative stress than liver as well as that the oxidative stress at co-exposure to these elements may be more markedly advanced than at separate exposure.

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