Antioxidant Biological Effects of Crude Oil and its Inorganic Component (Vanadium) in Liver and Kidney of Rats

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Abstract: Antioxidant defense were studied in liver and kidney of male albino rats which daily received deionized water to drink (group I, control) or solutions of ammonium metavanadate (AMV; 0.15 mg V mL^{-1}) groups II; crude oil (CO; 0.5 mL kg^{-1} bw ip) group III and AMV-CO at the same concentrations as in groups II, III and IV for 3 weeks period. The results revealed significant increase in lipid peroxidation (p<0.01) and concomitant decrease (p<0.001) in Superoxide Dismutase (SOD) activity in liver and kidney of rats treated with AMV or/and CO. Glutathione-S-Transferase (GST) activity significantly decreased (p<0.001) in liver and increased (p<0.001) in kidney after AMV or AMV-CO treatment whereas Glutathione (GSH) concentration decreased (p<0.001) in both these organs. In CO treatment, GST activity significantly (p<0.01) increased in liver and was unchanged in kidney while GSH concentration decreased in kidney and was unchanged in liver. Activities of serum aminotransferases were significantly (p<0.001) increased following AMV or/and CO treatment to normal rats. Vanadium administration at this dose showed signs of toxicity on the treated animals as evidenced by some deaths, decreased weight gain, the toxicity was more pronounced by combined vanadium and crude oil treatment whereas crude oil alone did not show any visible signs of distress or intoxication. 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.

Key words: Ammonium metavanadate, crude oil, antioxidant, lipid peroxidation, aminotransferases, rats

INTRODUCTION

The risk to humans from air pollutants and their constituent environmental chemicals is an important issue for human health. Many environmental pollutants have the potential to either directly or indirectly react with the genetic material (Kim *et al.*, 2003). In particular, petroleum hydrocarbons, diverse organic compounds contain complex mixture of chemicals, varying widely in composition of hydrocarbons, hydrocarbon-like chemicals (Albers, 1995), some trace elements like vanadium, nickel, iron, aluminium, copper and some heavy metals like lead and cadmium (National Research Council, 1985).

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 vanadium-containing crude oil and it's spilling into the sea (Sadiq and Zaidi, 1984; Kalogeropoulos *et al.*, 1989; Vazquez *et al.*, 1991; Moeller *et al.*, 1994). 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 (Zychlinski, 1980), the general population is also increasingly exposed to this metal (Flyger *et al.*, 1976), mostly as a result of increased utilization of vanadium-containing natural oil (Schiff and Graham, 1984). A growing body of evidence indicates that transition metals act as catalysts in the oxidative deterioration of biological macromolecules and therefore,

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the toxicities associated with these metals may be due at least in part to oxidative tissue damage (Stosh and Bagchi, 1995). 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 (Stosh and Bagchi, 1995; Chen *et al.*, 2001).

Exposure to crude petroleum (crude oil) or its complex chemical constituents can cause toxic effects in humans, livestock and other animal species (MacFarland *et al.*, 1984; Coppock *et al.*, 1995). Chemical toxicity and heavy metal poisoning lead to the accumulation of toxins in the 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 to take hold in the body. As the important roles of the liver in detoxification of toxins and the kidney in elimination of harmful toxins and metabolic wastes in the urine in the present study researchers have investigated the oxidative status of both organs in rats simultaneously exposed to vanadium and crude oil. If the lipid peroxidation in the liver and/or kidney is less or more intensified under co-exposure to ammonium metavanadate and crude oil, compared to separate intoxication, it can suggest that the risk of these organs damage may be modified due to ammonium metavanadate-crude oil interaction.

MATERIALS AND METHODS

Reagents: The kits for glutathione, superoxide dismutase, glutathione-S-transferase, aspartate aminotransferase and alanine aminotransferase were obtained from Cayman chemical, E. Ellsworth Road, Ann Arbor, USA. 2-Thiobarbituric Acid (TBA), Trichloroacetic Acid (TCA) and Ethylene Diamine Tetraacetic Acid (EDTA) were obtained from Sigma Chemicals, St. Louis, 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.

Animals: Healthy adult male albino rats, weighing between 240-250 g were obtained from the Animal House, Faculty of Biology and Biotechnology, Almaty, Kazakhstan. All the animals were acclimatized for 20 days prior to the commencement of the treatment. These were maintained on commercial pellets (protein 21%, fat 6.78% and fiber 3.26%) and water *ad libitum*. During the whole experiment food, fluids and water intakes were monitored daily. Moreover, all rats were weighted at the beginning of the exposure and then the body weight was checked weekly and again when killed. Throughout the 3 weeks, animal behavior was also observed.

Experimental design: The animals were divided into four groups and received daily: Deionized water to drink (group I, control); water solutions of: Ammonium metavanadate (AMV; 0.15 mg V mL^{-1}) group II; deionized water to drink in addition to crude oil injection (CO; 0.5 mL kg^{-1} bw ip 5 days in week) group III and AMV-CO (0.15 mg V mL^{-1} and 0.5 mL kg^{-1}) group IV for 3 weeks period. 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 kidney were removed immediately and one part of the lobe was processed immediately for the estimations enzymes activities and perfused with normal saline (0.9%, w/v) in order to take care of red blood cell contamination. The doses for ammonium metavanadate and crude oil were chosen on the basis of other researchers studies Russanov *et al.* (1994), Zaporowska (1994) and Khan *et al.* (2002), respectively.

Biochemical analysis: Lipid peroxidation was determined as Malondialdehyde (MDA) concentration in liver and kidneys by the method of Burlakova *et al.* (1975). Briefly, portions of liver and kidneys (250 mg) was homogenized in 2 mL of 0.1 Mice-cold potassium phosphate buffer (pH 7.4) then centrifuged at 6000 rpm for 40 min at 4°C. To 2 mL of the obtained supernatant 0.5 mL of 0.1 M potassium phosphate buffer was added. Then, the tube vigorously shakes immediately after adding 1 mL TCA. All samples were centrifuged (15 min, 4000 rpm, 4°C) then the supernatant was separated and 1 mL TBA (0.75-0.80%) was added to 2 mL of it and placed for 10-12 min in a boiling water bath. The content of MDA (nmol g^{-1} wet tissue) measured as the increase in absorbance at 532 nm. Glutathione (GSH) concentration was assayed in the liver and kidneys by the method of Baker *et al.* (1990) in the assay system, portions of liver and kidneys (200 mg) was homogenized in 2 mL of 50 mM cold phosphate buffer (pH 6.5) containing 1 mM EDTA then centrifuged at 10,000×g for 15 min at 4°C, the obtained supernatant was

deproteinized by 5% sulfosalicylic acid and used for assay by adding 150 µL from cocktail that freshly prepared by mixing the following reagent in 20 mL vial (11.25 mL of MES buffer, 0.45 mL of reconstituted cofactor mixture, 2.3 mL water and 0.45 mL reconstituted DTNB) to 50 µL of the obtained supernatant then the plate was shake for few seconds and the optical density was measured at 405 nm once every 5 min to obtain for at least 5 time points. Its content was expressed as µM.

Superoxide Dismutase (SOD) activity was assayed in the liver and kidneys by the method of Marklund (1980) in the assay system, portions of liver and kidneys (200 mg) was homogenized in 2 mL of 20 mM cold HEPES buffer (pH 7.2) containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose and then centrifuged at 1,500×g for 5 min at 4°C, 10 µL of the obtained supernatant was added to 200 µL tetrazolium salt solution the reaction initiated by adding 20 µL xanthine oxidase, the plate was shake for few seconds and incubated at room temperature for 20 min, the optical density was measured at 450 nm and its activity was measured as Units SOD mL^{-1}.

Total Glutathione-S-Transferase (GST) activity was assayed in the liver and kidneys by the method of Habig *et al.* (1974) in the assay system, portions of liver and kidneys (200 mg) was homogenized in 2 mL of 100 mM cold potassium phosphate buffer (pH 7.0) containing 2 mM EDTA then centrifuged at 10,000×g for 15 min at 4°C, 20 µL of the obtained supernatant was added to 150 µL assay buffer (100 mM potassium phosphate (pH 6.5) containing 0.1% triton X-100) after adding 20 µL reduced glutathione, the reaction was initiated by adding 10 µL CDNB and then, the plate was shake for few seconds and the optical density was measured at 340 nm once every min to obtained at least 5 time points and its activity was measured as nmol/min/ml.

Statistical analysis: Each value is expressed as Mean and Standard Error (SEM). One way Analysis of Variance (ANOVA) was used to compare each variable in the different studied groups. For all statistical comparisons a value of (p<0.05) was considered significant.

RESULTS

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

Health and clinical observations: Animals on multiple dosing with AMV alone or in combination with crude oil suffered from itching, conjunctivitis, congested facial vessels, dehydration, loss of appetite, weight loss, 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.

Changes in body weight and fluid intake: The variations in the body weight gain and fluid intake 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) decreased by 31.9, 10.3 and 22.3%, respectively compared to control.

The fluid intake consumption decreased significantly (p<0.001) in AMV and AMV-CO treated rats by 37.3 and 21.8% while in CO treated rats, there was a nonsignificant (p>0.05) increase in the fluid intake by 3.6%.

Lipid peroxidation and antioxidant enzymes in liver and kidneys of rats: Lipid peroxidation measured as Malondialdehyde (MDA) concentration significantly increased in liver and kidneys by 28.57, 22.86 and 34.29%, p<0.001 and 56.76, 27 and 70.27%; p<0.001, 0.01 and 0.001 at AMV, CO and AMV-CO treatment, respectively whereas the combined treatment had more impact (p<0.001) (Fig. 1). Superoxide Dismutase (SOD) showed a significant reduction in liver and kidneys by 30.26, 28.9 and 42.11%; p<0.001 and 43.37, 32.53 and 49.4%; p<0.001 at AMV, CO and AMV-CO treatment, respectively. The depletion was much more pronounced (p<0.001) by the combined treatment (Fig. 2). The level of Glutathione (GSH) had a significant decrease in kidneys following AMV, CO and AMV-CO treatment by 58.33, 36.1 and 60.9%; p<0.001, respectively while in the liver, GSH concentration decreased significantly by 49.83 and 53.26%; p<0.001 at AMV and AMV-CO treatment and was unchanged (p>0.05) in crude oil treatment (Fig. 3). Glutathione-S-Transferase (GST) activity significantly decreased in the liver by 33.07 and 33.88%, p<0.001 and increased in kidneys by 16.97 and 19.51%, p<0.001 at AMV and AMV-CO treatment, respectively while in crude oil treatment, GST activity increased significantly in the liver by 6.66 %, p<0.01 and was unchanged (p>0.05) in the kidneys (Fig. 4).

Table 1: Effect of ammonium metavanadate or/and crude oil on fluid and food intake and body weight gain of control and treated rats >21 days period

Treatments	Initial weight (g)	Final weight (g)	Fluid (mL kg^{-1} b.w./24 h)	Food (g kg^{-1} b.w./24 h)
Control	245.0±2.29 (100.0)	264.00±2.52 (100.0)	110±2.75 (100.0)	94.00±2.46 (100.0)
NH$_4$VO$_3$	240.0±2.93 (97.96)	179.75±3.34** (68.1)	69±3.02** (62.7)	40.00±2.67** (42.6)
Crude oil	250.0±3.20 (102.0)	236.75±2.90** (89.7)	114±1.87 (103.6)	86.00±2.31† (91.50)
NH$_4$VO$_3$+Crude oil	248.5±2.74 (101.4)	205.00±3.58** (77.7)	86±2.58** (78.2)	73.25±3.01** (77.90)

Values are significant in comparison with control mean±SEM; NH$_4$VO$_3$ = ammonium metavanadate, significant, †p<0.05; **p<0.001; Figures in parentheses indicate percent (%) values
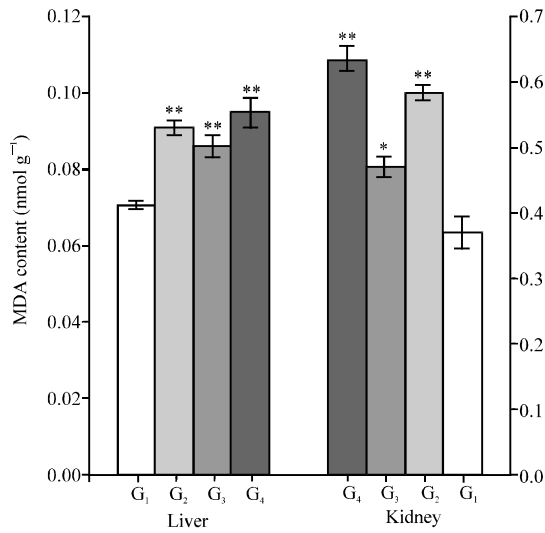
Fig. 1: Lipid peroxidation level (MDA) in liver and kidney of controls and treated rats >21 days period. Values are significant in comparison with control mean±SEM; G_1, Control; G_2, Ammonium metavanadate; G_3, Crude oil; G_4, Ammonium metavanadate and crude oil, significant, *$p<0.01$, **$p<0.001$
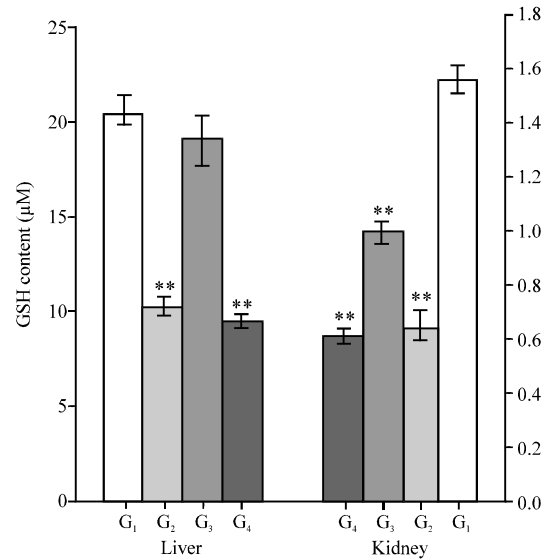

Fig. 3: Glutathione (GSH) concentration in liver and kidney of controls and treated rats >21 days period. Values are significant in comparison with control mean±SEM; G_1, Control; G_2, Ammonium metavanadate; G_3, Crude oil; G_4, Ammonium metavanadate and crude oil, Significant, **$p<0.001$
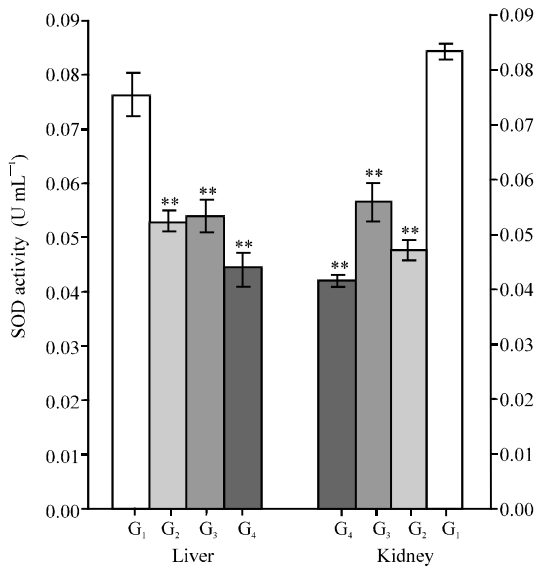

Fig. 2: Superoxide Dismutase (SOD) activity in liver and kidney of controls and treated rats >21 days period. Values are significant in comparison with control mean±SEM; G_1, Control; G_2, Ammonium metavanadate; G_3, Crude oil; G_4, Ammonium metavanadate and crude oil, Significant, **$p<0.001$
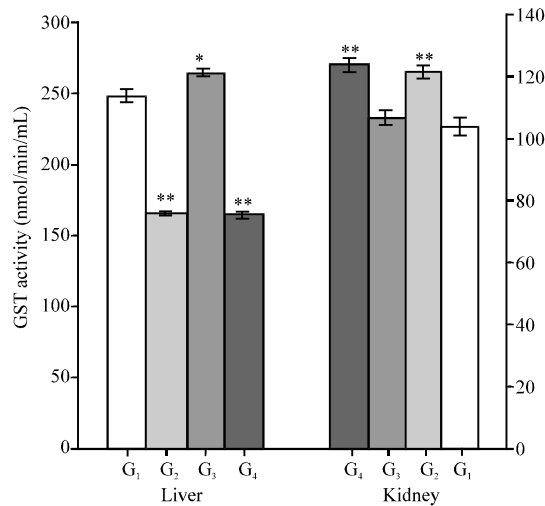

Fig. 4: Glutathione-S-Transferase (GST) activity in liver and kidney of controls and treated rats >21 days period. Values are significant in comparison with control mean±SEM; G_1, Control; G_2, Ammonium metavanadate; G_3, Crude oil; G_4, Ammonium metavanadate and crude oil, significant, *$p<0.01$, **$p<0.001$

Aminotransferases: The activities of serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were significantly ($p<0.001$) induced by (201.8,

144.6 and 217.9%) and (201.8, 150.9 and 228.1%) at AMV, CO and AMV-CO treatment, respectively. ALT and AST induction was more pronounced in the AMV-CO treated rats (Table 2).

Table 2: Effect of ammonium metavanadate or/and crude oil on the activities of serum aminotransferases (ALT and AST) of controls and treated rats >21 days period

Treatments	Control	NH_4VO_3	Crude oil	NH_4VO_3+Crude oil
ALT (nmol h L^{-1})	0.56±0.05 (100)	1.13±0.030** (201.8)	0.81±0.04** (144.6)	1.22±0.03** (217.9)
AST (nmol h L^{-1})	0.57±0.02 (100)	1.15±0.002** (201.8)	0.86±0.05** (150.9)	1.30±0.03** (228.1)

Values are significant in comparison with control mean±SEM, significant, **p<0.001

DISCUSSION

The purpose of this study was to investigate the effects of AMV or/and crude oil on the liver and kidney of male albino rats. In the present study, a significant reduction was observed in the body weight of the AMV or/and CO treated rats. This reduction in weights might be due to low food consumption. A consistent reduction in body weight by vanadium has also been reported by Thompson and McNeill (1993). The decreased body weight in the study is concomitant with that of Adedara et al. (2011) who has also reported decreased body weight in crude oil exposed rats. Changes in body weight have often been used as indices of toxicity of chemicals (Timbrell, 1991).

Significant decrease in fluid intake was observed in all vanadium treated rats. Decreased consumption of drinking water containing vanadium has been reported and discussed by Scibior and Zaporowska (2010). 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 (1996). 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 (0.15 mg V mL^{-1}) taken up by the rats with drinking water for 3 weeks may induce oxidative stress in kidney and to lesser degree in liver through increased MDA production and thereby, contribute to the enhancement of their susceptibility to oxidative injuries. Many researchers reported that vanadium administration enhance the lipid peroxidation in *in vivo* conditions (Russanov et al., 1994) and *in vitro* (Liochev et al., 1988; Younes et al., 1991). Furthermore, the MDA-enhancing effect of crude oil at a dose of 0.5 mL kg^{-1} b.w. 5 days in a week for 3 weeks that was supported by Adedara et al. (2011) who reported that BLCO induced lipid peroxidation in liver and kidney tissue. Achuba and Osakwe (2003)

Downs et al. (2002) and Khan et al. (2001) 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 (superoxide anion, hydroxyl radical and alkylperoxyl radical) has been implicated as a mediator in oxidative stress in animals (Halliwell, 1989, 1992; Liu and Mori, 1994). 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 Chandra et al. (2007), Oster et al. (1993), Russanov et al. (1994), Saxena et al. (1993), Soussi et al. (2006) and Zaporowska (1994) demonstrated that vanadium administration attenuate the antioxidant defense system. In order to evaluate if the defense 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 kidney 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 (Mates et al., 1999). 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^{-1}) had been produced through the redox metabolism by AMV or/and CO in liver and kidney of rats and the antioxidant defense system could not produce strong enough SOD activity to remove these O_2^{-1}. A decrease in SOD activity by vanadium has also been reported by Scibior and Zaporowska (2010). The decreased SOD activity in the study is concomitant with that of Adedara et al. (2011) who reported decreased SOD activity in the liver and kidney of rats treated with Nigerian bonny light crude oil. 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. The decreased hepatic GST activity after treatment with AMV alone or in combination with crude oil explained by AMV or AMV-crude oil intoxication led to generation of ROS which can oxidize -SH groups of the enzyme leading to disulfide bond formation and thereby, causing its inactivation (Uyanik *et al.*, 2001). The decrease in GST activity indicate organs damage as the level of GST expression is considered to be an important factor to protect organs against the deleterious effect of toxicants. Chiapotto *et al.* (1995) 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 a short string of amino acids called a peptide. It is composed of three amino acids: Glycine, glutamine and cysteine and made by all the cells in the body. Glutathione 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 kidney of rats following the treatment in this study indicate that they slowly become toxic, storing away poisons in their tissues. The decreased content of GSH by vanadium administration has also reported by Scibior and Zaporowska (2010) in support of the data.

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 (Ahmed *et al.*, 1999). Increased levels of these enzymes in the serum after AMV or/and CO treatment reflect destructive effect of these xenobiotic on the cell membrane, leading to increased permeability and increased seepage of intracellular enzymes.

CONCLUSION

Based on the results of the present study, it can be concluded that ammonium metavanadate 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 kidney is more vulnerable to the caused by the ammonium metavanadate

or/and crude oil-induced oxidative stress than the 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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