

# Antioxidant Responses to Different Exposure Regimes of Kazakhstan Light Crude Oil in Livers of Male Albino Rats

K. Mahmoud, T. Shalahmetova, B. Umbayev and Sh. Deraz

**Abstract**—Biochemical investigations were carried out to assess the effect of different exposure regimes of Kazakhstan crude oil (KCO) on hepatic antioxidant defense system in albino rats. Contaminants were delivered under two different dosing regimes, with all treatments receiving the same total contaminant load by the end of the exposure period. Rats in regime A injected with KCO once at a dose of 6 ml/kg bw while in regime B injected multiply at a dose of 1.5 ml/kg bw on day 1, 3, 5 and 8. Antioxidant biomarkers were measured in hepatic tissue after 1, 3, 5 and 8 days. Significant induction was observed in serum aminotransferases (ALT, AST) ( $p < 0.01$ ) and hepatic Glutathione-S-transferase (GST) ( $p < 0.05$ ) in the two exposure regimes, with the majority of significant induction occurring in regime A. Superoxide dismutase (SOD) increased 1-d after injection ( $p < 0.01$ ) but the increase was reduced time dependently thereafter while after 8-d induced again ( $p < 0.01$ ). Malondialdehyde (MDA) significantly induced after 3 and 5-d ( $p < 0.05$ ) in regime A while in regime B was not changed significantly ( $p > 0.05$ ) at short time after exposure. However, there was significant increase after 8-d ( $p < 0.01$ ). Histological examination indicates that crude oil induced pathologic changes from inflammatory cells infiltration to hemorrhage and necrosis of hepatocytes. Acute exposure to crude oil adversely affect hepatic cell so human must avoid such exposure.

**Keywords**—Kazakhstan crude oil, Antioxidant biomarkers, Histological examination, Dose regime, Rats

## I. INTRODUCTION

IN recent years, oil pollution has become a global environmental issue in that oceanic. Several of organisms in Kazakhstan are being affected by crude oil and hydrocarbons which are of anthropogenic contribution and a wide spectrum of petroleum contaminants are detected in water, sediment and animals. Crude oil has been described as a complex mixture of over 6000 potentially different hydrocarbons and metals [1]. They may be broadly characterized as paraffinic or naphthenic that contains alkanes, cycloalkanes and aromatic hydrocarbon containing low percentages of sulfur, nitrogen, oxygen compounds and trace quantities of many other elements [2]. Worldwide, about 500,000 workers are employed in crude oil exploration and production [2]. Accidental Exposure to crude

petroleum (crude oil) or its complex chemical constituents can cause toxic effects in humans, livestock and other animal species [3], [4]. Often such exposures are due to accidental spills. Following any oil spill, a number of simultaneous processes occur: spreading, dispersion, volatilization, evaporation, photo-oxidation, emulsification, sedimentation and biodegradation, which together determine the fate of the constituent hydrocarbons [5], other incidents by industrial operations. Furthermore, the type of exposure incidences in the field (i.e., single high-level or multiple low-level) could be an important factor for any associated impact on animal health. Crude oils of other geological origins also have been reported to cause various biochemical and/or toxicological effects in various animal species [4], [6], [7]. Thus the study presented in this paper aimed to investigate the clinical and biochemical effects to different exposure regimes of Kazakhstan crude oil with moderate or low dosage levels in rats using antioxidant enzymes as biochemical indicators.

## II. MATERIAL AND METHODS

Kits of superoxide dismutase (SOD), glutathione-S-transferase (GST), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Total protein were purchased from the BEN International Inc. (R.I. Milano, Italy). 2-thiobarbituric acid (TBA), Trichloroacetic acid (TCA) were obtained from Sigma-Aldrich., Ethylene diamine tetraacetic acid (EDTA), Hydrochloric acid (HCl), Heptane, Isopropyl alcohol and other chemicals reagents were purchased from high commercial company from Almaty, Kazakhstan. Fresh crude oil was obtained from the oil field Biikzhal, western Kazakhstan.

48 adult male albino rats with an average weight of ( $200 \pm 20$  g) were obtained from the Animal House, Faculty of Veterinary Medicine - Zagazig - Sharkia Gov. - Arab Republic of Egypt and acclimatized for ten days prior to the commencement of the experiment. The animals were grouped into three groups with each containing sixteen rats. The rats in Group 1 served as control. Animals in Groups 2 (regime A) injected intraperitoneally once with KCO at a dose of 6 ml/kg bw while in group 3 (regime B) injected with KCO multiply at a dose of 1.5 ml/kg bw on days 1, 3, 5 and 8. Rats fed on commercial pellets (protein 21%, fat 6.78% and fibre 3.26%) and water ad libitum throughout the experiment. Four rats from each group were sacrificed after 1, 3, 5 and 8-d following 1-d fasting before each day of sacrifice. Blood samples (5 ml) were collected from eye vein in heparin containing tubes just before sacrifice

K. Mahmoud is with faculty of biology, Al-Farabi Kazakh National University, Almaty, Kazakhstan. (Phone: 8705-554-2527; e-mail: kamal.mahmud@kaznu.kz).

T. Shalahmetova is with faculty of biology, Al-Farabi Kazakh National University, Almaty, Kazakhstan. (E-mail: t\_shalahmetova@kaznu.kz).

B. Umbayev is with faculty of biology, Al-Farabi Kazakh National University, Almaty, Kazakhstan (E-mail: baumhir@gmail.com).

Sh. Deraz is with Faculty of Agricultural Science, University of Minufiya, Minufiya, Egypt. (E-mail: shabanderaz@yahoo.com).

The activity of superoxide dismutase (SOD) was determined spectrophotometrically in the liver tissues at wave length 560 nm according to [8] and expressed in units of enzymes activities per gram of tissues wet wt.

The content of malondialdehyde (MDA) was determined according to the method of [9] Infers liver homogenated [1.0 g homogenates of liver prepared in 5 ml cold potassium phosphate buffer (pH 7.4) the homogenates were centrifuge at 5000 - 6000 rpm for 30 - 40 min at 0 - 4 °C. Then supernatant was separated]. The content of malondialdehyde (MDA) nmol/mg tissues was measured as the increase in absorbance at 532 nm.

The activity of glutathione-S-transferase (GST) was determined spectrophotometric at wave length 340 nm according to the method of [10] using 1-chloro-2-4 dinitrobenzene (CDNB) with reduced glutathione as substrate. Enzymes activities expressed in (Unit/g tissues).

Biopsies from each liver were processed for histology according to [11]. Mounted slides were examined under a light microscope and photographed. Serum activities of ALT and AST were determined as described by [12].

Statistical analysis each value is expressed as mean and standard error (SE). 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.

### III. RESULTS AND DISCUSSION

Under exposure to regimes A and B of crude oil both serum transaminases ALT and AST were insignificantly increased after 1-d while after 3, 5 and 8-d they were significantly elevated (table I).

TABLE I  
EFFECT OF DIFFERENT EXPOSURE REGIMES OF CRUDE OIL  
ON SERUM ALT AND AST ACTIVITIES

Enzymes	Exposure days	Control	1.5 ml/kg bw (regime B)	6 ml/kg bw (regime A)
ALT (U/L)	1	25.57±0.33	33.3±0.34***	26.63 ± 1.0
	3	23.97±0.43	26.79±0.74**	29.7±0.43***
	5	20.45±0.44	23.87±0.2***	26.5±0.79***
	8	22.64±0.46	24.9±0.055**	27.26±0.41***
AST (U/L)	1	101.8±0.58	104.9±0.42*	99.56±1.27
	3	95.7±0.82	101±0.36***	114.7±0.44***
	5	83.49±0.33	96.1±0.34***	110.2±0.44***
	8	57.43±0.36	80.9±0.16***	73.48±0.42***

Values represent means ± SE. Significant differences from control:

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

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. Similar results were observed in serum of rats administered 15 g k<sup>-1</sup> of crude petroleum (bonny light) [13]; in serum of fish exposed to 2, 3, 4-triaminoazo benzene resulting to the hepatocellular damage [14]. Other studies also indicated increase in the activities of the liver enzyme following liver damage in fish and albino mouse exposed to toxic substances [15], [16], the result of this study is in uniform to these findings.

TABLE II  
EFFECT OF DIFFERENT EXPOSURE REGIMES OF CRUDE OIL ON  
THE HEPATIC SOD, GST ACTIVITIES AND MDA CONTENT

Enzymes	Exposure days	Control	1.5 ml/kg bw (regime B)	6 ml/kg bw (regime A)
SOD (U/g)	1	33.2 ± 0.6	36±0.04**	47.2±0.5***
	3	29.6 ± 0.7	27.3 ± 0.4*	27.8 ± 0.8
	5	23.9±0.46	25.9±0.06**	20.21±0.6***
	8	5.2 ± 0.04	5.77±0.13**	7.2±0.16***
GST (U/g)	1	4.9 ± 0.2	5.05±0.25	5 ± 0.16
	3	4.5 ± 0.1	5.23±0.1**	4.95 ± 0.1*
	5	4.53±0.04	5.4±0.14***	5.44±0.07***
	8	4.76± 0.02	5.9±0.04***	5.4±0.037***
MDA (nmol/mg)	1	0.53±0.01	0.49 ± 0.01	0.58 ± 0.03
	3	0.3±0.012	0.33 ± 0.023	0.38 ± 0.013*
	5	0.45±0.01	0.46 ± 0.01	0.5 ± 0.01***
	8	0.7±0.014	0.81± 0.02**	0.74 ± 0.025

Values represent means ± SE. Significant differences from control:

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

Under normal physiological status the antioxidant defense systems can be induced by a slight oxidative stress as a compensatory response and thus the reactive oxygen species (ROS) can be removed to protect the organisms from oxidative damage [17]. Hepatocytes like other cells are dependent on antioxidant enzymes for the protection against reactive oxygen species produced during the biotransformation of xenobiotics [18]. Antioxidant enzymes in common use are SOD, GST.

SOD is responsible for the removal of hydrogen peroxide which is metabolized to oxygen and water [19]. In the present study under exposure to regimes A and B of crude oil, SOD activity was significantly higher than that in the control rats after 1-d ( $p < 0.01$ ) of intoxication. After 3 days, SOD activity decreased significantly ( $p < 0.05$ ) in rats of regime B and insignificantly ( $p < 0.05$ ) in rats of regime A. After 5 days, SOD activity increased significantly in rats of regime B ( $p < 0.01$ ) while in rats of regime A it decreased significantly ( $p < 0.001$ ). In generally SOD activity firstly induced then inhibited and this in agreement with [20]. With further increase in time SOD activity significantly induced 8-d in both regimes. SOD is inducible in mammals and the level of the enzyme increases with increased need of protection against toxic oxygen radicals [21], [22].

GST played an important part in the detoxification process of crude oil. GST was significantly induced ( $p < 0.001$ ) in both exposure regimes with increase in time after exposure in order to eliminate the intermediates produced by crude oil (table 2). The increase in GST activity in this work was uniform with those seen in rats administered kerosene and bonny light crude oil [13]; in *Carassium auratus* fish exposed to WSF of crude oil [23]; in *Prochilodus Lineatus* exposed to diesel oil for 15 days [24]. Some studies have indicated not only the importance of GST in detoxification of metabolites but also in regulation of stress [25].

Acute exposure (regime A) to crude oil in the present study resulted in significant induction in MDA after 3 and 5-d

( $p < 0.05$  and  $< 0.001$ ) respectively while in multiple exposure (regime B) it was not changed significantly at short time after exposure ( $p > 0.05$ ). However lipid peroxidation was evolved as a bi product of MDA after 8 days of exposure ( $p < 0.01$ ). The elevation of lipid peroxidation in the liver of rats as indicated by increased MDA production in the present study, suggested participation of free radical induced oxidative cell injury in mediating the toxicity of crude oil.

Histological analysis can also be used to examine the morphological changes in rats' liver to reflect possible effect of KCO on the hepatocytes. Analysis of the light micrographs revealed that KCO had no effect on rats' liver in regime A and B after 1 and 3-d. However, treatment-related histopathology was observed in liver biopsies from rats of regime A and B after 5 and 8-d of exposure in the form of apoptosis of hepatocytes, focal hepatic necrosis associated with inflammatory cells infiltration, kupffer cells activation and megakaryocytes in hepatic sinusoids compared with normal control.

#### IV. CONCLUSION

From data obtained we conclude that acute and multiple exposures to crude oil causes impairment in liver function as observed by changes in liver enzymes and induces structural damage to the hepatic tissue as revealed by histological study but acute exposure more serious so such exposure must be avoided.

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