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# Effect of Crude oil Intoxication on Antioxidant and Marker Enzymes of Tissue Damage in Liver of Rat

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

Abstract—The objective of the present study was to examine the dose-response relationships between antioxidant parameters and liver contaminant levels of Kazakhstan light crude oil (KLCO) in albino rats. The animals were repeatedly exposed, by intraperitoneal injection, to low dosages (0.5-1.5 ml/kg) of KLCO. Rats exposed to these doses levels did not show any apparent symptoms of intoxication. Serum aminotransferases increased significantly (p<0.05) while after 8-d recovered to control level. hepatic superoxide dismutase (SOD) activity significantly increased after 1 and 3-d except with 0.5 ml while with increased exposure time it deceased insignificantly after 5-d and significantly after 8-d. Dose and time-dependent increases were observed in the levels of hepatic glutathione-S-transeferase (GST) and conjugated diene (CD) while malondialdehyde (MDA) was evolved after 8 with the dose of 1.5 ml. the data obtained indicate impaired liver function in the rats intoxicated with KLCO as manifested by elevated liver enzymes.

**Keywords**—Crude oil; superoxide dismutase, glutathione-Stranseferase, malondialdehyde, conjugated diene, aminotransferases.

# I. INTRODUCTION

THE risk to humans from air pollutants and their **L** constituent environmental chemicals is an important issue for human health. Pollution by petroleum is a widespread and common problem that can arise either accidentally or operationally wherever oil is produced, transported, stored, processed, or used at sea or on land [1]. It has been described as a complex mixture of over 6000 potentially different hydrocarbons and metals. Accidental Exposure to crude petroleum (crude oil) or its complex chemical constituents can cause toxic effects in humans, livestock and other animal species [2]. Crude oil-induced pollution is dependent on the nature and type of crude oil, the level of oil contamination, type of environment, and selective degree of sensitivity of the individual organisms [3]. The toxicity of petroleum fraction is related to its hydrophobicity [4] because lipid solubility is an important factor in the passage of petroleum components through the plasma membrane of cells, as well as the degree of membrane disruption. Some components of petroleum have the potential to bioaccumulate within susceptible aquatic organisms and can be passed by trophic transfer to other levels of the food chain [5]. After being taken up by an organism, hydrocarbons and their metabolic products may enhance the

production of reactive oxygen species (ROS) by several mechanisms that can lead to cellular damage through protein oxidation, lipid peroxidation (LPO) [6]. Thus the influence of Kazakhstan light crude oil on hepatic antioxidant defense system at different doses level and various interval of times was planned to be carried in the present research dissertation.

### II. MATERIAL AND METHODS

Superoxide dismutase, glutathione S-transferase, Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Total protein kits 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.

Animals; 64 adult male albino rats weighing (200–220 g) obtained from the Animal House, Faculty of Veterinary Medicine, Zagazig, Egypt and acclimatized for ten days prior to the commencement of the experiment. Rats divided into four groups of sixteen rats each. Group 1 served as the control. The rats in Groups 2, 3 and 4 were injected intraperitoneally with KLCO at doses of (0.5, 1.0 and 1.5 ml/kg bw) respectively on days 1, 3, 5 and 8. After each day of injection four rats from each group were sacrificed. Blood samples (5 ml) were collected from eye vein in heparin containing tubes just before sacrifice. Animals fed on commercial pellets (protein 21%, fat 6.78% and fibre 3.26%) and water ad libitum throughout the experiment except 1 day fasting before sacrificed.

Serum activities of ALT and AST were determined as described by [7].

The activity of superoxide dismutase was determined spectrophotometrically in the liver tissues at wave length 560 nm according to [8] and expressed in (unit/g tissues).

The activity of glutathione-S-transferase was determined spectrophotometric at wave length 340 nm according to the method of [9] and expressed in (Unit/g tissues).

The content of conjugated diene expressed in (nmol/g tissues) was determined according to the method of [10] at wave length 233 nm

The content of malondialdehyde expressed in (nmol/g tissues) was determined according to the method of [10] at wave length 532 nm.

Statistical analysis each value is expressed as mean and standard error (SE). One way analysis of variance (ANOVA)

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\_shalakhmetova@kaznu.kz).

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

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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

In comparison with control, Serum activities of marker enzymes ALT and AST were significantly increased (0.01>p<0.001) after 1, 3 and 5-d of exposure to KLCO while finally after 8-d they recovered to control level (Fig. 1, 2). These marker enzymes are cytoplasmic in origin and are released into the circulation after cellular damage [11]. The level of serum ALT activity has been reported to be increased as a result of liver injury in patients developing severe hepatotoxicity [12]. ALT might have leaked from damaged cells, due to increased permeability of the hepatocellular membrane, or due to necrosis, indicating organ dysfunction

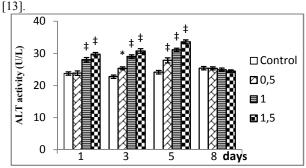


Fig. 1 Effect of KLCO on ALT activity after 1, 3, 5 and 8 days Values represent means  $\pm$  SE. Significant differences from control:  $^{\ddagger}$  P < 0.001, \* P < 0.01

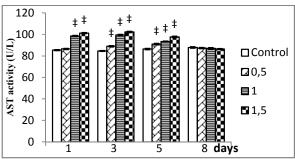


Fig. 2 Effect of KLCO on AST activity after 1, 3, 5 and 8 days Values represent means  $\pm$  SE. Significant differences from control:  $^{*}$  P < 0.001

Under normal physiological status, the antioxidant defense systems including SOD and GST (Fig. 3 and 4) 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 [14]. The activity of antioxidant may be increased or inhibited under chemical stress depending on the intensity and duration of stress applied as well as susceptibility of exposure species. In the present study SOD activity significantly (0.05>P<0.001) increased after 1 and 3-d except with 0.5 ml it increased insignificantly. With increased exposure duration it deceased insignificantly after 5 and 8-d except with all doses

except with the dose of 1.5 ml after 8-d it decreased significantly. Similar results have been observed in gilthead sea bream (*Sparus aurata*) and *Carassius auratus* exposed to polyaromatic hydrocarbons as phenanthrene [15]. The induction of SOD in the present study suggests that oxidative stress response still works well under the current conditions, and the increase of antioxidative enzymes may be a physiological adaptation for the elimination of ROS generation.

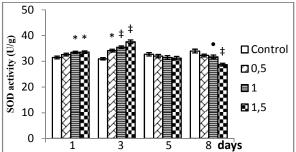


Fig. 3 Effect of KLCO on SOD activity after 1, 3, 5 and 8 days Values represent means  $\pm$  SE. Significant differences from control:  $^{\ddagger}$  P < 0.001,  $^{\ast}$  P < 0.01,  $^{\bullet}$  P < 0.05.

GST, a family of multi-functional enzymes involved in Phase II of biotransformation processes, has an important role in the detoxification processes of rat's liver and is known to be linked to their antioxidant defence system. In the present study, significantly (0.05>P<0.001) high levels of GST activity were found in the liver of rats after 3, 5 and 8-d, this Increase indicate that, the biotransformation pathway valid for crude oil used, a protective response in rat's liver toward exposure to an oxidative stress inducing xenobiotics and showed that GST activity can be a good biomarker for contamination by crude oil. Other authors also found that the activities of GST were increased in the presence of polycyclic aromatic hydrocarbon [16].

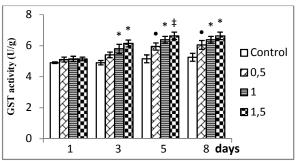


Fig. 4 Effect of KLCO on GST activity after 1, 3, 5 and 8 days Values represent means  $\pm$  SE. Significant differences from control:  $^{\ddagger}$  P < 0.001,  $^{*}$  P < 0.01,  $^{*}$  P < 0.05.

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 [17]. In the present study lipid peroxidation was increased significantly (P < 0.05) as a bi product of MAD after 8-d of exposure with the dose of 1.5 ml while after 1, 3

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and 5-d with all doses and after 8-d with 0.5 and 1 ml it changed insignificantly and CD that showed dose and time-dependent significantly increase (Fig. 5 and 6) The elevation of lipid peroxidation in the liver of rats as indicated by increased MAD and CD production in the present study, suggested participation of free radical induced oxidative cell injury in mediating the toxicity of crude oil.

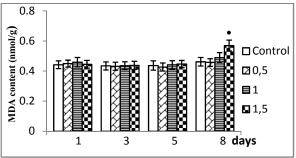


Fig. 5 Effect of KLCO on MDA content after 1, 3, 5 and 8 days. Values represent means ± SE. Significant differences from control: 'P < 0.05.

Conjugated dienes have transitory character as they are intermediate products of lipid peroxidation and in the presence of oxygen react very rapidly to form lipoperoxidic radicals [18]. The increase in the level of conjugated dienes in the present study was in agreement with [19] that reported significant increase (0.05>P<0.001) in conjugated diene content in the hepatic tissue of diabetic rats.

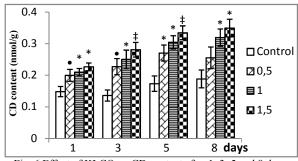


Fig. 6 Effect of KLCO on CD content after 1, 3, 5 and 8 days. Values represent means  $\pm$  SE. Significant differences from control:  $^{\ddagger}$  P < 0.001,  $^{\ast}$  P < 0.01,  $^{\bullet}$  P < 0.05.

## IV. CONCLUSION

From data obtained we conclude that multiple exposures to crude oil caused impairment in liver function and induced hepatic damage through disturbed antioxidant defense systems and increased lipid peroxidation overload of ROS cumulating in oxidative stress.

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