African Journal of Biotechnology Vol. 11(98), pp. 16455-16460, 6 December, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2228
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Effects of bonny light crude oil on anti-oxidative enzymes and total proteins in Wistar rats

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Accepted 30 November, 2012

Crude oil (CO) is a substance trado-medically used within some rural population as an antidote to poisoning and a cure for various gastro-intestinal disturbances among others. The ingestion of crude oil either orally or through polluted marine species represents a pathway for the delivery of potential toxicants to the human system. The study, therefore, analysed the effects of bonny light crude oil on the activities of anti-oxidative enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST)] and the concentration of total proteins (TP). The results show that SOD activity was significantly lower ($p<0.05$) in the control rats (0.97±0.01) compared to the rats administered 6 ml of crude oil/kg body weight (b.w) (1.31 ± 0.17), and 9 ml of crude oil/kg b.w (1.27 ± 0.03). There was however, no significant difference ($p>0.05$) between the SOD activity of the rats treated with 3 ml of crude oil/kg b.w (1.25 ± 0.27) and that of the control rats. CAT specific activity in the rats of the control group ($5.5 \pm 3.2 \times 10^{-3}$) was lower than that of the rats administered 3 ml of crude oil/kg b.w ($6.2 \pm 3.4 \times 10^{-3}$), 6 ml of crude oil/kg b.w ($7.5 \pm 4.8 \times 10^{-3}$) and 9 ml of crude oil/kg b.w ($12.5 \pm 8.3 \times 10^{-3}$); although the mean differences were not statistically significant ($p>0.05$). GST specific activity was higher in the rats of the crude oil-untreated group ($88.6 \pm 136.3 \times 10^{-3}$) compared to the rats of the group treated with 3 ml of crude oil/kg b.w ($82.7 \pm 32.3 \times 10^{-3}$), 6 ml of crude oil/kg b.w ($26.0 \pm 19.5 \times 10^{-3}$) and 9 ml of crude oil/kg b.w ($25.4 \pm 21.2 \times 10^{-3}$). Nevertheless, the mean differences were still not statistically significant ($p>0.05$). Total proteins concentration was significantly lower ($p<0.05$) in the rats given 9 ml of crude oil/kg b.w (0.33±0.08) compared to that of the control rats (0.05±0.02). In connection with the above results, the crude oil at high dose was found to have oxidative stress-inducing potential and hence, warrants that its use be discouraged or replaced with other less or non-toxic agents with similar therapeutic values as it.

Key words: Crude oil, trado-medically, bonny light, anti-oxidative enzymes and total proteins.

INTRODUCTION

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. On land, petroleum products can account for a large proportion of the chemicals at contaminated sites. Oil refineries discharge waste water containing some petroleum hydrocarbons. The receiving water is therefore, subject to low-level chronic pollution (Oliver et al., 2003).

The world's greatest demand of energy through fossil fuel strictly depends on crude oil which could be said to be the parent compound that serves as a source of derivatives of other products used for fuel or to generate energy such as paraffin wax, grease, diesel, methane, asphalt and tar. Nigeria is a country where crude oil exploration is the mainstay of the economy and constitutes about 90% of the foreign exchange earnings

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of the nation (Amadi et al., 2000). However, crude oil notwithstanding its benefits has a lot of negative impacts associated with it due to its possession of heavy metals (Deplege, 2002). In Nigeria, the exploration of crude oil brings about the pollution of our environment including our waterways (rivers and streams for instance) by a process called crude oil spillage and hence, exposure to crude oil presents a potential hazard to both aquatic and terrestrial species (Shore and Douben, 2001). Crude oil in various oil mineral-producing areas of Nigeria has caused devastating socio-economic problems and health hazards to communities involved and for this reason, the search for daily bread by oil workers may become a harvest of ill health, diseases and even in extreme cases death (Shertzer et al., 2005).

The ingestion of petroleum hydrocarbon has been reported to induce oxidative stress in bacteria (Onwurah, 1999). The survival of these organisms is a function of their defense mechanisms, which involve some enzyme systems. Petroleum hydrocabons or carbon-containing compounds are converted into free radicals or activated metabolites during their oxidation in the cells. These activated metabolites react with some cellular components such as membrane lipids and produce lipid peroxidation products which may lead to membrane damage. The consumption of petroleum hydrocarbon (PHC)-contaminated diets has been reported to cause liver enlargement, growth depression and histological changes in bacteria (Onwurah and Eze, 2000).

The toxicity of a petroleum fraction is related to its hydrophobicity because lipid solubility is an important factor in the passage of petroleum components through the plasma membrane of the cells and consequently, in the degree of membrane disruption (Freedman, 2000). The ingestion of crude oil either orally or through polluted marine species represents a pathway for the delivery of potential toxicants to the human system. There are indications that constant exposure of man and other animals that share common features with man to crude oil could lead to oxidative stress.

In view of the above, this research was aimed at evaluation of the effects of bonny light crude oil on the activities of anti-oxidative enzymes [superoxide dismutase (SOD) activity, catalase (CAT) and glutathione S-transferase (GST) specific activities] and the concentration of total proteins in Wistar (albino) rats. It was envisaged that the results of this research work might agree with the numerous researches already carried out vis-a-vis crude oil toxicity.

MATERIALS AND METHODS

Animal stock and administration

A total of 20 albino rats weighing between (150 to 205) g were obtained from the animal house of Veterinary Medicine Department, University of Nigeria, Nsukka and acclimatized in a wooden cage made of wire gauze for two months within which they were fed pelletised growers mash feed and water only under standard conditions of humidity. The rats were later divided into four groups of five rats each. The rats were administered the crude oil according to their body weights as follows: Group 1 (control): administered feed (grower's mash) and drinking water only; Group 2: administered 3 ml of crude oil per kg body weight of rats per day for seven days; Group 3: administered 6 ml of crude oil per kg body weight of rats per day for seven days; Group 4: administered 9 ml of crude oil per kg body weight of rats per day for seven days.

Collection of samples

The rats were sacrificed 24 h after the last day of crude oil administration and the liver of each animal was excised and homogenised with 5 ml (50 mM, pH 7.4) phosphate buffer to give a 20% (w/v) liver homogenate. The homogenate was centrifuged at 5000 *g* for 15 min and the supernatant obtained for further analyses.

Determination of total proteins

The total proteins was determined using the method of Lowry (1951).

Determination of superoxide dismutase (SOD) activity

The SOD activity was assayed using the method of Misra and Fridovich (1972).

Determination of catalase (CAT) specific activity

The CAT specific activity was assayed according to the method of Aebi (1984).

Determination of glutathione S-transferase (GST) specific activity

The GST specific activity was assayed using the method of Habig et al. (1974).

Statistical analysis

The results obtained from this study were analysed using the Statistical Package for Social Sciences (SPSS) version 17.0 for windows. Analysis of variance (ANOVA) and student's t-test were used to compare means and values were considered significant at $p<0.05$. Post hoc multiple comparisons for differences between groups were established by least significant difference (LSD). All the results were expressed as means ± standard errors of the means (SEM) and means ± standard deviation (SD).

RESULTS

The short term treatment with crude oil was found to cause significant increases ($p<0.05$) in the rat liver mean percentage inhibition of epinephrine oxidation at higher doses of 6 and 9 ml of crude oil/kg b.w compared to that of the rats in the control group (Group 1). There was

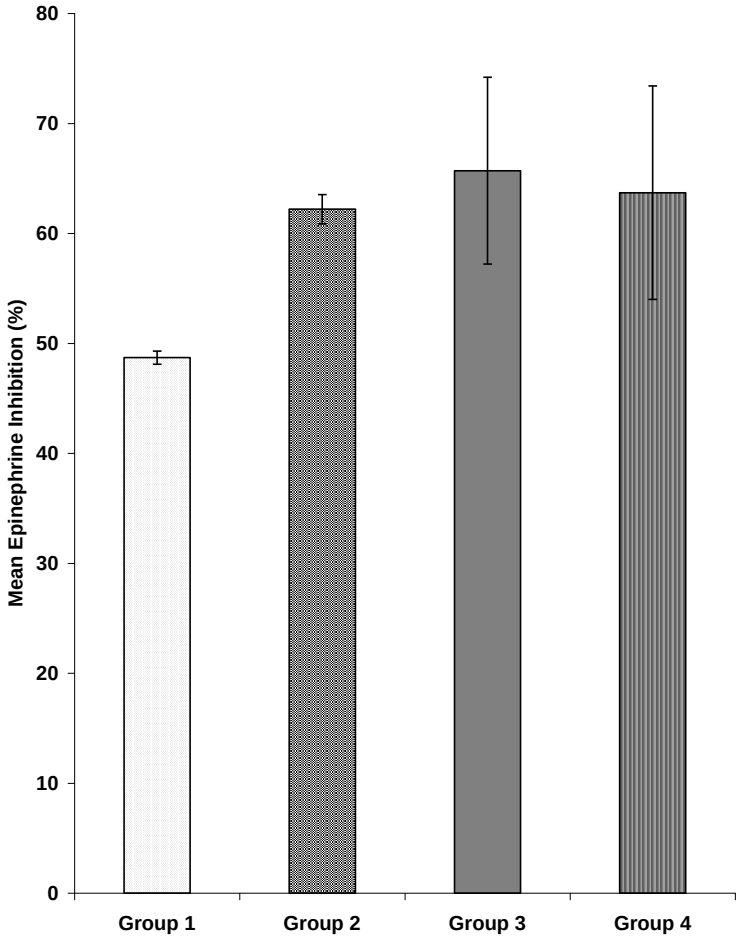

Figure 1. Liver homogenate percentage epinephrine inhibition in albino rats fed the crude oil. Group 1 (control), Administered feed (grower's mash) and water *ad libitum.* Group 2, Administered 3 ml of crude oil per kg body weight of rats per day for seven days. Group 3, Administered 6 ml of crude oil per kg body weight of rats per day for seven days. Group 4, Administered 9 ml of crude oil per kg body weight of rats per day for seven days.

however, no significant difference ($p>0.05$) between the mean percent of epinephrine oxidation of the rats fed 3 ml of crude oil/kg b.w and that of the control rats as shown in Figure 1.

Significant increases ($p<0.05$) in the rat liver mean SOD activity were recorded at higher doses of 6 and 9 ml of crude oil/kg b.w compared to the value obtained for rats in the control group. There was however, no significant difference ($p>0.05$) between the mean SOD activity of the rats fed 3 ml crude oil/kg b.w and that of the control rats as shown in Figure 2. There were non-significant increases ($p>0.05$) in the mean CAT specific activities of the rats administered 3, 6 and 9 ml of crude oil/kg b.w compared to that of the rats in the control group as shown in Figure 3. The crude oil did not significantly decrease ($p>0.05$) the mean GST specific activities in the rats administered 3, 6 and 9 ml of crude oil/kg b.w compared to that of the control rats as shown in Figure 4. Liver mean TP concentration was significantly decreased ($p<0.05$) in the rats at the dose of 9 ml of crude oil/kg b.w compared to the value obtained for rats in the control group. There were however, no significant differences ($p>0.05$) between the mean TP concentrations of the rats fed 3 and 6 ml of crude oil/kg b.w compared to that of the control rats as shown in Figure 5.

DISCUSSION

Consumption of feed contaminated with crude oil has been reported to induce lipid peroxidation in rats which is accompanied by increased generation of oxygen free radicals (superoxide anion, hydroxyl radical and alkylperoxyl radical) that may inactivate anti-oxidative enzymes (Anozie and Onwurah, 2001).

Crude oil was studied for its oxidative effects by

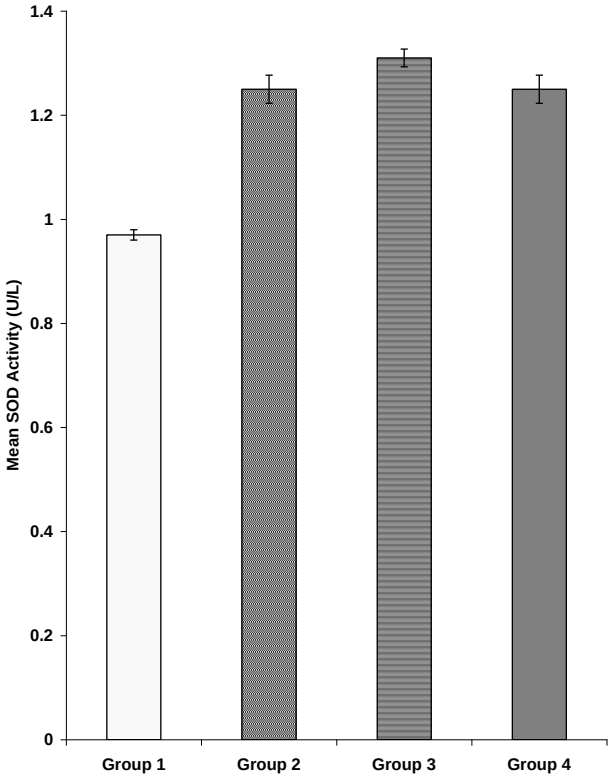

Figure 2. Liver homogenate superoxide dismutase activity in albino rats fed the crude oil. Group 1 (control), Administered feed (grower's mash) and water *ad libitum.* Group 2, Administered 3 ml of crude oil per kg body weight of rats per day for seven days. Group 3, Administered 6 ml of crude oil per kg body weight of rats per day for seven days. Group 4, Administered 9 ml of crude oil per kg body weight of rats per day for seven days.

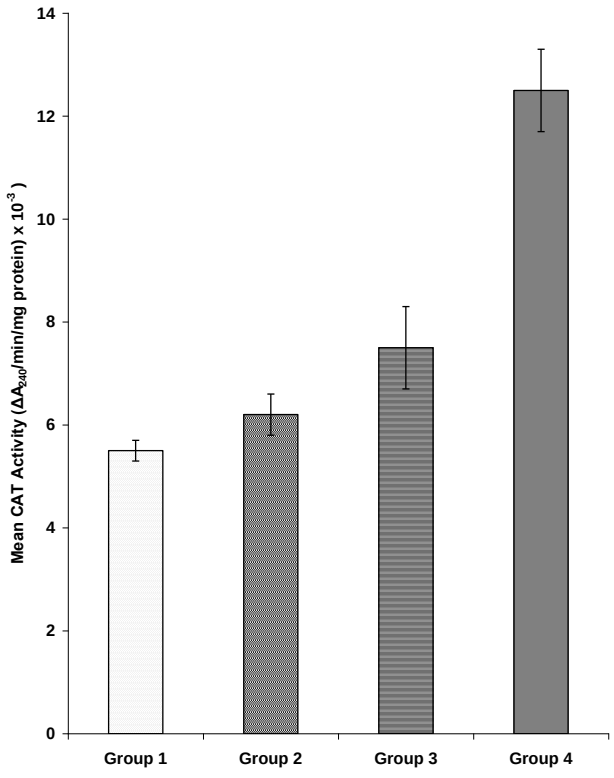

Figure 3. Liver homogenate catalase specific activity in albino rats fed the crude oil. Group 1 (control), Administered feed (grower's mash) and water *ad libitum.* Group 2, Administered 3 ml of crude oil per kg body weight of rats per day for seven days. Group 3, Administered 6 ml of crude oil per kg body weight of rats per day for seven days. Group 4, Administered 9 ml of crude oil per kg body weight of rats per day for seven days.

evaluating the activities of the anti-oxidative enzymes: SOD, CAT and GST in the liver of rats fed the substance.

The short term treatment with crude oil was found to cause a dose-dependent significant increase ($p<0.05$) in the liver mean SOD activity in the rats administered 6 and 9 ml of crude oil/kg b.w compared to that of the control rats. However, there was no significant difference ($p>0.05$) between the mean SOD activity of the rats fed 3 ml of crude oil/kg b.w and that of the rats in the control group. More so, the mean SOD activity in the rats fed 6 and 9 ml of crude oil/kg b.w were not significantly higher ($p>0.05$) compared to that of the rats fed 3 of crude oil/kg b.w although, the analysis suggests a general dose-dependent increase in the mean activity of SOD in the rats fed 3, 6 and 9 ml of crude oil/kg b.w compared to that of the control rats (evidencing a stressed environment). This increase in the mean activity of SOD in a dose-dependent manner may imply an increased production of superoxide anions in the treated rats in which case, the mean SOD activity rose correspondingly as an adaptive response to counteract the petroleum hydrocarbon-mediated production of oxygen radicals. SOD is inducible in mammals and the activity and/or concentration of the enzyme increases with increased need of protection against toxic oxygen radicals (Fridovich, 1974). This means that there is a direct relationship between the enzyme activity and the quantity of crude oil ingested.

The rats fed 3, 6 and 9 ml of crude oil/kg b.w showed increased mean specific activity of CAT compared to that of the control rats although, the mean differences were not statistically significant ($p>0.05$). Also, the mean specific activity of CAT in the rats fed 6 and 9 ml of crude oil/kg b.w were higher compared to that of the rats fed 3 ml of crude oil/kg b.w although, the mean differences were still not statistically significant ($p>0.05$). The general increase in the mean CAT activity in the rats fed 3, 6 and 9 ml of crude oil/kg b.w compared to that of the control rats however, not significant might be due to the production of more hydrogen peroxide by the SOD-mediated reaction in the liver (Fridovich, 1974).

The mean GST specific activities in the rats administered 3, 6, and 9 ml of crude oil/kg b.w were lower relative to that of the control rats though, the mean differences were not statistically significant ($p>0.05$). The

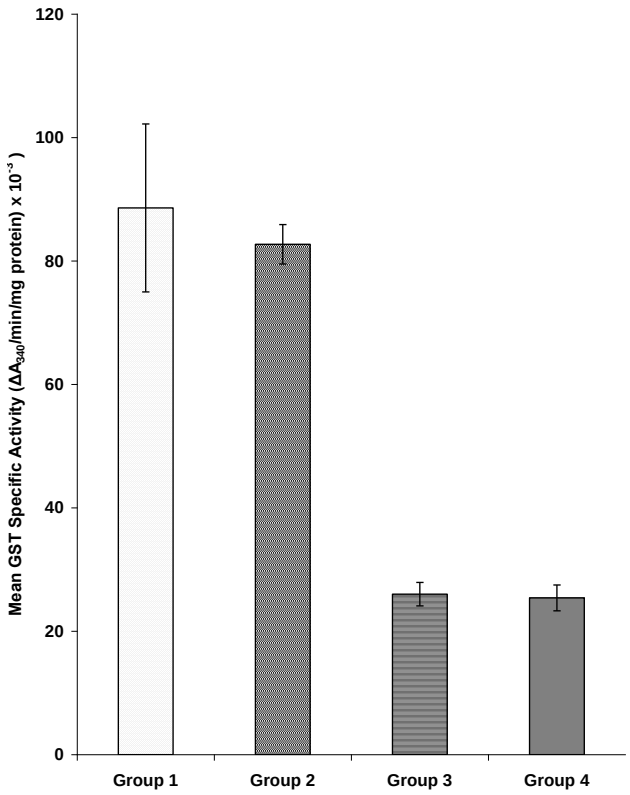

Figure 4. Liver homogenate glutathione specific activity in albino rats fed the crude oil. Group 1 (control), Administered feed (grower's mash) and water *ad libitum.* Group 2, Administered 3 ml of crude oil per kg body weight of rats per day for seven days. Group 3, Administered 6 ml of crude oil per kg body weight of rats per day for seven days. Group 4, Administered 9 ml of crude oil per kg body weight of rats per day for seven days.

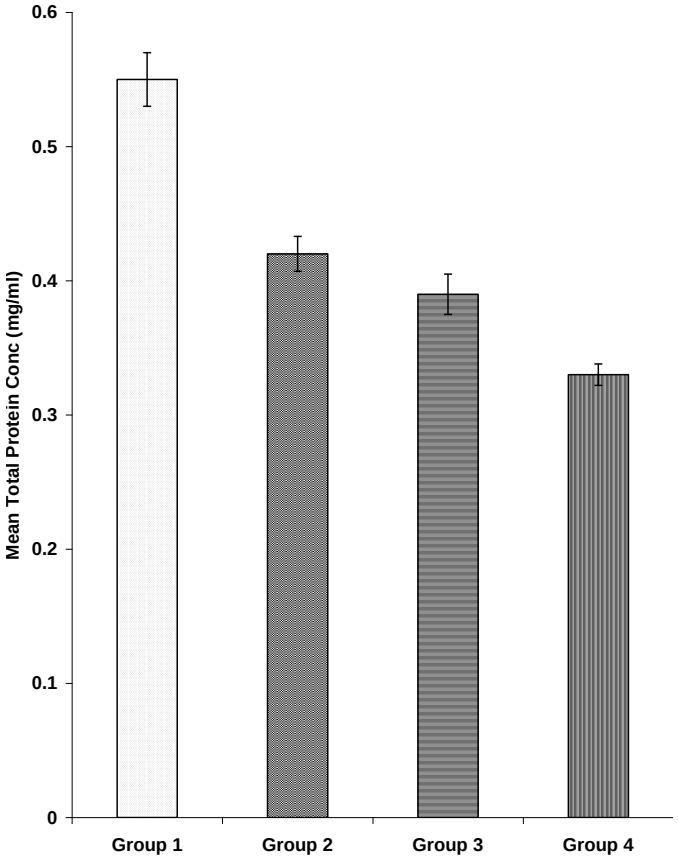

Figure 5. Liver homogenate total protein concentration in albino rats fed the crude oil. Group 1 (control), Administered feed (grower's mash) and water *ad libitum.* Group 2, Administered 3 ml of crude oil per kg body weight of rats per day for seven days. Group 3, Administered 6 ml of crude oil per kg body weight of rats per day for seven days. Group 4, Administered 9 ml of crude oil per kg body weight of rats per day for seven days.

mean specific activities of GST in the rats fed 6 and 9 ml of crude oil/kg b.w were lower compared to that of the rats fed 3 ml of crude oil/kg b.w although, the mean differences were still not statistically significant ($p>0.05$). The decrease in the mean GST specific activities in the treated rats compared to that of the control might possibly be as a result of the fact that as the concentration of the crude oil increased, the anti-oxidative capacity of this enzyme became overwhelmed and hence, the observed decrease in the enzyme activity.

Mean TP concentration in the rats administered 9 ml of crude oil/kg b.w was significantly lower ($p<0.05$) compared to that of the control rats. There were however, no significant differences ($p>0.05$) between the mean TP concentrations of the rats fed 3 and 6 ml of crude oil/kg b.w and that of the rats in the control group. The dose-dependent significant decrease in the mean TP concentration of the rats fed 9 ml of crude oil/kg b.w compared to that of the control rats might be due to hepato-cellular toxicity caused by the petroleum hydrocarbon-mediated oxyradicals which may have down-regulated protein expression in the liver cells. Previous results on biochemical studies by Anozie and Onwurah (2001) in the hepatic cells of rats fed crude oil-contaminated diet showed a significant increase and decrease of malondialdehyde (MDA) and reduced glutathione (GSH), respectively which are biomarkers of lipid peroxidation and oxidative stress. The present study clearly shows dose-dependent significant increases ($p<0.05$) in the rat mean SOD activity and catalase specific activity relative to that of the control. The dose-dependent increases in the hepatic mean SOD activity and catalase specific activity imply higher concentration of liver microsomal superoxide anion production. The enhanced superoxide anion production elicited a significant increase in the SOD activity at the two doses of 6 and 9 ml of crude oil/kg b.w. High SOD activity in conjunction with low glutathione peroxidase (GPX) activity will lead to increased concentrations of hydrogen peroxide and hydrogen peroxide-derived reactive oxygen

species (ROS) like hydroxyl radicals while high amount of hydrogen peroxide in turn, leads to elevation in the activity of CAT (Sabir and Vasudevan, 2007).

These results clearly indicate a dwindling state of anti-oxidative defense system in the hepatic cells which invariably might have exacerbated oxidative stress in the entire hepatic cells. The weakening anti-oxidative defense in the liver might favour a heightened state of oxidative stress which may precipitate genetic changes such as cancer. Although, there is no enough evidence to show the oxidant activity of crude oil, its ability to generate free radicals is attributable to its principal constituent (PAH) which when metabolised in the cells, generates ROS through the formation of redox labile metabolite (Anozie and Onwurah, 2001).

In conclusion, bonny light crude oil at high dose was found to have oxidative stress-inducing potential and therefore, demands that, its trado-medical uses be discouraged or replaced with other less or non-toxic agents with similar therapeutic values as it. This study also demonstrates an adaptive response of rats towards the oxidative stress triggered off by the interaction of crude oil with their liver cells on a short term basis. In view of these observations, a long term study that will evaluate all other anti-oxidative indices is hereby suggested in order to confirm the present study while at the same time, recommending the use of other safe substances with like therapeutic relevance as crude oil due to the fact that it (crude oil) may play a role in initiating and propagating oxidative stress as indicated in the present study.

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