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HAEMO-BIOCHEMICAL AND IMMUNOHISTOCHEMICAL CHANGES ASSOCIATED ADMINISTRATION OFCRUDE OIL TO THE MALE RATS

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ABSTRACT : Crude oil contain several poisonous compounds which can accumulate in the body and induce toxic symptoms sometimes result death .Therefore, this study performed to evaluate toxic effect of crude oil on hematological, biochemical parameters and immunohistopathological changes in experimental animals. Crude petroleum was obtained from the Petro china Oil Company Ltd (International Iraq FZE-Iraq Branch) sample. Experimental animals: Twenty four Albano Waster male rats weighting (150-170g)assigned into four groups (six rats for each group) Control group were orally administered corn oil a daily basis for 21 days,group two treated with 200ul of crude oil mixed with 0.5 ml corn oil,group three treated orally with 400ul of crude oil mixed with 0.5 ml corn oil andgroup four treated orally with 600ul of crude oil mixed with 0.5 ml corn oil. At the end of experimental period which contenous21days, blood samples were collected in clean class tubes with EDTA anticoagulant. Complete blood pictures (CBC)shown from collected blood samples by automatic method (Celltac X kx 021n automated hematology analyzer, Japan CARE Co, LTD). Serum samples were separated for estimation Alkaline phosphatase, Creatinine, Urea, Cholesterol and total protein. Kidney organ was immediately removed from experimental rats for immunohistochemical examination and KI67 immunoreactivity was performed using an Avidin-Biotin-Peroxidase (ABP) immunohistochemical method (Elite–ABC, Vector Laboratories, CA, USA) with KI67 monoclonal antibody (dilution 1:100; DAKO Japan Co, Tokyo, Japan).

The results observed significantly (P< 0.05) increase in serum alkaline phosphatase in rats treated with crude oil in different doses as compared with control group .high activity of alkaline phosphatase was reported in dose 600ul, significant increase in serum total cholesterol and creatinine concentrations, crude oil to the rats in dose 200ul observed insignificant effect on the serum urea concentration, while administration of the crude oil at doses 400ul and 600ul observed significant increase in serum urea concentration as compared with control group, significant decrease in total protein with increase doses of crude oil in different groups as compared with control group. Red blood cells (RBCs)were significantlydecrease with increase adminstreted doses of crude oil. Also the results obsrtved significant decrease in Haemoglubin (Hb), Packed cell volum (PCV) and Platelet (Plt) after adminstration crude oil as compared with control group. Ki-67 immunoreactivity (Ki-67-ir) in kidney tissue sections shown faint positive reactions for Ki-67-ir in glomrelous and renal tubules in control group,mild positive reactions for (Ki-67-ir) with kidney tissues in rats that administrated crude oil in dose (200ul), crude oil in dose 400ul observed positive reactions for (Ki-67-ir) in the renal tubules, while the rats administrated crude oil in dose 600ul observed moderate to strong positive reaction for (Ki-67-ir) in the renal tubules and glomrelous. Exposure to the crude oil may be lead to abnormal changes in biochemical and haematological parameters and attributed haemotoxic, nephrotoxicity and hepatotoxicity, dangerous to the kidney and liver functions.

Key words : Crude oil, hematological, biochemical parameters, Ki-67-ir, immunohistochemistry.

INTRODUCTION

Diesel and petrol are used for different purposes by human beings at various places such as homes, in petrochemical and manufacturing industries. The uses include fuels for vehicles, lighting and cooking fuels and as chemical for therapeutic reasons. Daily use of kerosene, diesel and petrol may expose the users to inhalation of these products (Edwards, 1989; Friday *et al*, 2015).

Crude oil is refined into fractions of petrol, diesel, kerosene, heavy gas and lubricating oils, among others. Petrol, diesel and kerosene are the frequently used fractionated products of crude petroleum. Petrol is reported to contain aromatic and aliphatic hydrocarbons, as well as different other branched unsaturated and

saturated hydrocarbons at variable concentrations (Kato *et al*, 1993).

The chemical pollutants from petrol vapour may be metabolically transformed into different metabolites in the body, these metabolites may be very reactive in various ways thereby interacting in different ways with the excreting and metabolizing tissues (mainly the liver and kidneys) to elicit toxic effects. Cellular injury may be caused by the interaction of these metabolites with the tissues causing damage to the tissues. Some composition of petroleum products such as volatile nitrates, benzene and lead have been reported to produce harmful effects on lymph nodes, bone marrow and spleen and exposure to different fractionated products of crude petroleum has been reported to cause impairment of renal function as a result of derangement of serum electrolytes (Friday *et al*, 2015). The toxic effects of the crude oil are lipid pneumonia, gastrointestinal irritation, and renal tubular nephritis, it also induces hepatocellular neoplasms and hepatotoxicity by alteration of biochemical metabolism (Patrick-Iwuanyanwu *et al*, 2011; Blahova *et al*, 2014).

Crude oil pollution at drilling sites and oil spills on lands used for agricultural purposes, as well as petroleum- or diesel-contaminated wastes poses serious exposure risks to occupational public, terrestrial wildlife mammals and livestock raised on these lands. Furthermore, some components of petroleum have the potential to bioaccumulation within susceptibleaquatic organisms and thus are passed by trophic transfer to other levels in the food chain (Gardener *et al*, 1991; Farid *et al*, 2016).

The toxic effects of petroleum hydrocarbon are exerted on variety of organs of living systems such as the lungs, liver and kidney and has been reported ability to accumulate in the body and induce toxic symptoms that sometimes result in death (Heintz *et al*, 1999; Akubue, 1997).

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, these activated metabolites react with some cellular components such as membrane lipids and produce lipid peroxidation products which may lead to membrane damage (Odo *et al*, 2012).

The consumption of petroleum hydrocarbon (PHC)-contaminated diets has been reported to cause liver enlargement, growth suppression and histological changes (Onwurah and Eze, 2000). Various studies have been reported that the crude oil can impact on reproductive systemand possible declining trend in fertility of man and wildlife animals as a result of exposure to various environmental pollutants such as estrogenic agents and aromatic hydrocarbons have been widely reported (Shittu *et al*, 2008; Raji and Hart, 2012).

The people who live nearly in the oil rich areas especially south-eastin Missan governorate are exposed to water from streams and ponds that have been polluted by oil spillage, this water is used for domestic activities such as drinking, cooking and washing by rural dwellers. Therefore, the present study was therefore designed to investigate the effects of crude oil on some haematological and biochemical parameters and histopathological changes in liver and kidney of experimental rats.

MATERIALS AND METHODS

Crude petroleum was obtained from the Petro china Oil Company Ltd (International Iraq FZE-Iraq Branch) sample type (No: 26D1). Experimental animals: Twenty four Albano Waster male rats weighting (150-170g) ,the rats assigned to four groups (six rats for each group),the test substances were administrated to the animals according to the following : Control group were orally administered corn oil via ball-tipped curved intubation needle on a daily basis for 21 days. Group two treated orally with 200ul of crude oil mixed with 0.5 ml corn oil for each rat for period 21 days. Group three treated orally with 400ul of crude oil mixed with 0.5 ml corn oil for each rat for period 21 days. Group four treated orally with 600ul of crude oil mixed with 0.5 ml corn oil for each rat for period 21 days.

At the end of experimental period, the rats were starved overnight, euthanized than dissected and blood samples taken from inferior venacava and collected in clean class tubes with EDTA anticoagulant.Complete blood pictures (CBC) were shown from collected blood samples by automatic method (Celltac X kx 021n automated hematology analyzer, Japan CARE Co, Ltd.), which included hemoglobin (Hb),Red blood cells (RBCs),White blood cells (WBCs), Platelet and Packed cell volume (PCV). Serum samples were separated by using centrifugation at 3000rpm for 15 minute and stored at -18^{0}C for estimation Alkaline phosphatase, Creatinine, urea, cholesterol and total protein. Alkaline phosphatase activity detected according to Principato *et al* (1985), creatinine and urea concentrations in serum were assayed by using commercial kit supplied by Diamond company, Egypt according to method of Henry *et al* (1974) and Patton and Crouch (1977). Serum total cholesterol was determined according to the method of Allain *et al*. (1974) using Kits of Linear Chemicals, S.L (Spain). Serum total protein was determined by method of Lowry *et al* (1951) using bovine serum albumin as a standard.Kidney organwas immediately removed from experimental rats

to immunohistochemical examination and put into 10% neutral buffer formalin as a fixative solution. Fixation time was limited to 24 hours and the fixed tissues were stored in 70% ethyl alcohol until they were processed. The fixed tissues were dehydrated through a graded series of ethanol and embedded in paraffin according to standard procedures (Latendresse *et al*, 2002).

KI67 immunoreactivity was performed according to Tousson *et al* (2011). Kidney KI67 distribution of receptor subunits were examined in deparaffinized sections (5 µm) using an Avidin-Biotin-Peroxidase (ABP) immunohistochemical method (Elite–ABC, Vector Laboratories, CA, USA) with KI67 monoclonal antibody (dilution 1:100; DAKO Japan Co, Tokyo, Japan).

Statistical analysis

The results were expressed as mean ± standard error (SE). Statistical analyses were made with one-way analysis of variance (ANOVA) using SPSS 17. Groups mean were compared forsignificance at $P<0.05$.

RESULTS

The results of oral administration of crude oil at doses 200ul, 400ul and 600ul mixed with corn oil to the male rats observed significant (P< 0.05) increase in serum alkaline phosphatase in rats treated with crude oil in different doses as compared with control group. High activity of alkaline phosphatase was reported in dose 600ul (Fig. 1).

Administration of crude oil at doses 200ul,400ul and 600ul to the experimental rats observed significant increase in serum total cholesterol and creatinine concentrations in (P< 0.05) as compared with control group (Figs. 2, 3).

Administration of crude oil to the rats in dose 200ul observed insignificant (P<0.05)effect on the serum urea concentration as compared with control group. While administration of the crude oil at doses 400ul and 600ul observed significant increase in serum urea concentration as compared with control group (Fig. 4).

Mean serum total protein in rats that administrated different doses of crude oil200ul, 400ul and 600ul for period 21 days showed significant decrease in total protein with increase doses of crude oil in different groups as compared with control group (Fig. 5).

The red blood cells (RBCs)were significantly (P< 0.05) decrease with increase adminstreted doses of crude oil to the expermintal rats as compared with control group (Fig. 6). Also the results obsrtved significant decrease in Haemoglubin (Hb), Packed cell volum (PCV) and Platelet (Plt) after adminstration gradual doses of crude oil to the

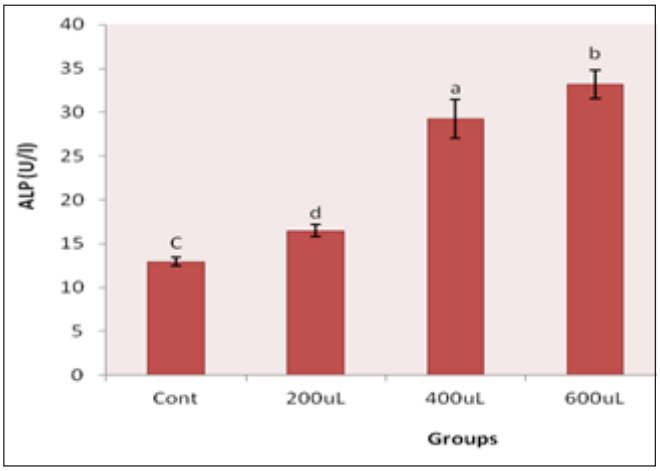

Fig. 1 : Changes in alkaline phosphatase (ALP) activity in serum of male rats treated different doses of crude oil . Values are expressed as means±SD of per group. Means with different letters are significantly different ($P<0.05$).

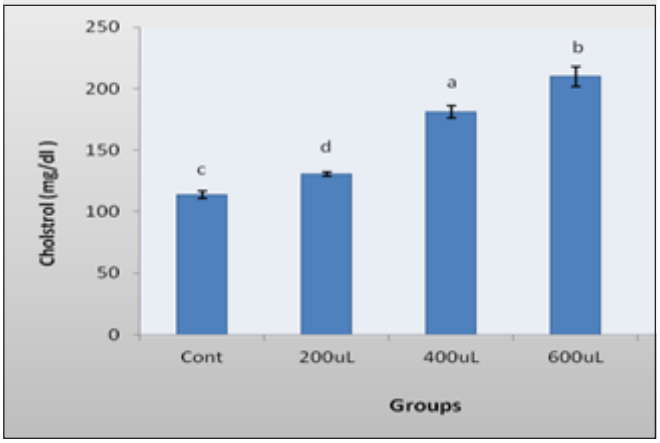

Fig. 2 : Changes in total cholesterol concentration in serum of male rats treated different doses of crude oil. Values are expressed as means±SD of per group. Means with different letters are significantly different ($P<0.05$).

expermintal animals as compared with control group (Figs. 7, 8, 9).

In the present study, the detection of Ki-67 immunoreactivity (Ki-67-ir) in kidney tissue sections in the different groups under study. Kidney tissue sections in control group (G1) shows faint positive reactionsfor Ki-67-ir in glomrelous and renal tubules (Fig. 10).

Fig. 11 shows mild positive reactions for Ki-67-ir with kidney tissues in rats that administrated crude oil in dose (200ul), with moderate atrophy in glomrelous and present in irregular shape, sever changes in the malpighian capsules.

Kidney tissue sections that obtained from rats administrated crude oil in dose 400ul observed positive reactions for Ki-67-ir in the renal tubules ,while the rats administrated crude oil in dose 600ul observed moderate to strong positive reaction for Ki-67-ir in the renal tubules and glomrelous, with sever atrophy in glomerulei, marked

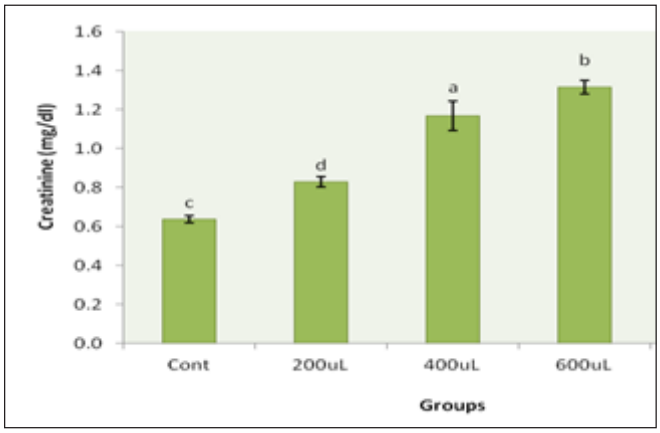

Fig. 3 : Changes in serum creatinine concentration of male rats treated different doses of crude oil. Values are expressed as means±SD of per group. Means with different letters are significantly different (P<0.05).

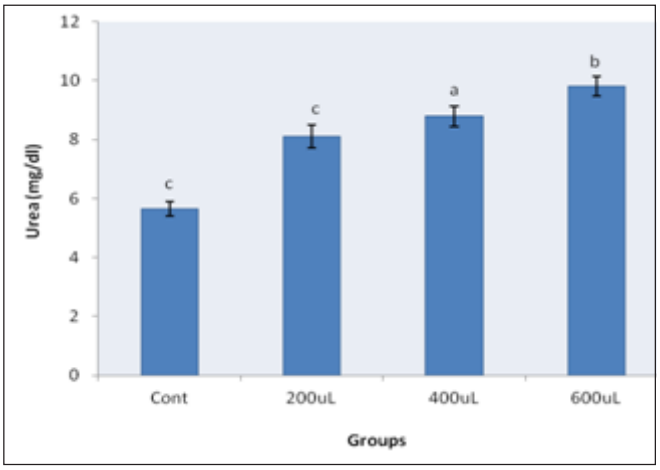

Fig. 4 : Changes in serum urea concentration of male rats treated different doses of crude oil. Values are expressed as means±SD of per group. Means with different letters are significantly different (P<0.05).

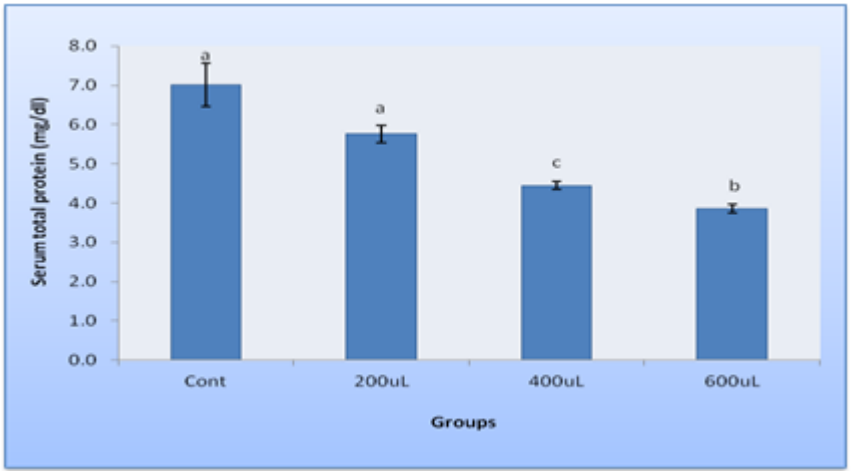

Fig. 5 : Changes in serum total protein of male rats treated different doses of crude oil. Values are expressed as means±SD of per group. Means with different letters are significantly different (P<0.05).

degeneration epithelium of renal tubules (Figs. 13, 14a,b).

DISCUSSION

The results of this study clearly observed oral administration of crude oil in different concentrations caused significant increase in the levels of some biochemical parameters such as alkaline phosphatase (ALP). This result agreement with Patrick-Lwuanyanwu *et al* (2010) that found increase in alkaline phosphatase, aspartate amino transferase (AST) and alanin aminotransferase (ALT) levels in the rats after exposure to different concentrations of Bonny light crude oil for 28 days and indicate arising these enzymes due to damage of hepatocytes and decrease their function. Rahman *et al* (2000) suggested that the increase in the activity of ALP in plasma and the decrease in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis. Also, they reported that the increase in the activity of ALP enzyme in blood might be due to the necrosis of liver, kidney and lung. Winter *et al* (1976) reported that elevation of alkaline phosphatase in the serum after exposure to the crude oil due to cellular leakage and attributed to the harmful metallic ions and dissolved hydrocarbon that present in crude oil which are capable to destroying cellular membranes.

Increase in cholesterol level in this study after administration different doses of crude oil may indicate liver damage. Golderberg *et al* (1982) reported that the lipids are thought to be most sensitive biological molecules to the oxidative stress, where increase reactive oxygen species can cause membrane lipid peroxidation and lead to degradation of unsaturated fatty acids located in the cell membrane, tissue and blood and lead to elevation serum cholesterol and triglycerides.

Kidney is the critical target organ for xenobiotic compounds which produce a variety of renal toxic effects involving tubular cells and glomerulus. These compounds

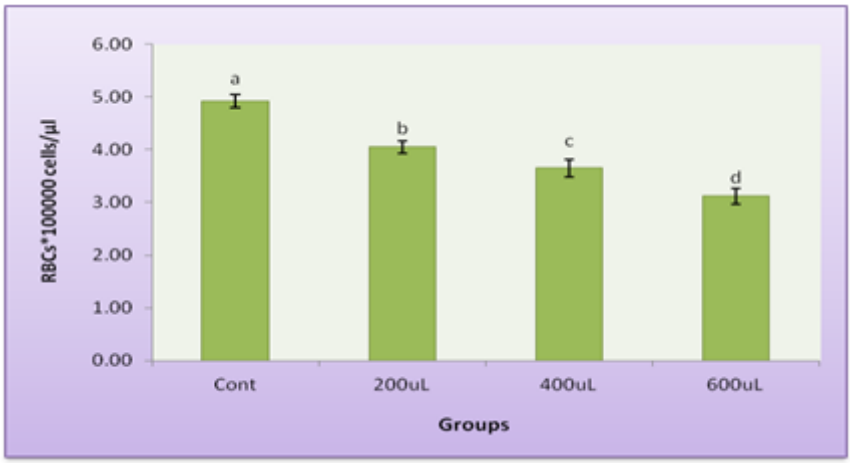

Fig. 6 : Changes in red blood cells (RBCs) level in male rats treated different doses of crude oil. Values are expressed as means±SD of per group. Means with different letters are significantly different (P<0.05).

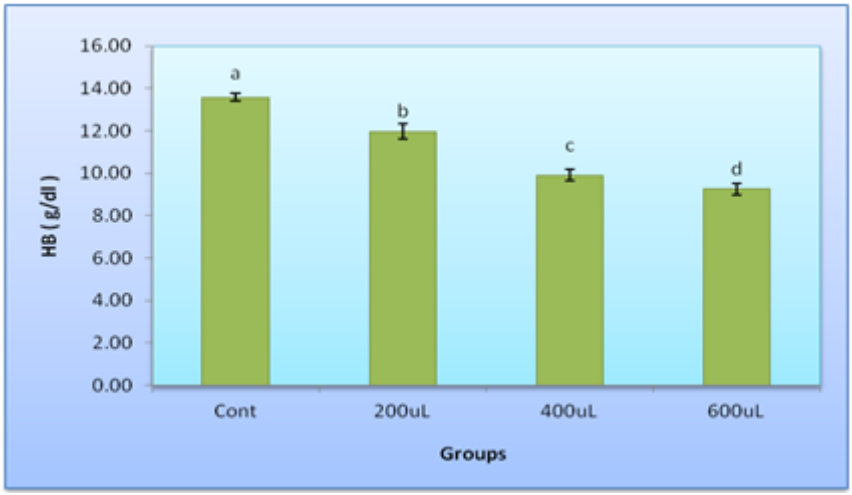

Fig. 7 : Changes in Hemoglobin concentration (Hb) in male rats treated different doses of crude oil. Values are expressed as means±SD of per group. Means with different letters are significantly different (P<0.05).

inhibit the incorporation of amino acid into protein causing an increase in urea levels which is the major nitrogen-containing metabolic product of protein metabolism (Pollak and Harsas, 1982; Mohamed *et al,* 2003).

Administration of crude oil at doses 200ul,400ul and 600ul caused significant increase in serum creatinine concentration as compared with control group. Similar results were reported by Owolabi *et al* (2010) that found significant increase in creatinine level in the experimental group after administration crude oil, which indicated to the adverse effect of crude oil on the kidney and caused kidney damage.

Serum urea concentration increased after administration different doses of crude oil (Fig. 4). This results agreement with Patrick-Lwuanyanwu *et al* (2013) that reported significant increase in urea concentration in group treated with 25, 50 and 100% of water –soluble frection of Bonny light crude oil WSF(BLCO) and concluded the WSF (BLCO) can interfere with renal function and cause renal damage.

Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage will lead to increased creatinine levels. In the present study, serum creatinine showed marginal increase in crude oil treated group in comparison to control animals and increase relates to renal failure. Serum creatinine and urea determine the glomerular filtration rate (GFR) improperly in renal failure. Serum creatinine has the potential to be a more precise marker for GFR. Similar results were reported in earlier studies in rats (Bhardwaj *et al*, 2010; Kapoor *et al*, 2014).

Serum total protein was observed increased in this study after administrated different doses of crude oil in experimental group as compared with control group .

Elevated blood urea is known to be correlated with an increased protein catabolism in mammals and/or the conversion of ammonia to urea as a result of increased

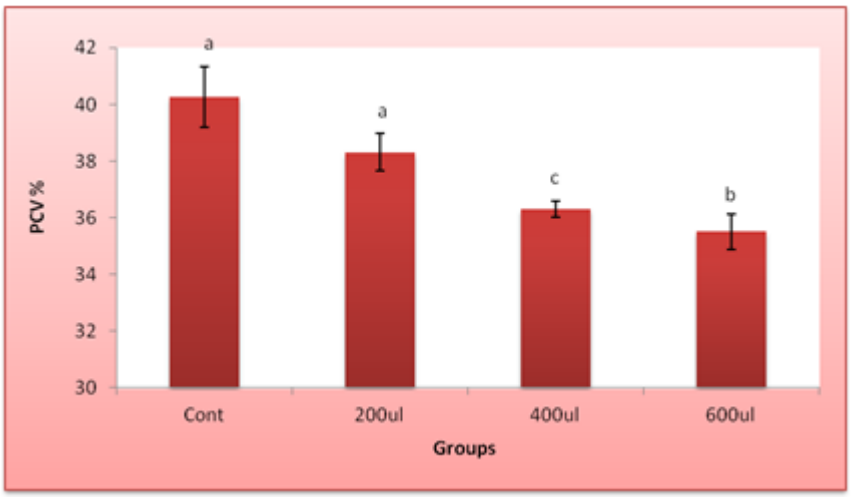

Fig. 8 : Changes in percentage of Packed cells volum (PCV) in male rats treated different doses of crude oil. Values are expressed as means±SD of per group. Means with different letters are significantly different (P<0.05).

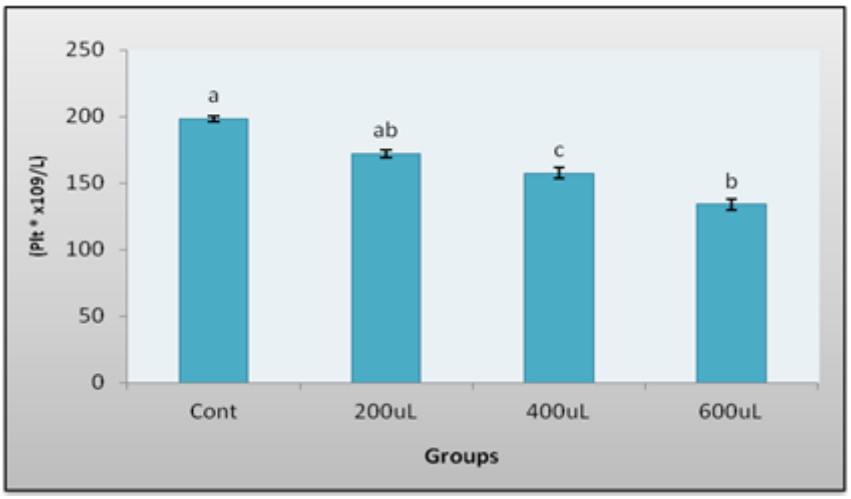

Fig. 9 : Changes in platelets (Plts) level in male rats treated different doses of crude oil. Values are expressed as means±SD of per group. Means with different letters are significantly different (P<0.05).

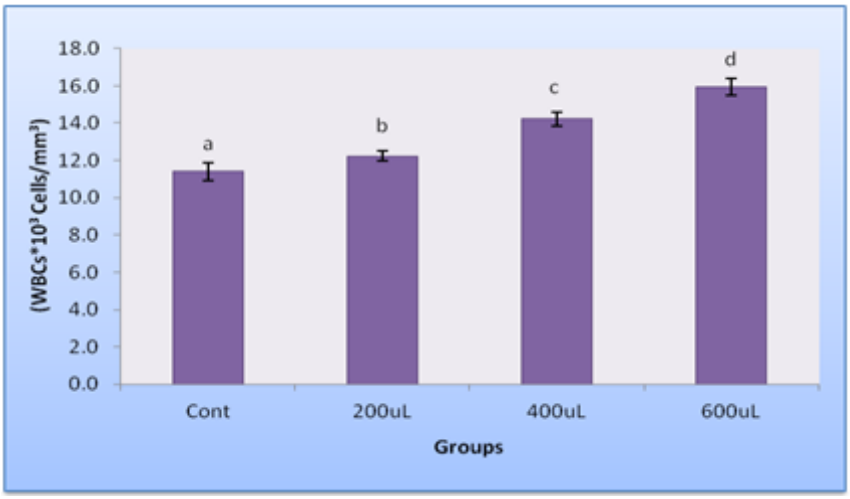

Fig. 10 : Changes in White blood cells (WBCs) level in male rats treated different doses of crude oil .Values are expressed as means±SD of per group. Means with different letters are significantly different (P<0.05).

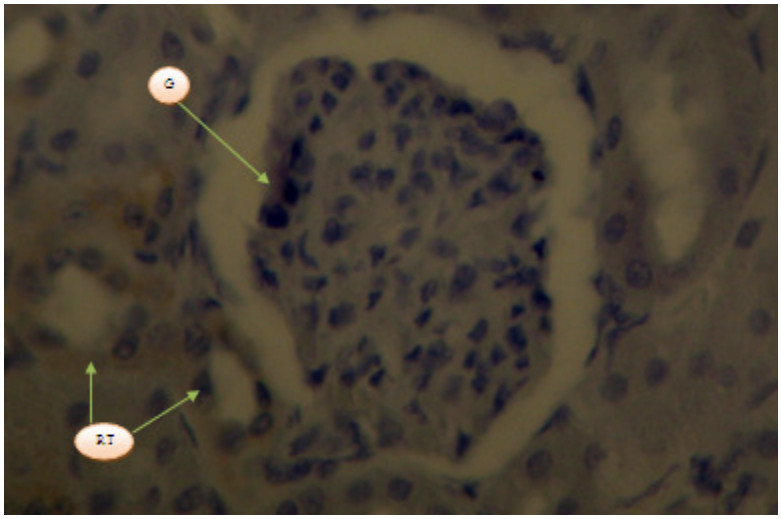

Fig. 11 : High power micrograph of rat kidney section in control group stained with (Ki-67-ir) revealed faint Positive reactions in the glomrelousand renal tubules.

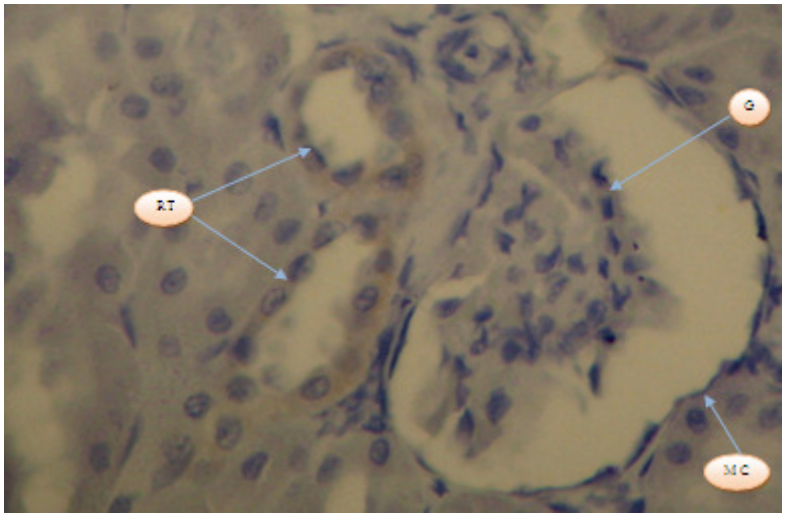

Fig. 12: High power micrograph of kidney tissue section for rat administrated 200ul of crude oil, observedmoderate reaction with (Ki-67-ir) and atrophy in glomerlous and changes in malpigian capsule.

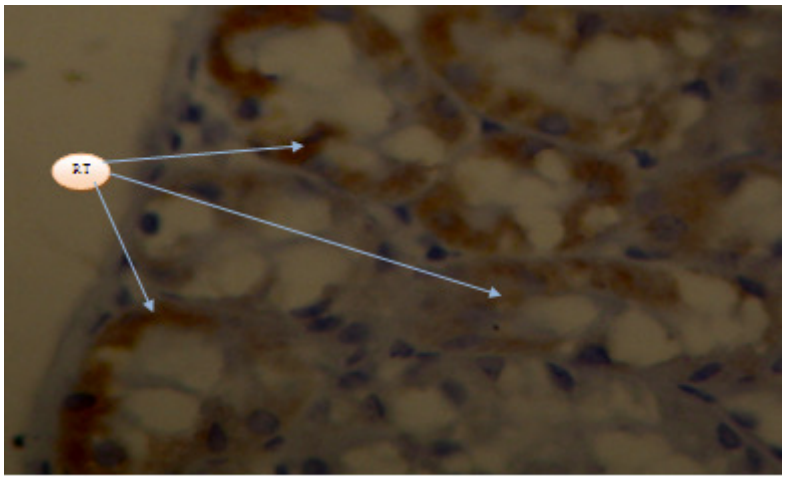

Fig. 13: High power micrograph of kidney tissue section for rat administrated 400ul of crude oil, shows moderate to strong positive reaction with (Ki-67-ir) in the renal tubules.

synthesis of arginase enzyme involved in urea production. The elevation in serum urea and creatinine levels in crude oil-treated rats is considered as a significant marker of renal dysfunction and it may be related to metabolic disturbances in liver function, as urea is the end-product of protein catabolism. Furthermore, xenobiotics intensify the acid-secretory function of kidney and change the transport of sodium (Rudenko *et al*, 1998).

The results observed significant decrease in red blood cells (RBCs) and haemoglubin concentration in the experimental groups with administration different doses of crude oil. This observation agreed with Ovuru and Ekweozor (2004) that reported significant decrease in red blood cells in rabbits with increasing concentration of crude oil in the diets toxic components in crude oil caused change in blood chemistry and induce anemia and interfere with platelet production in animals (Sudakov, 1992).

The haemotoxicity were notice clear after exposure to the crude oil due to binding of toxicants covalently with RBCs membrane and hemoglobinmolecules. Snyder (1987) found activation in bone marrow and cytotoxic effects with disturbance in DNA function after administration benzene to the rats, thus bone marrow failure in production RBCs and other formed elements.

The results of this study observed significant decrease in blood packed cell volume (PCV) and platelet (Plt) with increased administration different doses of crude oil in expermintal animals as compared with control group. This results agreement with Patrick-Lwuanyanwu *et al* (2013) that shown marginal decrease in PCV and platelet and Hb level after exposure to different concentrations of water –soluble fraction of Bonny light crude oil WSF (BLCO) in albino rats.

Krishna and Veena (1980) reported suppressive effect of petroleum samples on erythropoiesis and caused changes in blood chemistry and bone marrow hyperplasia which induced anemia.

Increase in total white blood cells (WBCs) observed in rats treated with crude oil in different doses. The main function of WBCs is defense against foreign bodies which achieved

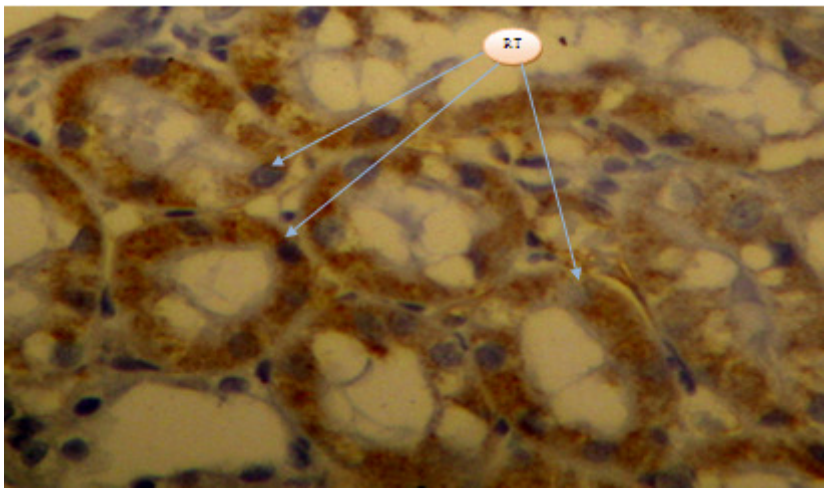

Fig. 14 a : High power micrograph of kidney tissue section for rat administrated 600ul of crude oil, shows strong positive reaction with (Ki-67-ir) in the renal tubules.

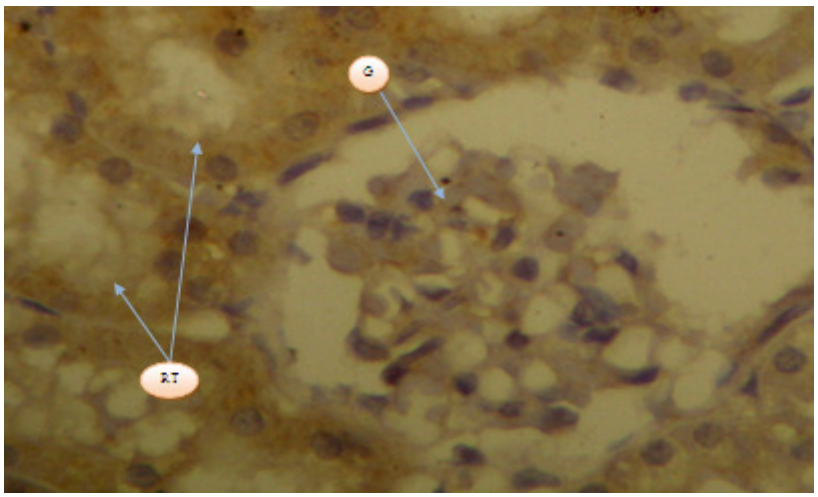

Fig. 14 b : High power micrograph of kidney tissue section for rat administrated 600ul of crude oil, shows strong positive reaction with (Ki-67-ir) in the renal tubules and glomrelous, with sever atrophy in glomerulei and abnormal changes in Bowman's capsule.

belencocytosis and antibody production ,therefore increase in the level of white blood cells can attributed to the defensive mechanism of immune system (Hoeney, 1985; Marieb, 1995).

Detection of Ki-67 immunoactivity (ki-67-ir) in kidney tissue sections after administration different doses of crude oil observed mild to strong positive reaction to (ki-67-ir) stain which mean that the toxicity of crude oil caused damage to the kidney cells and leading to change in the cells program.

The Ki-67 antigen anon histon protein was first describe by Gerdes when raising mouse monoclonal antibodies to the nuclei of Hodgkin's disease cell line, this work was performed at Kiel University in Germany hence the "Ki" the "67" refers to the clone number in 96 well plate . The KI-67 antigen can be identified by immune stain with a monoclonal antibody all phases of cell proliferation. The Ki-67 score or index is the percentage of positively stained cells among total number of cells in the tissue (Gerdosal *et al*, 1983). The proliferation-associated antigen Ki-67 is expressed in the nuclear matrix of cells during late G1-, S-, G2- and M phases of the cell cycle with a maximum in G2- and early M phases (Gerdes *et al*, 1984; Sasaki *et al*, 1987). The Ki-67 protein is absent in resting cells (G0-phase of the cell cycle) and in cells during early G1-phase, nor is it detectable during DNA repair processes (Hall *et al*, 1993; Kubbutat *et al*, 1994). The fact that the Ki-67 protein is present during all active phases of the cell cycle, but is absent from resting cells, makes it an excellent marker for determining the growth of cells population. Ki-67 is a protein associated with active cell proliferation and expressed in all phases of the cell cycle, except resting cells, with the highest expression seen in G2/M (Guzman *et al*, 2005).

In conclusion, the results of this study observed that repeated exposure to crude oil may be lead to abnormal change in biochemical parameters and attributed haemotoxic, nephrotoxicity and hepatotoxicity. Immunohistochemical observation in the kidney tissue sections of rats after exposure to the crude oil could be dangerous to the kidney and liver functions that proved the hematological and biochemical perturbations occurred due to crude oil toxicity. From the observation of this study, we recommended precautionary measures should be stratify by the workers in petroleum companies to avoid contamination or inhalation of crude oil.

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