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Ameliorating effect of extracted Ginger oil against toxic effects of crude oil in male rats

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Abstract

Aim of the study: Use extraction of medical herbal such as powder ginger extract observed to stimulate the hematological system as evidenced by a decrease in the total count of RBCs, platelets as well as hemoglobin percentage. This study aimed to determine the antioxidant activity and protective effect of ginger essential oil against the toxic effect of crude oil on the hematological parameters.

Method: Crude oil was taken from the medical laboratory center in Missan province. Crude oil obtained from Missan oil company (MOC) Ltd sample type (No:38DI) Ginger (Zanjabar offcienales) was obtained from the local market, Missan city, and classified in the Department of Biology, Faculty of Science.

Experimental animals: Twenty-four Albino Waster male rats weighing (150-200 g) were assigned into three groups (8 rats for each group). Group one was administered corn oil at a dose of 1ml for each rat daily basis for 30 days, group two was treated with ginger essential oil orally (50 mg/kg B.W.) once daily for 30 days, group three was treated orally with 600ul of crude oil mixed with 1 ml corn oil orally once daily for 30 days. 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). **The results:** The results observed that ginger essential oil is composed of 55 chemical components identified according to retention times and area parentage area in the extracted oil. The main active chemical components were Zingiberen in percentage (17.1), followed by Cyclohexene (α-Sesquiphellandrene) in percentage (12.1%), à-Farnesene in percentage (11.9%) and Benzene (7.9%). Red blood cells (RBCs) observed a significant decrease in the count of red blood cells (RBCs) when administrated crude oil orally as a compared control group, while the rats that were given ginger essential oil observed no significant changes in RBC count. Decrease in hemoglobin concentration (Hb), Packed cell volume (PCV), and Platelets(Plt)after administrated of crud oil in dose 600ul, while the rats that administrated GEO observed no significant changes in Hb, PCVand Plt as compared with the control group. A significant increase in White Blood Cells(WBCs), Granulocytes (Neutrophils), Monocytes, and Lymphocytes after administration of crude oil as compared with the control group, while administration of GEO orally improved activity of WBCs, Granulocytes, Monocytes, and Lymphocytes.

Conclusions: Exposure to crude oil may lead to abnormal changes in the hematological parameters and Ginger essential oil may have properties for protecting and ameliorating the toxic effects of crude oil even in high doses.

Keywords: Ginger essential oil, hematological parameters, Crude oil.

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Introduction

Ginger, (Zingiberacae) is one of the important medicinal plants which naturally occurs in various countries like India, China, Southeast Asia, the West Indies, Mexico, and other parts of the world. This natural gold has been consumed worldwide as a spice and flavoring agent since ancient times. The oil of ginger is a mixture of constituents, consisting of monoterpenes (phellandrene, camphene, cineole, citral, and borneol) and sesquiterpenes (zingiberene, zingiberol, zingiberenol, s-bisabolene, sesquiphellandrene, and others). Aldehydes and alcohols are also present (Tang and Eisenbrand, 1992). Ginger is grown primarily in Asia and tropical areas and, in addition to its culinary function, has been used since ancient times for a variety of conditions, including colds, fevers, and digestive problems, and as an appetite stimulant. It is categorized by the U.S. Food and Drug Administration as a food additive but has been studied as a treatment for nausea and vomiting, as well as for arthritis (Kamaliroosta *et al.*, 2013).

Ginger can probably reduce serum triglycerides and increase intestinal peristalsis and reduce fat absorption by inhibiting the lipase enzyme in the pancreas and intestine (Alizadeh-Navaei *et al.*, 2008). Another possible mechanism of the plant to reduce serum triglycerides is due to the increase in the expression and activity of the lipoprotein lipase enzyme in the vessels. This enzyme increases the breakdown of triglycerides in the blood vessels and reduces blood levels of triglycerides (Shirdel *et al.*, 2009). Ginger also inhibits hepatic fatty acid and triglyceride synthesis by lowering key enzyme activity (Kalaiselvi *et al.*, 2015).

Ginger can reduce serum total cholesterol because of this plant in increasing the activity of liver cholesterol 7-α-hydroxylase enzyme which can increase the conversion of cholesterol to bile acids and thus decrease serum levels of cholesterol (Srinivasan and Sambaiah, 1991). Ginger is used to improve the flow of body fluids. It stimulates blood circulation throughout the body by powerful stimulatory effects on the heart muscle and by diluting the blood. The improved circulation is believed to increase cellular metabolic activity, thus contributing to the relief of cramps and tension (Mowrey and Clayson, 1982). Ginger reduced the formation of pro-inflammatory prostaglandins and thromboxane thus lowering the clotting ability of the blood (Malhotra and Palsingh,2003). Ginger has a lowering effect on blood pressure which is mediated through the blockade of voltage-dependent calcium channels. Concluded that the blood pressure-lowering action of aqueous ginger extract was through a dual inhibitory effect mediated via stimulation of both muscarinic receptors and blockade of Ca_{2+} channels (Zadeh and Moradikor, 2014). Antioxidants affect the process of lipid oxidation at different stages due to the differences in their mode of action. Because of the complexity of the oxidation process itself, the diversity of the substrates, and the active species involved, the application of different test methods are necessary to evaluate antioxidants.

Oral administration of an ethanol extract of ginger (80 mg/kg) significantly decreases fasting blood glucose level after 1-hour treatment in an STZ-type 1 diabetic rat model, the effect peaked after 4 hours, with ginger producing a 24% to 53% reduction in blood glucose at doses ranging from 10 to 80 mg/kg (Mahluji *et al*, 2013). Long-term treatment with ginger not only affected blood glucose levels, but also decreased serum triglyceride and total cholesterol, increased insulin, and effectively prevented body weight, liver, and kidney weight loss in type 1 diabetic animals (Ali et al, 2008).

Using the extract of medical herbal such as powdered ginger extract was observed to stimulate the hematological system as evidenced by a decrease in the total count of RBCs, platelets as well as hemoglobin percentage (Ghosh et al,2006). Shakya. (2015) reported that administration powdered ginger rhizome diet for 12 weeks in male

rats showed a decrease in hematocrit, hemoglobin, erythrocyte, MCH, MCHC, and WBC values and an increase in neutrophil percentage. Administration of ginger rhizome can enhance the non-specific immune response in rainbow trouts. Non-specific immunity plays an especially important role in the defense by which the body can protect itself from diseases The inhibition of platelet aggregation by ginger is more than the similar effects observed with garlic and onion (Haghighi and Rohan, 2013).

The objectives of the present study are to determine the antioxidant activity and protective effect of ginger essential oil against the toxic effect of crude oil on the hematological parameters.

Material and methods

The study was performed in the medical laboratory center in Missan province. The crude oil was obtained from Missan oil company (MOC) Ltd sample type (No:38DI). Ginger (Zanjabar offcienales) was obtained from the local market, Missan city, and classified in the Department of Biology, Faculty of Science, Basrah University. Ginger roots in the dried status were used for the extraction process. Essential oils were extracted by hydrodistillation using the Clevenger-type apparatus according to the method of Guenther (1948). The obtained essential oil was collected in glass bottles covered with aluminum foil to avoid the negative effects of light. The chemical composition of the extracted essential oil(s) was identified using a Thermo scientific GC/MS version (5) 2009 system with TG-5MS column (30mX0.32mmID). Helium was used as a carrier gas at a flow rate of 1ml/min. Five µl essential oil was diluted to 1ml with dichloromethane, then 2 µl was injected on splitless mode for 1 min. followed by a split flow with a ratio of 1:10. The GC oven temperature was held at 45°C for 2 min then was programmed from 45°C to 165°C at 4°C /min; from 165°C to 280°C at 15°C /min. after which was kept constant at 280°C for 10 min. Both the interface and injection temperatures were adjusted at 250°C. The ionization voltage was 70eV with a mass range between 40-800 m/z. The essential oil components were identified by mass fragmentation patterns, which were compared with NIST mass spectral database (version 2) and their relative percentages were calculated based on GC peak areas.

Experimental animals (24) male albino rats weighing 150-200g were obtained from the animal house of the Faculty of Science, Basrah University. Animals were handled following the principles of laboratory animal care as contained in the NIH Guide for laboratory animal welfare and the experimental protocol was approved by the Local Ethics Committee and Animals Research. The rats were housed in stainless steel bottomed wire cages after grouping into control and treated groups (8 rats in each cage) and maintained at a temperature of 22 ± 2oC, relative humidity of 40-60%, with a 12 h/12 h light/dark cycle and allowed free access to food and water.

- Group I: Control rats were administered corn oil orally via a ball-tipped curved intubation needle daily for 30 days.
- Group II: The rats were treated with ginger essential oil orally (50 mg/kg B.W.) once daily for 30 days according to the protocol described by (Abd-El Azeim *et al.*, 2012).
- Group III: Rats were orally administered 600ul crude oil mixed with (1 ml) of corn oil /once daily for 30 days.

After the end period of the experimental study, the rats were starved overnight, euthanized then dissected and blood samples were taken from the inferior vena cava and collected in clean class tubes with EDTA anticoagulant. Blood pictures (CBC) shown from collected blood samples by automatic method (Celltac X kx 021n automated hematology analyzer, Japan CARE Co, LTD), which includes hemoglobin (Hb), white blood cells (WBCs)count, granulocytes (neutrophils), lymphocytes,

monocytes, red blood cells (RBCs), Platelets and Haematocrit or packed cell volume (PCV).

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. The criterion for statistical significance was (P<0.05).

Results

GC- Mass analysis of this study observed that ginger essential oil composed of 55 chemical components identified according to retention times and area parentage area in the extracted oil. The main active chemical components were Zingiberen in percentage (17.1), followed by Cyclohexene (α-Sesquiphellandrene) in percentage (12.1%), à-Farnesene in percentage (11.9%) and Benzene (7.9%), Table (1).

Kamaliroosta *et al* (2013) found that oxygenated monoterpenes found at moderate concentrations of extracted essential oil have more contributions to the flavoring characteristics of essential oil. Onyenekwe and Hashimoto (1999) reported that sesquiterpenes are the main constituents of West African ginger and zingiberene was the major compound of the essential oil.

The volatile oil consists of mainly mono and sesquiterpenes; camphene, beta phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, linalool, alpha-zingiberene (30-70%), beta-sesquiphellandrene (15-20%), beta bisabolene (10-15%) and alpha-farmesene (Banerjee et al, 2011). The zingberene was the major component that was present in the ginger essential oil of all 46-ginger accession except exotic ginger (Kintoki) in which α-curcumene was the major component (Ekundayo et al., 1988).

Kizhakkayil and Sasikumar (2012) recorded five major components of ginger essential oil identified such as Geranial (25.9%), à- Zingiberene (9.5%), (E-E)-α-Farnesene (7.6%), Neral (7.6%) and Ar-Curcumen (6.6%). Kizhakkayil and Sasikumar (2012) reported that Zangiberene was the highest percentage (245.5%), while the other major compounds identified were Z-Citral, Citral, Farnesene, β-Sesquiphellandrene, α-Curcumene, Camphene, and Nerolidola. Total polyphenols were found to be 1.21% in all components of ginger essential oil.

Kamaliroosta et al. (2013) found the total phenolic contents of aqueous ginger extract to be 23.5 mg gallic acid. In a study performed by Sultan et al. (2005) on the ginger rhizome imported from China and Thailand, the chemical analysis of ginger essential oil showed that the Thailand ginger contain α- Pinene (3.59%), α-Phallendrene (2.8%) and Zangiberene (30.8%), while essential oil of China ginger contains α- Pinene (0.30%), à- Phallendrene (1.02%) and Zangiberene (8%), therefore they concluded that Thailand ginger essential oil was better in quality than the Chinese ginger essential oil due to high percentage of Zangiberene (70%).

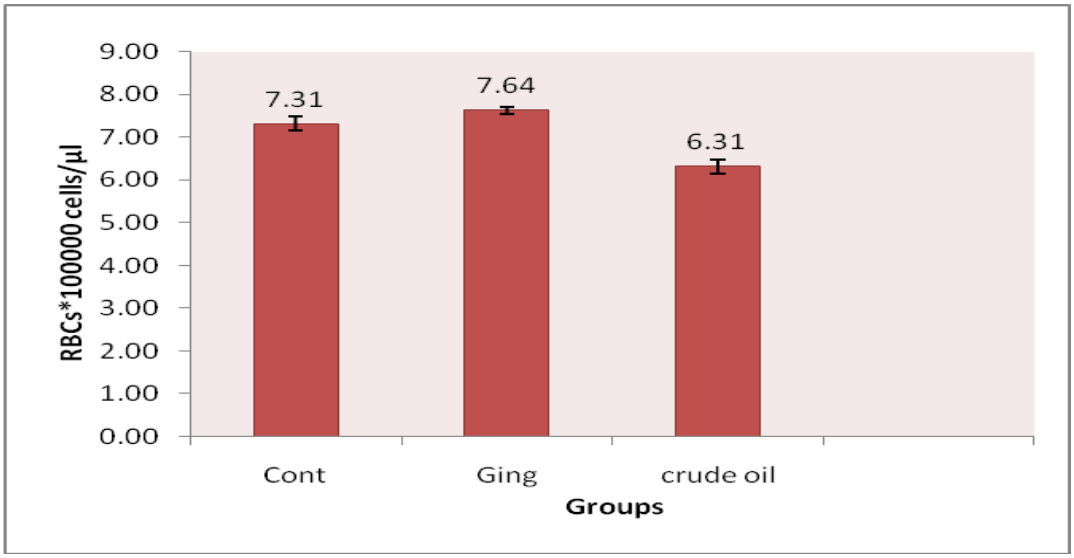
The results of this study observed a significant decrease in the count of red blood cells (RBCs) when administrated crude oil orally as a compared control group, while the rats that were given ginger essential oil observed no significant changes in RBCs count as compared with control group, figs(1).

Also, the results observed a decrease in hemoglobin concentration (Hb), Packed cell volume (PCV), and Platelets(Plt)after administrating crud oil in dose 600ul mixed with corn oil, while the rats that administrated GEO observed no significant changes in Hb, PCVand Plt as compared with a control group, fig (2,3,4).

The results also recorded an increase in White Blood Cells(WBCs), Granulocytes (Neutrophils), Monocytes, and Lymphocytes after administration of crude oil in dose 600ul as compared with a control group, while administration of GEO orally improved the activity of WBCs, Granulocytes, Monocytes, and Lymphocytes, Fig(5,6,7,8).

Table (1): Active components found in ginger essential oil detected by GC-Mass

Chemical group	Active compound	Retention time	Area %
Monoterpenes	Borneol	15.32	3.08
	Eucalyptol	9.47	2.71
Sesquiterpenes	Cyclohexadiene (Zingiberene)	30.43	17.16
	Cyclohexene (Sesquiphellandrene)	31.48	12.14
	à-Farnesene	30.87	11.93
	Isocaryophillene	25.45	2.51
Aldehydes Aliphatics	Cyclohexane	25.45	2.51
Ketones Aliphatics	Benzene	29.66	7.96



Fig(1): The changes in red blood cells (RBCs) levels in rats treated orally with crude oil and ginger essential oil. Values are expressed as means±SD of per group.

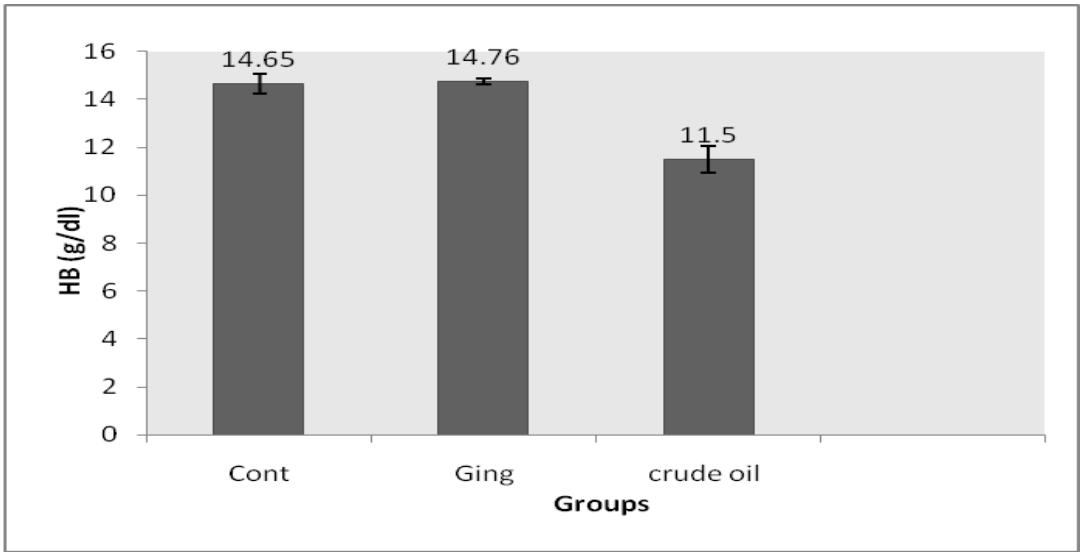
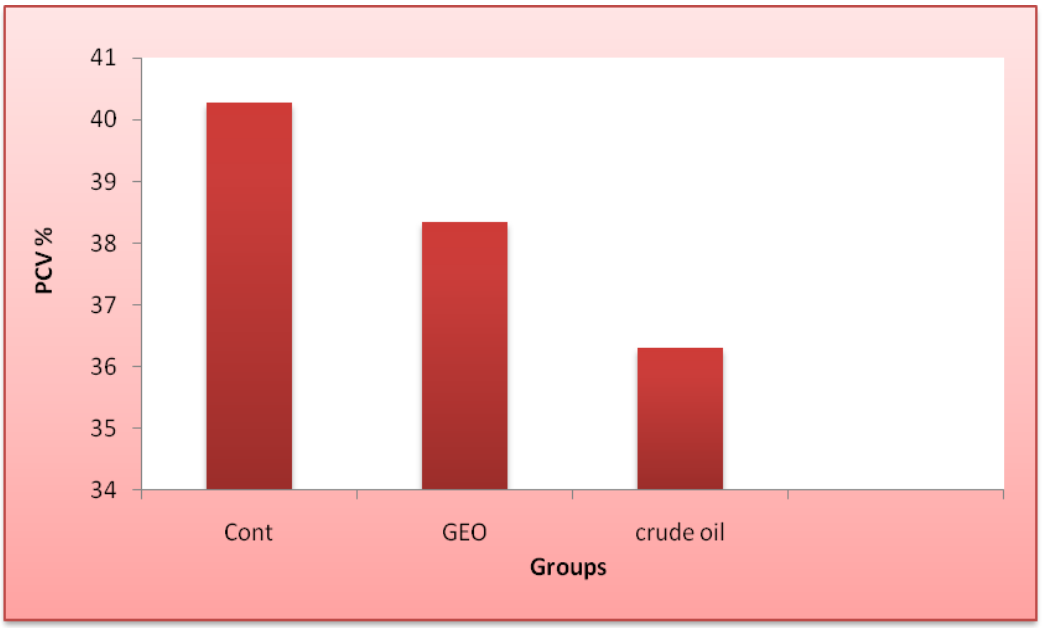
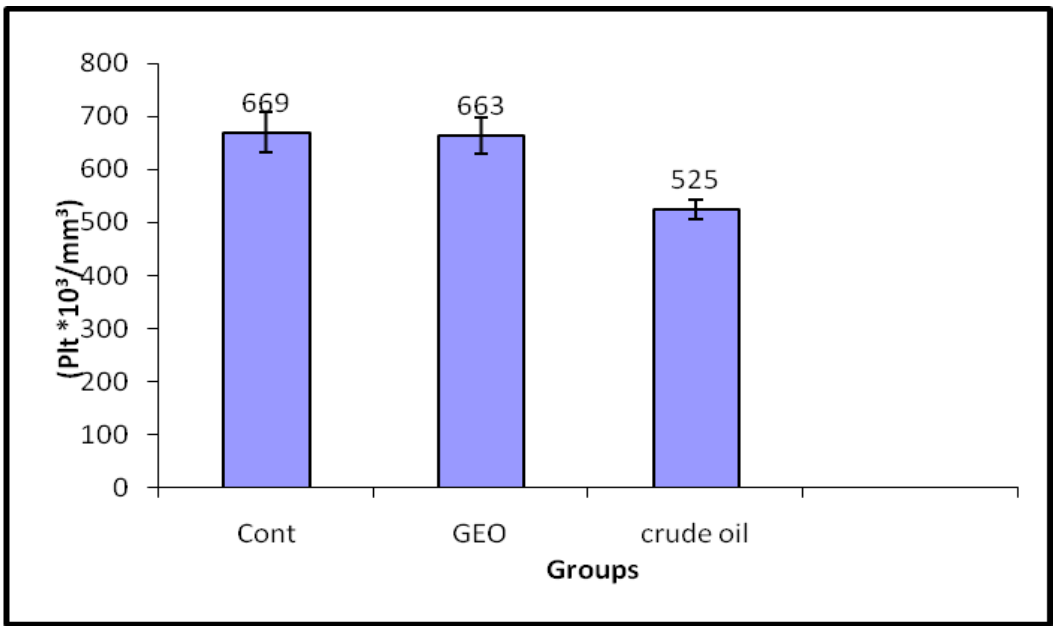


Fig (2): Hemoglobin concentration (Hb) in male rats treated orally with crude oil and ginger essential oil. Values are expressed as means±SD of per group.



Fig(3): Observed percentage of Packed cell volume (PCV) in male rats treated orally with crude oil and ginger essential oil. Values are expressed as means±SD of per group.



Fig(4): Reveal platelets (Plts) level in male rats treated orally with crude oil and ginger essential oil. Values are expressed as means±SD of per group.

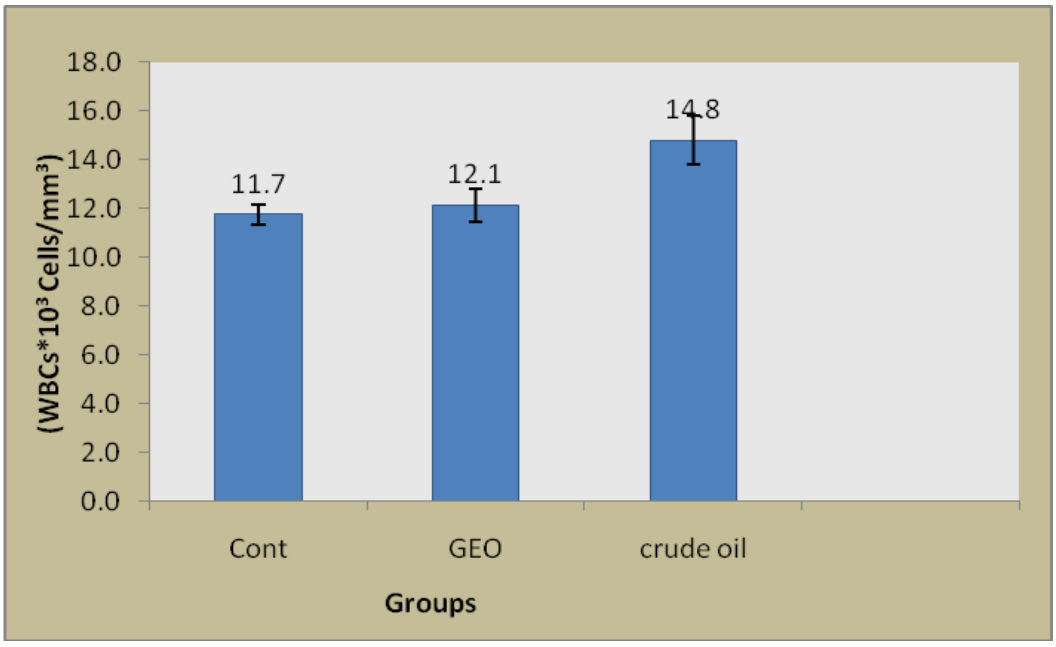
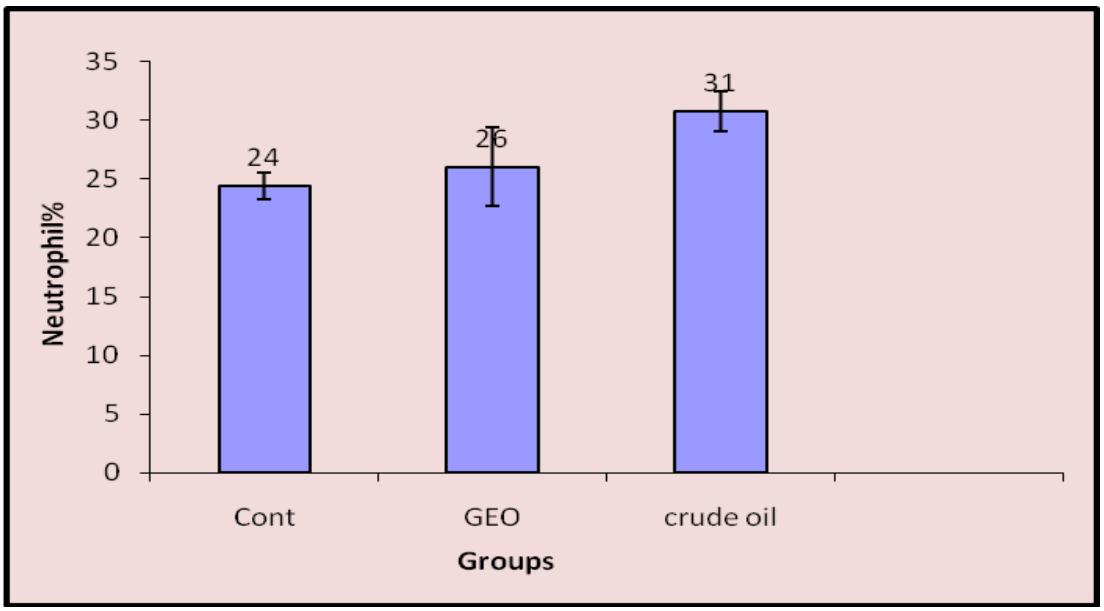
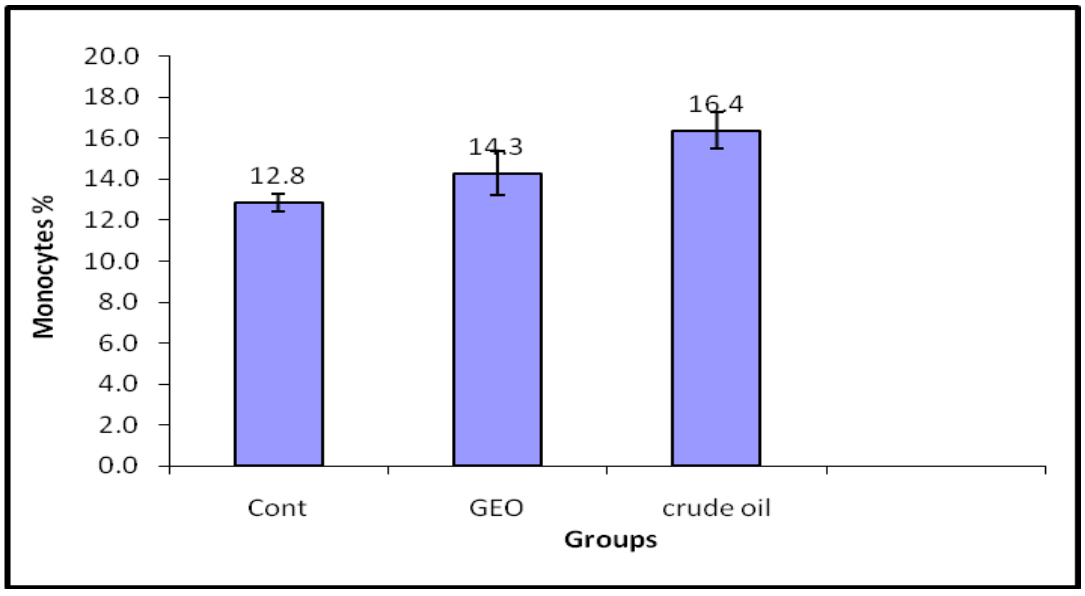


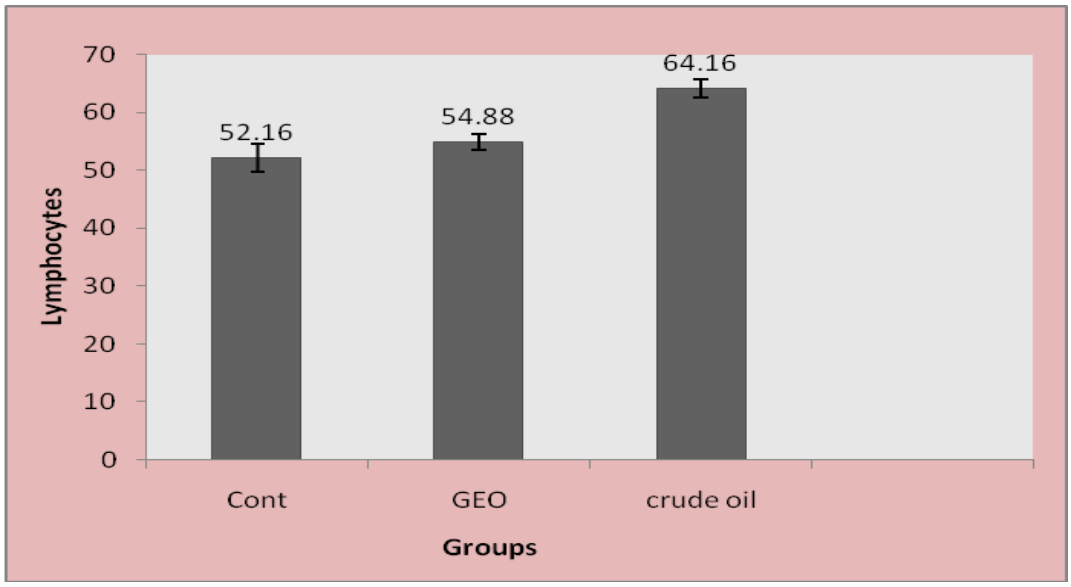
Fig (5): Observed White blood cells (WBCs) level in male rats treated orally with crude oil and ginger essential oil. Values are expressed as means±SD of per group.



Fig(6): Reveal the granulocytes (neutrophils) percentage in male rats treated orally with crude oil and ginger essential oil. Values are expressed as means±SD of per group.



Fig(7): Observed the monocyte percentage in male rats treated orally with crude oil and ginger essential oil. Values are expressed as means±SD of per group.



Fig(8): Reveal the lymphocytes in male rats treated orally with crude oil and ginger essential oil. Values are expressed as means±SD of per group.

Discussion:

Blood is one of the specialized body fluids, responsible for the transport of nutrients, oxygen, hormones, chemical substances, and other metabolites to the body's cells, also, the elimination of the waste products away from those cells to sites of excretion (Xutian and Yuan,2014). Exposure to different organic solvents such as gasoline, benzene, toluene, hexane, carbon disulfide, insecticides, and pesticides has been reported to cause adverse effects on the hematological profiles in animals and humans (Ita and Udofia, 2011).

Ovuru and Ekweozor (2004) found a significant decrease in RBCs with increasing concentrations of crude oil in rabbits when taking diets containing toxic components of crude oil which caused a change in blood chemistry and induce anemia and interfere with platelet production in animals. Haemotoxicity was observed after administration of the crude oil due to the binding of toxicants with RBCs membrane and hemoglobin molecules and found activation in the bone marrow and cytotoxic effects with disturbance in DNA function after administration of benzene to the rats, thus bone marrow failure in production RBCs and other formed elements (Snyder,1987).

The results observed a significant decrease in blood-packed cell volume (PCV) and platelet (Plt) with increased administration of the crude oil into the laboratory animals as compared with a control group. Patrick-Lwuanyanwu *et al* (2013) found a significant decrease in PCV and platelet and Hb levels after exposure to different concentrations of crude oil soluble in water.

Krishna and Veena (1980) reported a decrease in erythropoiesis because of petroleum samples and caused changes in blood chemistry and bone marrow hyperplasia which induced anemia.

The study performed by Farroqui and Ahmed (1983) observed a decrease in red blood cells account; hematocrit and hemoglobin after acute exposure to the toxicant, and the mechanism of haemotoxicity were not clear but may be due to the toxic materials covalently with RBCs membranes and hemoglobin molecules. Hounkpatin *et al.* (2013) showed a significant increase in WBCs and a decrease in RBCs,

Hb concentration, mean corpuscular, and platelets, while the lymphocytes found in high concentration in rats after being exposed to the cadmium, mercury, and their combination for 28days and these results are similar to the results observed in the present study.

The increase in total white blood cells observed in rats treated with a high dose of crude oil due to stimulated lymphopoiesis or enhanced release of lymphocytes from lymph myeloid tissue T, this lymphocyte response might be a direct stimulatory effect of toxic substances on lymphoid tissues. Alternatively, this response may be assumed to be associated with the toxicant-induced tissue damage and disturbance of the non-specific immune system leading to increased production of leukocytes (Das and Mukherjee, 2003).

Ghosh *et al.* (2006) reported that the used extract of medical herbal such as powdered ginger extract was observed to stimulate the hematological system as evidenced by a decrease in the total count of RBCs, platelets as well as hemoglobin percentage, while Shakya (2015) reported that administration powdered of ginger rhizome diet for 12 weeks to the male rats showed a decrease in hematocrit, hemoglobin, erythrocyte, MCH, MCHC, WBC values and increase neutrophils percentage. The powdered ginger rhizome can enhance the non-specific immune response and the non-specific immunity plays important role in the defense by which the body can protect itself from diseases (Haghighi and Rohan, 2013). The inhibition of platelet aggregation by ginger is more than the similar effects observed with garlic and onion.

In conclusion, results observed exposure to crude oil in high doses for long periods may be attributed to haemotoxic and cause a dangerous effect on the hematological parameters. The ginger essential oil may have properties to protect and ameliorate the toxic effects of crude oil even in high doses.

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