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Full Length Research Paper

Effects of Crude and Degummed Seed Oil Extracts of *Citrullus lonatus* on Carbon Tetrachloride (CCl_4) Induced Liver Damage in Wistar Rats

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ABSTRACT: Qualitative and quantitative phytochemical screening, antioxidant activity, biochemical and haematological effects of crude and degummed seed oil extracts of *Citrullus lonatus* on carbon tetrachloride-induced liver damage in rats were determined. Qualitative phytochemical screening showed the presence of saponins, terpenoids, flavonoids, alkaloids and steroids in both crude and degummed seed oil extracts while tannins, glycosides and phenols were absent in both samples. Quantitatively, saponins (4.02 ± 0.01 and 2.60 ± 0.01), terpenoids (1.71 ± 0.06 and 2.17 ± 0.05), flavonoids (2.91 ± 0.01 and 3.49 ± 0.02), alkaloids (1.11 ± 0.01 and 0.74 ± 0.01) and steroids (5.13 ± 0.01 and 4.43 ± 0.01) were determined for the crude and degummed seed oil extracts respectively. Crude and degummed seed oil extracts possessed antioxidant activities compared to L-ascorbic acid. The results were expressed in percentage inhibition (IC_{50}) (mg/ml). Degummed seed oil extract exhibited better antioxidant activity with IC_{50} 38.22 mg/ml, 38.66 mg/ml and 39.32 mg/ml for FRAP, DPPH and TBARS compared to crude seed oil extract 38.38 mg/ml, 38.86 mg/ml, 39.39 mg/ml and L-ascorbic acid 41.25 mg/ml, 48.14 mg/ml and 58.15 mg/ml respectively. Crude and degummed seed oil extracts at 100 to 300 mg/kg body weights both exhibited their ameliorative effects on the deleterious effects of carbon tetrachloride on the experimental animals by significantly ($p< 0.05$) decreasing serum levels of ALT, AST, ALP TC, TG and LDL and significantly ($p<0.05$) increasing total protein, albumin, bilirubin and HDL levels. Haematological indices observed showed that the crude and degummed seed oil extracts at 100 to 300 mg/kg body weights significantly ($p< 0.05$) decrease the levels of WBCs, MCV, MCH and MCHC while RBC, PCV, Hb and PLT levels significantly ($p< 0.05$) increased. Even though these extracts exhibited their effects positively on the various biochemical parameters, the degummed seed oil extract's effects were to some extent more pronounced compared to the crude seed oil extract's effects.

Keyword: *Citrullus lonatus,* L- ascorbic acid, carbon tetrachloride, haematological indices, crude and degummed seed oil extracts

INTRODUCTION

Citrullus lonatus of the family Cucurbitaceae is commonly known as water melon in English and "Kankana" in Hausa. The ripe fruits are edible and largely used for making confectionary. Its nutritive values are also useful to the human health. The fruit is used in cooling, strengthening, aphrodisiac, astringent to the bowels, expectorant, diuretic, and stomachic, purifies the blood, allays thirst, cures biliousness, good for sore eyes, scabies and itches and as brain tonic (Rahman *et al*., 2008). Its roots and leaves are also reported to have analgesic and anti-inflammatory activities (Deng and Wang, 2010), antimicrobial activity (Loiy *et al*., 2011), laxative activity of fruit (Swapnil, 2011), anti-oxidant and anti-ulcerative activities (Naresh, 2011) (Figures 1 and 2).

Oils extracted from plant sources have a rich history of use by local people as a source of food, energy, medicine and for cosmetic applications. It has been used in the production of lubricants, soaps and personal care products, as well as in the topical treatment of various conditions such as hair dandruff, muscle spasms,

Figure 1: Watermelon plant and fruit.

Figure 2: Watermelon seeds.

varicose veins and wounds (Zimba *et al*., 2005). In recent years, demand for seed oils as ingredients for food, cosmetics and bio fuel has greatly increased as industries seek natural alternatives. The global production of seed oils increased dramatically, creating pressure on countries providing the raw material to meet the growing demand. In 2004–2005, the global production of seed oils was approximately 113 million metric tonnes (MMT). Currently, the global supply is obtained from only about 15 plant species out of nearly half a million known to man, highlighting the greater potential (Mitei *et al*., 2008). The liver is the largest organ in the abdominal cavity and the most complex with more functions than any other human organ. It consists of a myriad of individual microscopic functional units called lobules. The liver performs a variety of functions including the removal of endogenous and exogenous materials from the blood,

complex metabolic processes including bile production, carbohydrate homeostasis, lipid metabolism, urea formation, and immune functions and is the main target of a number of toxicants (Mayer and Kulkarni, 2001). Transformations that occur in the liver that render many drugs water soluble and they readily excreted by the kidneys. The physiological response to injury results such as necrosis, cholestasis, steatosis, inflammation and fibrosis.

Carbon Tetrachloride (CCl_4)

Carbon tetrachloride is a colourless liquid with a sweetish odour (NLM, 2003; Lewis, 1979). Synonyms include tetrachloromethane and perchloromethane (NLM, 2003; O'Neil and Smith, 2001). In the United States, carbon tetrachloride is most commonly prepared by chlorinating methane or by a chlorinating cleavage reaction with less than or equal to C3 hydrocarbons or chlorinated hydrocarbons (Rossberg, 2002). Prior to the late 1950s, carbon tetrachloride was produced primarily by carbon disulphide chlorination (NLM, 2003; Rossberg, 2002). Carbon tetrachloride has been used as a dry-cleaning agent, fabric-spotting fluid, solvent, reagent in chemical synthesis, fire extinguisher fluid, and grain fumigant (NLM, 2003; Holbrook, 1993), but its primary use was in chlorofluorocarbon (CFC) production (NLM, 2003; Rossberg, 2002). The Consumer Product Safety Commission banned the use of carbon tetrachloride in consumer products in the 1970s.

MATERIALS AND METHODS

Water melon (*Citrullus lonatus*) seeds were obtained from a local market in Sansani village in Bali L. G. A. of Taraba State, Nigeria. It was identified and authenticated in the Department of Plant Science, Modibbo Adama University of Technology, Yola. The sample was carefully washed under running tap water followed by distilled water. It was shed dried for some days at room temperature and put into sterile sample bottles to avoid contamination. Fifty-four Wistar strain rats weighing between 100±10g were obtained from National Veterinary Research Institute Vom, Jos, Plateau state, Nigeria. They were housed in polypropylene cages, and were given standard grower diet (vital feeds) and water *ad libitum* for some days before the commencement of the experiment. Guide for the Care and Use of Laboratory Animals was strictly followed.

Extraction

Two hundred grammes of pulverized sample were mixed with n-Hexane 1000ml and placed on a magnetic stirrer with stirring for 24h. The extract was filtered using a sterile Whatman No. 1 filter paper to obtain a particle free extract. The sample was re-extracted with the solvent three times, filtered and the extract pooled. The extract was concentrated using rotary evaporator at less than 50^{o}C. Extract was made solvent free by finally drying in a vacuum oven at < 50^{o}C to obtain the oil material. Watermelon seed oil blends well with other nutty oils because of its slightly nutty flavour. It is light, very stable and is used as carrier oil for any essential oil during aromatherapy massage.

Phytochemical Screening

The extract was subjected to qualitative phytochemical screening for the presence of some phytoconstituents like alkaloids, saponins, tannins, steroids, flavonoids and phenols. They were identified by their characteristics colour change using standard procedures as described by (Trease and Evans, 1989; Harborne, 1998).

Test for alkaloids

For alkaloids, 2ml of picric acid was added to 0.5g of the extract, the appearance of orange colour indicated the presence of alkaloids.

Test for saponins

For saponins, 0.5g of the plant extract was shaken with water in a test tube and heated to boil. Frothing was observed, indicating the presence of saponins.

Test for tannins

For tannins, 0.5g of the plant extract was added to10ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride were added and observed, no visible colour was seen indicating the absence of tannins.

Test for steroids

For steroids, 2ml of acetic anhydride was added to 0.5g of extract and 2 ml sulphuric acid added. Violet to blue colour was observed indicating the presence of steroids.

Test for flavonoids

For flavonoids, 10ml of ethylacetate was added to small portion of the extract and it was held over steam bath for 3min. The mixture was filtered and 1ml of dilute ammonia solution was added to 4ml of the filtrate and then shaken. Yellow colour was observed indicating the presence of flavonoids (Sofowora, 1993).

Test for phenols

For phenols, 0.5g of the extract material was boiled with 10ml of sulphuric acid and filtered while hot, and the filtrate was shaken with 5ml of chloroform. The chloroform layer was pipetted into another test tube and 1ml of diluted ammonia was added to it. No colour change indicating the absence of phenols.

Quantitative Determination of Phenol, Flavonoid and Tannin

Estimation of total phenols

The total phenolics content was estimated using the Folin-Ciocalteau procedure described by Siddhuraju and Becker (2003). About 1g of seed oil extract was used and made up to 1ml, with distilled water. Then 0.5ml of diluted Folins-phenols reagent (1:1 ratio with water) and 2.5ml of (20%) sodium carbonate was added. The mixture was shaken and incubated for 40min at room temperature for the development of colour. Distilled water was used as blank. Absorbance was measured at 760nm using Thermo Fisher double beam spectrophotometer. Gallic acid was used as standard and the result was expressed as microgramme of gallic acid equivalents per gramme dry mass of extract (µg GAE/gDM).

Estimation of total flavonoids

The total flavonoids content was estimated using the procedure described by Zhishen *et al* (1999). One millilitre of crude and degummed seed oil extracts were diluted with 0.2ml, of distilled water separately followed by the addition of 0.15ml of 5% sodium nitrite solution. The mixture was incubated for 5min and then 0.15ml of 10% aluminium chloride solution was added and allowed to stand for 6min. Then 2ml of 4% sodium hydroxide solution was added and made up to 5ml with distilled water. The mixture was well shaken and allowed to stand at room temperature for 15min. The absorbance was read at 510nm against the blank. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as rutin equivalent mg/g extract on a dry weight basis using standard curve.

Estimation of total alkaloids

A total of 200ml, of 20% acetic acid was added to 1g of crude and degummed seed oil extracts taken in separate 250ml beaker, covered and allowed to stand for 4h. The mixture was filtered and the volume of the filtrate was reduced to one quarter using water bath. To the sample concentrated ammonium hydroxide was added drop-wise until a precipitate was formed. The solution was allowed to settle and the precipitate was collected by filtration and weighed (Obadoni and Ochuko, 2001). The percentage of total alkaloids content was calculated as:

$$\text{Percentage of total alkaloids} = \frac{\text{Weight of residue} \times 100}{\text{Initial weight of sample.}}$$

Estimation of total tannins content

Tannins content of the crude and degummed seed oil extract was estimated by the method described by Siddhuraju and Manian (2007). A total of 0.5ml the extracts were taken in test tube separately and treated with 100mg of polyvinyl polypyrrolidone and 0.5ml of distilled water. The solution was incubated at 4^{o}C for 4 h. Then the sample was centrifuged at 5000rpm for 5min and 0.02ml of the supernatant was taken. This supernatant only contains simple phenolics free of tannins (tannins were precipitated along with the polyvinyl polyprrolidone). The phenolics content of the supernatant was measured at 725nm and expressed as the content of free phenolics on a dry matter basis. From the above result, the tannins content of the extract was calculated as follows:

$$\%\ \text{Tannins (mg GAE/g)} = \text{Total phenolics (mg GAE/g)} - \text{Freephenolics (mg GAE/g).}$$

Estimation of total saponins content

Estimation of total saponins content was determined by the method described by Makkar *et al.,* (2007). Zero point zero five millilitres (0.05ml) of the crude and degummed seed oil extracts was taken and 0.25ml of distilled water was added. To this mixture 0.25ml of vanillin reagent (800mg of vanillin in 10ml of 99.5% ethanol) was added. Then 2.5ml of 72% sulphuric acid was added and mixed well. This solution was kept in a water bath at 60^{o}C for 10min. After 10min, it was allowed to cool in ice cold water and the absorbance was read at 544nm. The value was expressed as diosgenin equivalent (mg DE/g extract derived from a standard curve.

Estimation of total terpenoids content

Two grammes of crude and degummed seed oil extracts of *Citrullus lonatus* was weighed and soaked in 50ml of 95% ethanol for 24h. The extract was filtered and filtrate was extracted with petroleum ether (60-80%) and concentrated to dryness. The dried ether extract was treated as total terpenoids (Ferguson, 1956).

In vitro antioxidant activity assays

Ferric reducing antioxidant power (FRAP) assay

The antioxidants activities of the crude and degummed seed oil extracts were estimated using FRAP assay modified method as described by Sutharsingh *et al.* (2011). A stock solution of the extract and standard ascorbic acid was prepared in the concentration of 10 mg/ml. In ferric reducing antioxidant power assay, 1ml of test sample of both extracst in different concentration was mixed with 1ml of 0.2M sodium phosphate buffer (pH 6.6) and 1ml of 1% potassium ferricyanide in separate test tubes. The reaction mixture was incubated in a temperature-controlled water bath at 50^{o}C for 20min followed by addition of 1ml of 10% trichloroacetic acid. The mixtures were centrifuged for 10min at room temperature. The supernatant obtained (1ml) was added to 1ml of distilled water in a test tube and 0.2ml of 0.1% $FeCl_3$ also added. The blank was prepared in the same manner as the samples except that the extract will be replaced by distilled water. The absorbance of the reaction mixture was measured at 700nm. The reducing power was expressed as an increase in absorbance after blank subtraction.

2, 2'-diphenyl-1-picrylhydrazyl (DPPH) Assay

The antioxidant activity of the plant extract was estimated using the DPPH radical scavenging assay as described by Sutharsingh *et al.* (2011). DPPH solution (0.04% w/v) was prepared in 95% ethanol. A stock solution of the extract and standard ascorbic acid was prepared in the concentration of 10 mg/ml. From stock solution 2ml, 4ml, 6ml, 8ml and 10ml of this solution were taken in five test tubes respectively. With same solvent made the final volume of each test tube up to 10ml whose concentration were 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml respectively. Then 2ml of freshly prepared DPPH solution (0.04% w/v) was added to each of these test tubes. The reaction mixture was incubated in the dark for 15min and thereafter the optical density recorded against the blank at 523nm. For the blank, 2ml of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution recorded after 30 min. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (IC_{50}) of DPPH radical.

Thiobarbituric acid reactive substances (TBARS) Assay

TBARS method was used for evaluating the extent of lipid peroxidation at low pH, and high temperature (100^{o}C). Malondialdehyde (MDA) binds Thiobarbituric acid to form a red complex that can be measured at 532nm. A 2ml of 20% trichloroacetic acid (TCA) and 2ml of 0.67% TBA solutions were added to 2ml of the mixtures containing 4mg of the sample in 4ml of 99.5% ethanol. This mixture was kept in water bath 100^{o}C for antioxidant activity which was based on the absorbance of the supernatant at 532 nm on the final day of the assay (Sutharsingh *et al.*, 2011). The percentage of antioxidant activity was calculated by the following formulae for all the methods.

$$\text{Percentage antioxidant} = \frac{\text{Absorbance standard} - \text{Absorbance of sample} \times 100}{\text{Absorbance standard}}$$

Experimental design

After acclimatization, 54 rats were randomly divided into nine groups of six rats per group and the average weight of the rats was taken and recorded for each group. Group I were injected intraperitoneally with sterile normal saline (2ml) daily for seven days in addition to their normal diet and water and was kept as a normal control group. Group II were given CCl_4 per day for seven days to induce liver damage in addition to their normal diet and water and were kept as negative control. Group III were given CCl_4 per day for seven days to induce liver damage and subsequently treated with a standard drug (silymarin 100mg/kg) in addition to their normal diet and water and were kept as positive control. Groups IV, V and VI were given CCl_4 per day for seven days to induce liver damage and subsequently received 100mg/kg body weight per day, 200mg/kg body weight per day and 300mg/kg body weight per day respectively of the crude seed oil extract by intubation for twenty one (21) days in addition to their normal diet and water. Groups VII, VIII and IX were given CCl_4 per day for seven days to induce liver damage and subsequently received 100mg/kg body weight per day, 200mg/kg body weight per day and 300mg/kg body weight per day respectively of the degummed seed oil extract through intubation for twenty one (21) days in addition to their normal diet and water.

Collection of blood samples for analysis

The administration of the extract lasted for the period of three weeks after which the animals were sacrificed twelve hours after the last administration in accordance with guidelines of the European Convention for the protection of vertebrate animals and other scientific purpose European Treaty Series-123 (ETS-123). At the end of the study period, the rats were anaesthetised with chloroform, followed by a surgical exposure of the heart and blood samples collected from the experimental animals via cardiac puncture. About 2ml of blood sample for haematological studies was transferred into an EDTA

bottles. While the remaining blood samples were transferred into clean sterile test tubes and incubated in an upright position at room temperature for 30min to allow clotting. The clotted blood was centrifuged at 3000rpm for 15min after which the serum was transferred into clean sterile sample bottles and stored in the refrigerator until use.

Determination of Aspartate transaminase (AST)

The AST activity was determined according to the method of Reitman and Frankel, (1957).

Principle

The principle for the determination of AST is based on the rate of formation of glutamic acid. In AST, the amount of Oxaloacetic acid formed is proportional to the transaminase activity. The amber colour which developed in an alkaline medium is the measure of the amount of glutamic acid formed.
α-ketoglutaric acid + Aspartic acid Glutamic acid + Oxaloacetic acid

Procedure

Substrate for AST: 29.2mg α-ketoglutarate and 2.66g of aspartate were placed in a beaker. 1M sodium hydroxide was added until it was completely dissolved. The pH was adjusted to 7.4 with sodium hydroxide. It was transferred quantitatively to 100ml volumetric flask with phosphate buffer pH 7.4 (R1). To this mixture, 1ml of 2, 4-Dinitrophenylhydrazine (R2) was added, mixed well and allowed to stand for 10min and optical densities measured at 540nm. This was used to obtain a standard curve by plotting optical densities against concentrations. For AST, two test tubes (reagent blank and sample) were set. For reagent blank, 0.5ml of R1 and 0.1ml of distilled water was added incubated for 30min. For sample, 0.1ml of sample and 0.5ml of R1 was added and incubated for 30min. After incubation, 0.5ml of R2 was added to both reagent blank and sample, mixed and allowed to stand for 20min. Their optical densities were measured at 540nm.The activities of both AST and ALT were determined by extrapolation from the standard curve.

Determination of Alanine transaminase (ALT)

The ALT level was estimated using a method proposed by Reitman and Frankel, (1957).

Principle

The principle for the formation of ALT is based on the rate of formation of glutamic acid. In ALT, the amount of pyruvate formed is proportional to the transaminase activity.

α-ketoglutarate + L-alanine ⟶ L-glutamate + pyruvate

Procedure

The procedure for AST is the same for ALT except that aspartate is replaced by alanine in the preparation of the substrate.

Determination of Alkaline phosphatase (ALP)

The ALP level was determined according to the method described by Reitman and Frankel, (1957).

Principle

Phosphatases are enzymes that split off the phosphate moiety from various substrates. Phosphatases vary in their specificity towards substrates. The alkaline phosphatase is active in an alkaline medium, which is its major distinction from phosphatases that perform catalysis in neutral and acid media; the specificity of alkaline phosphatase is not high. This method is based on the photocolorimetric determination of inorganic phosphate that is split off from p-nitrophenylphosphate with phosphatase of the blood serum in an alkaline medium.

P-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ p-nitrophenol + phosphate.

Procedure

R1a Buffer (diethanolamine buffer, 1mmol/l, pH 9.8 and $MgCl_2$. 0.5mmol/l). R1b Subtrate (p-nitrophenylphosphate). Reagent was reconstituted by mixing one vial of R1b with 3ml of R1a. After reconstitution of the reagent, 0.05ml of sample and 3ml of the reagent were put into cuvette. Mixed and initial absorbance read. After taking the initial absorbance the timer was set on and absorbances were read against reagent blank at 1, 2 and 3 minutes interval respectively. ALP activity was calculated as:
U/l = 3300 x Absorbance at 405nm/min.

Determination of serum bilirubin

The bilirubin level in serum was determined by method described by Dangerfield and Finlayson, (1953).

Principle

Sulfanilic acid reacts with sodium nitrite to produce deoxidized sulfanilic acid. Total bilirubin couples with deoxidized sulfanilic acid in the presence of methylsulfoxide to produce azobilirubin which is

measured at 532nm. In the absence of methylsulfoxide, only direct (conjugated) bilirubin forms azobilirubin complex.

Procedure

To 1.0ml total bilirubin reagent, 0.02ml of activator and 0.1ml of serum were added, mixed well and incubated for exactly 5min at room temperature. Sample blank was prepared by mixing 1.0ml total bilirubin reagent with 0.1ml of distilled water; mixed and incubated for exactly 5min at room temperature. The absorbance of each sample blank and test was measured at 532 nm against distilled water blank. Total bilirubin and direct bilirubin level was express as:

$$\text{Total bilirubin concentration (mg/dl)} = \frac{\text{Absorbance of sample x conc. of standard}}{\text{Absorbance of standard.}}$$

Determination of total protein

Determination of total protein was carried out according to the method described by Fine, (1935).

Principle

Protein in serum forms a violet coloured complex when reacted with cupric ions in an alkaline solution. The intensity of the violet colour is proportional to the amount of protein present when compared to a solution with known protein concentration.

$$\text{Protein} + Cu^{++} \longrightarrow \text{Coloured Complex}$$

Procedure

For total protein, 0.1ml of serum was diluted with 0.9ml of distilled water and 4.5ml of alkaline copper reagent was added and kept in room temperature for 10min. And then 0.5ml of Folin's reagent was added and the colour formed was read after 20 min at 640 nm.

$$\text{Total Protein concentration (g/dl)} = \frac{\text{Absorbance of sample x conc. Standard.}}{\text{Absorbance of Standard}}$$

Determination of serum cholesterol

Principle

Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase (Trindar, 1969).

Procedure

Zero point zero one millilitre (0.01ml) of distilled water, cholesterol standard and test serum was dispensed into test tubes labelled reagent blank, standard and sample respectively. Exactly 0.001ml of the cholesterol reagent (4-aminoantipyrine) provided was added to each of the test tubes and mixed. The mixture was incubated at 37°C for 10 min the absorbance of the sample and that of the standard were measured against the reagent blank at 500nm.The values obtained were used to calculate the total cholesterol concentration using the formula:

$$\text{Total cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of sample x conc. of standard}}{\text{Absorbance of standard}}$$

Determination of triglycerides in serum using calorimetric method

Principle

The triglyceride was determined after enzymatic hydrolysis with lipase. The indicator quinoneimine was formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase (Stein, 1987).

Procedure:

Exactly 0.01ml each of distilled water, triglycerides standard and test serum were dispensed into test tubes labelled reagent blank, standard and sample respectively. To each of the test tubes, 0.001ml of the randox TG enzyme reagent provided was added and mixed. The mixture was incubated at 37°C for 5min in a water bath. After the incubation period, the absorbance of the sample and that of the standard were read against the reagent blank within 60 min at 500nm.

$$\text{Triglycerides concentration (mg/dl)} = \frac{\text{Absorbance of sample x Conc. of standard}}{\text{Absorbance of standard}}$$

Determination of HDL-cholesterol in serum using calorimetric method

Principle

Low density lipoprotein (LDL and VLDL) and chylomicron fractions are precipitated qualitatively by the addition of phosphotungstic acid in the presence of magnesium ions.

After centrifugation, the cholesterol concentration in the high density lipoprotein (HDL) fraction, which remains in the supernatant, was determined (Trindar, 1969).

Procedure

Zero point five millilitres (0.5ml) of standard and 0.5ml of test serum were dispensed into test tubes labelled standard and sample respectively. Then 1ml of the precipitant provided was added to each of the test tubes and mixed thoroughly, then incubated for 10min at room temperature. After incubation, it was centrifuged at 4000rpm for 10min. The clear supernatant was separated from the precipitant and the cholesterol concentration was determine using the cholesterol reagent by dispensing 0.05ml of distilled water, 0.05ml of HDL-cholesterol standard and 0.05ml of sample were dispensed into the test tubes labelled reagent blank, standard and sample respectively. To each of these test tubes, 1ml of the cholesterol reagent (4-aminoantipyrine) provided was added and mixed. The mixture was incubated at 37^{o}C for 10min. After the incubation the absorbance of the sample and that of the standard were read against the reagent blank at 500nm.

$$\text{HDL-cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of sample x Conc. standard}}{\text{Absorbance of standard}}$$

Determination of LDL-cholesterol

The LDL-cholesterol was determined in accordance with the method described by Friedwald *et al.*(1972).

$$\text{LDL-cholesterol concentration (mg/dl)} = \frac{\text{Total cholesterol} - \text{Triglycerides}}{5 - \text{HDL cholesterol}}$$

Haematological parameters

Haematological profile of the experimental animals which includes white blood cell (WBC), red blood cell (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets, lymphocytes, neutrophils, monocytes and haemoglobin was determined using auto-haematology analyser (RT 7200, UK) (Sheth and Shah, 2015).

Principle

The Coulter Principle was used in every haematology analyser. Whole blood was passed between two electrodes through an aperture so narrow that only one cell can pass through at a time. The impedance changes as a cell passes through. The change in impedance is proportional to cell volume, resulting in a cell count and measure of volume.

Statistical analysis

Numerical data obtained from the study was expressed as Mean value ± standard error of mean (SEM). Differences among the means of control and test groups was determined using Statistical Package for Social Sciences (SPSS 24.0) and the data was further analysed using student t-test to compare them. The result was considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Qualitative phytochemical screening of the crude and degummed seed oil extracts of *Citrullus lonatus* showed that saponins, terpenoids, flavonoids, alkaloids and steroids were present in both extracts while tannins, glycosides and phenols were absent in both samples (Table 1). The same observation was documented by Rabiu and Muhammad, (2015) where they reported the presence of saponins, terpenoids, flavonoids, alkaloids and steroids in *Citrullus lonatus.*

Antioxidant activities were evaluated using ferric reducing antioxidant power, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and thiobarbituric acid reactive substances (TBARS) at concentration of 20 – 100mg/ml as shown in (Figures 3, 4, 5, and 6). It was observed that degummed seed oil extract exhibited slightly higher antioxidant activity compared to the crude seed oil extract and L-ascorbic acid. The scavenging activity can be explained by the presence of phenolic compounds which can undergo redox reaction and can be reducing agents, neutralizers of singlet oxygen and hydrogen donors which are relevant in free radical scavenging. Total phenolic content is a good indicator of antioxidant potency (Iqbal *et al*., 2005).The findings in this study seemed to agree with what was reported by Mogotlane, (2015) for lower percentage average antioxidant activity as 45.90% while the present study reported 41.25% having lower IC_{50} with higher percentage inhibition.

On the biochemical assessment, a sharp elevation of biochemical parameters such as ALT, AST, and ALP was observed in the negative control group compared to the positive group. Co-treatment of the test groups with the crude and degummed seed oil extracts of *Citrullus lonatus* showed a significant decrease in ALT, AST and ALP levels. This observation was similar to what was reported by Ahsan *et al.* (2009) that ALT and AST are present in high concentrations in CCl_4 damaged hepatocytes. Liver damage is linked with leakage of membrane contents of the cytosolic hepatocyte which causes elevation in serum levels of liver marker enzymes

Table 1: Phytochemical components detected in the crude and degummed seed oil extracts of *Citrullus lonatus*.

Phytochemicals	Crude seed oil extract	Degummed seed oil extract
Saponins	+	+
Tannins	_	_
Terpenoids	+	+
Flavonoids	+	+
Alkaloids	+	+
Glycosides	_	_
Steroids	+	+
Phenols	_	_

Key: + = Presence of phytochemical component; - = Absence of phytochemical component.

Table 2: Effect ofcCrude extract and Degummed Oil from seed of *Citrullus lanatus* on some biochemical parameters in CCl_4-induced liver Damage in Rats.

Group	AST (iu/L)	ALT (iu/L)	ALP (iu/L)	Total Proteins (g/L)	Albumin (g/L)	Bilirubin (mg/L)
Normal	271.50 ± 1.68	41.67 ± 1.73	85.67 ± 1.37	82.67 ± 0.66	42.33 ± 1.06	1.43 ± 0.02
Negative Control	370.00 ± 3.12^{a}	70.50 ± 1.44^{a}	115.00 ± 0.58^{a}	52.67 ± 1.73^{c}	28.00 ± 1.00^{c}	2.80 ± 0.05^{a}
Positive Control	275.50 ± 2.36^{b}	43.50 ± 1.36^{ab}	84.50 ± 1.54^{b}	81.00 ± 1.00^{e}	43.00 ± 1.00^{e}	1.30 ± 0.04^{b}
100 mg/kg bw CE	320.50 ± 1.68^{ab}	68.00 ± 2.24^{ab}	93.33 ±2.06^{ab}	53.00 ± 1.20	32.00 ± 2.65^{e}	2.60 ± 0.01^{ab}
200 mgkg /bw CE	310.00 ± 2.73^{ab}	61.50 ± 3.54^{ab}	90.50 ±1.61^{ab}	58.50 ± 0.71^{e}	35.33 ± 1.53^{e}	2.30 ± 0.06^{ab}
300 mg/kg bw CE	300.50 ± 1.95^{ab}	56.00 ± 0.00^{ab}	89.50 ± 0.71^{ab}	66.00 ± 2.57^{e}	37.33 ± 1.15^{ae}	1.90 ± 0.00^{ab}
100 mg/kg bw DE	290.00 ± 2.83^{abd}	55.67 ± 0.73^{abd}	90.50 ± 0.70^{abd}	70.00 ± 1.46^{ef}	38.00 ± 2.00^{ef}	1.80 ± 0.05^{abd}
200 mg/ kg bw DE	285.50 ± 3.36^{abd}	52.67 ± 0.04^{abd}	87.50 ± 1.54^{abd}	75.00 ± 0.00^{ef}	40.33 ± 2.08^{ef}	1.50 ± 0.02^{abd}
300 mg/kg bw DE	272.50 ± 1.09^{abcd}	40.47 ± 1.58^{abcd}	84.50 ± 2.19^{abd}	82.43 ± 0.0^{ef}	44.33 ± 0.93^{ef}	1.20 ± 0.00^{abd}

Key: Values are Mean ± SD, (n = 3)
[a]Significantly (p< 0.05) higher compared to normal control
[b]Significantly (p< 0.05) lower compared to negative control
[c]Significantly (p< 0.05) lower compared to positive control
[d]Significantly (p< 0.05) lower compared to crude extracts at the same concentration
[e]Significantly (p< 0.05) higher compared to negative control
[f]Significantly (p< 0.05) higher compared to crude extracts at the same concentration
CE= Crude Extract; DE= Degummed Extract

(AST, ALT and ALP) (Bhattacharyya *et al.,* 2003). It could be stated here that higher level of AST, ALT and ALP in the serum is an indication of the degree of damage to the liver caused by CCl_4. Administration of the crude and degummed seed oil extracts of *Citrullus lonatus* on the rats at various concentrations remarkably reduced carbon tetrachloride induced elevation of serum levels of ALT, ALP and AST as depicted in (Table 2). The reduction in the serum levels of these liver marker enzymes is an indication of the degree of protection against the hepatotoxic effect of CCl_4. The findings in this study agreed with results of Anthony, (2010) for effect of aqueous seed extract of *Citrullus lonatus* on ethanol induced liver damage. The findings also agree with was earlier reported by Kolawole *et al.* (2016) for elevated level of liver marker enzymes and subsequent decrease after administration of methanolic extract of *Citrullus lonatus* studied in alloxan induced diabetes in experimental animals. Increased ALT enzymes in CCl_4-induced liver toxicity were due to the loss of structural integrity of liver. Since this enzyme is localized in the cytoplasm, it will be released into the blood circulation after cellular damage resulting in its elevation. Increased level of AST enzymes indicated that CCl_4 ingestion to rats causes both plasma membrane and organelle membrane (mitochondria) damage (Sutha *et al*., 2010).

In this study hepatocellular damage induced by carbon tetrachloride in rat was also established based on significant decrease in total protein and albumin in the negative control compared to the positive control and treatment group as shown in (Table 2). Significant increment in serum levels of total protein and albumin was observed in animals treated with 100, 200 and 300 mg/kg body weight of degummed seed oil extract compared to animals treated with 100, 200 and 300 mg/kg body weight of crude seed oil extract. The elevation in plasma bilirubin level in the test group as compared to normal observed in this study suggested abnormal conjugation of bilirubin by the liver due to generalized hepatocellular damage which agreed with earlier report by El-Sherbiny *et al*., 2003. Bilirubin level was observed to decrease in carbon tetrachloride hepatotoxic rats after being treated with the standard drug and *Citrullus lonatus* seed oil extracts. A decrease in bilirubin levels in animals treated with 100, 200 and 300 mg/kg body weight of degummed seed oil extract as compared to those treated with 100, 200 and 300 mg/kg body weight of the crude seed oil extract was also

Table 3: Effect of crude and degummed seed oil extract of *Citrullus lonatus* on Lipid profile in Wistar Rats.

Group	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	High Density Lipoproteins (mg/dl)	Low Density Lipoproteins (mg/dl)
Normal	123.11 ± 1.32	46.89 ± 2.08	63.01 ± 1.04	50.72 ± 1.86
Negative Control	184.13 ± 0.39[a]	104.22 ± 0.58[a]	54.50 ± 1.58[e]	108.78 ± 0.00[a]
Positive Control	124.42 ± 1.15[b]	50.01 ± 1.53[b]	62.80 ± 2.83[f]	51.62 ± 1.00[b]
100 mg/kg bwt CE	165.41 ± 2.31[b]	80.22 ± 1.04[b]	56.41 ± 2.65	92.96 ± 0.06[b]
200 mg/kg bwt CE	150.14 ± 2.52[b]	70.14 ± 0.71[b]	57.91 ± 0.73[cf]	78.20 ± 1.41[b]
300 mg/kg bwt CE	145.39 ± 1.04[b]	65.61 ± 1.53[b]	59.22 ± 0.71[f]	75.05 ± 0.54[b]
100 mg/kg bwt DE	135.01 ± 2.83[bd]	60.24 ± 5.13[bd]	55.44 ± 0.51	67.38 ± 1.51[bd]
200 mg/kg bwt DE	130.51 ± 1.12[bd]	54.36 ± 2.65[bd]	61.31 ± 2.51[f]	58.33 ± 1.07[bd]
300 mg/kg bwt DE	123.37 ± 2.65[bd]	46.48 ± 1.29[bcd]	64.09 ± 1.93[f]	49.31 ± 1.00[bd]

Key: CE = Crude extract; DE = Degummed extract
Values are Mean ± SD, (n = 3)
[a]Significantly increased (p< 0.05) compared to normal control
[b]Significantly decreased (p< 0.05) compared to negative control
[c]Significantly decreased (p< 0.05) compared to positive control
[d]Significantly decreased (p< 0.05) compared to crude extract at the same concentration
[e]Significantly decreased (p< 0.05) compared to normal control
[f]Significantly increased (p< 0.05) compared to Negative Control

Table 4: Effects of crude and degummed seed oil extracts of *Citrullus lonatus* on Haematological parameters in Wistar Rats.

Group	WBC (x109/l)	RBC (x1012/l)	PCV (%)	Hb (g/dl)	PLT (x109/l)	MCV (fl)	MCH (Pg)	MCHC (g/dl)
Normal	10.50 ± 0.61	10.50 ± 0.56	58.47 ± 1.36	19.33 ± 1.00	680.00 ± 5.57	52.90 ± 0.61	18.33 ± 0.25	31.70 ± 1.53
Negative Control	21.15± 0.31[e]	6.91 ± 0.06[a]	38.43 ± 2.00[a]	12.10± 0.36[a]	450.50 ± 2.92[a]	67.70 ± 2.88[e]	28.70±0.60[e]	42.47 ± 1.15[e]
Positive Control	11.50 ± 0.50[f]	10.90± 0.69[b]	57.30 ± 2.49[b]	19.43± 0.74[b]	681.00 ± 2.70[b]	53.77 ± 1.95[f]	18.87±0.81[f]	32.10 ± 0.44[f]
100 mg/kgbwt CE	20.17 ± 0.16	7.18 ± 0.75	40.53 ± 1.50	13.20 ± 0.95	513.00 ± 3.60[b]	65.73 ± 0.85	26.40 ± 0.62	40.30 ± 0.69
200 mg/kgbwt CE	18.97 ± 0.57	7.30 ± 0.56	44.40 ± 1.08[b]	14.09± 1.33[b]	530.00 ± 3.15[b]	64.63 ± 1.01	25.33 ± 0.70	38.33 ± 0.81
300 mg/kgbwt CE	17.45 ± 0.46	8.23 ± 0.46[b]	47.80 ± 1.21[b]	15.55± 0.76[b]	570.33 ± 1.10[bc]	63.97 ± 1.53	23.30 ± 0.42	37.27 ± 0.23
100 mg/kgbwt DE	16.87± 0.40[h]	8.83 ± 0.12[b]	52.50± 2.16[bd]	16.10±0.50[bd]	601.50 ± 2.75[bd]	60.23 ± 2.37[h]	21.57±0.45[h]	35.50 ± 0.66[h]
200 mg/kgbwt DE	13.10± 0.96[h]	9.10 ± 0.50[bd]	56.23± 1.01[bd]	17.80±1.17[bd]	630.00±3.36[bcd]	56.90 ± 2.30[h]	19.83±0.80[h]	33.00 ± 1.01[h]
300 mg/kgbwt DE	11.51± 0.17[h]	10.54±0.78[bd]	58.53± 1.66[bd]	19.50±1.40[bd]	670.50 ± 3.13[bd]	51.97 ± 2.14[g]	16.83±0.64[gh]	31.47 ± 0.99[h]

Key: CE = Crude extract; DE = Degummed extract
Values are Mean ± SD, (n = 3)
[a]Significantly decreased (p<0.05) compared to normal control
[b]Significantly increased (p<0.05) compared to negative control
[c]Significantly increased (p<0.05) compared to positive control
[d]Significantly increased (p<0.05) compared to crude extracts at the same concentration
[e]Significantly increased (p<0.05) compared to normal control
[f]Significantly decreased (p<0.05) compared to negative control
[g]Significantly decreased (p<0.05) compared to positive Control
[h]Significantly decreased (p<0.05) compared to crude extract at the same concentration

observed. These findings were in agreement with studies reported by Damilola *et al.* (2016) for elevated level of bilirubin in paracetamol-induced hepatotoxicity in rats and decreased level after treatment with *C. lonatus* seed extract. Treatment with crude and degummed seed oil extracts of *Citrullus lonatus* revealed a dose dependent response in which pronounced responses are seen more in groups administered with 300mg/kg body weight as compared to 200 and 100 mg/kg body weight in this manner: 300mg/kg body weight>200mg/kg body weight>100mg/kg body weight (Table 3).

Further findings showed abnormally high concentration of lipids in carbon tetrachloride induced hepatotoxic rats (negative control group). This could be due to the increase in the mobilization of free fatty acids from the peripheral fat depots (Bopama *et al.,* 1997). The results showed that *Citrullus lonatus* crude and degummed seed oil extracts significantly (p<0.05) reduce TC, TG and LDL concentrations and significantly (p<0.05) increase the HDL concentration in carbon tetrachloride induced hepatotoxic rats in a dose dependent manner. The results also showed that *Citrullus lonatus* degummed seed oil extract showed higher concentrations of these lipids when compared with the crude seed oil extract. The mechanism of action of the seed oil extract of *Citrullus lonatus* on fat metabolism is uncertain. However, studies have shown that nitric oxide synthesized from arginine plays an important role in regulating the oxidation of fatty acids (Jobgen *et al.,* 2006) and *Citrullus lonatus* extract has been reported to serve as a bioavailable source of the precursor (i.e. citrulline) for arginine synthesis. Furthermore, this study seemed to agree with the findings by Damilola and Adekunle, (2016) whereby there was a significant (p<0.05) decreased in TG, TC and LDL with an increase level of HDL in acetaminophen intoxicated rats treated with *Citrullus lonatus* seed oil extracts (Table 4).

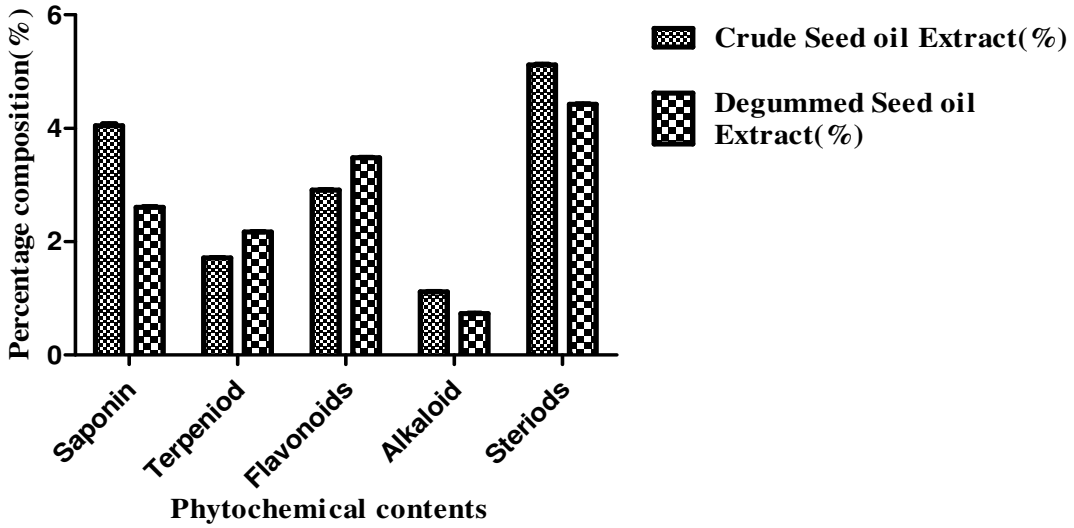

Figure 3: Concentration of phytochemical components in the crude and degummed seed oil extracts of *Citrullus lonatus.*

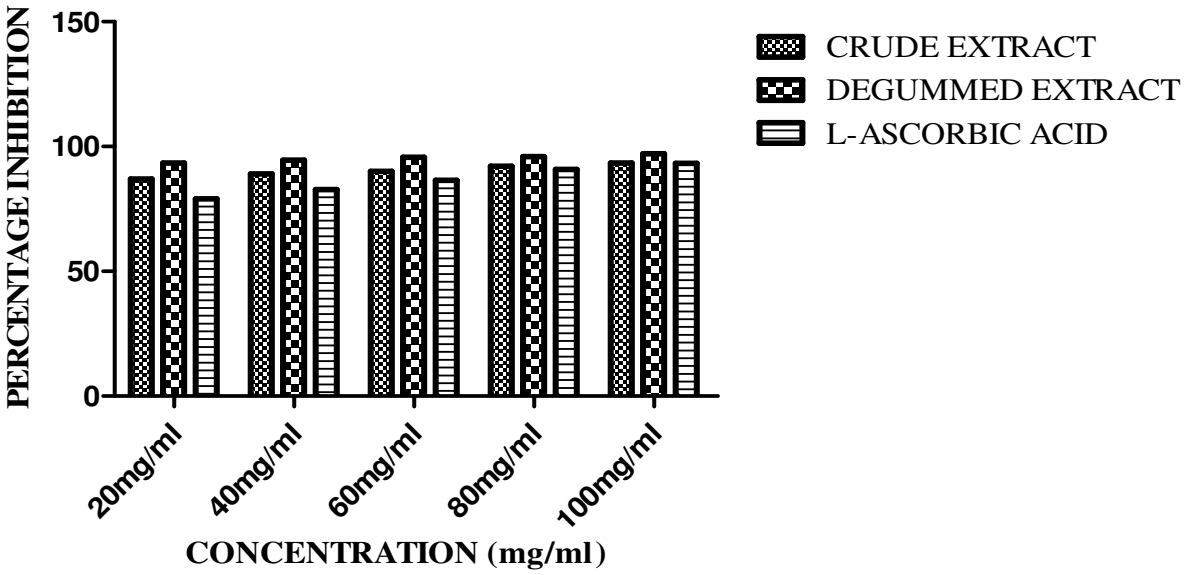

Figure 4: Ferric Reducing Antioxidant Power of crude and degummed seed oil extracts of *Citrullus lonatus.*

Studies on the haematological parameters (Table 4) showed significant increase in WBCs, PCV, MCH and MCHC carbon tetrachloride hepatotoxic rats. Treatment of the rats with the crude and degummed seed oil extracts showed a significant decrease in these parameters in a dose dependent manner. The results also showed significant decreased on RBC, PCV, Hb and PLT in the experimental animals which on treatment with the crude and degummed seed oil extracts showed significant increase in their levels in a dose dependent manner. The results also showed that degummed seed oil extract in all treatment groups significantly ($p<0.05$) exhibited higher effect when compared to the crude seed oil extract. The result in this study was also in agreement with findings by Adedeji *et al.* (2017) for significant increase in WBCs, MCV, MCH and MCHC and significant decreased in RBC, PCV, Hb and PLT of rats treated with acetaminophen and retreated with *Citrullus lonatus* seed

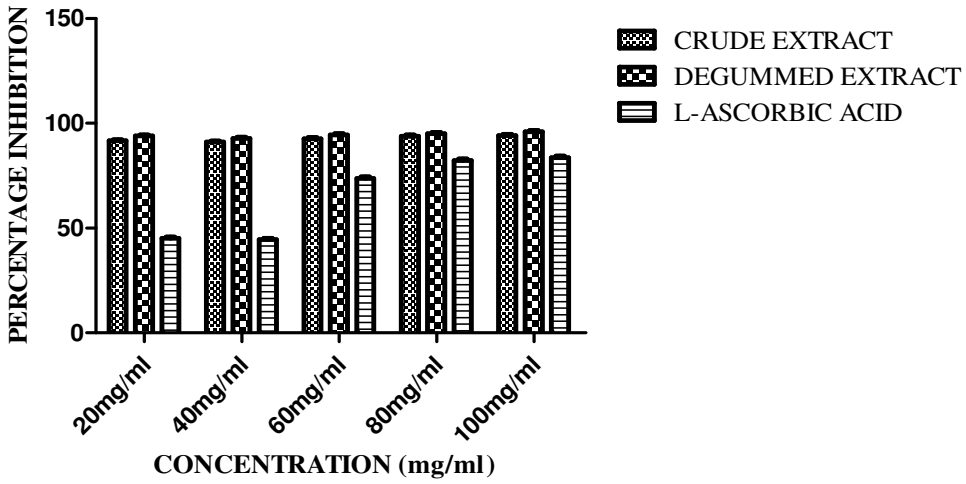

Figure 5: DPPH Radical Scavenging Activity of crude and degummed seed oil extracts of *Citrullus lonatus*.

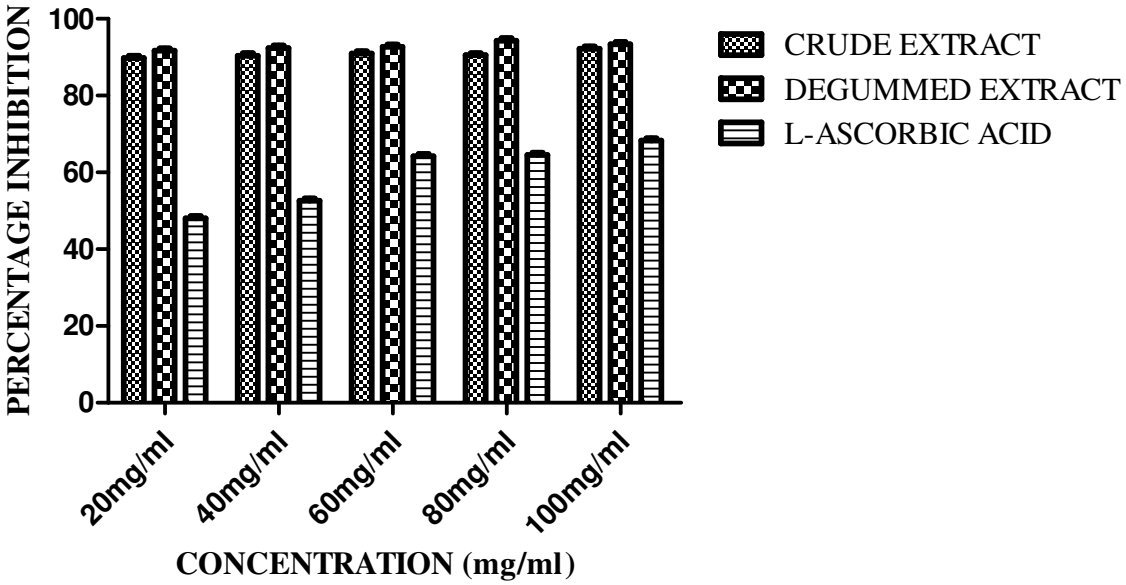

Figure 6: Thiobarbituric Acid Reactive Substances (TBARS) of crude and degummed seed oil extracts of *Citrullus lonatus*.

extract. Increased in WBCs, MCV, MCH and MCHC observed were probably due to harmful effects of the carbon tetrachloride on bone marrow and haematopoietic organs. White blood cells function primarily in body defence against foreign bodies and this is often achieved through leukocytosis and antibody production. The blood parameters Hb, PCV, RBC, WBC, MCH, MCV and MCHC provide information on the general state of the blood on the experimental animals used in the study. MCV, MCH and MCHC are also used in measuring the average size and haemoglobin composition of red blood cells (Ghosh *et al.,* 2012). However, *Citrullus lonatus* extract showed remarkable effects on all treatment groups administered with crude and degummed seed oil extracts. The observed effects of *Citrullus lonatus* seed oil extracts may be attributed to the presence of the different phytochemical components in the seed oil extracts such as saponnins, terpenoids, flavonoids, and alkaloids. The possible mechanism of action of *C. lonatus* seed oil extracts may also be through their anti-oxidative

effect. This is because *Citrullus lonatus* possesses bioactive compounds that are capable of free radical scavenging in living system.

Conclusion

In conclusion, this study has demonstrated that the crude and degummed seed oil extracs of *Citrullus lonatus* contained various secondary metabolites such as saponins, terpenoids, flavonoids, alkaloids, and steroids. Degummed seed oil exhibited slightly higher antioxidant activity when compared to the crude seed oil extract and L-asccorbic acid. Both crude and degummed seed oil extracts of *Citrullus lonatus* exerted their ameliorative effects by significantly improving the status of the liver marker enzymes, lipid profile and haematological parameters in carbon tetrachloride hepatotoxic rats in a dose dependent manner.

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