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Chemical Composition, *In vivo* Immunomodulatory and Anti-Hyperlipidaemic Properties of R*hinoceros* (Rhino) Oil in Lead-Induced Immunocompromised Models

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ABSTRACT

A knowledge of the chemical content of *Rhinoceros* (Rhino) oil and its activity on selected biomolecules of experimental models would help boost the immune system against an immunocompromised COVID-19 status. The study seeks to evaluate the chemical and biomolecular profile of Rhino oil. Chemical profile was done using standard methods of analysis. 25 rats were assigned in five groups (A-E) (n=5). Animals in group A (control) were administered 0.5 ml of distilled water while those in groups B-E which were immunocompromised (by intraperitoneal administration of 5 mg/kg body weight (b.w) of lead [Pb]) were also administered distilled water, immunomodulatory drug (5 mg/kg body weight of zinc [Zn]), 2 and 5 mg/kg b.w of Rhino oil extract respectively, once daily for 8 days followed by biomolecular assay. Proximate analysis gave moisture content (14.37±0.29), among others. FAMEs analysis showed hexadecanoic acid (12.80%) and other esters. Lipid profile of the oil gave LDLC to contain (32.90±0.53 mg/L), and others. The physicochemical properties gave iodine value as (115.80±0.40 mg/g), among others. The metal composition revealed Zn (0.28±0.06) plus others. The amnio acid profile of the oil gave ten essential amino acids and non-essential amino acids respectively. The levels of biomolecules in serum of the animals were altered at specific doses of the oil extract. Altogether, the chemical content of the oil was significantly high, with altered biomolecular effect. The rich content of vital nutrients and chemicals of Rhino oil may boost the white blood cells against COVID-19. The isolation and characterization of the active principles of the oil is encouraged.

Keywords: *Rhinoceros* (Rhino) oil, *Rhinocerotidae*, COVID-19, Immunosuppression, Biomolecules, American Oil Chemists' Society (AOCS).

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INTRODUCTION

The kingdom animalia is a large group that consists of eukaryotic, multicellular organisms that are heterotrophic in nature. Animals are divided into two main groups namely, vertebrates (animals with a backbone) and invertebrates (animals that lack a backbone). Vertebrates include all animals classified under the subphylum Vertebrata [1]. They belong to the phylum Chordata and possess a backbone/vertebra (where the spinal cord is located). Vertebrate animals are classified into seven main classes including mammalia, aves/birds, reptilia, amphibia, agnatha, osteichthyes, chondrichthyes while the invertebrates comprise of only one phylum, invertebrates make up the remaining phyla of the kingdom animalia. As the name suggests, invertebrates lack a backbone and internal skeletons [2]. However, some of the species have an external skeletal system, the exoskeleton, that provides structural support. Currently, invertebrates are suggested to make up over 97 percent of all animals in the animalia kingdom [3].

Rhinoceros (rhinos), Family: *Rhinocerotidae* are thought to be the second largest land animal, with the elephant being the largest. They have a robust cylindrical body with large head, relatively short legs and short tail. When a person mentions a rhinoceros, the first image that comes to mind is the animals' glorious horn. This horn is made up of strong fibres known as keratin. This same protein can be found in human skin, hair and nails [4, 5]. Most rhinos use their horns for territorial combat or as a tool to reach sought after morsels. Most rhinos have characteristic flaps of skin that cover their necks and hips. This skin is very thick and tough, to protect against sharp foliage and the occasional predatory attack. In the larger rhinoceros, this skin also protects a layer of subcutaneous fat. This additional layer protects these massive creatures from overheating and freezing in extremely cold temperatures [6]. This "armor" has also been the prize of poachers for centuries. Two lip separate lip structures offset the black rhino from the rest of the "herd". Black rhinos, or hook lipped, are identified by their pointed lip use to cut branches and grabbing saplings. White rhinos have a square or flat lip more appropriately suited grazing, though the sumatran rhino actively browsed with this mastication method [7].

Rhinoceroses (rhinos) are among the most primitive of the world's large mammals, and in prehistoric times were common large herbivores in North America. Five extant species exist in four genera; in Africa, the white (*Ceratotherium simum*) and black (*Diceros bicornis*) rhinos and in Asia, the Sumatran (*Dicerorhinus sumatrensis*), Indian (*Rhinoceros unicornis*), and

Javan (*Rhinoceros sondaicus*) rhinos. The Sumatran rhino is the most primitive and predates the extinct woolly rhino (*Coelodonta antiqitatis*), which inhabited in northern Europe and Asia during the last ice age [8].

Biomolecules are substances generated by cells and living organisms for specific biological processes, such as cell division, morphogenesis and development. The four major types of biomolecules are carbohydrates, proteins, lipids and nucleic acids [9].

The novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the pandemic chiefly called coronavirus disease 2019 (COVID-19) and first identified in December 2019 in Wuhan, China from cluster of patients [10]. The novel coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 has raised plethora of global concerns. Despite these concerns, there is currently no specific treatment regimen for COVID-19 [11]. Therefore, identifying chemical constituents, from organisms, with immune boosting capacity could serve as a breakthrough in the fight against COVID-19. This study which was born from the folklore use of *Rhino oil*, without scientific backing seeks to investigate for the first time, the probable capacity of the chemical constituents of *Rhinoceros* oil to manage covid-19 and its effect on biomolecules of rats.

MATERIALS AND METHODS

MATERIALS

Study Area

The study area for the research is Bingham University, Karu. Karu is one of the thirteen Local Government Areas of Nasarawa State, Nigeria and lies between latitude 9.0167 of the equator and longitude 7.5833, 111 N 9’ 0’ E 7 3 4’ 60" of the Greenwich Median [12].

Assay Kits, Chemicals and Drugs

The assay kit for serum lipid profile of Rhino oil and those of the animals as well as those of total and cojugated bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were products of Randox Laboratories Ltd., Co-Antrim, UK. Lead (Pb), Zinc (Zn) and other chemicals were products of Healthy Life Pharm Pvt. Ltd, Thane, India; Merck Sante, France and Agappe Diagnostics Ltd, Kerala, India respectively.

Reagents, Chemicals and Apparatus

All the chemicals and reagent used were of JHD China and were mostly of analytical grade. The apparatus used includes sample bottles, measuring cylinder, beakers, hot plate, cover glass, crucible and thermometer.

METHODS

Extraction of Rhino oil

The rhino fat was extracted locally by villagers, from the body of the rhino. After a few minutes of exposure to heat, the fat turns to oil and later processed as described by [13, 14]. 5 cm^3 of Conc. HNO_3 were added for preservation.

Determination of Metal Composition in Rhino Oil

Preparation of Sample

All plastics and glass wares were washed with tap water, soaked in a 10% mixture of HCl – HNO_3 for two days, then rinsed with tap and deionized water. Stock solutions of the various heavy metals to be determined were prepared using 10 cm^3 of nitric acid and dilute to the mark in a 1liter flask from which the working standard solution of the metals were prepared by serial dilution; for the various heavy metals according to their various working standard.

Sample Digestion

Digestion of oil was carried out by adopting the procedure described by [15, 16]. A known volume (3 cm^3) of concentrated HNO_3 was added to 100 cm^3 of the preserved oil, covered with a glass, heated gradually on hot plate and continuously added until digestion was completed. The solution was thereafter evaporated to near dryness and cooled. 5 cm^3 of 1:1 Conc. HCl was then added to the digested oil sample, warmed, filtered in 25 cm^3 volumetric flask and made up to the mark with deionized water.

Sample Measurement

The digested oil sample was subsequently determined for the presence of heavy metals, by atomic absorption spectrophotometer (AAS VGB 210 system). Analysis of the digested oil sample was done in triplicate and was carried out by AAS. Internally added standards were used for calibration of the AAS. A blank was used in the AAS quantitative determination of the heavy metals. A recovery study was also carried out on two of the oil samples understudy by spiking one of the oil samples with known amount of solution (1 cm^3) of the heavy metal to be determined before digestion, in order to determine the recovery [17]. All the values were recorded.

Principle: Atomic absorption spectrophotometer can be used to determine presence of metals using light absorption and wavelength.

Fatty Acid Methyl Esters (FAMEs) Composition of Rhino Oil Using GC/MS

Fatty Acid Methyl Ester Preparation: Fatty acid methyl esters (FAMEs) were prepared according to [18]. An aliquot (1 mL) of total lipids was evaporated in a tube of methylation. Fatty acids were saponified with 10mL of

methanolic sodium hydroxide solution (0.5M) for 15 min in a boiling water bath at 65 ^{0}C. For transmethylation, the mixture was homogenized with 10mL of methanolic solution of BF3 (20%, w/v), and the reaction was allowed to proceed for 5 min. FAMEs were extracted twice with 10mL of petroleum ether and 10mL of water being added to the mixture.

Gas Chromatography and Gas Chromatography-Mass Spectrometry Analyses: FAMEs were analyzed using gasl-iquid chromatography (model HP 6890; Agilent, Palo Alto, CA, USA) equipped with a flame ionization detector. An SP-2560 fused silica capillary column (i.d., 100m× 0.25 mm; film thickness, 0.2 μm; Supelco, Inc., Bellefonte, PA, USA) was used. The column parameters were as follows: initial column temperature was held at 40 ^{0}C for 5min after injection, 2 ^{0}C/min to 220 ^{0}C, and finally the temperature was held at 220 ^{0}C for 30 min. Helium was the carrier gas, with the column inlet pressure set at 17 psi. The detector temperature was 250^{0}C. For identification purposes, these analyses were also performed with a gas chromatograph (model HP 6890; Agilent) coupled with a 5973mass spectrometer detector (Agilent) by using the same column described previously.

FAMEs were identified by using standards (Supelco 37 Component FAME mix; Supelco Bellefonte, PA, USA) and comparing their mass-spectral data with the mass-spectral database in the Wiley 7.0 library (HP Mass Spectral Libraries, Palo Alto, CA, USA) [19]. The conjugated linoleic acid peaks were identified by comparison with the retention times of the reference standard (conjugated linoleic acid methyl ester, a mixture of *cis*-9, *trans*-11 octadecadienoic acid methyl ester and *cis*-10, *trans*-12 octadecadienoic acid methyl ester; Sigma Chemical Co.). Fatty acid contents were expressed as the proportion of each individual fatty acid to the total amount of all fatty acids present in the sample.

Physicochemical Analysis of Rhino Oil

The determination of physicochemical properties in rhino oil for the presence of saponification value, ester value, peroxide value, free fatty acid value, acid value and iodine value were conducted using the methods of [20].

Saponification Value

The saponification value is the number of mg of potassium hydroxide (KOH) to saponify the esters in 1 g of the sample and neutralize the free acids in 1 g of a sample.

Procedure

A total of 1 g of sample was weighed accurately and transferred into a conical flask, added with 40 ml of ethanol, and dissolved while warming if necessary. 20 ml of ethanolic potassium hydroxide was added. The flask was equipped

with a reflux condenser, and heated in a water bath for 30 minutes while shaking the flask occasionally. It was allowed to cool, few drops of phenolphthalein TS were added, and immediately titrated using excess potassium hydroxide and 0.5 M hydrochloric acid. A blank test was performed, and the saponification value was calculated by the formula:

Saponification value =

$$\frac{(a - b) \times 28.05}{\text{Weight (g) of the sample}}$$

Where:

a = volume of 0.5 M hydrochloric acid consumed in the blank test;

b = volume (ml) of 0.5 M hydrochloric acid consumed in the test.

Acid Value

Is the number of mg of potassium hydroxide (KOH) required to neutralize 1 g of a sample.

Procedure

A total of 1 g of sample was weighed accurately; 50 ml of an ethanol-ether mixture was added (1:1). Dissolve while heating if necessary and the solution was used as test solution. It was allowed to cool, few drops of phenolphthalein TS were added, titrated with 0.1 M ethanolic potassium hydroxide until the pink colour of the solution persists for 30 seconds, and the acid value was calculated by the formula below. Before using the solvent, 0.1 M ethanolic potassium hydroxide was added to the solvent until its pink colour persists for 30 seconds, using phenolphthalein TS as an indicator.

Acid value =

$$\frac{\text{Vol. (ml) of 0.1 Mol/L ethanolic potassium hydroxide consumed} \times 5.611}{\text{Weight (g) of the sample}}$$

Ester Value

Is the number of mg of potassium hydroxide (KOH) required to saponify the esters in 1 g of a sample?

Procedure

Unless otherwise specified, the saponification and the acid values were determined, and the ester value was calculated by the following formula:

Ester value = Saponification value – Acid value

Peroxide Value

Is usually applied to edible product including tallow and associated product such as margarine shortenings and fried fats. It is also applied to the fat in meat and meat product. It is used as an indicator of the extent of oxidative rancidity. It

is affected by the age of raw material as well as oxidation of fat during processing and storage. It reflects the palatability of the oil.

Procedure

5.0 g of melted fat was added in a 250 ml stoppered conical flask. While the fat was still liquid, 30 ml of acetic acid-chloroform solution was added and the flask was swirled to dissolve the fat in the solvent. 0.5 M of saturated KI solution was added from a 1 ml graduated pipette. Stopper the flask and allow the mixture to stand with occasional shaking for exactly 1 min. After 1 min, few drops of distilled water were added. About 5 drops of starch indicator solution was also added. Titrate with 0.01 M $Na_2S_2O_4$ solution. The flask was shaked vigorously during the titration to extract iodine from the chloroform layer. The end point occurred when the purple colour disappeared. For peroxide values > 10 meq/1000 g, it was titrated with 0.1 M $Na_2S_2O_4$. A blank determination of the reagents was performed. The blank titration should not exceed 0.1 M of 0.01 M $Na_2S_2O_4$. The calculation is shown below:

Peroxide value (meq/1000 g) =

$$\frac{(A - B) \times M \times 1000}{W}$$

Where:

Titration value of sample (ml of thiosulphate) = A

Titration value of blank (ml of thiosulphate) = B

Molarity of $Na_2S_2O_4$ = M

Weight of sample (g) = W

Iodine Value

Procedure

0.5 g of the sample was accurately weighed into a tarred 250 ml iodine flask. Using the dispensing device, 10 ml chloroform was added. Dissolution was done by swirling (NOTE: 50% aqueous products will not dissolve completely until Hanus solution was added). 25.0 ml Hanus solution was accurately added as well as the stopper which was immediately added. It was swirled to mix thoroughly and placed in the dark. By Simultaneous preparation, a corresponding reagent blank containing 10 ml chloroform and 25 ml Hanus solution was added. The stopper was immediately used and the flask was placed in the dark with the test sample. After 60 minutes, using the dispensing device, 20 ml 10% potassium iodide was added. By graduated cylinder, 100 ml water was used in rinsing the neck and side walls of the flask during addition of the water. Immediately, the solution was titrated using 0.1 N sodium thiosulfate until the aqueous layer's colour

began to lighten. Using the dispensing device, approximately 3 ml starch solution was added and the titration continued until the blue colour of the aqueous layer began to disappear. Towards the end of the titration, at intervals, stopper of the flask was done and shaken vigorously to extract any iodine remaining in the chloroform layer. When the end point appears to have been reached, it was again stoppered and shaken vigorously, the layer was allowed to separate and two drops of starch solution was added to verify that no blue colour was formed in the aqueous layer. The burette was read accurately.

Iodine value =

$$\frac{\text{(ml blank - ml sample) x N sodium thiosulfate x 12.69}}{\text{Weight (g) of the sample}}$$

Free Fatty Acids

Procedure

The free fatty acids were measured by extracting the sample with hot ethyl alcohol and titrating the supernatant alcohol layer with standard alkali. For python fat, the free fatty acids were normally calculated as oleic acid.

Free fatty acids as oleic acid (%) =

$$\frac{\text{mL of 0.1 N Na0H x 0.0282 x 10}}{\text{Weight of sample (g)}}$$

NOTE: For the ethyl alcohol, 95% must give a sharp end point with phenolphthalein. The alcohol was immediately neutralized before using. Heated 15mL of alcohol containing 0.4ml of phenolphthalein indicator was adopted as the incipient boiling and 0.1 N sodium hydroxide solution was drop-wisely added until a faint pink colour persisted.

Determination of AminoAcids in Rhino Oil

Rhino oil aliquots containing around 8–12 mg of proteins was placed in a 20-mL cuvette and mixed with 9mL of 6M HCl [21]. After sealing the cuvette, the samples were hydrolyzed at 110^0C for 24h under N_2. The hydrolysates were transferred into a 100mL volumetric flask, mixed with 9mL of 6M NaOH, and diluted with 0.02N HCl. Then, all the samples were filtered and loaded in a Hitachi L-8800 amino acid analyzer (Tokyo, Japan) for amino acid analysis [22, 23].

Derivatized amino acids were eluted from the column with increasing concentrations of acetonitrile. The eluate was monitored at 254 nm and the areas under the peaks were used to calculate the concentrations of the unknowns using a Pierce Standard H amino acid calibration mixture (Rockford, IL).

Determination of the Proximate Composition of the Rhino Oil

Moisture Content

The moisture content was determined according to of the Association of Official Analytical Chemists [24, 25]. Here, 2 g of the oil sample was transferred into a pre-weighed dish. The sample was then placed into an oven at 105 ^{0}C until a constant weight was obtained. After drying, the sample was removed and transferred to a desiccator and cooled to room temperature before reweighing. Triplicate results were obtained for each sample and the mean value was reported to two decimal points according to the following formula:

$$Moisture\ Contents\ (\%) = \frac{W1 - W2\ X\ 100\%}{W1}$$

where;

W1= Sample weight before drying

W2 =Sample weight after drying.

Ash Content

The ash content of the desert date was determined according to the method described by [26] and adopted by [27]. Here, 5 g of the dried oil sample was transferred into a pre-weighed and tarred porcelain crucible and placed into a muffle furnace (Yamato, DX-302C) at 550^{0}C until a white-gray ash was obtained. The crucible was the transferred to a desiccator where it was allowed to cool to room temperature and the reweighed. The ash content was then calculated as a percentage based on the initial weight of the sample using the following formula:

$$Ash\ Contents\ (\%) = \frac{(Weight\ of\ Crucible + Ash) - (Weight\ of\ Crucible)\ X\ 100\%}{Initial\ Weight\ of\ the\ Sample}$$

Fat Content

The fat content of the oil sample was determined according to the official method of [25] AOAC (2008). Here 5 g of the oil sample was transferred into an extraction thimble. The extraction thimble was then positioned into the Soxhlet attachment, with the pre-weighed round-bottomed flask containing 90 ml of petroleum ether. The flask was then fitted under the Soxhlet attachment and the whole set-up was placed on a heating mantle for 6 hours at 100 ^{0}C. At the end of the extraction period, the flask was disconnected from the unit and the solvent was redistilled. Later, the flask with the remaining crude petroleum ether extract was cooled to room temperature in a desiccator and then reweighed. The fat content was then calculated using the following formula:

$$Fat\ Content\ (\%) = \frac{(Weight\ of\ Flask + the\ Extract) - (Weight\ of\ Empty\ Flask)\ X\ 100\%}{Initial\ Weight\ of\ the\ Sample}$$

Crude Protein Content

The crude protein content of the oil sample was determined by the micro-Kjeldahl method using a copper sulphate-sodium sulphate catalyst in accordance with the official method of the [25] AOAC (2008). Here, 2 g of the sample, 4 g of the Kjeldahl catalyst (Na_2SO_4) and 25 ml were transferred into a Kjeldahl digestion flask. The flask was then placed into a Kjeldahl digestion unit for about 2 hours until a colorless digest was obtained, and then the flask was left to cool down to room temperature. Distillation of ammonia was carried out into 25 m1 of 2% boric acid using 20 m1 distilled water and 70 m1 of 45% sodium hydroxide solution. Finally, the distillate was titrated with standard solution of 0.1 N HCl using 3 drops of bromocresol green as indicator, until a brown reddish color was observed. Using the same procedure, a blank test was analyzed using water instead of the test sample. The percentage nitrogen content of the sample was calculated as follows, as adopted by [28]:

$$\% \text{ Nitrogen by Weight} = \frac{\dfrac{(Vs - Vb)\ HCl\ consumed\ x\ N\ HCl\ x\ 1.4007\ X}{100}}{Sample\ Weight}$$

Percentage Crude Protein = % N x 6.38

Crude Fiber Content

The percentage crude fiber of the desert date kernel was determined according to the official method of the [25] AOAC (2008). Here, 2 g of the oil sample was placed into a conical flask containing 20 m1 of 2% H_2SO_4, and the flask was then fitted to a condenser and allowed to boil for 30 minutes. After this digestion, the flask was removed and the digest filtered under vacuum

through a proclain filter crucible, with the precipitate repeatedly rinsed with distilled boiled water followed by boiling in 20 ml 2% NaOH solution for 30 minutes under reflux condenser and the precipitate was filtered, rinsed with hot distilled water, followed by 20 m1 0f 96% ethyl alcohol and 20 ml diethyl ether. The crucible was finally dried overnight to a constant weight at a temperature of 105 ^{0}C, then cooled in a desiccator, reweighed, and then ashed in a muffle furnace at a temperature of 550 ^{0}C until a constant weight was obtained, and the difference in weight was considered as the crude fiber content. The percentage fiber content was calculated using the formula as reported by [29].

$$Fiber\ Content\ (\%) = \frac{(Wt.\ of\ Cruc. + Dry\ Residue) - (Wt\ of\ Cruc. + Ignited\ Residue)\ X\ 100\%}{Weight\ of\ the\ Sample}$$

Experimental Animals

Albino rats (*Rattus norvegicus*) weighing 151.23±2.72 g was obtained from the animal

house of National Veterinary Research Institute, VOM, Jos, Plateau State, Nigeria. The animals which were housed in aluminium cages placed in well ventilated standard housing conditions (temperature: 28-31 ^{0}C; photoperiod: 12 hours; humidity: 50-55%) were was allowed free access to rat pellets (Vital Feed®, Grand Cereals Ltd, Jos, Plateau State, Nigeria) and tap water.

Preparation of Aqueous Extract of *Rhinoceros* (Rhino) Oil

A solution comprising Rhino oil (50 ml) and distilled water (150 ml) to give an aqueous-rhino oil solution was transferred into a steam bath (Model: NL-420S, NEWLIFE® Medical Instrument, England) to concentrate. This gave a brownish-black residue (extract). The extract was then reconstituted in distilled water to give the required doses (2 and 5 mg/kg body weight) used in the present study.

Induction of Immunosupression and White Blood Cell (WBC) Count Determination

After eight hours of fast (without food, but water), white blood cell count of the animals was determined before the administration of lead (Pb) by drawing blood samples (0.6 µL) from the sharply cut tail vein using Auto Haematology Analyzer (Model RT-7200, Rayto Life & Analytical Sciences Co., Ltd, China). Thereafter, Pb (5 mg/kg body weight), in normal saline, was administered intraperitoneally to induce immunosuppression in rats [30] (Nwokocha *et al*., 2012). WBC count was then determined after 48 hours of Pb administration. Only animals with WBC count lower than those of the normal control rats were declared immunocompromised and used for the immunological study as well as lipid profile and other related assays.

Animal Grouping and Administration of Extract

Twenty-five rats weighing 151.23±2.72 g was assigned in five groups (A-E) (n=5). Animals in group A (control) were administered 0.5 ml of distilled water while those in groups B-E which were immunocompromised (by intraperitoneal administration of 5 mg/kg body weight of lead [Pb]) were also administered distilled water, immunomodulatory drug (5 mg/kg body weight of zinc [Zn]), 2 and 5 mg/kg body weight of Rhino oil extract respectively, once daily for 8 days during which monitoring of WBC count, lipid profile, function indices and other haematological assays of the animals was done.

Determination of Full Blood Count in Rhino oil

Blood collected in EDTA bottles were investigated for red blood cell (RBC) and their indices: haemoglobin (Hb), and packed cell volume (PCV) among others as well as white

blood cell (WBC) and their differentials: platelet (PLT), and lymphocytes (Lym) among others using Auto Haematology Analyzer.

Collection of Blood, Preparation of Plasma and Serum

The rats were anaesthesized in a glass jar containing cotton wool soaked in diethyl ether. Two mls of blood was collected into sample bottles containing Ethylene Diamine Tetraacetic Acid (EDTA) for the analysis of haematological parameters. Blood (5 ml) collected in plain bottles was centrifuged (using High Speed Centrifugal Machine, Model: YXJ-2, Essex, England) at 2000 g for 10 minutes and serum used for the biochemical analyses.

Determination of Selected Serum Biomolecules in Animals

The levels of some biomolecules were determined by adopting the protocols previously described for serum total and conjugated bilirubin [31], serum total cholesterol [32], serum triglyceride [33], high-density lipoprotein cholesterol [34], low-density lipoprotein cholesterol [35]. The activities of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase of the animals were determined according to the procedures described by [36] and [37] respectively.

Data Analysis

Results were expressed as the mean of three determinations for chemical profile and five determinations for *in vivo* study ± SEM. Significant difference was determined by analysis of variance and Duncan's Multiple Range Test at 5% confidence level using SPSS 23.0 Software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

RESULTS

The spectrum profile of GC-MS for rhino oil confirmed the presence of four major fatty acid methyl ester components namely; hexadecanoic acid (12.80%), 6-octadecenoic acid (82.29%), n-tridecan-1-ol (2.72%) and phenol-2,2-methylenebis [6-(1,1-dimethyl ethyl)-4-methyl-1-ol] (2.91%) (Table 1).

Table 1: Fatty acid methyl ester (FAMEs) of *Rhinoceros* oil

FAMEs composition	% of FAMEs
Hexadecanoic acid	12.80
6-octadecenoic acid	82.29
n-Tridecan-1-oil	2.72
Phenol-2,2-methylenebis [6-(1,1-dimethyl ethyl)-4-methyl-1-ol]	2.91

Data are mean ± SEM of three determinations

Lipid profile of Rhino oil, all in mg/l, gave low density lipoprotein cholesterol to contain (32.90±0.53), high density lipoprotein cholesterol (51.90±0.15), triglyceride (13.91±0.40), and total cholesterol (87.60±0.32) (Table 2).

Table 2: Lipid profile of *Rhinoceros* oil

Parameter	Value (mg/L)
LDL Cholesterol	32.90±0.53
HDL Cholesterol	51.90±0.15
Triglyceride	13.91±0.40
Total Cholesterol	87.60±0.32

Data are mean ± SEM of three determinations

The physicochemical properties of the oil showed its iodine value to be (115.80±0.40 mg/g), peroxide value (358.04±0.50 mEq/kg), acid value (12.90±0.20 mgKOH/g), free fatty acid value (10.70±1.01 % oleic acid), saponification value (244.04±0.80 mgKOH/g) and ester value (231.14±0.60 mgKOH/g) (Table 3). The values of iodine, acid, free fatty acid, saponification, ester and peroxide were found to be higher than FAO/WHO standard. The saponification value (244.04±0.80 mgKOH/g) occurred within the FAO/WHO limit (Table 3).

Table 3: Physicochemical properties of *Rhinoceros* oil

Parameter	Value	FAO/WHO standard
Iodine value (mg/g)	115.80±0.40	80-106
Peroxide value (mEq/kg)	358.04±0.50	<10
Acid value (mgKOH/g)	12.90±0.20	4
Free fatty acid value (% oleic acid)	10.70±1.01	5.78-7.28
Saponification value (mgKOH/g)	244.04±0.80	195-250
Ester value (mgKOH/g)	231.14±0.60	150-170

Data are mean ± SEM of three determinations

The proximate content of Rhino oil showed moisture content to be (14.37±0.29), crude fibre (9.24±0.40), crude protein (3.86±0.15), ash (13.64±0.91), fat (6.13±0.52) and carbohydrate (52.75±1.03) (Table 4). Carbohydrate had the highest value while crude protein recorded the least.

Table 4: Proximate analysis of *Rhinoceros* oil

Parameter	Value
Moisture content	14.37±0.29
Crude fibre	9.24±0.40
Crude protein	13.86±0.15
Ash	3.64±0.19
Fat	6.13±0.52
Carbohydrate	52.75±1.03

Data are mean ± SEM of three determinations

The metal composition of Rhino oil, in mg/g of oil, showed Mn (1.10±0.98), Mg (58.38±1.60), Ni (0.66±0.28), Fe (50.64±0.73), Na (757.14±3.57), Cr (8.68±0.91), Zn (0.28±0.06), Cu (5.34±0.03) and Co (0.06±0.01) while lead (Pb) and cadmium (Cd) were not detected (Table 5). There was significant ($p< 0.05$) increase in the mean concentration value of Mn, Mg, Ni, Fe and Cr when compared with the WHO standard while Cu and Zn was lower. Cobalt was within the WHO standard (Table 5).

Table 5: Metal composition of *Rhinoceros* oil

Metal	Amount (mg/g of oil)	WHO standard (mg/L)
Mn	1.10±0.98	0.4
Mg (ppm)	58.38±1.60	5.0
Ni (ppm)	0.66±0.28	0.05
Pb	ND	0.01
Fe	50.64±0.73	0.5
Na	757.14±3.57	NA
Co	0.06±0.01	0.05
Cr	8.68±0.91	0.05
Zn	0.28±0.06	3.0
Cd (mg/m^3)	ND	0.05
Cu	1.34±0.03	2.0

Data are mean ± SEM of three determinations
WHO = World Health Organization**; Mg** = Magnesium**; Co** = Cobalt**; Zn** = Zinc**; Fe** = Iron**; Pb** = Lead**; Na** = Sodium**; Ni** = Nickel**; Mn** = Manganese**; Cr** = Chromium**; Cd** = Cadmium**; ppm** = Part per million**; ND** = Not detected**; Cu** = Copper**; NA**= Not available
WHO (2006)

The amino acid profile of the oil gave ten essential amino acids including arginine (1.12%), isoleucine (1.30%) leucine (1.19%), lysine (0.98%), methionine (0.45%), phenylalanine (0.72%), threonine (0.89%), tryptophan (0.95%), histidine (0.48%) and valine (0.37%) as well as ten non-essential amino acids which include serine (0.18%), proline (1.77%), alanine (0.54%), asparagine (1.25%), trimethyl serine (0.63%), glutamate (0.23%), glutamine (0.58%), glycine (1.06%), aspartate (0.57%) and tyrosine (0.28%) (Table 6).

Table 6: Amino acid profile of *Rhinoceros* oil

S/N	Amino Acid	Percentage Composition (%)
1	*Arginine	1.12
2	Serine	0.18
3	*Isoleucine	1.30
4	*Leucine	1.19
5	*Lysine	0.98
6	*Methionine	0.45
7	*Phenylalanine	0.72
8	*Threonine	0.89
9	*Tryptophan	0.95
10	Proline	1.77
11	Alanine	0.54
12	Asparagine	1.25
13	*Histidine	0.48
14	Trimethyl serine	0.63
15	Glutamate	0.23
16	Glutamine	0.58
17	Glycine	1.06
18	*Valine	0.37
19	Aspartate	0.57
20	Tyrosine	0.28
TEAA		8.45 (50.86%)
TNEAA		7.09 (49.14%)

* Essential amino acids, **TEAA** = Total essential amino acids, **TNEAA** = Total non-essential amino acid

Table 7: Enzyme activities as well as total and conjugated bilirubin of immunocompromised rats following oral administration of Rhino oil extract

Treatment Group	Total Bilirubin (mg/dL)	Conjugated Bilirubin (mg/dL)	Alkaline Phosphatase (U/L)	Aspartate Aminotransferase (U/L)	Alanine Aminotransferase (U/L)
NR + DW	0.13±0.02[a]	2.59±0.26[a]	3842.51±1.55[a]	1560.70±1.59[a]	1502.01±1.47[a]
IR + DW	7.24±0.56[b]	12.63±0.41[b]	1241.24±1.24[b]	825.04±0.03[b]	649.54±0.14[b]
IR + Zn	0.35±0.14[c]	2.56±0.21[a]	3124.32±1.12[c]	1242.24±0.23[c]	1004.21±0.05[c]
IR + 2 mg/kg b.w	0.09±0.02[d]	1.81±0.17[c]	3034.33±2.83[d]	1458.70±3.26[d]	1006.02±0.29[c]
IR + 5 mg/kg b.w	0.27±0.21[e]	0.89±0.03[d]	3205.62±1.48[e]	1193.30±2.25[e]	1005.71±1.02[c]

Data are mean ± SEM of five determinations. Test values with superscript different from their respective control down the column are significantly different (p<0.05)

NR = Normal rats**; IR** = Immunocompromised rats**; DW** = Distilled water**;** Zn = Zinc sulphate**; b.w. =** Body weight

The administration of Lead (Pb) substantively ($p<0.05$) raised the concentration of total and conjugated bilirubin when matched with the distilled water treated normal control animals while it reduced the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum of the animals. Treatment of immunocompromised rats with 2 and 5 mg/kg body weight of Rhino oil notably ($p<0.05$) lowered the levels of total and conjugated bilirubin with zinc sulphate producing conjugated bilirubin values that flowed ($p>0.05$) well with the distilled water treated normal control animals (Table 7). Both doses of Rhino oil meaningfully ($p<0.05$) heightened serum activities of ALP, AST and ALT in the immunocompromised rats, although the 2 and 5 mg/kg body weight of the oil produced ALT activities that compared ($p>0.05$) favourably with the zinc treated immunocompromised rats (Table 7).

Table 8: Serum lipids of immunocompromised rats after oral administration of Rhino oil extract

Treatment Group	Total Chol. (mg/dL)	TRIG. (mg/dL)	HDLC (mg/dL)	LDLC (mg/dL)
NR + DW	1252.87±5.05[a]	9.27±0.45[a]	569.57±5.92[a]	681.45±3.55[a]
IR + DW	2314.08±6.15[b]	31.84±1.36[b]	214.56±0.45[b]	851.11±5.08[b]
IR + Zn	1250.74±5.16[a]	10.04±0.43[a]	571.25±1.34[a]	678.56±3.02[a]
IR + 2 mg/kg body weight	1090.57±4.95[c]	14.40±0.56[c]	807.69±2.48[c]	280.09±0.69[c]
IR + 5 mg/kg body weight	1065.27±4.72[c]	16.53±0.64[c]	826.70±1.56[d]	235.26±3.54[d]

Data are mean ± SEM of five determinations. Test values with superscript different from their respective control down the column for each day are significantly different ($p<0.05$)

NR = Normal rats; **IR** = Immunocompromised rats; **DW** = Distilled water; **Zn =** Zinc sulphate; **TRIG.** = Triglyceride; **Chol. =** Cholesterol; **HDLC** = High density lipoprotein cholesterol; **LDLC** = Low density lipoprotein cholesterol

The levels of serum total cholesterol, TRIG and LDLC in the immunocompromised animals were remarkably ($p<0.05$) increased after Pb administration when liken with the distilled water treated normal control animals, however it decreased HDLC concentration. Treatment of the immunocompromised rats with Rhino oil at both doses (2 and 5 mg/kg body weight) glaringly ($p<0.05$) lowered serum levels of serum total chol., TRIG and LDLC when juxtaposed with the immunocompromised rats, with the zinc sulphate producing total chol., TRIG and LDLC values that matched ($p>0.05$) well with the distilled water treated normal control animals. However, both doses of the oil lowered serum HDLC level with zinc sulphate giving values comparable with the normal control animals (Table 8).

Table 9: Haematological parameters of immunocompromised rats following oral administration of Rhino oil extract

				mg/kg body weight of the Rhino oil	
Parameter	NR + DW	IR + DW	IR + Zn	IR + 2	IR + 5
RBC ($x10^6/\mu L$)	0.35±0.02[a]	0.05±0.01[b]	0.34±0.02[a]	0.31±0.12[c]	0.28±0.02[c]
PCV (%)	43.03±0.24[a]	32.12±0.35[b]	43.25±0.46[a]	42.71±0.25[a]	43.04±0.34[a]
Hb (g/dL)	35.20±0.41[c]	27.42±0.12[b]	34.87±0.72[a]	36.71±0.48[a]	37.42±0.02[a]
MCV (fl)	56.34±0.48[a]	30.79±0.26[b]	54.63±0.16[a]	56.04±0.23[d]	57.12±0.35[d]
MCH (pg)	17.12±0.72[a]	11.39±0.25[b]	18.05±0.32[a]	16.89±0.56[a]	17.61±0.42[a]
MCHC (g/dL)	31.52±0.16[a]	19.62±0.19[b]	30.04±0.13[a]	32.12±0.62[a]	31.26±0.81[a]
WBC ($x10^3/\mu L$)	13.45±0.28[a]	0.87±0.14[b]	12.46±0.21[a]	5.81±0.11[c]	11.26±0.16[a]
PLT ($x10^3/\mu L$)	412.07±1.06[a]	93.12±0.21[b]	172.53±0.44[c]	224.46±0.54[d]	365.72±0.29[e]
Lym ($x10^3/\mu L$)	15.06±0.45[a]	3.18±0.11[b]	10.42±0.16[c]	7.04±0.12[d]	11.74±0.31[c]
Neutro (%)	62.09±0.22[a]	12.54±0.14[b]	29.14±0.21[c]	54.72±0.28[d]	48.12±0.11[e]

Data are mean ± SEM of five determinations. Test values with superscript different from their respective control across the row are significantly different ($p<0.05$)

NR = Normal Rat; **IR** = Immunocompromised rats; **DW** = Distilled water; **Zn** =Zn sulphate; **RBC** = Red blood cell; **PCV** = Packed cell volume; **Hb** = Haemoglobin; **MCV** = Mean corpuscular volume; **MCH** = Mean corpuscular haemoglobin; **MCHC** = Mean corpuscular haemoglobin concentration; **WBC** = White blood cell; **PLT** = Platelet; **Lym** = Lymphocytes; **Neutro** = Neutrophils

The levels of RBC, PCV, Hb, MCV, MCH and MCHC in the distilled water treated lead-induced immunocompromised animals were notably ($p<0.05$) lowered when stacked up with the distilled water treated normal animals. However, treatment of immunocompromised rats with Rhino oil at both doses (2 and 5 mg/kg body weight) substantively ($p<0.05$) raised the levels of RBC, PCV, Hb, MCV, MCH and MCHC when weighed up with the distilled water treated normal control animals. The oil produced PCV, Hb, MCV, MCH and MCHC values that matched ($p>0.05$) favourably with the zinc-treated immunocompromised and distilled water treated normal control animals (Table 9).

The administration of Pb glaringly ($p<0.05$) lowered the levels of WBC, PLT, Lym and Neutro in the distilled water treated lead-induced immunocompromised animals when placed side by side with the distilled water treated normal animals. However, treatment of the immunocompromised animals at both doses of the oil notably ($p<0.05$) heightened the levels of WBC, PLT, Lym and Neutro. Treatment of the immunocompromised rats with zinc sulphate and the oil at 5 mg/kg body weight produced WBC values that matched well with that of the distilled water treated normal control animals (Table 9).

DISCUSSION

Physicochemical Properties of Rhino Oil

The chemical properties of oils are amongst the most important properties that determine the present condition of the oil.

Acid value is a measure of the free fatty acids in oil. Normally fatty acids are found in the triglyceride form; however, during the processing of fatty acids, it may get hydrolysed into free fatty acids. The higher the acid value, the higher the amount of free fatty acids (which indicates decreased oil quality). Acceptable levels for all oil samples should be below 0.6mg KOH/g (measured in potassium hydroxide per gram) [38] or 4 mgKOH/g of the FAO/WHO standard. This observation supports previous study that unrefined vegetable oils had higher acid value than refined oils [39]. The high acid value (12.90±0.20 mg KOH/g) in rhino oil implies that much free fatty acid is produced and leads to a tendency to become rancid, that is, off-flavour [40]. Similarly, rhino oil the acid value in rhino oil in the present study has higher acid value than the Codex standard (0.6 mg KOH/g) [41]. Free fatty acid (oleic) of 10.70±1.10% present in rhino oil which was higher than the FAO/WHO standard indicates that the oil can undergo deterioration or rancidity if exposed for a long time. Free fatty acid and peroxide values are valuable measures

of oil quality. These free fatty acid levels have high probability for increasing the ability of liver to store sugars [42]. Overall, the high acid and free fatty acid value, compared to the FAO/WHO standard, shows the inability of the oil to resist hydrolytic rancidity [43].

Peroxide value is a measure of oxidation during storage and the freshness of lipid matrix. In addition, it is a useful indicator of the early stages of rancidity occurring under mild conditions and it is a measure of the primary lipid oxidation products. It is an indicator of oxidation level and the greater the peroxide value, the more oxidized the oil. The high peroxide value (358.0±0.5 mEq/kg) of rhino oil implies high degree of unsaturation. Peroxides are the primary reaction products formed in the initial stages of oxidation of oil and therefore give an indication of the process of lipid peroxidation [44]. Lipid peroxidation depends on the reaction between the unsaturated fatty acids and oxygen [45]. The implication is that high inherent rhino oil peroxidation will facilitate ability to withstand long time storage without undergoing oxidative peroxidation. The iodine value is the mass of iodine in gram that is consumed by 100 grams of a chemical substance. Iodine numbers are often used to determine the amount of unsaturation in fatty acids. This unsaturation is in the form of double bonds, which react with iodine compounds. The higher the iodine number, the more C=C bonds present in the fat. It can be seen from the high iodine value of rhino oil (115.80±0.40 mg/g) that it is unsaturated, meaning it would be useful for soap making [45, 46]. Saponification value is an estimation of oxidation during storage and is also an indication of the decomposition level of the oils [46]. It is an important parameter for characterizing the industrial use of oil, specifically for soap production [44]. Oils with high saponification value can be used for the production of soap, candle, and raw materials for lubricants [45]. The high saponification value (244.04±0.80 mg KOH/g) of rhino oil might indicate high proportion of lower fatty acids since saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids [47]. Therefore, the shorter the average chain length, the higher the saponification number [46], hence is suitable for soap making.

Fatty Acid Methyl Esters of Rhino Oil

Palmitic acid, or hexadecanoic acid, is the most common saturated fatty acid found in animals, plants and microorganisms [48, 49]. Its chemical formula is $C_6H_{22}O_2$. It makes up 12.80% of the fatty acid methyl esters of rhino oil. Excess carbohydrates in the body are converted to palmitic acid. Palmitic acid is the first fatty acid produced during fatty acid synthesis and is the precursor to longer fatty acids. As a

consequence, palmitic acid is a major body component of animals. In humans, it was reported to make up 21–30% (molar) of human depot fat, [50] and it is a major, but highly variable, lipid component of human breast milk [51, 52]. Palmitate negatively feeds back on acetyl-CoA carboxylase (ACC), which is responsible for converting acetyl-CoA to malonyl-CoA, which in turn is used to add to the growing acyl chain, thus preventing further palmitate generation [50]. According to WHO, evidence is "convincing" that consumption of palmitic acid increases the risk of developing cardiovascular disease, based on studies indicating that it may increase LDL levels in the blood.

Oleic acid, also known as 6-octadecenoic acid methyl ester is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is an odourless, colourless oil, although commercial samples may be yellowish. In chemical terms, oleic acid is classified as a monounsaturated omega-9 fatty acid, abbreviated with a lipid number of 18:1 *cis*-9. It has the formula $C_{18}H_{34}O_2$ and it makes up 82.29% of the fatty acid methyl esters of rhino oil. It is the most common fatty acid in nature [53]. Fatty acids (or their salts) do not often occur as such in biological systems. Instead fatty acids like oleic acid occur as their esters, commonly triglycerides, which are the greasy materials in many natural oils. The principal use of oleic acid is as a component in many foods, in the form of its triglycerides. It is a component of the normal human diet as a part of animal fats and vegetable oils. Oleic acid as its sodium salt is a major component of soap as an emulsifying agent. It is also used as an emollient [54]. Small amounts of oleic acid are used as an excipient in pharmaceuticals, and it is used as an emulsifying or solubilizing agent in aerosol products [55]. Oleic acid is a common monounsaturated fat in human diet. Monounsaturated fat consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol [56, 57]. The 6-octadecenoic acid methyl ester [z] has cancer preventive and insecticide activities according to [“Dr. Duke's Phytochemical and Ethnobotanical Database”].

n-Tridecan-1-ol or 1-Tridecanol is a chemical compound from the group of alcohols (specifically, the fatty alcohols). It is in the form of a colourless, flammable solid. It usually occurs as a mixture of different isomers to compounds such as 2-tridecanol, 3-tridecanol, 4-tridecanol, 5-tridecanol, 6-tridecanol and isotridecanol with the chemical formula $C_{13}H_{28}O$ and makes up 2.72% of the fatty acid methyl esters of rhino oil. It is used as a lubricant and for the manufacture of surfactants and plasticizers.

Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- or 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl, also known as MBMBP, is an industrial chemical with molecular formula $C_{23}H_{32}O_2$ and it makes up 2.91% of the fatty acid methyl esters of rhino oil. It is used in industry as an antioxidant in thermoplastic polymers, rubber, latex and adhesives and as a stabilizer in polymers.

Metal Analysis of Rhino Oil

Sodium (757.14±3.57mg/g) being the predominant element in the present study plays an important role in electrolyte balance of the body and can serve as a source to balance high potassium intake resulting from food additives as well as maintenance of normal cellular homeostasis and in the regulation of fluid and electrolyte balance and blood pressure (BP) [58-61].

Magnesium (58.38±1.60mg/g) is essential for enzyme activity because Mg^{2+} ion tends to bind only weakly to proteins and this can be exploited by the cell to switch enzymatic activity. Magnesium also plays a role in regulating the acid-base balance in the body. Magnesium is an essential mineral [62].

Chromium (8.68mg/g) is also present in rhino oil. The biologically beneficial effects of Chromium (III) continue to be debated. Some experts believe that they reflect pharmacological rather than nutritional responses, while others suggest that they are side effects of a toxic metal [63]. Chromium is accepted by the U.S. National Institutes of Health as a trace element for its roles in the action of insulin, a hormone critical to the metabolism and storage of carbohydrate, fat and protein [64]. The precise mechanism of its actions in the body, however, has not been fully defined, leaving in question whether chromium is essential for healthy people [65]. In contrast, hexavalent chromium (Cr (VI) or Cr^{6+}) is highly toxic and mutagenic when inhaled [66-67].

Manganese (1.10mg/g) is also present in rhino oil. In biology, manganese(II) ions function as cofactors for a large variety of enzymes with many functions [68]. Manganese enzymes are particularly essential in detoxification of superoxide free radicals in organisms that must deal with elemental oxygen. Manganese also functions in the oxygen-evolving complex of photosynthetic plants. While the element is a required trace mineral for all known living organisms, it also acts as a neurotoxin in larger amounts [69-71].

Nickel (0.66mg/g) is also present in rhino oil. There is evidence that nickel is an essential trace element in several animal species, plants and prokaryotic organisms [72]. Nickel may

precipitate autoimmune phenomena and induce immunosuppression *in vitro*; the clinical importance of such effects has not been reported [73, 74].

Cadmium was also not detected in the oil. Administration of cadmium to cells causing the oxidative stress and increase the levels of oxidants causing cellular damage [75-76].

Lead (Pb) is a highly poisonous metal (whether inhaled or swallowed), affecting almost every organ and system in the human body. It was not detected in the oil.

The presence of iron (50.64±0.73mg/g) in rhino oil shows that the oil will prevent the occurrence of haemolytic anaemia hence it will keep the concentration of serum bilirubin normal. To maintain the necessary levels, human iron metabolism requires iron in the diet. Iron is also the metal at the active site of many important redox enzymes dealing with cellular respiration and oxidation and reduction in plants and animals [77]. Iron deficiency is the most common nutritional deficiency in the world [78]. When loss of iron is not adequately compensated by adequate dietary iron intake, a state of latent iron deficiency occurs, which over time leads to iron-deficiency (anaemia) if left untreated, which is characterised by an insufficient number of red blood cells and an insufficient amount of haemoglobin [79].

Zinc (0.28±0.06 mg/g of oil) was also present in rhino oil. Zinc is an essential trace element for humans, other animals, plants and for microorganisms [80]. Zinc is required for the function of over 300 enzymes and 1000 transcription factors, and is stored and transferred in metallothioneins. It is the second most abundant trace metal in humans after iron and it is the only metal which appears in all enzyme classes [81] but in rhino oil it is just 0.28mg/g of the oil. In humans, the biological roles of zinc are ubiquitous. It interacts with "a wide range of organic ligands", and has roles in the metabolism of RNA and DNA, signal transduction, and gene expression. It also regulates apoptosis. Zn and Fe which were present in rhino oil are potent immune boosting metals [82, 83].

Lipid Profile of Rhino Oil

Rhino oil was found to have 87.6mg/dl of total cholesterol. Cholesterol is a type of fat found in the blood. It is produced by the body and also comes from the foods we eat (animal products). Cholesterol has essential functions in the body such as providing essential components of membrane and serving as a precursor of bile acids, steroid hormones and vitamin D. Cholesterol is needed by the body to maintain the health of the cells but too much of it leads to coronary artery disease. Live is inevitable

without cholesterol ingestion. It helps cellular function and the performance of certain hormones. Cholesterol consumption increases the level of low-density lipoprotein.

The triglyceride value of rhino oil was found to be 13.9mg/dl. Triglycerides are the main constituents of animal fats found in the blood. The blood level of this type of fat is most affected by the type of food consumed such as sugar, fat or alcohol but can also be due to overweight, thyroid or liver diseases and genetic conditions. High levels of triglycerides are related to higher risk of heart and blood vessel disease.

The lipid function test determines how much of a patients' cholesterol is LDL, which helps illuminate the risk factors for coronary disease. These problems do not usually have symptoms until the blockage is severe, which can result in a fatal heart attack.

The high high-density lipoprotein (HDL) (51.9mg/dl), observed in this study, which is a good cholesterol helps to remove the LDL and excess cholesterol from the blood and takes it to the liver. The higher the HDL, the better. A high HDL level is related to lowering the risk of heart and blood vessel disease. A high LDL level on the other hand is related to increase in the risk of heart and blood vessel disease. Therapeutic interventions such as increased production or infusion of high-density lipoproteins may sever the links between cholesterol accumulation and inflammation, and have beneficial effects in patients with immune and metabolic disorders [84]. This occurs because of the relatively high concentration of HDLC in the present study. Lipids observed in this study (HDLC), in addition to serving as fuel stores and structural components of cell membranes, act as effectors and second messengers in a variety of biological processes including those associated with the immune system. These lipid mediators and regulators differ in structural composition and exert a diverse array of effects on cellular functional activities including those linked to homeostasis, immune responsiveness, and inflammation. They function as intercellular mediators and at the intracellular level act as critical conduits of external stimuli in signal transduction cascades [85]. Overall, lipids, (lysophospholipids) have been reported to serve as mediators of immunity (Lin and Boyce, 2006) or as bioeffectors in the immune system [85].

Proximate Analysis of Rhino Oil

Carbohydrates are one of the three macronutrients in our diet concerned with energy provision to the body. Carbohydrates come in many different forms, ranging from sugars (polysaccharides, oligosaccharides, disaccharides and monosaccharides) to dietary fibres, and in many different foods, such as

whole grains, as well as fruit and vegetables [87]. The high carbohydrate and protein contents in the oil indicates that rhino oil is energy giving, highly nutritious and good for human consumption.

Protein is one of the key macronutrients in our diet. The high protein content of the oil is very important in animals for the building and repair of worn-out tissues in the body. Moisture content is one of the most important components of food preservation and testing. The amount of dry matter in a food is always inversely proportional to the amount of moisture it contains as such moisture content is of direct economic importance to both the consumer and processor. The high moisture content accounts for its short shelf life as it deteriorates easily after oil extraction if preservative measures are not employed. This high-water content promotes susceptibility to microbial growth and enzymes activities. However, moisture content of the oil depends on their animal body/skin and environmental conditions such as humidity and temperature in animal's niche [60]. Crude fiber is usually a measure of the quantity of indigestible lignin, cellulose and other components. It consists largely of 60-80% cellulose and 4-6% lignin, in addition to other mineral matter. Fibers also are essential in treating or preventing diverticulosis, hemorrhoids, coronary heart diseases and constipation [88]. The high crude fibre content obtained in the present study implies that rhino oil is a potential source of dietary fibre (roughages). High level of fibre is known as anti-tumorigenic and hypocholestrolaemic agent [89]. This implies that the oil may be recommended for people with cholesterol related problems [90].

Fats, one the major macronutrients are a vital food class for many forms of life, and serves both structural and metabolic functions. They are a necessary part of the diet of both humans and animals, and the most efficient form of energy storage [91]. The relatively high crude fat recorded from this study in comparison to protein suggests that this oil could be recommended as good source of food supplement for patient with cardiac problems or at risk with lipid induced heart disorders.

Ash content refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in the food sample. It is also an important quality attribute for some food ingredients, as well as the first step in the preparation of a sample for specific elemental analysis [92]. The low ash content of rhino oil obtained in this study agrees with the investigation of [93] who published low ash content value.

Amino Acid Profile of Rhino Oil

A deficiency of dietary protein or amino acids has long been known to impair immune function

and increase the susceptibility of animals and humans to infectious disease [94]. Findings from recent studies indicate an important role for amino acids in immune responses by regulating: (1) the activation of T-lymphocytes, B-lymphocytes, natural killer cells and macrophages; (2) cellular redox state, gene expression and lymphocyte proliferation; and (3) the production of antibodies, cytokines and other cytotoxic substances. Increasing evidence shows that dietary supplementation of specific amino acids to animals and humans with malnutrition and infectious disease enhances the immune status, thereby reducing morbidity and mortality. Arginine, glutamine and cysteine precursors, which were present in Rhino oil, are the best prototypes [95].

Adequate methionine prevents disorder of hair, skin and nail; reduces liver fat and protect the kidney. Arginine is a factor for maintaining the nitrogen balance in muscles; and can enhance the lean tissue to fat tissue body fat ration; a great factor for weight management. Arginine has antioxidant activity suggesting a disease preventive role against cardiovascular disease [96]. Aspartate deficiency decreases cellular energy and may like be a factor in chronic fatigue. Essential amino acids in rhino oil contribute to good health and well-being. Deficiency of lysine leads to physical and mental handicap. The antioxidant activity of these amino acids suggests a disease preventive role as exemplifies by arginine which is beneficial for prevention of cardiovascular disease [97] (Perumal *et al*., 2001). Rhino oil were also found to be rich in essential amino acids which are necessary as precursors of many other amino acids and vital cellular substances such as hormones and important proteins [98].

In vivo Immunological Study

The decrease in levels of RBC and its indices (Hb, PCV, MCV, MCH, MCHC and RCDW) in the distilled water treated immunocompromised animals may indicate anaemia [104]. The occurrence of anaemia in immunosuppression has been reported due to the increase in non-enzymatic glycosylation of RBC membrane proteins which correlates with defective immune system [105]. Oxidation of these glycosylated membrane proteins and altered immunity in immunosuppressive status causes an increase in the production of lipid peroxides, which leads to haemolysis of RBC and affects its production [106]. The non-significant effect in levels of MCV, MCH, MCHC and RCDW following administration of oil extract suggest that the animals may not be predisposed to anaemia which is a condition that characterize immunosuppression [107]. Increase in RBC and PCV by the extract may also imply increased rate of production of RBCs (erythropoiesis). The increase in Hb by the extract may be adduced to

increase in the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues. The correction offered in terms of reduction of the elevated RBC, Hb and PCV and as well as the non-significant effect of MCV, MCH and MCHC and RCDW suggests that the extract may possess the ability to correct haematological disorders associated with immunosuppression.

Lead is a well-known chemical that suppresses the immune system by damaging WBC and certain organs in the body [105]. In this investigation, the intraperitoneal injection of lead into the animals significantly reduced the WBC count and its related indices such as platelets, basophils, monocytes, eosinophils, lymphocytes and neutrophils. The reduction of these indices could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection [108]. Consequentially, they might have effect on the immune system and phagocytic activity of the animals [109]. Upon extract treatment, the WBC count and its related indices were significantly increased when compared to the distilled water treated immunosuppressed animals. The increase in WBC count by the extract may suggest improved defensive mechanisms against infection resulting to increase in ability of the body to respond to infection. This increase by the extract also indicates a boost in the immune system. The increase in platelet may imply better blood clotting ability. It may also be due to stimulatory effect on thrombopoietin [110]. The significant increase in percentage neutrophils by the extract may be adduced to increased ability of neutrophils to phagocytose (cellular ingestion of offending agents) [108, 111-112]. Lymphocytes are the main effectors cells of the immune system. The increase in lymphocytes by the Rhino oil extract suggests stimulatory effect on the effectors cells of the immune system. The activity of the extract with regards to elevation of the reduced WBC indices suggest stimulatory effect of the extract on effector cells of the immune system in immunocompromised animals.

In vivo Lipid Profile

Immunosuppression is associated with profound alterations in lipids, triacylglycerol and lipoprotein profile resulting in increased risk of coronary heart disease [99]. The increase in serum triacylglycerol (TAG) concentration in immunocompromised rats may be adduced to ability of insulin to activate lipoprotein lipase to hydrolyze lipids leading to increased TAG. It may also be due to insulin ability to increase uptake of fatty acids into adipose tissues which increase TAG synthesis and inhibit lipolysis [99]. The non-significant effect of Zinc with normal control group is an indication that it does that affect triacylglycerol metabolism. The

reduction mediated by Rhino oil in immunosuppressed rats suggests its inactivation of lipoprotein lipase on lipid thereby decreasing TAG synthesis [99].

Diabetes mellitus is associated with high levels of circulatory cholesterol and other lipids and this account for the atherosclerosis, arteriosclerosis and severe coronary heart diseases [100]. The increase in serum cholesterol in immunosuppressive state has been reported to be associated with hypercholesterolemia due to inactivation of lipoprotein lipase to hydrolyze lipids [101]. Therefore, the increase in serum total cholesterol in the distilled water treated diabetic animals in the present investigation may be due to inactivation of lipoprotein lipase to hydrolyze lipids causing elevated serum cholesterol [102]. The reduction in serum total cholesterol by all doses of the extract may be due to its ability to manage the immunosuppressive damage of the white blood cells by correcting abnormalities of lipid metabolism [103].

The increase in serum low density lipoprotein cholesterol (LDLC) in immunocompromised animals may be due to insulin deficiency since insulin has inhibitory action on HMG CoA reductase, a key rate limiting enzyme responsible for LDLC particles [99] (Subramanian and Trence, 2007). The decrease in LDLC level in rhino oil treated immunosuppressed animals may be attributed to insulin availability which facilitates activation of the activity of HMG CoA reductase [103]. High density lipoprotein Cholesterol (HDLC) transports excess cholesterol from the peripheral tissues and carry them to the liver for degradation or for excretion in bile [101]. The reduced serum concentration of HDLC in the zinc treated immunosuppressed animals which is harmful as it can predispose the animals to atherosclerosis was elevated by Rhino oil extract in the treated immunocompromised rats. This further corroborates the beneficial effect of the Rhino oil. The findings from this study which reduced elevated levels of TC, TAG and LDLC and increased the reduced level of HDLC may be beneficial as it may not predispose the immunocompromised treated animals to atherosclerosis and its related coronary artery diseases [99].

In vivo Function Indices

Liver is a vital organ of metabolism, detoxication, storage and excretion of xenobiotices and their metabolites. The elevation in levels of total and conjugated bilirubin in the serum of the distilled water treated immunocompromised animals may be due to necrosis of the liver in Pb-induced immunosuppression [113-114]. The reduction in levels of serum total and conjugated bilirubin in the Rhino oil extract treated

immunocompromised animals, in a manner similar to zinc sulphate, suggest that Rhino oil extract may attenuate disturbances in bilirubin metabolism that characterizes immunosuppression [115-116].

Alkaline phosphatase (ALP) is a ubiquitous enzyme localized within the plasma membrane where it helps in the hydrolysis of phosphate esters. It is also a 'marker' enzyme for the plasma membrane and endoplasmic reticulum, where it is often used to assess the integrity of the plasma membrane. The decrease in activity of liver ALP in the distilled water treated immunosuppressed animals may be due to toxicity of lead (Pb) on cell membrane. The increase in liver ALP activity following treatment of immunocompromised animals with all both doses of Rhino oil extract suggests the ability of the extract to facilitate *De novo* synthesis in enzyme molecule and maintenance of cell membrane integrity. However, the increase in serum ALP activity in the distilled water treated immunocompromised animals may be adduced to lysis of the animal's cell membrane, leading to leakage of this enzyme, an indicator to immune damage (such as obstructive leukemia, bone marrow disease) [117-119].

The decrease in activities of liver AST and ALT in the distilled water treated diabetic animals may be attributed to insulin deficiency caused by Pb, which would inhibit *De novo* synthesis of the enzyme molecule [120]. The increase in serum AST and ALT activities in immunocompromised animals treated may be adduced to ability of the extract to facilitate insulin release for enzyme production [121]. Elevated serum activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) is the most frequently measured indicator of liver disease, and occurs more frequently in immunosuppressive state [122]. Therefore, the increase in serum activities of AST and ALT of the distilled water treated immunocompromised animals may be due to Pb toxicity on liver causing leakage of enzyme molecule into the extracellular fluid [120]. The decrease in serum AST and ALT activities in extract treated animals may be adduced to hepatoprotective potential offered by the extract on liver injury.

LIMITATION

The scope of this work covered the quantitative determination of the chemical profile in Rhino oil as well as the effect of degraded doses of the oil extract on selected haematology, lipid profile and function indices of lead-immunocompromised rats.

CONCLUSION

It is evident from the investigation that Rhino oil possess a robust chemical profile which confers

it an immune boosting capacity an against immunocompromised Covid-19 state.

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Conflict of Interests

The authors have not declared any conflict of interests.

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