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CYTOTOXICITY AND *IN VIVO* TOXICOLOGICAL SCREENING OF A POLYHERBAL PRODUCT, *NEFANG*.

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ABSTRACT

Nefang is a polyherbal product composed of *Mangifera indica* (bark and leaf), *Psidium guajava*, *Carica papaya*, *Cymbopogon citratus*, *Citrus sinensis* and *Ocimum gratissimum* (leaves). It is often used in Cameroon traditional medicine as an antimalarial. Previous studies have demonstrated its *in vitro* antiplasmodial activity, synergistic interactions between its constituents, antioxidant properties and *in vivo* antiplasmodial efficacy. This study aimed at evaluating its safety. Cytotoxicity screening was evaluated against Hep G2 hepatoma and U2OS osteosarcoma epithelial cell lines. Systemic acute oral toxicity was assessed in Swiss albino mice, while sub-acute toxicity was studied in wistar rats at doses of 250, 500 and 1000 $mgkg^{-1}$ body weight. Physiological, biochemical and hematological parameters were analysed to determine its effects on the liver, kidney, lipid profile, nitrogen balance, glycemic levels and the hematological systems. *Nefang* showed no significant cytotoxic effects (CC_{50}>2000 µg/mL). No observed adverse effects levels (NOAEL) in acute toxicity > 5000 $mgkg^{-1}$ bwt. Repeated dose toxicity produced no observed adverse effects on the organ-system profiles. Oral administration of *Nefang* significantly ($p<0.05$) lowered the glucose levels. We also observed a dose-dependent

significant ($p<0.05$) increase in white and red blood cell counts, hemoglobin, hematocrit and platelet densities. These findings suggest that *Nefang* is relatively safe when administered orally at doses tested. *Nefang* exhibited potentials of maintaining the integral functioning of the liver and kidney while boosting the immune system. It also showed hypoglycemic effects. This demonstrated safety of *Nefang* upholds knowledge on its folk use, thereby paving the way for clinical development.

KEYWORDS: Nefang, medicinal plants, cytotoxicity, in vivo toxicity, no observed adverse effect level (NOAEL), hepatotoxicity, renal toxicity, lipid profile, blood glucose level.

1.0 BACKGROUND

Herbs and herb-derived products have played a significant role in health maintenance, disease prevention and treatment for centuries. There is documented evidence of use from many ancient civilizations and they continue to be widely used in most countries, especially low and middle income countries (LMICs).[1] Apart from being used as remedy for disease, herbs have also been a reliable source of biomolecules in drug development. The world's population has been estimated to increase to more than 7.5 million in the next 10 -15 years. This increase is expected to occur mostly in the Southern hemisphere, where about 80% of the population relies on herbal-drugs based traditional system of medicine for primary healthcare.[2] This has led to increased concerns about potential harmful effects of plant-derived products and implications of their use in therapy.

In an attempt to integrate the use of plant-based products towards improving health and patient autonomy, there is the need to harness the potential contribution of traditional and complementary medicine (TCM) to health, wellness and people-centred health care. The safe and effective use of TCM requires international regulation, analytical approaches for safety assessment, application of modern technologies towards establishing safety, efficacy and quality and communicating safety to consumers.[3]

Nefang is a polyherbal product composed of the aqueous extracts of *Mangifera indica* (bark and leaf), *Psidium guajava, Carica papaya, Cymbopogon citratus, Citrus sinensis* and *Ocimum gratissimum* (leaves). Though not yet in commercial form, the folkloric information on its formulation and use in the treatment of malaria has been documented in which some respondents reported adverse side effects such as nausea and dizziness.[4] Evaluation of the *in vitro* antioxidant activities of its constituent plants revealed potential free radical scavenging

and antioxidant activity of some of its constituent extracts. These activities correlated with their phenolic content and are believed to contribute to the demonstrated in vivo antioxidant activity of *Nefang*.[5] The *in vitro* antiplasmodial activities of *Nefang* and its constituents have been reported, showing the presence of both antiplasmodial and non-antiplasmodial components. The synergistic interactions between some constituents extracts are believed to contribute favorably towards the antiplasmodial activity of *Nefang*.[6] Its antimalarial efficacy during early and established *Plasmodium* infection has also been demonstrated, revealing good chemosuppressive and chemotherapeutic activities. Despite the demonstrated efficacy, there is no documented data on the safety of *Nefang*. In order to exploit the potentials of plant-based traditional system of medicine, toxicity testing of herbal products is essential in the development of new drugs and for the extension of the therapeutic potential of existing molecules derived from them.[7, 3]

This study aimed at evaluating the *in vitro* and *in vivo* toxicities of *Nefang* using cell lines and rodent models respectively.

2.0 METHODS

2.1 Collection and Extraction of Plant Material

Fresh parts of the constituent plants of *Nefang*: bark and leaves of *Mangifera indica* (*MiB* and *MiL*, respectively), and leaves of *Psidium guajava* (*Pg*), *Carica papaya* (*Cp*), *Cymbopogon citratus* (*Cc*), Citrus *sinensis* (*Cs*), *Ocimum gratissimum* (*Og*) were harvested from their natural habitat in Cameroon between July and August 2011. Plant identification and voucher specimen referencing were done at the Institute of Medical Research and Medicinal Plants Studies (IMPM) herbarium in Yaoundé, Cameroon by a botanist, Dr. Tsabang Nole. The freshly harvested plant parts were air dried and pulverized. An equi-weight mixture of the constituents was formulated and an aqueous co-extraction was performed. Research evidence shows that ethanol extracts are as effective as the aqueous extracts[8] and therefore ethanol co-extraction was also performed. Weighed quantities of each plant mixture were exhaustively macerated in water and ethanol respectively. Each of the macerate was transferred to a conical percolator for 72 h and the extracts were filtered.[9] The ethanol filtrate was first concentrated using a rotary evaporator. Both filtrates were then concentrated in an air oven at 60^{o}C. The extracts were weighed and stored in labeled sealed plastic containers at 4^{o}C until use.

2.2 Cytotoxicity screening

Cytotoxicity screening of the aqueous and ethanol extracts of *Nefang* and its constituents was carried out using the Resazurin Fluorimetric Cell Viability Assay method[10, 11] on Hep G2 hepatoma and U2OS osteosarcoma epithelial cell lines.

The cells were maintained in Dulbecco's MEM glutamax-1 containing sodium pyruvate, glucose, pyridoxine and supplemented with 10% FCS. The ethanol extract (200 mg/mL) was dissolved in 100% DMSO and diluted 1/10 in DMEM to obtain 20 mg/mL in 10% DMSO while a solution of the aqueous extract (20 mg/mL) was prepared in DMEM. Serial dilutions were prepared for each extract in an intermediate DRC plate (384-well format). Each well contained 25 µL of dilutions of extracts (in 10% DMSO and DMEM respectively) with concentrations ranging from 2000 – 0.061 µg/mL. Each dose response experiment comprised a 2-fold dilution of the extracts (2000 µg/mL maximum concentration, 16 dose-response points). Hep G2 hepatoma and U2OS osteosarcoma epithelial cells in log phase of growth were harvested by trypsinization (0.05% trypsin-treatment for 10 min) and then seeded at 5 $x10^3$ cells/per 100 µL of media in the DRC 384-well plate followed by a 24-h culture at 37^{o}C in a 5% CO_2 incubator to allow for cell attachment. To each well, 10 µL of each concentration of polyherbal extract was added in triplicate. Each plate contained an untreated cell control, a blank control and puromycin standard. Prepared plates were incubated at 37^{o}C for 72 h in a 5% CO_2 environment. After incubation, 10 µL of Resazurin solution was added to each well and plates incubated for further 12 h. Fluorescence of the formed resorufin product in each well was measured at an excitation wavelength of 530 nm and emission wavelength at 590 nm using a micro-titer plate reader (Victor; PerkinElmer, USA). Fluorescence signal from each sample was obtained after background fluorescence subtraction.

2.3 *In vivo* toxicological screening

2.3.1 Experimental Animals

Swiss albino mice (25-30 g) were used for the acute oral toxicity testing while wistar rats (160 – 180 g) were used for *in vivo* sub-acute toxicity testing.

All experimental animals were housed under standard environmental conditions of temperature at 22-24°C under a 12 h dark-light cycle, and allowed free access to drinking water and standard pellet diet.

Ethical approval for the study was obtained from Kenyatta National Hospital/University of Nairobi Ethics and Research committee, Nairobi-Kenya and IMPM Institutional Review Board, Yaoundé, Cameroon.

2.3.2 Acute Toxicity Testing (Single Dose Toxicity Testing)

The acute oral toxicity of the aqueous and ethanol extracts was evaluated in Swiss albino mice according to the procedures outlined by the Organization for Economic Co-operation and Development.[12] Following the fasting period, the mice were weighed and the dose was calculated with reference to the body weight. The crude extract was suspended in a vehicle (distilled water and corn oil for the aqueous and ethanol extracts respectively). Single male and female adult Swiss albino mice (25-30 g) were dosed in a stepwise procedure using fixed doses of 5, 50, 300, 1200 and 2000 $mgkg^{-1}$ bwt of the aqueous and ethanol extracts respectively and animals were observed for signs of toxicity. No mortality or signs of toxicity was observed at the highest dose, so an upper limit dose of 5000 $mgkg^{-1}$ bwt was administered orally. Each crude extract was administered to three male (Test 1) and three female (Test 2) mice in the treatment groups, whereas the control groups received the vehicle. Food was provided to the mice approximately an hour after treatment. The animals were observed 30 min after dosing, followed by hourly observation for 8 h and once a day for the next 13 days. All observations were systematically recorded with individual records maintained for each animal. Surviving animals were weighed and visual observations for mortality, behavioral pattern, changes in physical appearance, injury, pain and signs of illness were conducted daily during the period.

2.3.3 Sub-acute Toxicity Testing (Repeated Dose Toxicity Testing)

Sub-acute toxicity of the aqueous and ethanol extracts was evaluated in wistar rats. Wistar rats were divided into 8 groups (A - H) of 6 rats each. Groups A and E served as control and received the vehicle only (water and corn oil for aqueous and ethanol extracts respectively), while groups B, C, D and F, G, H served as test groups and were administered graded doses of 250, 500 and 1000 $mgkg^{-1}$ bwt of the aqueous and ethanol extracts respectively. At the end of 28 days, experimental animals were fasted overnight and blood was collected through the jugular vein, under diethyl ether anaesthesia into separate EDTA tubes, one for immediate hematological analysis and the other for collection of plasma after centrifugation (3000 rpm × 10 minutes), to be used for biochemical assays respectively. All the rats were sacrificed. The liver, kidney and heart were harvested immediately cleaned of blood using physiological

saline and weighed. The liver and kidney were then fixed in 10% formalin for histopathological examination.

Hematological analysis was done using a Hospitex Diagnostics Hema Screen 18 automatic analyser and parameters analysed were full white blood cell (WBC) count, red blood cell (RBC) count and platelet count as well as their indices.

Safety endpoints for biochemical assays included total proteins (TP), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), urea (BUN), uric acid (URIC), creatinine (CRE), triglycerides (TGY), cholesterol (CHOL) and glucose (GLU). All plasma biochemical assays were performed using standard analytical diagnostic kits from Fortress Diagnostics Ltd, U. K., while optical densities were read using a light spectrophotometer.

The fixed tissues were then dehydrated with 100% ethanol solution and embedded in paraffin. They were processed into 4-5 µm thick sections and stained using hematoxylin-eosin and observed under light microscope as earlier described by Gabe.[13]

2.4 Statistical Analysis

To determine half-maximal cytotoxic concentration (CC50) values for each extract, the obtained data were analyzed using GraphPad Prism 6.0. The logarithm of the extract concentration was plotted against its activity represented by the fluorescence reading to obtain a nonlinear regression curve-fitting and a variable slope sigmoidal dose-response curve.

All other data are expressed as mean ± standard deviation (SD). Data were analyzed using SPSS Version 20.0. One-way analysis of variance (ANOVA) followed by Neuman-Keuls multiple comparison test to identify the differences between treated groups and controls. The data was considered significant at $P < 0.05$.

3.0 RESULTS

3.1 Cytotoxicity screening

Details of the constituent plants of Nefang are summarized in **Table 1**. Both the aqueous and ethanol extracts of *Nefang* were screened against Hep G2 hepatoma and U2OS osteosarcoma epithelial cell lines. Compared to puromycin which exhibited CC_{50} values of 0.91 and 0.18 µM/mL (Hep G2 and U2OS respectively), *Nefang* did not affect cell viability when tested at

up to 2000 µg/mL dose-response concentration point. These findings suggest that *Nefang* is non-toxic against the above tested human cell lines.

3.2 *In vivo* toxicity Testing

3.2.1 Acute (Single Dose) Oral Toxicity Testing

There were no observed adverse effects at all doses tested (5, 50, 300, 2000 $mgkg^{-1}$ bwt) for both the aqueous and ethanol extracts of *Nefang*. All the mice survived.

No toxic effects were observed throughout the 14 days study period in mice. None of the mice showed any signs of toxicity such as changes on skin, eyes and mucus membranes, behavioral patterns, trembling, diarrhea, falling of the fur, sleep or coma. No significant changes were observed in their body weights. The estimated maximum tolerable dose (MTD) was > 5000 $mgkg^{-1}$ bwt for both extracts tested.

Table 1. Constituent Plants of *Nefang*: voucher numbers, common names, parts used and place of collection

Plant Family and Species	Common Name (Voucher specimen Number)	(Part Used)	Place of Harvest
Anacardiaceae *Mangifera indica* Linnaeus	Mango (TN6225)	Bark and Leaves	Mballa II, Yaoundé
Myrtaceae *Psidium guajava* Linnaeus	Guava (TN6226)	Leaves	Nkomo, Yaoundé
Caricaceae *Carica papaya* L. papaya	Pawpaw (TN6227)	Leaves	Nkoabang, Yaoundé
Poaceae *Cymbopogon citratus* (DC. Ex Nees) Stapf	Lemon Grass or Fever Grass (TN6228)	Leaves	Kombone, Kumba
Rutaceae *Citrus sinensis* (Linnaeus) Osbeck (pro sp.) [maxima reticula]	Sweet Orange (TN6229)	Leaves	Mamfe
Lamiaceae *Ocimum gratissimum* Linnaeus	Wild Basil or Mosquito Plant (TN6230)	Leaves	Buea

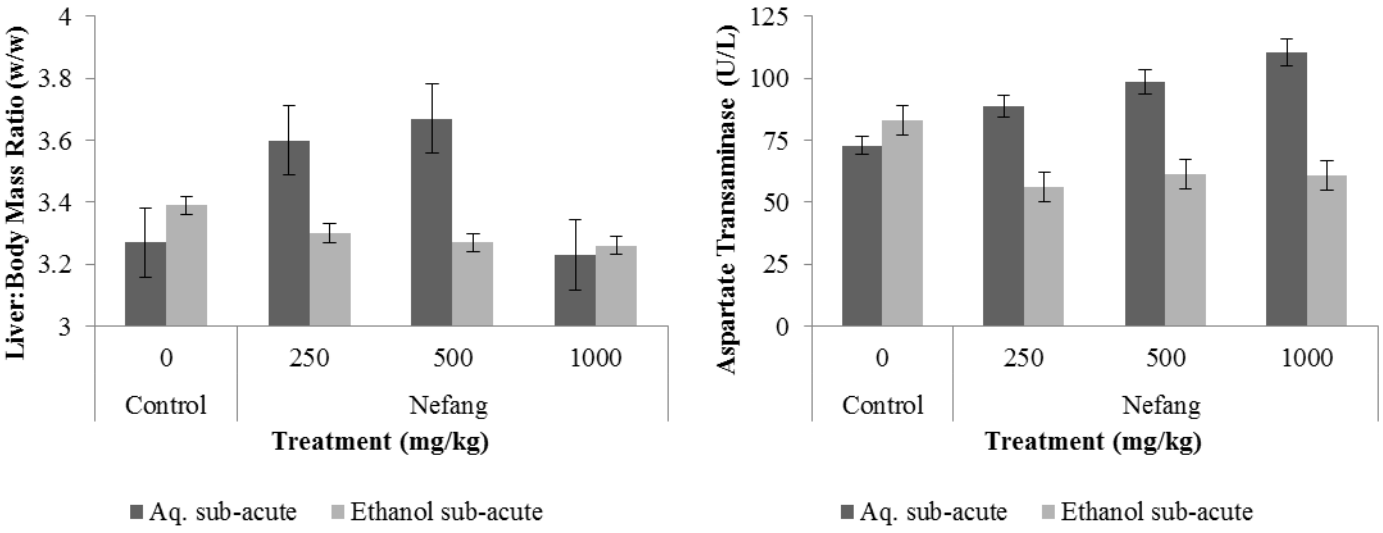
3.2.2 Sub-acute (Repeated Dose) Oral Toxicity Testing

The effects of the oral administration of *Nefang* aqueous and ethanol extracts on some plasma biochemical parameters analysed in experimental animals were summarized using the organ system approach.

3.2.2.1 Hepatotoxicity/ Effects on the liver

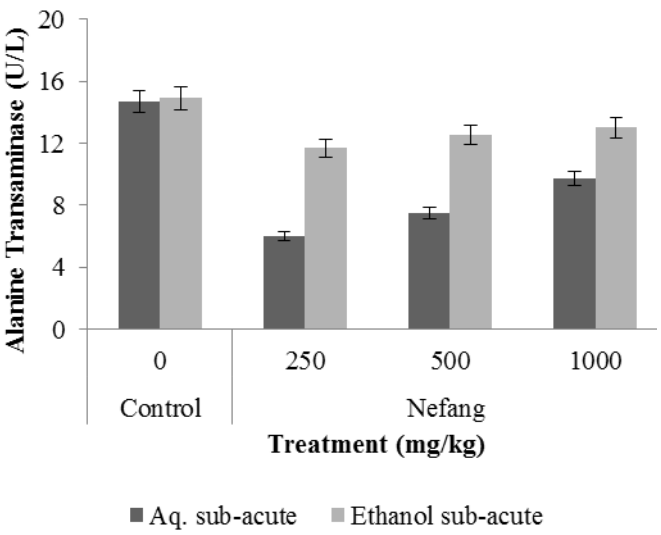
After sub-acute administration of the aqueous and ethanol extracts of *Nefang*, the effects on the liver were evaluated by determining some physiological, biochemical (**Figure 1)** and histopathological parameters.

The liver: body mass ratio (Relative organ weight) of each group of animals was calculated and no significant differences were observed in weights of the liver of test animals when compared to the control.

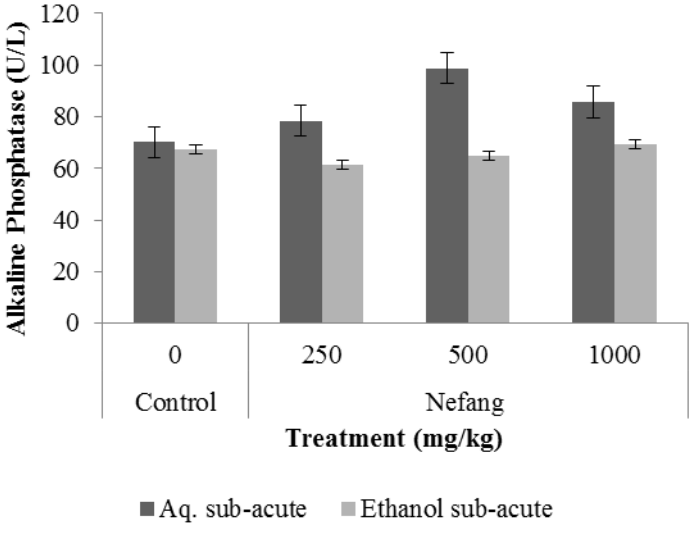


(a) Relative liver weight

(b) Aspartate Transaminase



(c) Alanine Transaminase



(d) Alkaline Phosphatase

Figure 1. **Effects of the sub-acute and sub-chronic administration of the aqueous and ethanol extracts of *Nefang* on the liver of experimental rats. Parameters: (a)** Relative liver weight **(b)** Aspartate Transaminase (AST **(c)** Alanine Transaminase (ALT) **(d)** Plasma Alkaline Phosphatase (ALP).
Each bar represents the Mean ± SD for each group of rats, n=6.

Biochemical parameters for hepatotoxicity evaluated included plasma AST, ALT and ALP. After sub-acute administration, we observed a significant ($p<0.05$) increase in AST values at 1000 mgkg-1 after sub-acute administration of the aqueous extract whereas there was a decrease in AST and ALT values after administration of the ethanol extract when compared to the control.

Histopathological examination did not reveal any distortion in the architecture of the liver of *Nefang*-treated animals when compared to the control.

3.2.2.2 Renal Toxicity/ Effects on the kidneys

The effects of *Nefang* on the kidneys were investigated by determining the relative kidney weight, blood urea nitrogen (BUN), creatinine (CRE), uric acid (**Figure 2**) and histopathological analysis.

The calculated kidney: body mass ratio of test animals after sub-acute administration of the aqueous and ethanol extracts of *Nefang* did not reveal any significant changes at doses of up to 1000 $mgkg^{-1}$, when compared to the control groups.

Administration of *Nefang* showed a significant ($p<0.05$) decrease in BUN values after sub-acute aqueous extract administration at a dose of 250 $mgkg^{-1}$. This was dose-dependently restored to normal relative to the control. All other values of test groups stayed within normal ranges. Histopathological examination revealed no adverse effects in experimental animals.

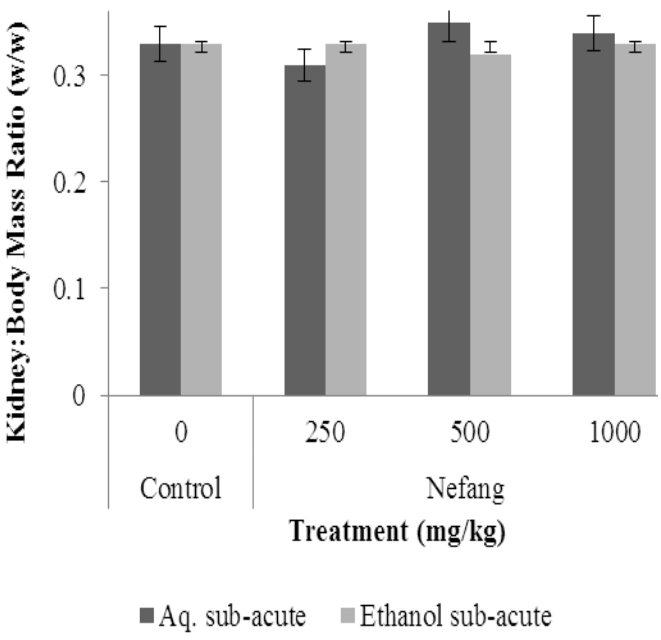
3.2.2.3 Effects on the Lipid profile

The effects of the administration of *Nefang* on the lipid profile were evaluated by determining plasma triglycerides and cholesterol levels in extract-treated animals relative to the control (**Figure 3**). Administration of *Nefang* extracts showed a significant ($p<0.05$) dose-dependent increase in TGY values in sub-acute ethanol extract-treated rats as well as CHOL levels (at 1000 $mgkg^{-1}$) when compared to the control.

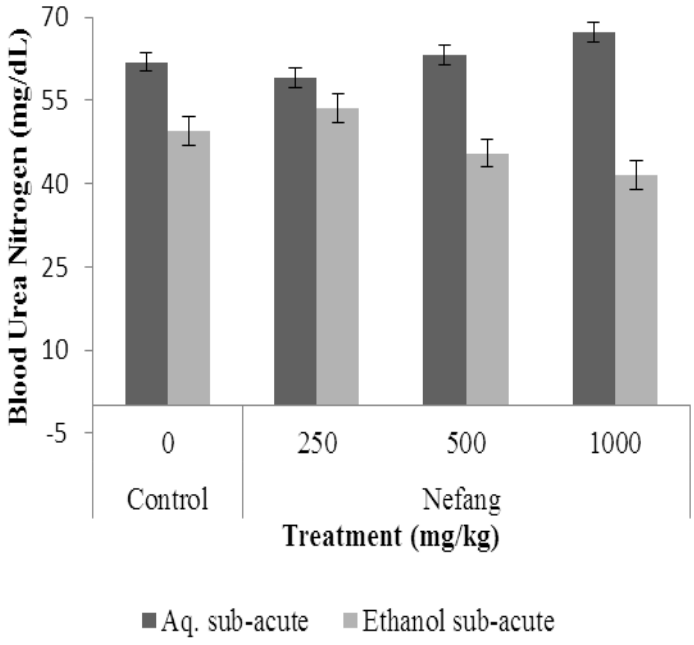
3.2.2.4 Effects on Nitrogen Balance and Glycemic levels

The effects of *Nefang* extracts on the nitrogen balance and glycemic levels were determined by analyzing plasma total proteins (TP) and glucose (GLU), as well as the overall body weight of experimental animals. While there was no significant difference in total proteins level in experimental animals when compared to the control, *Nefang* exhibited significant hypoglycemic effects on experimental animals after sub-acute aqueous extract administration.

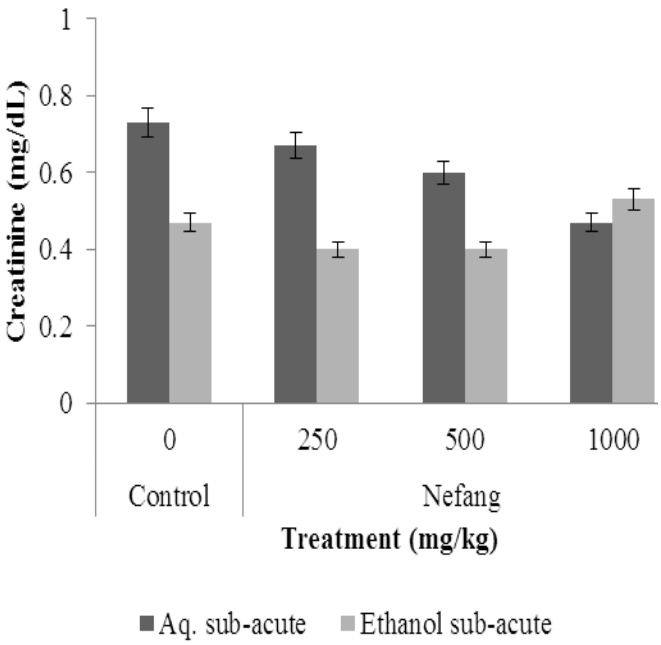
Nefang did not have any adverse effect on the overall body weight of experimental animals when compared to the control. **(Figure 4).**



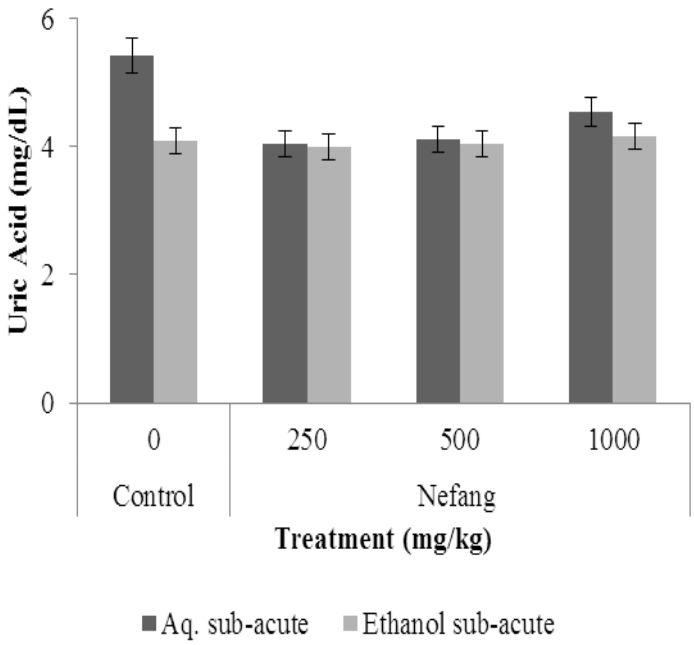
(a) Relative kidney weight



(b) Blood Urea Nitrogen

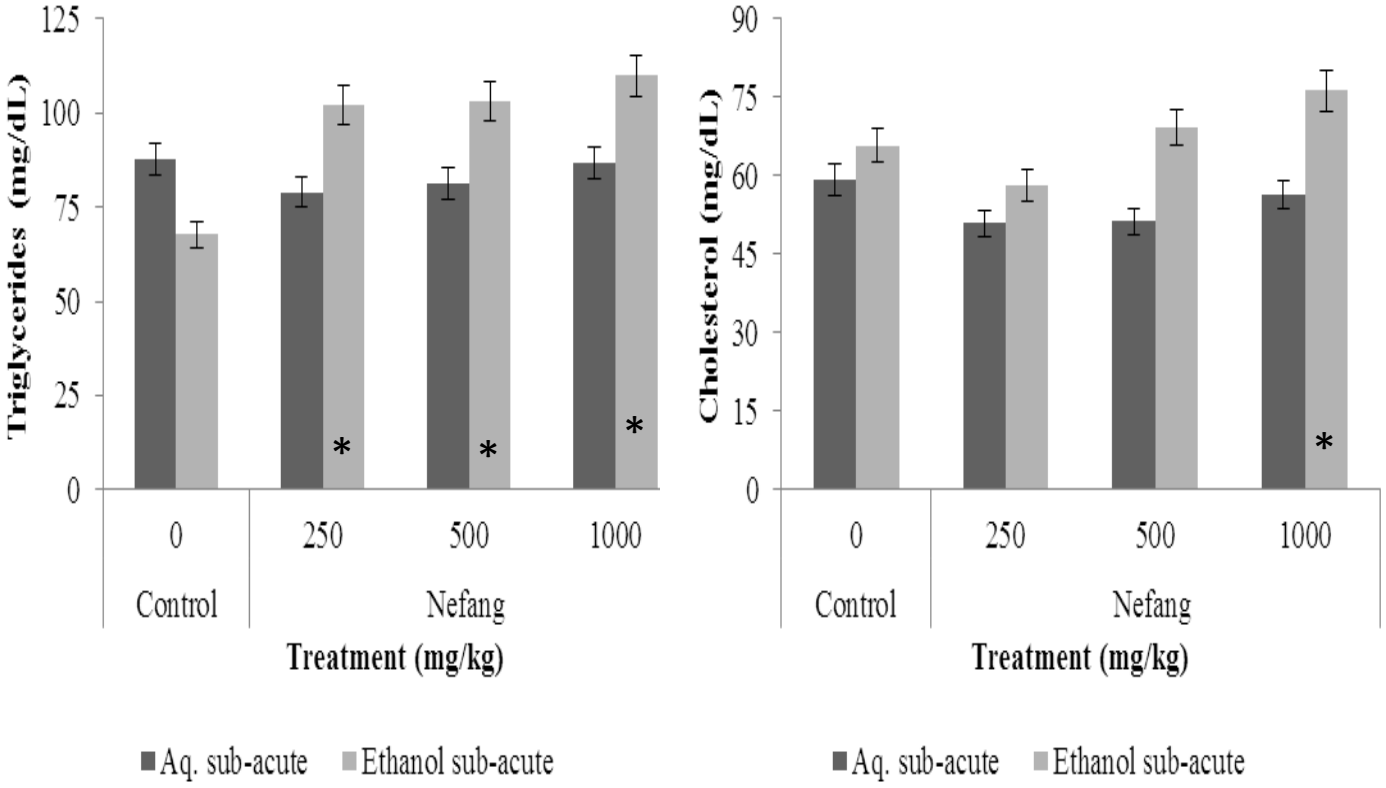


(c) Creatinine



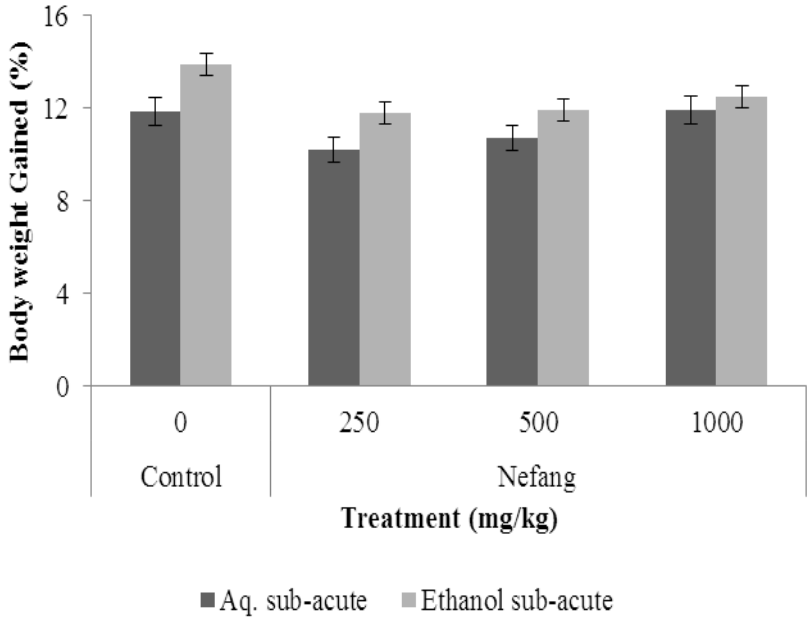
(d) Uric acid

Figure 2. Effects of the sub-acute and sub-chronic administration of the aqueous and ethanol extracts of *Nefang* on the kidney of experimental rats. Parameters: (a) Relative kidney weight **(b)** Blood Urea Nitrogen **(c)** Creatinine **(d)** Uric acid.
Each bar represents the Mean ± SD for each group of rats, n=6.

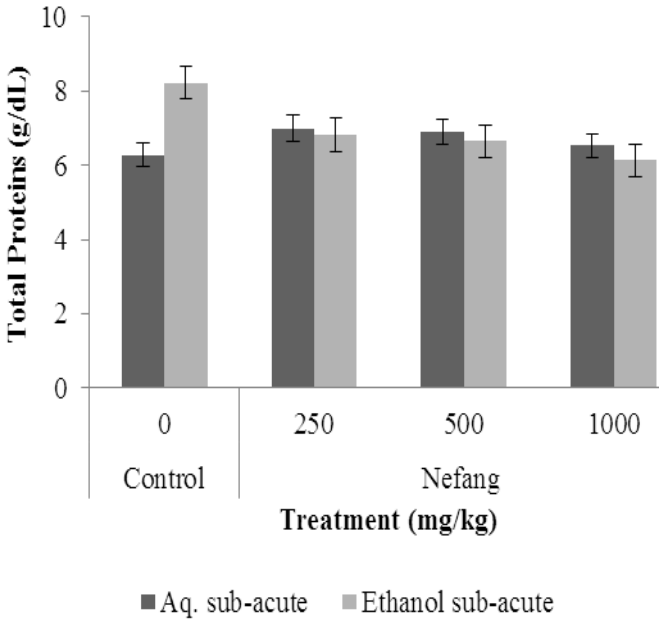


(a) Triglycerides (b) Cholesterol

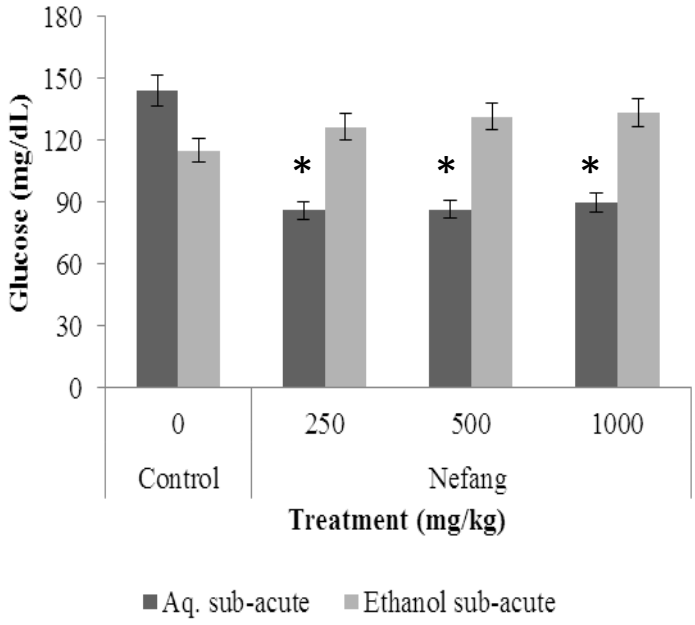
Figure 3. **Effects of the sub-acute and sub-chronic administration of the aqueous and ethanol extracts of *Nefang* on the lipid profile of experimental rats. Parameters: (a)** Triglycerides **(b)** Cholesterol.
Each bar represents the Mean ± SD for each group of rats, n=6.
**-TGY and CHOL values significantly ($p<0.05$) higher than the control.*



(a) Overall Body weight



(b) Total Proteins

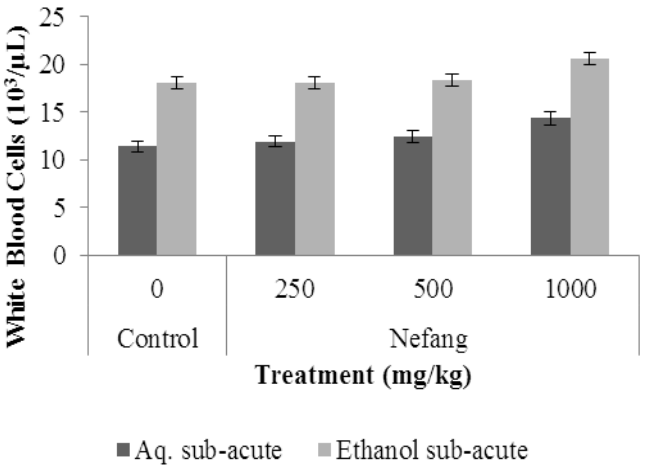


(c) Blood Glucose

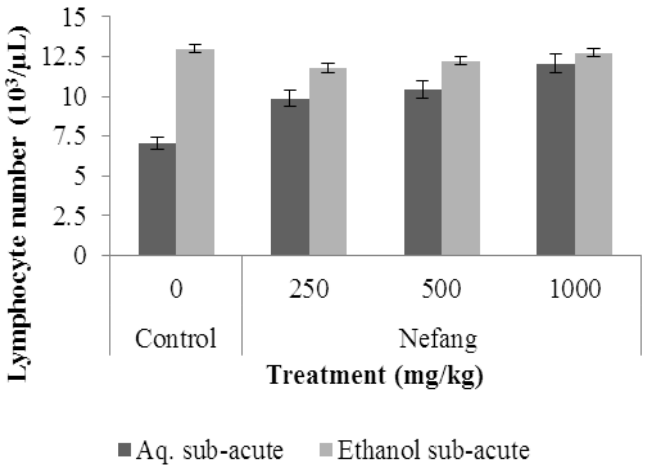
Figure 4. Effects of the sub-acute and sub-chronic administration of the aqueous and ethanol extracts of *Nefang* on Nitrogen Balance and glycemic levels of experimental rats. Parameters: (a) Overall body weight (b) Total proteins (c) Blood glucose levels.

Each bar represents the Mean ± SD for each group of rats, n=6.

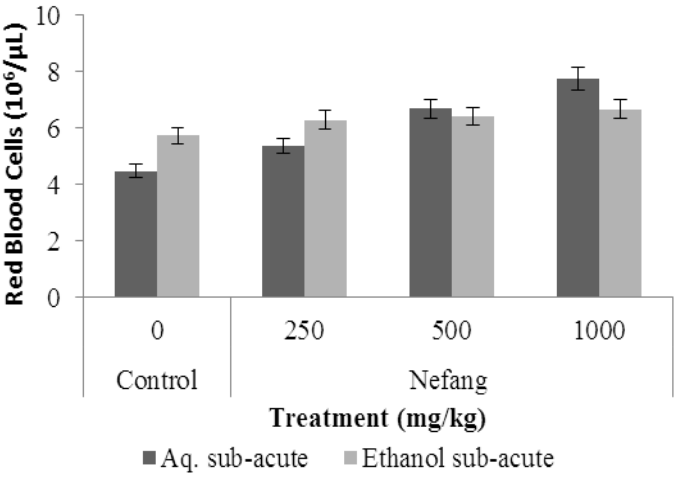
****-GLU values significantly (p<0.05) lower than the control***



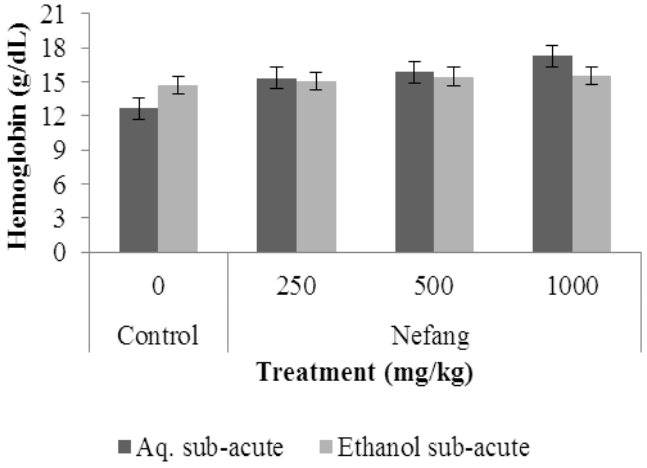
(a) White Blood cells count



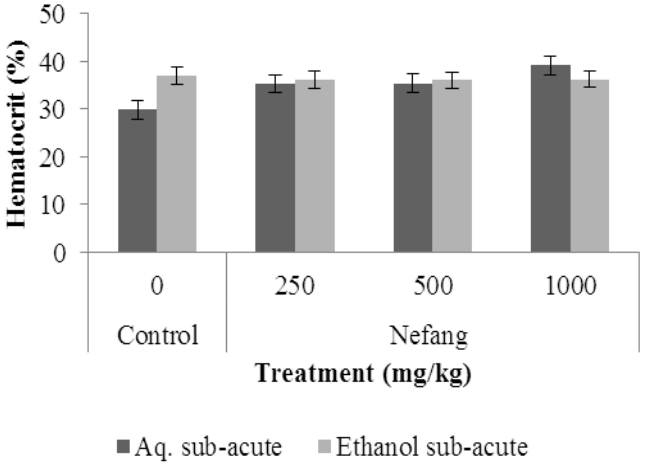
(b) Lymphocyte number



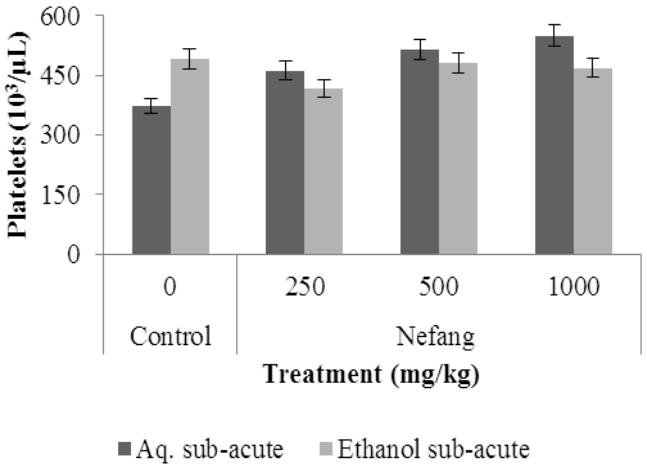
(c) Red Blood cells count



(d) Hemoglobin



(e) Hematocrit



(f) Platelets count

Figure 5. Effects of the sub-acute and sub-chronic administration of the aqueous and ethanol extracts of *Nefang* on hematological system of experimental rats. Parameters: (a) White Blood cells count **(b)** Lymphocyte number **(c)** Red Blood cells count **(d)** Hemoglobin **(e)** Hematocrit **(f)** Platelets count.

Each bar represents the Mean ± SD for each group of rats, n=6.

3.2.2.5 Effects on the Hematological systems

The effects of sub-acute administration of the aqueous and ethanol extracts of *Nefang* on the hematological systems in experimental animals were studied by analyzing the white blood cells (WBC), lymphocyte number (LYM #), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT) and platelet (PLT) counts. These revealed dose-dependent increases in LYM #, RBC, HGB and PLT in experimental animals after aqueous administration of *Nefang* compared to the control. However, these values were found to be non-significant **(Figure 5).**

4.0 DISCUSSION

Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules and herbal drugs. Evaluation of the safety of *Nefang* revealed overall decrease in adverse effects in experimental animals upon exposure to it. In acute toxicity testing, doses higher than 5000 $mgkg^{-1}$ bwt are generally not considered as dose related, which is in accordance with the Organization for Economic Corporation and Development (OECD) Guidance Document for Acute Oral Toxicity Testing.[12, 14] Compounds with LD_{50} values lower than 2000 $mgkg^{-1}$ bwt are generally considered to be relatively safe, since values above this are non-classified. This signifies that *Nefang* aqueous and ethanol extracts can be considered as non-toxic at acute oral administration since the extracts were well tolerated and there was no observed adverse effect at a dose of 5000 $mgkg^{-1}$.

After sub-acute oral administration, body and organ weights of experimental rats relative to the control did not show any significant difference. In as much as the percentage weight gained of the experimental animals was lower than that of the controls, the difference was not significant and the relative organ weights (ROWs) of experimental animals did not show any significant difference when compared to the control. Comparison of body and organ weights between treated and untreated groups of animals have conventionally been used to evaluate the toxic or adverse effects[15, 16] and as an assessment of therapeutic response to test articles or drugs.[17] This suggests that the extracts did not have any adverse effects on experimental animals that would cause them to loose appetite[18] and is indicative of an adaptive response of the organs to the accumulation of the extracts.[19] Hence, organ weights did not indicate any toxic or adverse effects from *Nefang* administration.

Assay of plasma biochemical parameters was performed in order to evaluate the hepatic, renal, lipid and glycemic profiles of experimental animals and to give insight into

pathological changes and nature of disease or other effects upon exposure to drugs. Lipid peroxidation is induced when drugs are taken resulting in the release of cytosolic enzymes into the blood stream such as ALT, AST, ALP which when observed in increased quantities in the plasma are indicative of liver and cellular damage.[20] Serum transaminases (AST, ALT) and alkaline phosphatase (ALP) are indicators of hepatic function[21], URIC is the end product of purine metabolism[22] and it is an indicator of cardiovascular and renal diseases [23], BUN and CRE are indicators of glomerular filtration rate (GFR), which is an indicator of the renal function[24] while blood lipids such as TGY and CHOL are some of the factors associated with atherosclerosis and cardiovascular diseases.[25] The significantly decreased values observed in the values of ALT, AST and normal values in ALP, URIC and BUN in experimental animals may suggest a potential hepatoprotective and enhanced renal function activities.

The lipid (TGY, CHOL) and glycemic (GLU) parameters are indications that *Nefang* aqueous extract does not present any risk of hypercholesterolemia or artherosclerosis and shows hypoglycemic potentials contrary to hyperlipidemic effects observed in ethanol extract treated groups. In this study, corn oil was used as the vehicle for the ethanol extracts. Previous studies reported significantly increased values in TGY and CHOL levels upon administration of corn oil diet in Swiss mice[26] suggesting that observed hyperlipidemia might be as a result of the corn oil used as the vehicle. The hypolipidemic activity of the aqueous extract might be as a result of the antioxidant property of the extracts, which lowers the level of cholesterol in the blood by increasing LDL catabolism. *Nefang* might also inhibit cholesterol synthesis and delay its absorption.[27]

Blood glucose levels in experimental animals suggest that *Nefang* aqueous extract exhibited great hypoglycemic tendencies. The hypoglycemic effects of the aqueous extract may be due to the presence of hypoglycemic substances in *Nefang*[28, 29], stimulation of ß cells to produce more insulin[30], increasing glucose metabolism or regenerative effect on pancreatic tissue [31] Hence, *Nefang* could be used in the regulation of blood sugar and lipid levels.

Hematological parameters analyzed included the complete blood count of all animals, in an effort to understand the risk alterations in the hematopoietic system upon exposure to drugs.[32] Hematopoiesis is the process of blood cell formation. All blood cells are believed to be derived from the pluripotential stem cell, an immature cell with the capability of becoming an erythrocyte (RBC), a leukocyte (WBC), or a thrombocyte (platelet). In healthy adults,

stem cells in hematopoietic sites undergo a series of divisions and maturational changes to form the mature cells found in the blood. The hematological parameters are mediators of immunity and play a vital role in immune protection and tissue repair.[22, 33] The WBCs protect the body from infection by foreign organisms, the RBCs boost the immune system and the platelets protect blood vessels from endothelial damage as well as initiate repair of these vessels. The mean corpuscular volume (MCV) and mean cell hemoglobin (MCH) give the volume and weight of the HGB in each RBC while the mean corpuscular hemoglobin concentration (MCHC) gives a valuable indicator of HGB deficiency. In this study, we observed a general increase and/or normal values of all parameters analysed in experimental rats when compared to the control, indicating that *Nefang* had no observed adverse effect on the hematopoietic system. This serves as an important index of the physiological and pathological status [34] and suggests a stimulation of the hematopoietic system, leading to the production of these blood cells and hence a strong immuno-modulatory, antioxidant and endothelial protection and repair activity by *Nefang*.[35] These results were upheld by earlier studies on some constituent plant extracts of *Nefang* which reported the membrane-protective activity and protection against hemolysis of the RBC[36] and wound healing potential of *C. papaya*[37], anti-inflammatory, free radical scavenging and antioxidant activity of *C. citratus* [38, 39], antioxidant activities of the *M. indica* bark and leaf, *P. guajava* and *O. gratissimum* leaf extracts [5] and substances isolated from *C. sinensis*.[40] However, this is the first study on the effect of *Nefang* on the hematopoietic system.

5.0 CONCLUSION

This study provides information on the toxicological profile of the aqueous and ethanol extracts of *Nefang*. The results obtained suggest that *Nefang* does not present any adverse effects on the cell lines used and in experimental animals at the doses tested. *Nefang* exhibited potentials for boosting the components of the hematopoietic system, maintaining the integral functioning of the liver, kidneys and showed some hypoglycemic effects. These uphold indigenous knowledge on its safe folkloric use in humans and provide justification for specifically designed studies to investigate other beneficial pharmacological effects and clinical studies in humans.

Competing interests

None

AUTHOR'S CONTRIBUTION

PAT conceived the study, carried out cytotoxicity, in vivo toxicity and drafted the manuscript; GAA participated in the design/execution of the in vivo toxicity studies and co-supervision; LSA participated in the design and execution of the cytotoxicity screening; FAO participated in the design, co-supervision and review of the manuscript; ANG participated in the conception, design and general supervision of the studies. All authors read and approved the final draft of the manuscript.

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