British Journal of Pharmaceutical Research
5(6): 392-405, 2015, Article no.BJPR.2015.038
ISSN: 2231-2919

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Effect of Aqueous and Ethanol Leaf Extracts of *Landolphia dulcis* on Haematology and Histopathology of Swiss Albino Mice

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Authors' contributions

This work was carried out in collaboration between both authors. Author AFC designed the study, wrote the protocol, wrote the draft of the manuscript and performed the statistical analysis. Author BB managed the analysis and literature searches. Both authors read and approved the final manuscript.

Article Information

DOI:10.9734/BJPR/2015/7098
Editor(s):
(1) Nan Mei, Division of Genetic and Molecular Toxicology, National Center for Toxicological Research (NCTR), U.S. Food and Drug Administration (FDA), USA.
(2) Wenbin Zeng, School of Pharmaceutical Sciences, Central South University, Hunan, China.
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Reviewers:
(1) Anonymous, South Korea.
(2) Anonymous, Nigeria.
(3) Anonymous, Mexico.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=884&id=14&aid=7972

Original Research Article

Received 24th September 2013
Accepted 28th March 2014
Published 31st January 2015

ABSTRACT

Aims: To evaluate toxicological effect of aqueous and ethanol leaf extracts of *Landolphia dulcis* on haematology, liver and kidney physiological changes in mice.
Study Design: *In-vivo*.
Place and Duration of Study: Microbiology Department, Federal University of Technology, Akure, Ondo State, Nigeria, between April 2012 and July, 2012.
Methodology: Thirty five mice of both sexes weighing between 23-35 g were divided into seven groups of five per group after six hour fasting period. The mice in group 1 received normal saline (10 ml/kg oral) while the mice in groups 2-6 received oral doses of the extracts (200, 400, 800,

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1000, 2000 mg/kg respectively) for 14 days on a single daily dose. The animals were observed for obvious toxic symptoms and mortality in each group within 24 hr.
Results: Though slight differences in values were observed between the control and extract treated mice, insignificant different in values (P>0.05) were observed among the aqueous and ethanol extract treated mice. Hematological indices of the mice showed slight differences in decreased values from the control to the extract treated group of mice. Results observed were on dose dependence of the extract where no significant difference occurred and changes below or above standard range to suggest ill health. The histopathology of the liver and kidney provided supportive evidence for the unaltered haematological parameters observed.
Conclusion: With the results obtained, composition of drugs, which have the polyherbal formulations of *L. dulcis* plant extract, should be encouraged for its therapeutic importance, proposed useful remedy in hepatoprotective renal protection.

Keywords: Plant extracts; solvents; hematology; histology; toxicity; mice.

1. INTRODUCTION

The treatment and control of diseases by the use of the available medicinal plants in a locality has been helpful and of a priority to majority urban and rural duelers in healing various diseases because of the reliability and stability in plant products for healing. Nearly, all cultures and civilization from ancient times to the present day depended fully or partially on herbal medicines because of their effectiveness, affordability, availability, low toxicity and acceptability [1]. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological studies [2]. In plants, the synthesized aromatic substances (metabolites) are used as defensive weapons against predation by microorganisms, insects and herbivores. However, some of these metabolites are involved in pigmentation (tannins and quinines) and formation of plant odour (terpenoids) and flavour (capsaicin). These defensive molecules give plants their medicinal value which is appreciated by human beings because of their importance in health care of individuals and communities [1].

Liver, an important organ actively involved in metabolic functions, is a frequent target of a number of toxicants [3]. The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins, abused by poor drug habits, alcohol and prescribed over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease [4,5]. Thus liver diseases are some of the fatal disease in the world today. They pose a serious challenge to international public health. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders. But there are not much drug available for the treatment of liver disorders [6,7].

Landolphia dulcis is a climber widely dispersed in dense forest from Guinea to south Nigeria and extending to Congo. The leafy twigs and powdered back decoction is use in the treatment of serious wounds while the trunk-bark and root decoction are used as a galactogogue by application to the breast [8]. The root decoction is used traditionally in south eastern Nigeria to enhance sexual performance [9]. Among the traditionally used sex enhancement natural remedies in south eastern Nigeria, *L. dulcis* root is very popular because of its quick onset of action.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples and Extracts Preparations

Healthy looking leaves of *Landolphia dulcis* were collected from forest in Akure, Ondo State, Nigeria and identified in Department of Forestry and Wood Technology, Federal University of Technology, Akure, Nigeria. The leaves were rinsed in clean water and air dried for 3 weeks at room temperature of 25±2ºC on side bench in the laboratory and then ground to powder with a mechanical grinder. 1.5 kg each of the powder obtained was extracted with cold water and ethanol at room temperature (25±2ºC). The resulting crude extracts were filtered with sterile muslin cloth and the ethanol extract was concentrated using a rotary evaporator (RE-52 A Union Laboratories, England) at 40-45ºC, while

the aqueous extract was evaporated using water bath regulated at 55ºC.

Final percentage yield of the extracts was calculated thus:

$$\frac{\text{Weight of extract recovered after extraction}}{\text{Initial weight of dried plant samples}} \times 100$$

The final volume obtained from the dried crude extracts from water and ethanol were contained in plastic containers and labeled appropriately as aqueous extract (AE) and ethanol extract (EE).

2.2 Acute Toxicity Study

The bioassay was conducted according to the World Health Organization guideline for the evaluation of the safety and efficiency of herbal medicine [10] and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals [11]. Thirty five mice comprising both male and female were divided into seven groups of five after six hour fasting period. The mice in group 1 received normal saline (10 ml/kg oral) while the mice in groups 2-6 received oral doses of the extracts (200, 500, 1000, 2000, 3000, 4000, mg/kg respectively). The animals were observed for obvious toxic symptoms and mortality in each group within 24 h based on [12] method. The median lethal dose of the extract (LD_{50}) was estimated using probit analysis [13]. From the acute toxicity study, the LD cut-off dose for extracts was found to be 3000 mg/kg body weight. Hence, the therapeutic doses were taken as 200-2000 mg/kg body weight. The mice were weighed before and after the experiment.

2.3 Experimental Animals

Hence the experimental study is not aimed at antitumor neither effects of the extracts on reproductive organs of mice but on haematology and histopathology, apparently healthy male and female Swiss albino mice weighing between 23-35 g were used. The animals were contained in a cage and maintained under standard laboratory conditions. They were given rodent pellets (Vital feeds) and water *ad libitum*. The mice were acclimatized for 2 weeks and were fasted over night with free access to water prior the experiments. The animals were conducted in compliance with NIH Guide for Care and Use of Laboratory Animals. Before the experiment, the mice were divided into seven groups of five mice each.

2.4 Administration of Crude Extracts of *L. dulcis*

A total of 35 mice divided into seven groups with 5 mice per group were used. While group 1 served as control which was only allowed to normal rat feed and water, 0.5ml each of varying doses of 200, 400, 800, 1000 and 2000 mg/kg^{bw} was given orally to animals in groups 2, 3, 4, 5 and 6, respectively, as a single daily dose using wash bottle whose dispenser was directly laid on the mice throat. After 14 days of treatment a wide period enough for the extracts to manifest possible physiological changes or clinical signs of toxicity such as respiratory pattern, color of body surfaces, frequency and nature of movement, marked involuntary contraction or seizures of contraction of voluntary muscle, and loss of reflex among other signs, each rat was bled through the orbital sinus for red blood cell count, white blood cell count, haemoglobin estimation, total differential white blood cell count and packed cell volume.

2.5 Haematological Studies

Red blood cells (RBC) and White Blood Cells (WBC) were counted using Neubauer haemocytometer and hemoglobin was estimated using Sahli's Hemoglobinometer by standard procedures; differential counts was estimated by the criteria of [14,15].

2.6 Histopathological Examination

Liver and kidney tissues were collected from the controls, the extract and bacterial treated and the satellite groups and then washed in normal saline. The tissues were cut to small sizes and dehydrated with grades of ethanol starting from 50% - absolute. Thereafter were cleared in xylene for two changes and impregnated with paraffin wax in oven at 60ºC for 1h. The impregnated tissues were embedded in paraffin wax and sectioned with a microtome (Bright, England) at 4-7 µm. The sectioned tissue films on glass slides were de-waxed with xylene, hydrated, cleared in xylene and stained with haematoxylin and eosin; mount with DPX and photographed. The photographed images were observed with microscope and interpreted according to the level of damages or protective safety.

2.7 Statistical Analysis

The results are expressed as mean $\pm$ standard error of means (SEM). The Dunnett's test was

used to make a statistical comparison between groups. Result with P<0.01 and P<0.05 were considered significant.

3. RESULTS

Red blood cells mean values of control group was 7.67±0.92 m/cu.mm and was higher than the values obtained from the ethanol and aqueous extract concentrations dosage. However, ethanol extract concentrations of 200 and 400 mg/kg of extract per body weight resulted to counts of 6.83±0.64 m/cu.mm each while the aqueous extract valued at 5.32±0.54 and 5.67±0.36 m/cu.mm respectively. The counts obtained with ethanol extract concentrations of 800, 1000 and 2000 mg/kg of extract per body weight were 6.86±0.36, 6.95±0.33 and 7.46±0.17 m/cu.mm respectively. The aqueous extract at 200 to 800 mg/kg of extract per body weight were 5.32, 5.67 and 6.26 m/cu.mm and was significantly different (P≤ 0.05) from the results exhibited by 1000 and 2000 mg/kg of extract per body weight where values were 7.18 and 7.27 m/cu.mm respectively. These results apart from the obtained from aqueous extract of 200 to 800 mg/kg, significant difference was not observed among the extracts treatment and the negative control group. Meanwhile, the results obtained from the ethanol extract concentrations and aqueous extract at 1000 to 2000 mg/kg of extract per body weight were within the standard range (7-10 m/cu.mm) for evaluation of normal health status Fig.1.

The haematological changes observed in the white blood cell counts with the ethanol extract treatment was 4.10, 4.06, 3.31, 3.32 and 3.41 t/cu.mm respectively for 200, 400, 800, 1000 and 2000 mg/kg of extract per body weight. The aqueous extract treatment resolved at 4.43, 4.20, 4.08, 3.67 and 3.54 t/cu.mm respectively for 200, 400, 800, 1000 and 2000 mg/kg body weight while the control valued at 3.50 t/cu.mm Fig. 2.

Effects of ethanol and extract treatments in haemoglobin interaction are presented in Fig. 3. Though the values obtained from both ethanol and aqueous extracts are within the standard range for normal health status, significant differences (P≤0.05) occurred between ethanol and aqueous extracts at 200, 400 and 800 mg/kg body weight extract where higher values of 14.45, 14.43 and 14.36% were observed over ethanol extract values of 11.40, 11.53 and 11.50%. However, significant differences was not observed (P>0.05) in the 1000 and 2000 mg/kg body weight of extract in both extract treatments Fig. 3.

Differential monocyte count in control group was 2.72%. Observed in ethanol extracts at 200, 400, 800, 1000 and 2000 mg/kg of extract per body weight were 1.48, 1.60, 2.48, 2.50 and 2.53% respectively while in that order it was 1.35, 1.43, 1.78, 2.37 and 2.46% in aqueous extract treatments. Though the values obtained in ethanol extract concentrations seemed a bit of better status than aqueous extracts treatment, both were within the standard range of normal health status (1-6%) Fig. 4.

Differential neutrophil count is as shown in Fig. 5. In the control group, a value of 13.83% was observed which was lesser than the values obtained in the extract concentrations treatment. In ethanol extract treatment with 200 and 400 mg/kg body weight, values of 26.18 and 20.21% were observed respectively while in aqueous extract at same concentrations were 28.14 and 26.28% respectively. However, decreased values were observed from 800 to 2000 mg/kg in both ethanol and aqueous extracts treatment. Notwithstanding the variations in values obtained in the extracts treatment, promising values within standard range of between 5-49% were obtained to evaluate the extract concentrations not harmful to alter negative changes for ill health proposal Fig. 5.

Decreased values in 200 to 2000 mg/kg body weight of extract in lymphocyte counts was observed in both ethanol and aqueous extracts treatment. In ethanol extract, lymphocyte values were 67.20, 67.45, 66.01, 64.03 and 63.11% at 200, 400, 800, 1000 and 2000 mg/kg body weight respectively while it was 70.05, 68.66, 67.21, 65.13 and 63.05% respectively with aqueous extracts treatment. Meanwhile, control group valued at 63.42% Fig. 6. With ethanol extract concentration treatment increase in values were observed in eosinophil count determining better status in order of extract concentration Fig.7.

Dose dependent increase in PCV determination was observed which was statistically significant (P<0.05) on compared with control group. While the control group PCV was 42.05±0.13%, ethanol extract at 200, 400, 800, 1000 and 2000 mg/kg were 38.06±0.15, 39.18±0.21, 40.11±0.18, 40.26±0.27 and 42.36±0.27% respectively. Aqueous extract in that order was 36.40±0.15, 38.42±0.34, 39.64±0.42, 40.68±0.25 and 40.25±0.18 respectively Fig. 8. However, standard range of between 40-56%) was not exceeded by the extract concentrations.

Male and female mice in each group were separated and weighed where average weights were obtained Table 1. Progressive increase in weights was observed from the control and extracts treated groups. However, administration of the extracts was dependant on average weights of mice in order to appropriate weight gain in the different extracts treatment. The weight gain observed in the extracts treated in comparison with the control group Table 1, further suggested the good role *L. dulcis* leaf ethanol and aqueous extract as a therapeutic agent could play in traditional medicine.

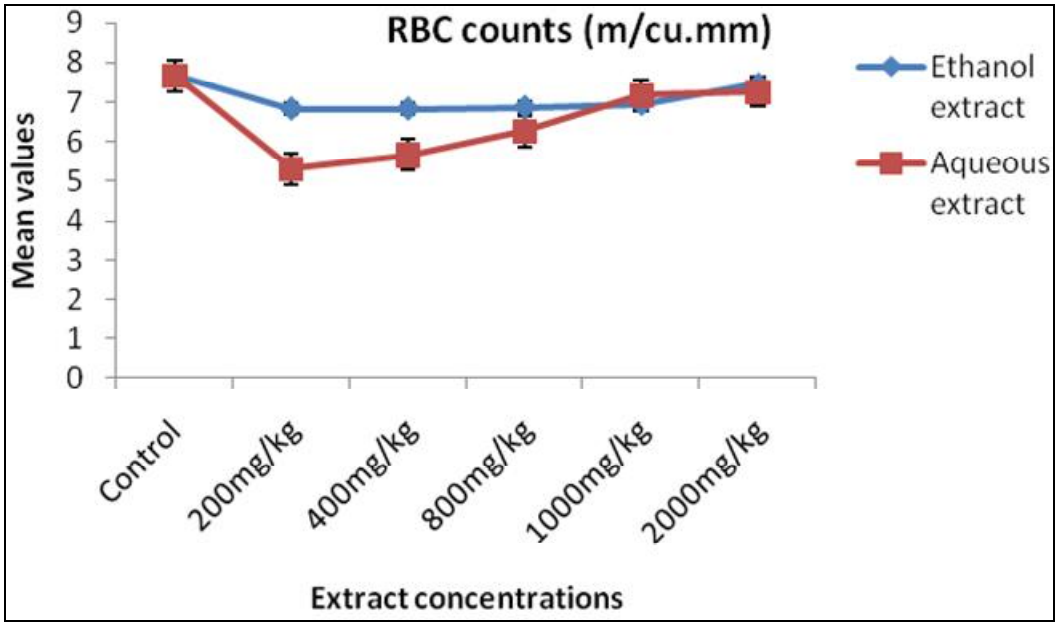

Fig. 1. Red blood means values of ethanol and aqueous extracts of *L. dulcis*
Values are expressed as mean ± SE, for five animals in each group

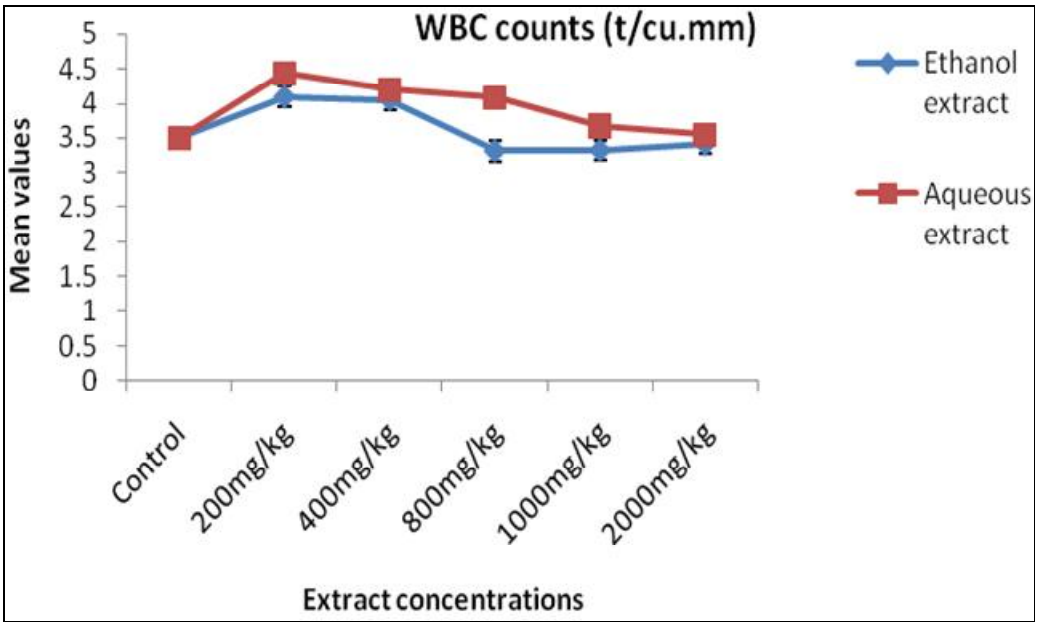

Fig. 2. Means counts value of white blood of ethanol and aqueous extracts of *L. dulcis*
Values are expressed as mean ± SE, for five animals in each group

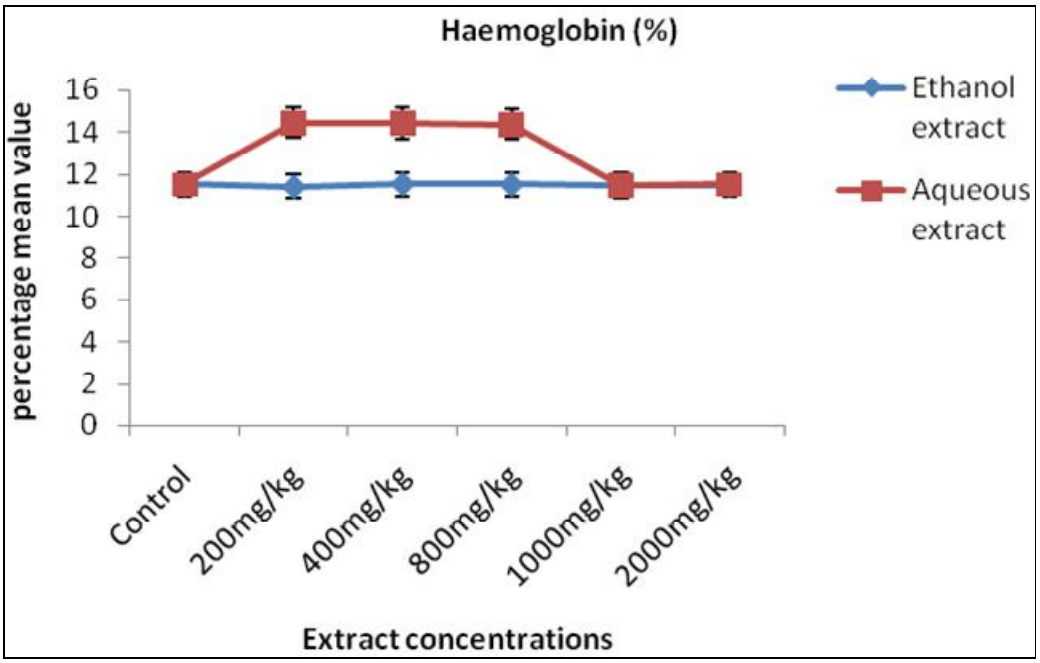

Fig. 3. Percentage means values of *L. dulcis* extracts on haemoglobin of Swiss albino mice
Values are expressed as mean ± SE, for five animals in each group

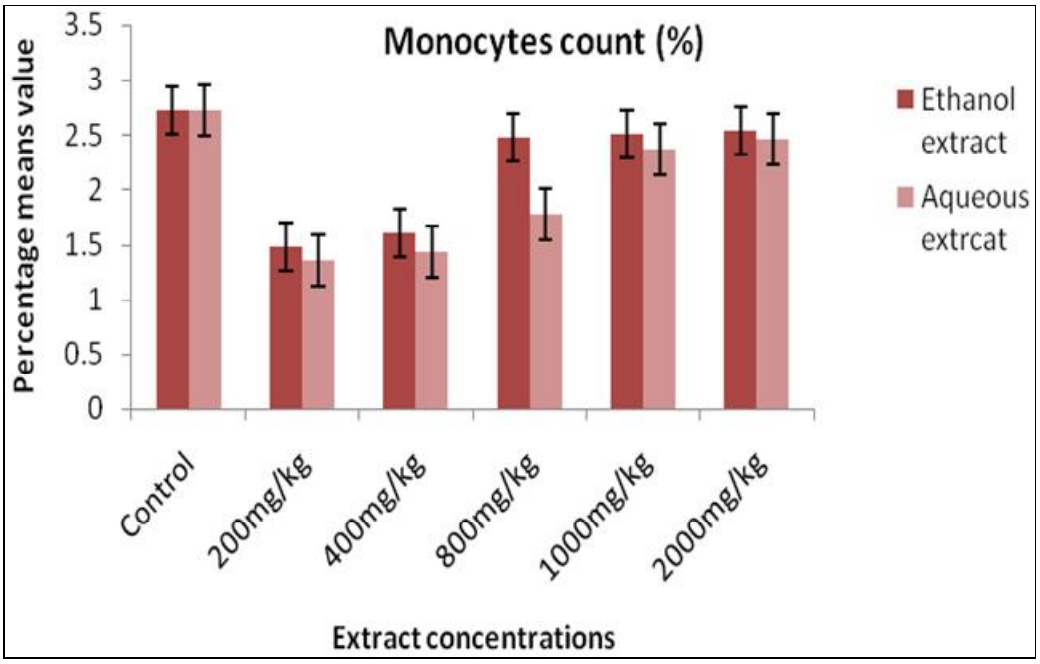

Fig. 4. Percentage means values of *L. dulcis* extracts on monocyte counts of Swiss albino mice
Values are expressed as mean ± SE, for five animals in each group

Plates 1 and 2 illustrates the histopathology of liver and kidney respectively of mice treated with the extracts and control group where no alteration to describe dysfunction of the organs were observed.

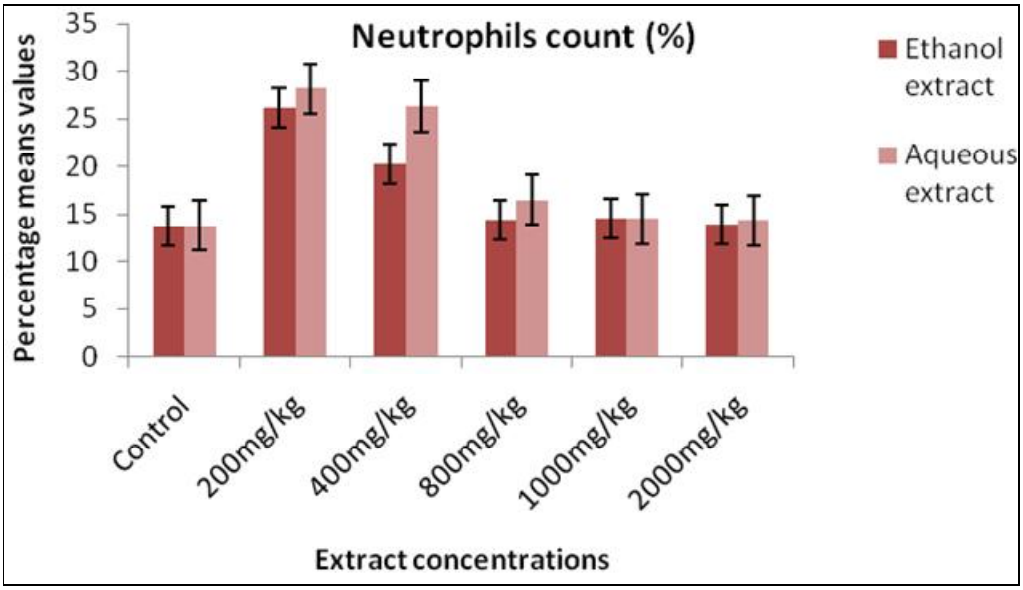

Fig. 5. Percentage means values of *L. dulcis* extracts on Neutrophil counts of Swiss albino mice

Values are expressed as mean ± SE, for five animals in each group

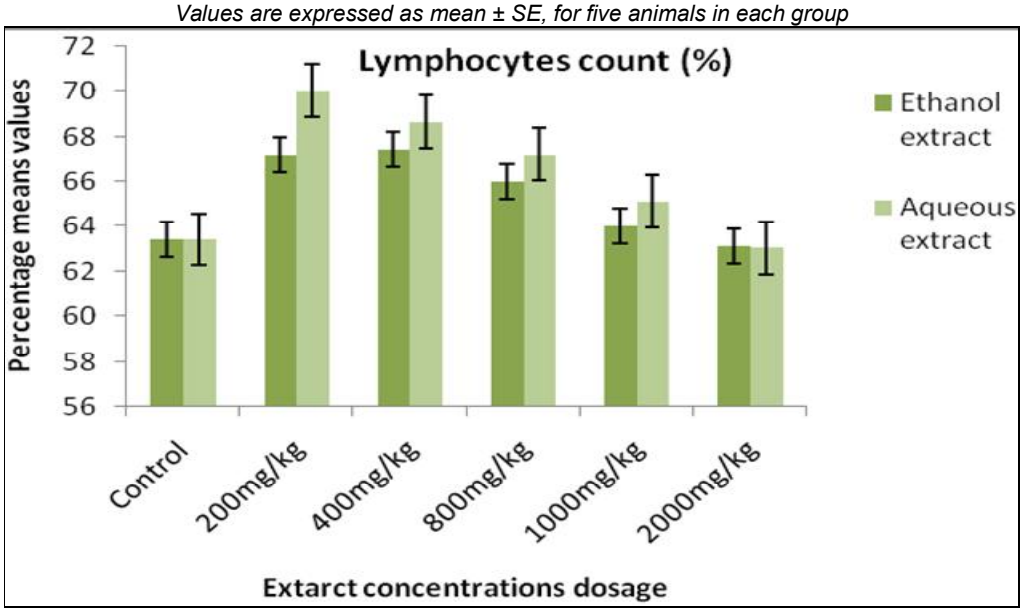

Fig. 6. Percentage means values of *L. dulcis* extracts on Lymphocytes counts of Swiss albino mice

Values are expressed as mean ± SE, for five animals in each group

Table 1. Body weight (g) of mice in acute toxicity for 14 days of extracts treatment

Parameters	Control		200mg/kg		400mg/kg		800mg/kg		1000mg/kg		2000mg/kg	
	F	M	F	M	F	M	F	M	F	M	F	M
Day 0	60*	79**	54*	66**	61*	70**	71*	76**	73*	81**	80*	90**
Day 7	65*	85**	62*	71**	67*	78**	72*	84**	77*	92**	87*	107**
Day 14	76*	96**	70*	82**	75*	86**	86*	93**	89*	100**	97*	110**
Weight gained at 14th day	16	17	16	16	14	16	15	17	16	19	17	20

** Average weight of two mice; **Average weight of three mice*

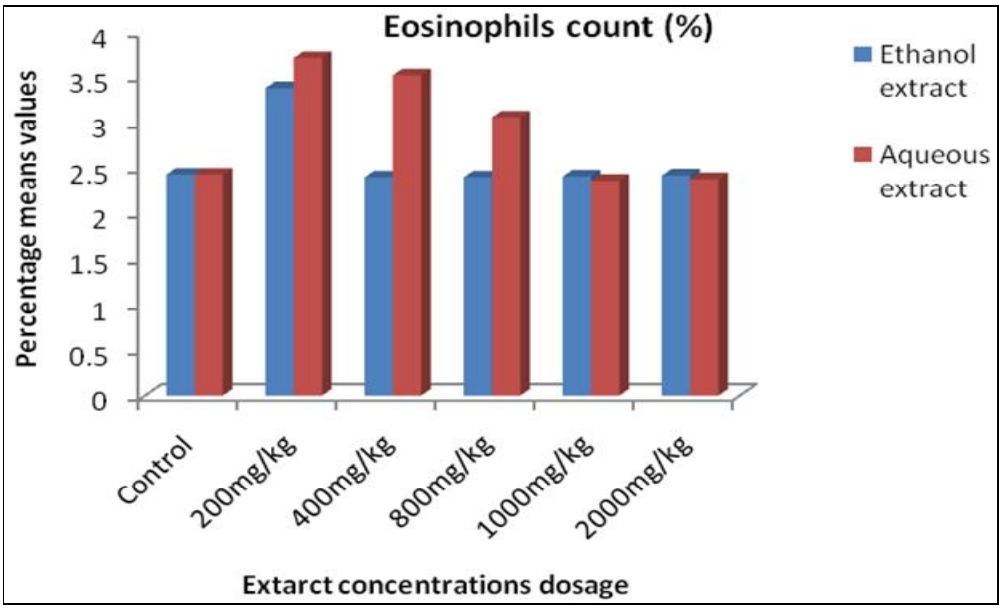

Fig. 7. Percentage means values of *L. dulcis* extracts on eosinophil counts of Swiss albino mice

Values are expressed as mean ± SE, for five animals in each group

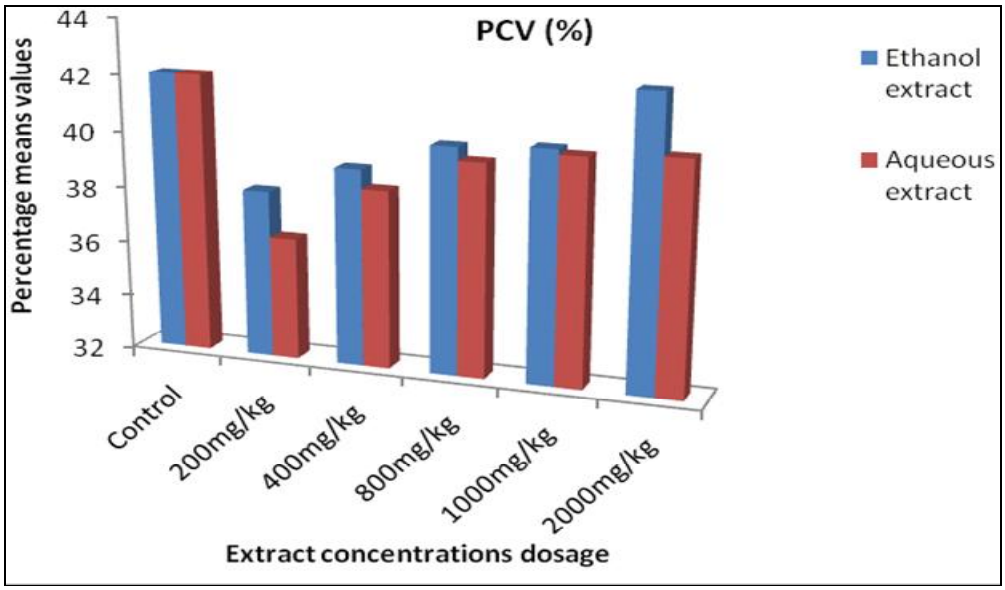

Fig. 8. Percentage means values of *L. dulcis* extracts on eosinophil counts of Swiss albino *mice*

Values are expressed as mean ± SE, for five animals in each group

4. DISCUSSION

Animals model have been useful in preclinical studies and clinical trials in the evaluation to proposing safe drugs for human use. Effects of toxicity are revealed in haematological profiles and vital organs such as liver, kidney and intestine. In this study possible hepatoprotective or toxicity of *L dulcis* extract concentrations at 200, 400, 800, 1000 and 2000 mg/kg body weight were investigated where effect on haematology, liver and kidney were monitored. Herbal drugs have become important in medication because they are readily available,

accessible by the common man, highly efficient and safe in use. One of the important and well-documented uses of plant-products is their use as hepatoprotective agents and cure for other diseases of non microbial and microbial origin hence; there is an ever in-creasing need for safe hepatoprotective agent [16]. Herbal preparations for cure and prevention of liver diseases are practiced by many cultures all over the world. However, only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy [5]. Slight depletion in the number of RBCs count alongside Hb concentration was detected in mice treated with ethanol and aqueous extract concentrations. The less RBCs count and Hb content recorded in the extract treated mice than the control group could be attributed to disturbed hematopoiesis, destruction of erythrocyte, and reduction in the rate of their formation and/or their enhanced removal from circulation. The reduction in the values of blood parameters (PCV, RBC and Hb) may be attributed to the hyperactivity of bone marrow, which leads to production of red blood cells with impaired integrity that are easily destroyed in the circulation. The higher RBC observed in the control group than the extract treated mice could be as a result of the normal diet and water which were free from toxic substances having no expression of changes in the physiological and pathological status of the mice. However, the minor physiological changes in the extract treated mice resulted to lower RBC mean count in values. These changes could result when there is temperature rise in the mice which might lyse some of the red blood cells that can lead to reduction in PCV, increase in WBC and increase in haemoglobin. Increase in temperature is experienced mainly when the body system observed foreign compounds which it tries to combat and absorbed for its eradication from the body. In most cases the rise in temperature is lowered to normal when the foreign compounds are suppressed by the body mechanisms. The reduced level of haemoglobin could be associated with haemolysis or disturbances in heme biosynthesis as a result of inhibiting the link of iron with heme and drop in activity of enzymes necessary for heme biosynthesis [17]. The presence of such changes suggested that the alterations of the erythrocyte parameters with the extract treatment may be related to enhance intravascular haemolysis as a result of oxidative stress and lipid peroxidation in the circulating erythrocytes [18]. This occurrence might probably be due to normal response to foreign bodies or stress associated with the chronic toxicity studies which might necessarily not result to anaemia because the changes observed was slight and there is tendency for recovering from the stress after a while hence the extracts were not found to be of actual toxic form in the mice.

Though reduction in RBC was observed in extract treated mice, signs of illness (es) such as anxiety, fatigue, reduction of food intake, tremor, dizziness, posterior paralysis, diarrhea, aggressiveness, ruffled hair coat, salivation etc were not noticed in mice, weight loss was not also recorded. This indicates that the extracts lethal dose (LD_{50}) is higher than 2000 mg/kg for the mice. This could be responsible for the non significant difference observed in the values of the control and extracts treated groups of mice. The non alteration of haematological indices of the extract treated mice RBC, hepatic and renal functions in relationship to the control value in dose dependant could approve of the extract concentrations valuable in therapeutic application.

Higher WBC count was observed in the aqueous extract treated mice than the ethanol extract treated. This signifies that the ethanol extract has lesser effect of physiological changes in the mice. On general note, both the ethanol and aqueous extracts treatment values were found higher than the control value up to 400 mg/kg in ethanol extract and 800 mg/kg body weight in the aqueous extract treated group and thereafter drop in values to normal health status correlation as observed with the control mice values. Therefore, the insignificant changes observed could be due only to normal response to foreign compounds or stress associated with the toxicity study even as observed by [9].

Hence aqueous extract treatment had more slight effect in the physiological changes of the mice, improvement in values were found better in the ethanol extract treated where increase in values was observed to be on dose dependant. Despite the alterations in haematological changes in the mice with the extracts treatments, the varied values observed were within the standard range. This implies that the extract concentrations employed in this study does not possess any significant adverse effect on haematology of mice. Similar observation was recorded by [19] in the use of *Cucurbita maxima* on acute and sub chronic toxicity.

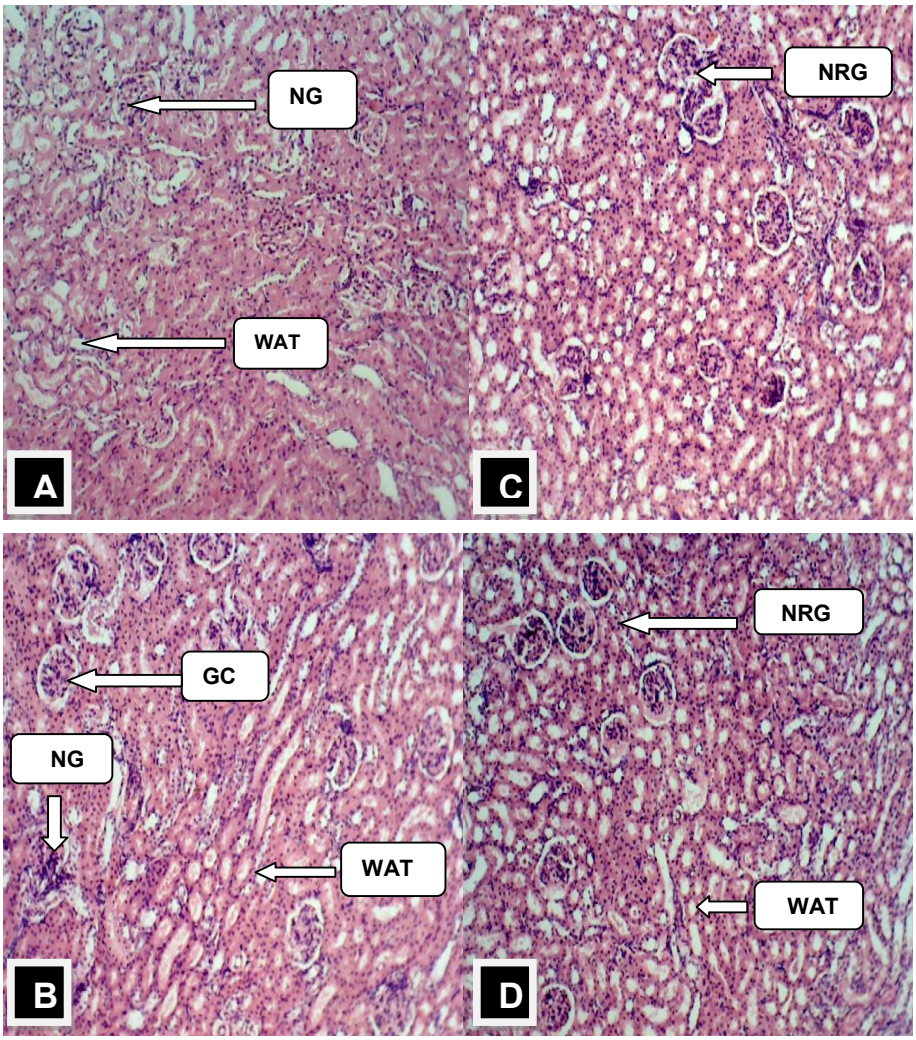

Plate 1. Histopathology of kidney of the experimental mice treated with 200 mg/kg body weight

(A): showing Necrotised glomeruli (NG) and well arranged tubules (WAT); 400 mg/kg (B): showing glomeruli capsule (GC), Necrotised glomeruli (NG) and Well arranged tubules (WAT); 800 mg/kg (C): showing normal round glomeruli (NRG) and 2000 mg/kg (D): showing normal round glomeruli (NRG) and well arranged tubules (WAT). Magnification = X100

For all haematological data, 200 and 400 mg/kg extracts slightly decreased or increased cell counts while there were no any differences between control and higher dose groups. This could be as a result of some toxic compounds which were neutralized at higher dose of 800 through 2000 mg/kg. The effects produced on heamatology count were within the normal range and there is the tendency for the mice body mechanism to regulate the observed effects in a period of time. The male and female mice treated with the extract concentrations of 200 to 2000 mg/kg body weight extract had gradual increase in body weight for the period of 14 days observation. The continuous increase in body weight for the period of 14 days study explained regular feeding and improvement of nutritional state in the mice. In this study ethanol and aqueous extracts of *L. dulcis* at the employed concentrations was found not toxic and safe for use on mice for a period of 14 days treatment.

Toxins from plants have been known to induce regenerative anemia [20]. Regenerative anemia is seen as an increase in MCH. It was observed

that when RBC precursors mature in the bone marrow their volume decreases as the Hb content increases. However, reticulocytes which are released into circulation during regenerative anaemia have a higher MCH [20]. The acute administration of aqueous and ethanol extracts of *L. dulcis* did not have any much effect on the WBC value to ascertain as been significant. [21] Reported that toxic substances caused decrease in total white blood count (TWBC) through either bone marrow depression or competition with folic acid utilization to cause leucopenia. In this study, all the erythrocyte and leucocyte values were not affected when the mice were treated for a period of fourteen days with 200-2000 mg/kg extract per body weight. This emphasized that *L. dulcis* will not induce anaemia when given for a long duration. Hence the extracts could not have any related effect on haemoglobin estimation and RBC count; it indicates that the extracts may have no possibility to manifest anaemia.

The histopathological study with the aqueous and ethanol extract of *L. dulcis* in possible aspect of toxicity activity in liver of mice was as well studied alongside effects on haematology, With the studied extracts dose of both aqueous and ethanol extract of the plant, effect no damage to liver architectural structure of the mice liver and ultra structure of hepatocytes, few lipid globules, and normal glycogen deposits into cytoplasm was found in the extracts treated mice on comparison with the control group with regular aspect of nuclear shape and rER's profiles. This result obtained is in correlation with [22,23]. The envisaged hepatoprotective role of *L. dulcis* could be attached to many co-factors that may modify the pharmacological activity of the plant extract such as vitamins E, A, C, glutathione and phytochemicals thus its possible effectiveness in hepatoprotective and therapeutic management of several diseases. Other possible mechanisms of hepatoprotective action of *L. dulcis* extract may be due to its free radical scavenging activity as indicated by non increase in TWBC and the total differential count values which were within standard range.

Kidney lesions due to toxic damage were not observed which could not have made the tubular epithelium to be confined and therefore resulting to no suppression of tubular reabsorbing function as evidenced by the normal architectural structure in the kidney tubules. These observations are similar to the reports of [24]. [25] Stated that the functional studies in toxicology should be coupled with the appropriate histological studies, because appropriate morphological studies are useful especially during the anatomical localization of action of toxin. Based on the histological studies conducted the ethanol and aqueous leaf extract of *L. dulcis* seems to be devoid of any toxic effects in mice up to 2000 mg/kg body weight.

The present study showed for the first time, *L. dulcis* aqueous and ethanol leaf extract could possess hepatoprotective and renal protective activity as expressed by histological studies and the encouraging haematological indices which were within standard range. The aqueous and ethanol leaf extract of *L. dulcis* concentrations orally dosed at (200, 400, 800 & 2000 mg/kg) once daily for 14 days showed dose dependent hepatoprotective and renal protective activity, where highly significant effectiveness of the extracts was seen with 2000 mg/kg extract per body weight. Though this study had few limitations under the view that the extracts were not tested against toxic induced substances such as chemicals and drugs, effects to predict toxicity on haematology, hepatic and renal dysfunctions were not observed in mice. Solvents preparation of plant products for therapeutic use is necessary to evaluate their efficacy, safety, durability and choice of preference for acceptability. The histopathological aspect of this work provided supportive evidence for the unaltered haematological analysis observed. The liver section histology of control mice showed normal hepatic cells each with well preserved cytoplasm, prominent nucleus and nucleolus and a well defined central vein while also the kidney histology showed well arranged tubules, glomeruli in well defined architectural structure, renal tubules lined with thick cubic epithelium and tubules with regular distinct lumen without noticeable alterations when compared to the control groups of liver and kidney histology.

With the results obtained, composition of drugs, which have the polyherbal formulations of *L. dulcis* plant extract, should be encouraged for its therapeutic importance, envisaged useful remedy in hepatoprotective and renal protection.

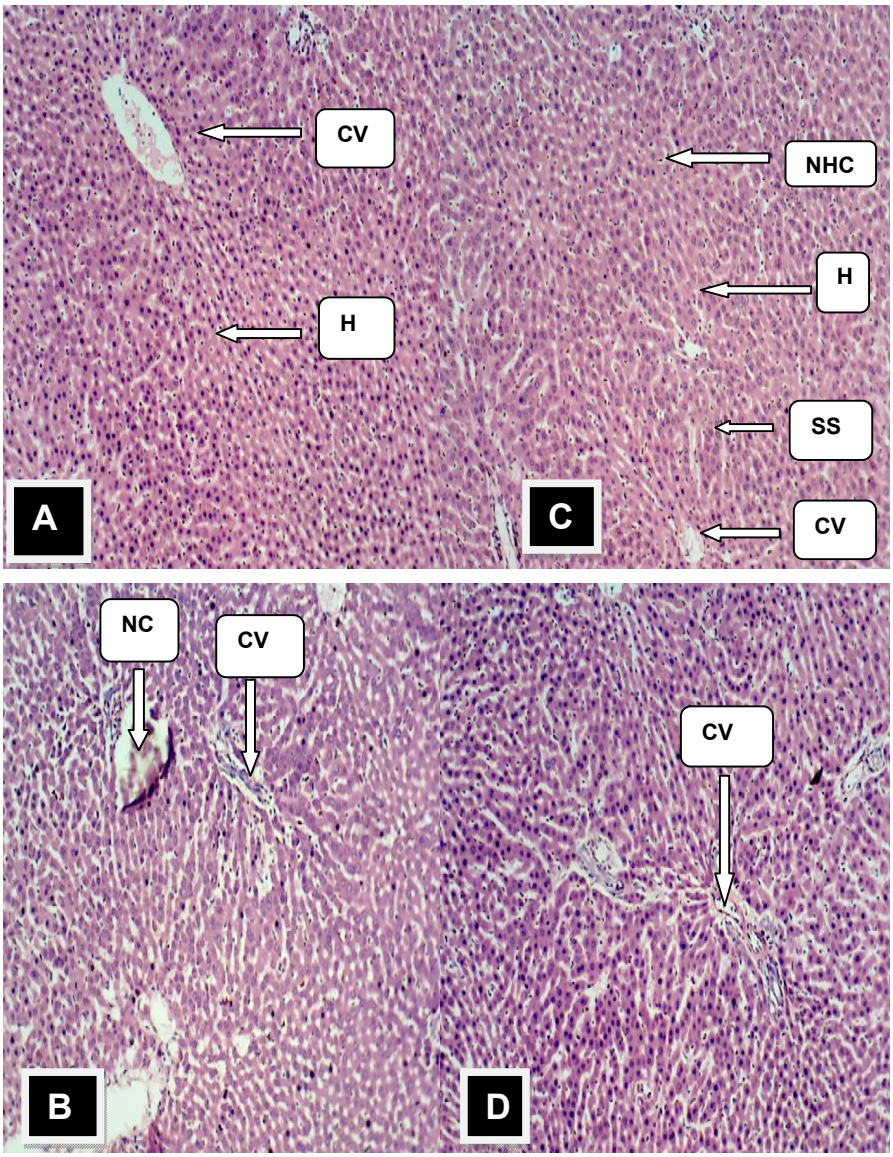

Plate 2. **Histopathology of liver of the experimental mice treated with 200 mg/kg body weight**
(A): showing Central vein (CV) and hepatocytes (H); 400 mg/kg (B): showing necrotic cell (NC) and central vein (CV); 800 mg/kg (C): showing normal hepatic cells, (NHC), hepatocytes (H), sinusoidal spaces (SS) and central vein; 2000 mg/kg (D): central vein (CV). Magnification = X100

5. CONCLUSION

In this study we employed two methods of solvent extraction of *L*. *dulcis* leaf as a trial for therapeutic function in disease control or prevention using animal model. The extract concentrations at 200 10 2000 mg were found not toxic and safe for administration in mice for 14 days. Effects to be considered as hazardous were not found in the studied heamatology parameters as values recorded were within standard range. Histology of animal liver and kidney tissues showed well defined architectural structures and improvement of health status of the extract was on dose dependant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-

23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

ACKNOWLEDGEMENTS

We highly appreciates Prince J. A. Adesida for his technical assistance and Prof. F. A. Akinyosoye for his valuable suggestions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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