Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 9 (2): 60-71, 2015
ISSN 1819-3455 / DOI: 10.3923/rjmp.2015.60.71

Sub-Acute Evaluation of Extract of *Syzygium malaccense* in Albino Rats

[1]Abiodun Humphrey Adebayo, [1,2]Oyinlade Cecilia Ogundare and [1]Oluwatobi Samuel Adegbite

[1]Biochemistry and Molecular Biology Unit, Department of Biological Sciences, College of Science and Technology Covenant University, PMB 1023, Canaan Land, Ota, Ogun State, Nigeria

[2]Biochemistry Unit, Department of Science and Laboratory Technology, School of Technology, Lagos State Polytechnic Ikorodu, PMB, 21606, Ikeja, Lagos State, Nigeria

Corresponding Author: Abiodun Humphrey Adebayo, Biochemistry and Molecular Biology Unit, Department of Biological Sciences, College of Science and Technology Covenant University, PMB 1023, Canaan Land, Ota, Ogun State, Nigeria

ABSTRACT

The study was aimed at investigating the sub-acute evaluation of the extract of *Syzygium malaccense* in albino rats. Five groups of eight rats per group were orally administered with graded 50, 100, 250 and 500 mg kg^{-1} b.wt. doses of the extract for 28 days. Blood samples of the sacrificed rats were collected for biochemical and haematological studies while liver and kidney tissues were used for histopathological assessment. The results showed an LD_{50} of 1224.75 mg kg^{-1} b.wt. with no significant ($p>0.05$) changes in weight of organs tested. Biochemical parameters such as AST, ALP, protein and albumin levels in all the treated animals did not change significantly, however, there was significant ($p<0.05$) change in the activity of ALT as well as haematological parameters such as RBC, WBC, HGB, platelet counts, MCV and MCH when compared with the control group. The results from histopathology showed an inflammation of the liver cells at doses beyond 100 mg kg^{-1} b.wt. but there was no significant damage to the kidney tissue. It may be concluded that the extract of *S. malaccense* possesses the tendency of affecting the haematopoietic elements and may also alter the structural integrity of the liver tissue if ingested at higher doses.

Key words: *Syzygium malaccense*, myrtaceae, biochemical parameters, haematological indices, histopathology

INTRODUCTION

Many people preferred medicinal plants because orthodox treatments are not readily available, expensive and often associated with serious side effects (Taiwo *et al*., 2005). Several herbal extracts are available in markets of most developing countries like Nigeria, where medicinal plant extracts are dispensed indiscriminately by personnel who lack toxicological knowledge of such extracts and their side effects (Dhiman and Chawla, 2005; Mohamed *et al*., 2010). However, the effectiveness of the extracts for different therapeutic purposes including cancer, oxidative and purgative diseases, anti-diabetic, topically emollient, anti-inflammatory, antimicrobial properties and precursors for the synthesis of metabolites are due to their bioactive constituents (Abolaji *et al*., 2007; Adebayo *et al*., 2010). *Syzygium malaccense* (L.) belongs to the family of Myrtaceae and it is popularly called "Mountain or Malay apple" among the natives of India and

Malaysia. Its parts including seeds, bark, fruits and leaves are used traditionally as anti-inflammatory, antiviral, antifungal, antibacterial, antibiotic, remedy for itching, diuretic, as skin lotion and anti-edema (Locher *et al*., 1995; Dunstan *et al*., 1997; Pulliah, 2006). Studies reported the presence of a hydro distilled essential oil from *S. malaccense* leaves containing monoterpenes (61.1%) and sesquiterpenes (30.8%) (Karioti *et al*., 2007). The plant reportedly exhibited strong antioxidant activity due to higher phenolic and flavonoid contents than aqueous extract (Savitha *et al*., 2011). The recent use of medicinal plants as an alternative to clinical therapy has greatly increased, therefore, experimental screening could be an important basis for scientific documentation on the safety and toxicity profiles of medicinal plants through an extensive assessment of their protective and toxicological effects, hence, the need to investigate the oral toxicity of the leaf extract of *S. malaccense* in albino wistar rats has become necessary.

MATERIALS AND METHODS

Plant collection and identification: Fresh leaves of *Syzygium malaccense* were obtained from Ikorodu in Lagos metropolis, Lagos State, Nigeria. These were identified by a botanist at the Herbarium unit, Department of Botany University of Lagos, Lagos, Nigeria.

Sources of animals: Albino Wistar rats weighing between 170-210 g were purchased from the animal house of the Department of Pharmacology, University of Lagos, Lagos State, Nigeria. These animals were allowed to acclimatize for two weeks. Grower feed mash from Konsult Nigeria Limited and water were given *ad libitum* to the animals. The animals were handled in compliance with the good ethical practice of animal ethic committee rules and the recommendations of National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH., 1985).

Extraction of plant sample: Fresh leaves of *S. malaccense* were collected, air dried in laboratory for about four weeks and pulverized into fine powder by a homogenizer. The powdered leaves (500 g) were extracted in ethanol using soxhlet extractor according to the procedure described by Adebayo *et al*. (2006) and concentrated at 50°C in a rotary evaporator before further concentrated in laboratory oven at 50°C. The yield of the extract was determined as 18.2%.

LD_{50} investigation: The lethal dose value which will cause death in half of the total animals (LD_{50}) was used to ascertain the extent of the extract toxicity at different dosages using an established method (Lorke, 1983). Following the period of fasting, the animals were weighed in other to determine their fasted body weight. The results of the phase one determined the dosages of phase two. In the first phase, nine mice were divided equally into three groups and received the ethanolic extract of *S. malaccense* leaf at an oral dose of 50, 100 and 200 mg kg^{-1} b.wt. respectively. In the second phase, another three groups of three mice were administered with extract at the dose of 1000, 1500 and 2000 mg kg^{-1} b.wt. respectively. Animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 h. The LD_{50} value was calculated as the square root of the geometric mean of highest non lethal dose for which the animal survived and the lowest lethal dose for which the animal died (Danmalam *et al*., 2012).

Sub-acute toxicity study: Twenty five rats were used for the sub-acute study. The rats were divided into five groups of five rats per group. Animals in each group were treated orally with extract of *S. malaccense* for 28 days via a cannula. Animals in group A which received only distilled

water served as the control group while the animals in groups B, C, D and E were administered 50, 100, 250 and 500 mg kg^{-1} b.wt., respectively for 28 days. They were observed daily for general signs and symptoms of toxicity.

Blood collection and preparation of sample: The rats were anaesthetized using diethyl ether prior to dissection on the 29th day. The blood samples were collected by cardiac puncture into serum separator tubes and centrifuged at 10,000 revolution per minute for 10 min to obtain their sera. These were stored at -20°C until required for biochemical assays according to method described by Adebayo *et al*. (2014). The remaining blood samples were collected in EDTA tubes in an iced bath for haematological assays. The liver and kidney tissues were also collected, rinsed in normal saline and fixed with 10% formaldehyde for histopathological examination.

Biochemical studies: The under listed biochemical parameters as described by each procedures were determined using biochemical kits (Randox Laboratories, UK): Aspartate aminotransferase, AST (Reitman and Frankel, 1957); alanine aminotransferase, ALT (Deneke and Riiffersdorf, 1984); alkaline phosphatase, ALP (Reitman and Frankel, 1957); total protein (Bradford, 1976) and albumin (Doumas *et al*., 1971).

Determination of haematological studies: The blood samples were analyzed using haematology automated analyzer (Sysmex KX-21N, Japan) to determine the haematocrit (HCT), White Blood Cell count (WBC), Red Blood Cell count (RBC), Haemoglobin Count (HB), Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Platelet Count (PLT) and strictly followed the method of Olagunju *et al*. (2000).

Histopathological studies of selected vital organs: The fixed liver samples were treated using detailed procedures for tissue processing and staining as described by Aliyu *et al*. (2007). After the blood was rinsed in normal saline, the organs were fixed in 10% formalin, dehydrated with 100% ethanol solution and embedded in paraffin. Sections were taken from each selected samples and processed into 4-5 μm thick, stained with haematoxylin and eosin and observed under a microscope.

Statistical analysis: Statistical analysis software package, SSPS version 14 and Graph Pad Prism version 5.0.1 were used to carry out data entry and validation. One way ANOVA was used to compare treatment groups. The results were expressed as Means±standard deviation and Tukey's multiple comparison test was used to determine the significant difference between the mean of paired groups.

RESULTS

The calculated mean lethal dose (LD_{50}) of 1224.75 mg kg^{-1} b.wt. was obtained. The *S. malaccense* leaf extract treated rats neither experienced behavioral changes, CNS responses, convulsion nor death during this experiment. It was observed as shown in Fig. 1, that there was a remarkable change in body weight of tested rats where the initial significant ($p<0.05$) increase in body weight of rats in groups treated with 100 and 250 mg kg^{-1} b.wt. extract observed as at 21st day of treatment became normal at the end of the toxicity testing.

The effects of *S. malaccense* on some biochemical parameters of tested albino rats are shown in Fig. 2. There was no observed significant difference ($p>0.05$) in the level of serum albumin, ALP, AST and ALT in all treated groups when compared with the control group.

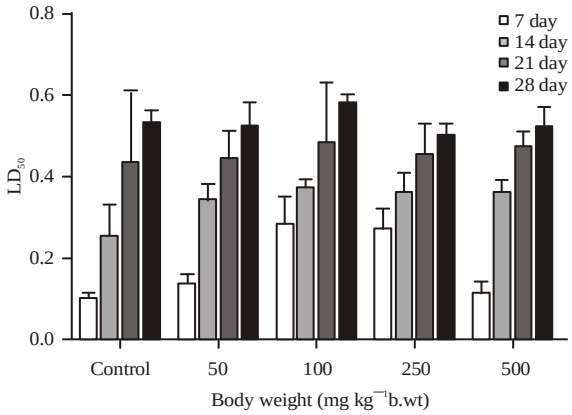

Fig. 1: Effect of *S. malaccense* on body weight of albino rats. Values represent Mean±SD of 5 replicates. p>0.05 compared to control group

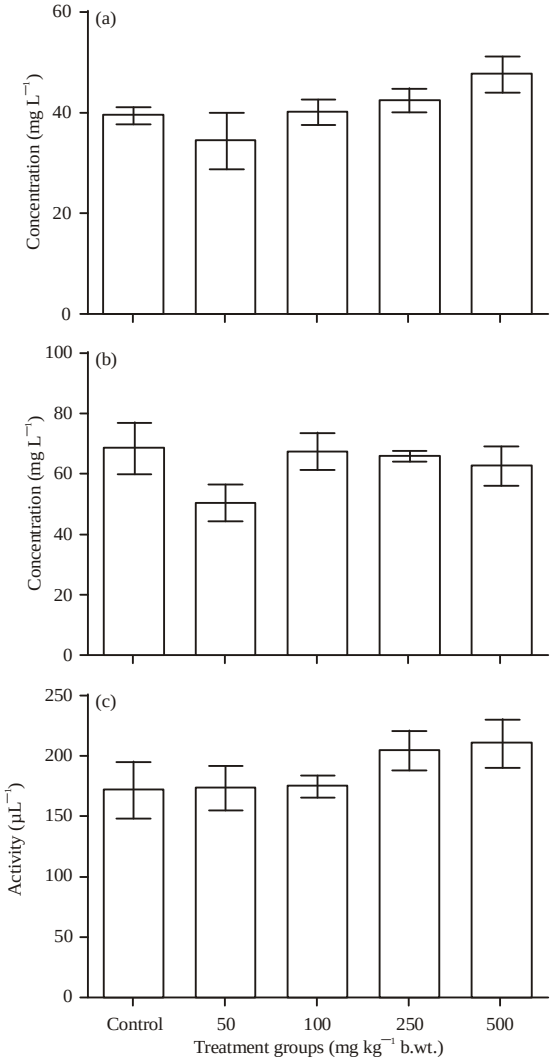

Fig. 2(a-e): Continue

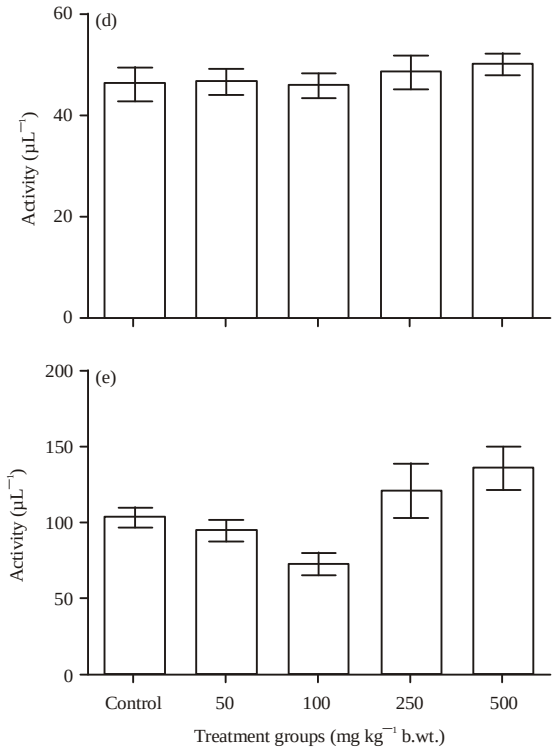

Fig. 2(a-e): Effect of extract of *S. malaccense* on selected biochemical parameters in albino rats. Values represent Mean±SD of 5 replicates. p>0.05 compared to control group. Effect of the extract on (a) Serum albumin, (b) Total protein, (c) Serum ALP, (d) Serum AST and (e) Serum ALT

Figure 3 shows the effect of *S. malaccense* on haematological indices in albino rats. The leaf extract significantly ($p<0.05$) depleted Red Blood Cells (RBC) count in group treated with 50 mg kg^{-1} b.wt. when compared to the normal control group. Similarly, HCT level was significantly ($p<0.05$) decreased across the groups except the group treated with 100 mg kg^{-1} b.wt. Moreover, there were significant ($p<0.05$) differences across all the treated groups in WBC and HB counts except in the group administered with 250 mg kg^{-1} b.wt. Furthermore, there were significant ($p<0.05$) reductions in PLT, MCV and MCH counts in all the groups treated with the extract when compared with the animals in the control groups.

The results from liver histological study showed that the groups treated with 250 and 500 mg kg^{-1} b.wt. of the extract indicated a mild to chronic inflammation of the liver cells (Fig. 4d-e). There were no visible damages to the hepatocytes of rats treated with the lower doses (Fig. 4a-c). Similarly, there was no significant damage to the kidney tissues in all the treated groups when compared with the control group (Fig. 5a-e).

DISCUSSION

Investigation of the acute toxicity is often the initial step in all toxicological investigations of unknown substances. Most acute toxicity data are of limited clinical application because cumulative harmful effects occur even at very low doses therefore, multiple dose studies are almost always

valuable in evaluating the safety profile of phytomedicines (Abotsi *et al*., 2011). The inability of the rats to experience behavioral changes, CNS responses, convulsion nor death during the study could be a pointer to the fact that the extract probably had no action on the nervous system (Ogwal-Okeng *et al*., 2003; Mabeku *et al*., 2007).

The observed normalcy in weight (Fig. 1) despite initial variation in weight gained by the treated rats might be due to initial suppression of appetite at higher dose as recorded in the group treated with 100 mg kg^{-1} b.wt. of the leaf extract of *S. malaccense*. Subsequent increased in food and water intake led to a non-significant weight change throughout the period of study. *S. malaccense* was able to maintain a healthy body weight especially in management of obesity, dyslipidemia, management, cardiovascular risk and hypertension (Pieme *et al*., 2006; Ikewuchi *et al*., 2011).

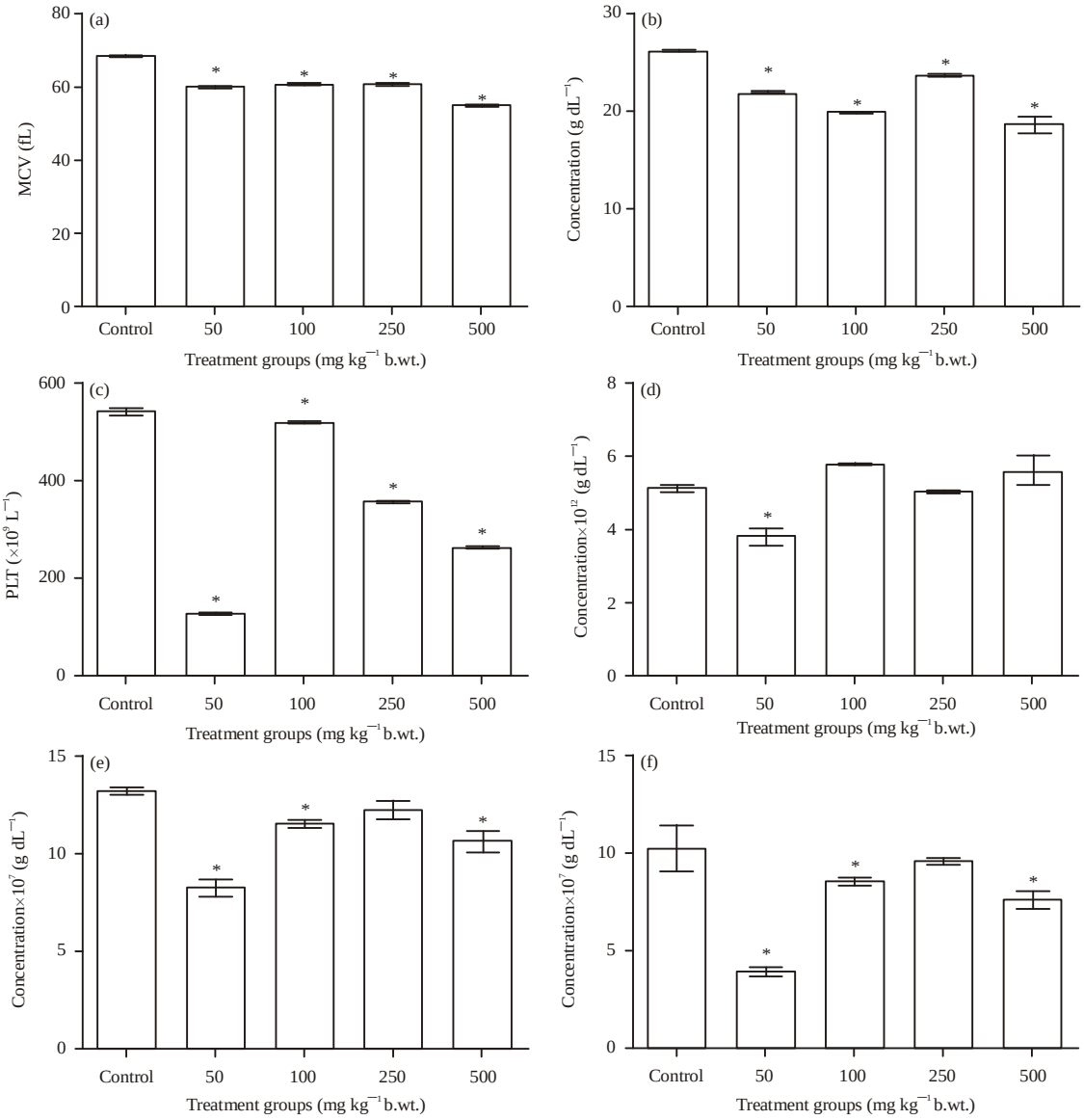

Fig. 3(a-g): Continue

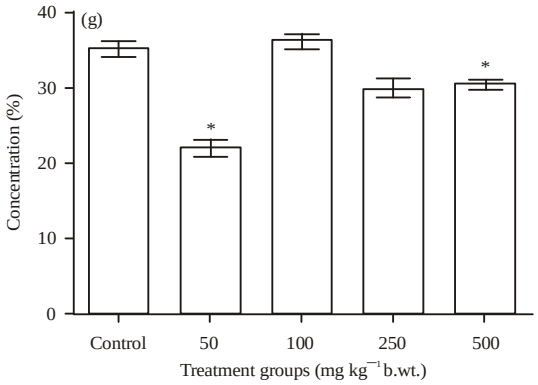

Fig. 3(a-g): Effect of *S. malaccense* on hematological parameters of albino rats. Values represent Mean±SD of 5 replicates. *p<0.05 compared to control group. Effect of the extract on: (a) MCV, (b) MCH (c) PLT, (d) RBC, (e) Hb, (f) WBC and (g) HCT

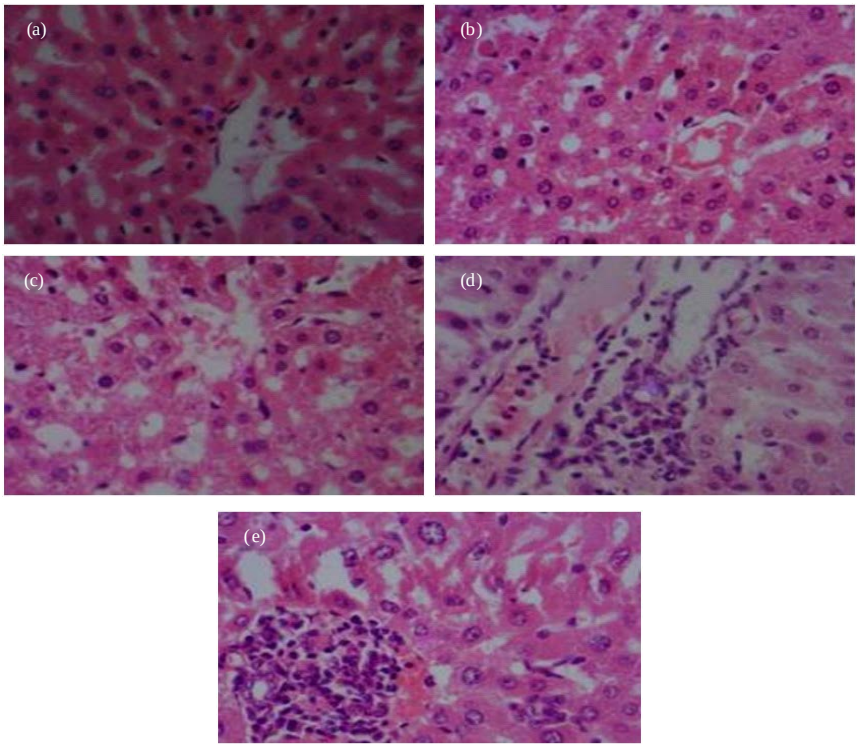

Fig. 4(a-e): Photomicrographs of liver sections in normal and *S. malaccense* extract treated albino Wistar rats (HE stain, x400). (a) Liver tissues of normal rats and (b, c, d and e) Rats treated with a daily dose of 50, 100, 250 and 500 mg kg^{-1} b.wt. extract of *S. malaccense* extract, respectively for 28 days. Figure 4a-e show normal liver architecture where the hepatocytes surround the central vein of liver in rats. Figure 4d and e indicate liver sections of rats treated with 250 and 500 mg kg^{-1} b.wt. of *S. malaccense* extract respectively for 28 days. The liver sections show mild to chronic inflammatory parenchyma liver cells where the hepatocytes surround the chronic inflammatory cells beside a central vein

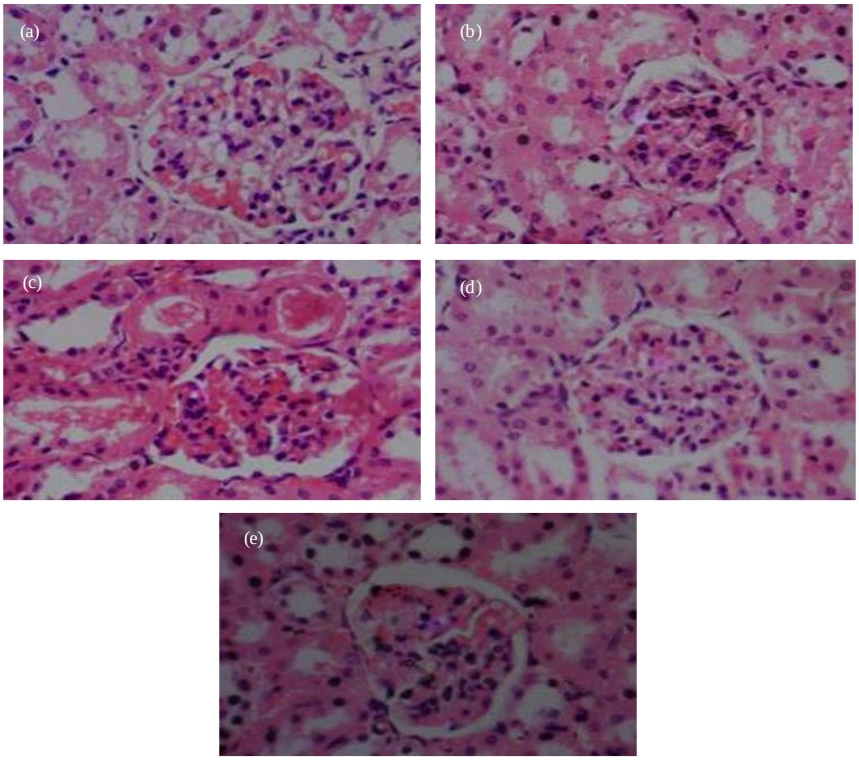

Fig. 5(a-e): Photomicrographs of kidney sections in normal and *S. malaccense* extract treated albino Wistar rats (HE stain, x400). Sections of the kidney in (a) Normal control rats and (b, c, d and e) Rats respectively treated with 50, 100, 250 and 500 mg kg^{-1} b.wt. extract of *S. malaccense* for 28 days

Our investigations have shown that ethanolic leaf extract of *S. malaccense* is non toxic to the liver at low doses. Mean lethal dose (LD_{50}) value is often used as the basis for assessing acute toxicity. However, LD_{50} values often differ from one laboratory to another due to both endogenous and exogenous variables such as: animal species and strain, age, gender, diet, bedding, ambient temperature and time of the day (Lorke, 1983). Hence, there are considerable uncertainties in extrapolating LD_{50} value obtained from species to species, consequently, recognizing LD_{50} test as providing, at best, only a ballpark estimate of human lethality has been advocated (Zbinden and Flury-Roversi, 1981). The calculated LD_{50} of 1224.75 mg kg^{-1} b.wt. was obtained. This shows that the extract is relatively safe or may be slightly toxic like any xenobiotics and may not cause any form of hepatotoxicity since it falls within the range of 1000-5000 mg kg^{-1} b.wt. (Lorke, 1983; Clarke and Clarke, 1997). Results from biochemical parameters suggest that the plant extract may not pose any risk to the liver and kidney when consumed within range of minimal concentration. Consequently, there was no significant change ($p>0.05$) in the activities of serum albumin, AST, ALT and ALP (Fig. 2). Since, the liver is the major organ of detoxification and mostly exposed to different levels of ingested xenobiotics which can adversely affect hepatic cells (Prosper *et al*., 2010). This result suggests that the extract is not likely to exert damage to liver cells. The liver re-synthesizes proteins to maintain the fluid and electrolyte balance in the plasma. With respect to that, *S. malaccense* leaf extract did not alter the level of total protein content of the treatment groups, although there was an initial decline in total protein content at

50 mg kg^{-1} which later increased as the doses of the extract increases, however it remains in contrast with the level of albumin in isolation which spiked at 500 mg kg^{-1} b.wt. of extract although not significant. Albumin, a major protein synthesized in the liver is an important biomarker for liver diseases (Adebayo *et al*., 2014). Our study showed no significant elevation of albumin at in all treated groups. This result is similar to the effects of *O. subscorpioidea* on biochemical parameters of albino rats (Adebayo *et al*., 2014). Albumin maintains osmotic pressure, protein reservoir and transportation of endogenous and exogenous substances (Saidu *et al*., 2007). However, results of MCV obtained from haematological studies suggest possible loss of fluid in treated rats since increased albumin and reduced MCV values are markers of dehydration (Byock, 1995). Dehydration may not be caused by cellular reactions but could result from the distress that resulted initially from introduction of the plant extract. Alterations in the levels of liver function parameters are dependent on the physiological state of the liver during the course of administration and metabolism of the introduced plant extract. Causes of the alteration could be attributed to increased metabolic rate of the liver which required increased induction of several drug metabolizing enzymes such as phase 1 and phase 2 drug metabolizing enzymes, in addition, mild degeneration/regeneration cycle of hepatic cells can also lead to altered but there was no significant ($p>0.05$) changes in ALT, ALP and AST levels of the serum. The evaluation of haematological parameters could be used to reveal the deleterious effect of foreign substances including plant extracts on the blood constituents of animals and to determine possible alterations in the levels of biomolecules such as enzymes, metabolites, haematology, normal functioning and histomorphology of the organs (Oyedemi *et al*., 2010; Magalhaes *et al*., 2008). *S. malaccense* has been reported to contain antioxidant properties which can efficiently arrest the deleterious activities of free radicals since, free radicals are implicated in various forms of hepatic injuries. Presence of polyphenols in this extract can help modulate the activities of redox sensitive transcription factors which are involved in mitogenic and apoptogenic pathways. Alteration of haematological parameters in human is responsible for several blood related disorders. Some plant extracts are reportedly used to reverse these conditions even in a diseased state because this extract might contain some phytochemicals that enhances the formation of erythropoietin which is a glycoprotein that stimulates stem cells in the bone marrow of experimental animals to produce red blood cells (Ohlsson and Aher, 2006). *S. malaccense* being able to significantly decrease MCV in all treated groups makes the plant material efficient in the treatment of macrocytic anaemia (Kasper *et al*., 2005) but could also make normocytic patients microcytic which is a dangerous blood disorder. However, it has been reported that MCH levels mirror the MCV level, thus, justifying the significant decrease observed in the level of MCH in all treated groups (Davidson and Hamilton, 1978). A significant ($p<0.05$) reduction in packed cell volume and hemoglobin counts (Fig. 3) implies that increased doses of the extract is dangerous to anaemic patients. Suppression of PCV and haemoglobin can be linked to effects of saponin in the extract. Similar results were observed in hematological experiments involving *Gongronema latifolium* (Akinnuga *et al*., 2011). However, no significant change was observed in RBC counts of rats treated with high concentration of the extract. A significant decrease in serum White Blood Count (WBC) counts also known as total leukocyte count in all treatment groups might result to a decrease stimulation of immunity where a form of attack and interaction with foreign antigens may initiate a primary immune response as reported (Evans, 1989; Helal *et al*., 2008). This result is similar to studies carried out on the leaf extract of *Acalypha wilkesiana* (Iniaghe *et al*., 2013). The extract elicited the same effect on WBC at the highest given dose. Platelet reducing agents are potential anti-sickling agents, because of

their ability to prevent platelet aggregation during sickle cell disease crisis. However, the values plant extract which reduced significantly the haematocrit, haemoglobin, MCV values and MCH may not be classified as a potential anti-sickling agent. Relative reduction of these haematological parameters has suggested that *S. malaccense* might possess certain anti-proliferative activities since, it has successfully reduced the quantity of rapidly proliferating cells of the vascular system. Our lab is presently carrying out the analysis of its pro-apoptotic activities. Podophyllotoxin which is an anti-cancer agent has been reported to reduce WBC of treated subjects (Ogwal-Okeng *et al*., 2003).

Histological studies (Fig. 4 and 5) show mild to chronic inflammatory parenchyma liver cells at higher dose of the extract. The observed derangements do not correlate with biochemical findings possibly because the inflammations of the liver parenchyma cells were not sufficient to elicit significant alterations on the liver markers. This result also explains the mild degeneration observed by fluctuating level of liver function test parameters although not significant (Kotue *et al*., 2013).

CONCLUSION

Results of this study confirmed the traditional usage of *S. malaccense* in management of several disease conditions to be safe as a result of its non toxicity at low doses. It is therefore, recommended that the prolong intake of the extract should be done with caution since, it could elicit inflammation of the liver cells. Further studies should be done to determine their phytochemical constituents responsible for the plants medicinal actions.

REFERENCES

Abolaji, O.A., A.H. Adebayo and O.S. Odesanmi, 2007. Nutritional qualities of three medicinal plant parts (*Xylopia aethiopica*, *Blighia sapida* and *Parinari polyandra*) commonly used by pregnant women in the western part of Nigeria. Pak. J. Nutr., 6: 665-668.

Abotsi, W.K., G.K. Ainooson, E.B. Gyasi and W.K.M. Abotsi, 2011. Acute and sub-acute toxicity studies of the ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae) in rodents. West Afr. J. Pharm., 22: 27-35.

Adebayo, A.H., R. Aliyu, D. Gatsing and I.H. Garba, 2006. The effects of ethanolic leaf extract of *Commiphora africana* (Buseraceae) on lipid profile in rats. Int. J. Pharmacol., 2: 618-622.

Adebayo, A.H., A.O. Abolaji, T.K. Opata and I.K. Adegbenro, 2010. Effects of ethanolic leaves extract of *Chrysophyllum albidum* G. on biochemical and haematological parameters of albino wistar rats. Afr. J. Biotechnol., 9: 2145-2150.

Adebayo, A.H., O.S. Adegbite, J.A. Olugbuyiro, O.O. Famodu and K.B. Odenigbo, 2014. Toxicological evaluation of extract of *Olax subsorpioidea* on albino Wistar rats. Afr. J. Pharm. Pharmacol., 8: 570-578.

Akinnuga, A.M., O. Bamidele, P. Ekechi and O.S. Adeniyi, 2011. Effects of an ethanolic leaf extract of *Gongronema latifolium* on haematological some parameters in rats. Afr. J. Biomed. Res., 14: 153-156.

Aliyu, R., A.H. Adebayo, D. Gatsing and I.H. Garba, 2007. The effects of ethanolic leaf extract of *Commiphora africana* (Burseraceae) on rat liver and kidney functions. J. Pharmacol. Toxicol., 2: 373-379.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Byock, I.R., 1995. Patient refusal of nutrition and hydration: Walking the ever-fine line. Am. J. Hosp. Palliative Care, 12: 8-13.

Clarke, E.G.C. and M.L. Clarke, 1977. Veterinary Toxicology. Cassel and Collier Macmillan Publishers, London, pp: 268-277.

Danmalam, U.H., P.K. Allahmagani, N. Ilyas, E.M. Abdurahman and A.H. Yaro *et al*., 2012. Phytochemical and anticonvulsant studies on the aqueous ethanol extract of the root-back of *Ficus Abutilifolia* (Miq.) Miq. (Family: Moracea). J. Appl. Pharm. Sci., 2: 234-237.

Davidson, R.J. and P.J. Hamilton, 1978. High mean red cell volume: Its incidence and significance in routine haematology. J. Clin. Pathol., 31: 493-498.

Deneke, U. and W. Riiffersdorf, 1984. Diagnostic enzymology. J. Clin. Chem., 30: 1009-1009.

Dhiman, R.K. and Y.K. Chawla, 2005. Herbal medicines for liver diseases. Digestive Dis. Sci., 50: 1807-1812.

Doumas, B.T., W.A. Watson and H.G. Biggs, 1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta, 31: 87-96.

Dunstan, C.A., Y. Noreen, G. Serrano, P.A. Cox, P. Perera and L. Bohlin, 1997. Evaluation of some Samoan and Peruvian medicinal plants by prostaglandin biosynthesis and rat ear oedema assays. J. Ethnopharmacol., 57: 35-36.

Evans, W.C., 1989. Trease and Evans' Pharmacognosy. 13th Edn., University Press, Cambridge, UK., Pages: 546.

Helal, E., S. Zahkok, G.Z.A. Soliman, M. Al-Kassas and H. Abdel Wahed, 2008. Biochemical studies on the effect of sodium nitrite and/or glutathione treatment on male rats. Egypt. J. Hosp. Med., 30: 25-38.

Ikewuchi, J.C., E.N. Onyeike, A.A. Uwakwe and C.C. Ikewuchi, 2011. Weight reducing and hypocholesterolemic effects of aqueous extract of the leaves of *Tridax procumbens* Linn on sub-chronic salt-loaded rats. Int. J. Biol. Chem. Sci., 5: 680-687.

Iniaghe, O.M., O. Egharevba and E.B. Oyewo, 2013. Effect of aqueous leaf extract of *Acalypha wilkesiana* on hematological parameters in male wistar albino rats. Br. J. Pharm. Res., 3: 465-471.

Karioti, A., H. Skaltsa and A.A. Gbolade, 2007. Analysis of the leaf oil of *Syzygium malaccense* Merr. et Perry from Nigeria. J. Essential Oil Res., 19: 313-315.

Kasper, D.L., E. Braunwald, A.S. Fauci, S.L. Hauser, D.L. Longo and J.L. Jameson, 2005. Harrison's Principles of Internal Medicine. 16th Edn., McGrow-Hill, New York, USA.

Kotue, T.C., M. Ndomou, A.C. Pieme, P.C. Oumdoum and Y.J. Ngogang, 2013. Acute and subacute toxicity studies of Hemodya, aqueous extract of three medicinal plants, used to fight against sickle cells disease in albino rats. J. Biotechnol. Pharm. Res., 4: 22-28.

Locher, O.P., M.T. Burch, H.F. Mower, J. Berestecky and H. Davis *et al*., 1995. Anti-microbial activity and anti-complement activity of extracts obtained from selected Hawaiian medicinal plants. J. Ethnopharmacol., 49: 23-32.

Lorke, D., 1983. A new approach to acute toxicity testing. Arch. Toxicol., 54: 275-287.

Mabeku, L.B.K., V.P. Beng, J. Kouam, O. Essame and F.X. Etoa, 2007. Toxicological evaluation of ethyl acetate extract of *Cylicodiscus gabunensis* stem bark (Mimosaceae). J. Ethnopharmacol., 111: 598-606.

Magalhaes, P.M., H.J. Appell and J.A. Duarte, 2008. Involvement of advanced glycation end products in the pathogenesis of diabetic complications: The protective role of regular physical activity. Eur. Rev. Aging Phys. Activity, 5: 17-29.

Mohamed, T.S., S. Maadhusudhana, V. Ramkanth, V. Rajan, K. Kumar and K. Gauthman, 2010. Hepatoprotective herbs a review. Int. J. Res. Pharm. Sci., 1: 1-5.

NIH., 1985. Guide for the care and use of laboratory animals. DHEW Publication No. (NIH) 85-23, Office of Science and Health Reports, DRR/NIH, Bethesda, MD., USA.

Ogwal-Okeng, J.W., C. Obua and W.W. Anokbonggo, 2003. Acute toxicity effects of the methanolic extract of *Fagara zanthoxyloides* (L.) root-bark. Afri. Health Sci., 3: 124-126.

Ohlsson, A. and S.M. Aher, 2006. Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Cochrane Lib. 10.1002/14651858.CD004863.pub2

Olagunju, J.A., O.W. Kazeem and O.O. Oyedapo, 2000. Further studies on the mechanism of carbohydrate intolerance induced in the rat by an ethanolic root extract of *Plumbago zeylanica*. Pharmaceut. Biol., 38: 362-366.

Oyedemi, S.O., G. Bradley and A.J. Afolayan, 2010. *In-vitro* and *-vivo* antioxidant activities of aqueous extract of *Strychnos henningsii* Gilg. Afr. J. Pharm. Pharmacol., 4: 70-78.

Pieme, C.A., V.N. Penlap, B. Nkegoum, C.L. Taziebou, E.M. Tekwu, F.X. Etoa and J. Ngongang, 2006. Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L.) Roxb (Ceasalpiniaceae). Afr. J. Biotechnol., 5: 283-289.

Prosper, B.N., K.R. Jules, W. Modeste and D. Ntiokam, 2010. Acute and subacute toxicity studies of *Zinginber officinalis* roscoe essential oil on mice (swiss) and rats (wistar). Afr. J. Pharm. Sci. Pharm., 1: 39-49.

Pulliah, T., 2006. Encyclopedia of World Medicinal Plants. Regency Publications, New Delhi, ISBN: 9788189233426, pp: 1082.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.

Saidu, Y., L.S. Bilbis, M. Lawal, S.A. Isezuo, S.W. Hassan and A.Y. Abbas, 2007. Acute and sub-chronic toxicity studies of crude aqueous extract of *Albizzia chevalieri* harms (Leguminosae). Asian J. Biochem., 2: 224-236.

Savitha, R.C., S. Padmavathy and A. Sundhararajan, 2011. *In vitro* antioxidant activities on leaf extracts of *Syzygium malaccense* (L.) merr and perry. Ancient Sci. Life, 30: 110-113.

Taiwo, V.O., O.A. Olukunle, I.C. Ozor and A.T. Oyejobi, 2005. Consumption of aqueous extract of raw *Aloe vera* leaves: Histopathological and biochemical studies in rat and Tilapia. Afr. J. Biomed. Res., 8: 169-178.

Zbinden, G. and M. Flury-Roversi, 1981. Significance of the LD_{50}-test for the toxicological evaluation of chemical substances. Arch. Toxicol., 47: 77-99.