FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 3 No. 2, June, 2019, pp 124 - 134

AQUEOUS LEAF EXTRACT OF *ZIZIPHUS MAURITIANA* IS POTENT ON CARBON TETRACHLORIDE-INDUCED LIVER DAMAGE IN ALBINO RATS

[1*]Gadanya, H. S., [2]Gadanya, M. A., [3]Ibrahim, I. M., and [4]Wudil, A. M.

[1]Department of Chemistry, Sa'adatu Rimi College of Education, Kano, Kano State, Nigeria.
[2]Department of Community Medicine, Bayero University/Aminu Kano Teaching Hospital, Kano, Kano State, Nigeria.
[3]Department of Histopathology, Bayero University/Aminu Kano Teaching Hospital, Kano, Kano State, Nigeria.
[4]Department of Biochemistry, Bayero University, Kano, Kano State, Nigeria.

Corresponding Author's Email: halisasg@yahoo.com

ABSTRACT

Globally and locally there is an increase in burden of liver diseases arising from toxins, infectious and non-infectious conditions. This research work was aimed at studying the effects of aqueous leaf extract of *Ziziphus mauritiana* on Carbon Tetrachloride-induced liver damage in albino rats in comparison with currently used drug Livolin. Liver damage was induced by intramuscular injection of 120 mg/kg by weight of CCl_4. The effects of aqueous leaves extract of *Z. mauritiana* were determined in groups of liver toxicity model rats. Different groups of rats (total of 36 rats) were administered with three different doses of aqueous leaves extract of *Z. mauritiana* (50 mg/kg, 100 mg/kg and 150 mg/kg), with assessment of liver functions indices at intervals of two and four weeks. The allocation was as follows: control group (6 rats), test-control (6 rats), 50 mg/Kg extract dose (6 rats), 100 mg/Kg extract dose (6 rats), 150 mg/kg dose (6 rats) and Livolin group (6 rats). Dose and time-dependent responses were found in the normalisation of the liver functions indices and histological, with the best performing extract (150 mg/kg) been less efficacious than Livolin in liver damage. The findings of this study shows that aqueous leaf extract of *Z. mauritiana* has phytochemical components that can potentially be useful in treating liver damage, with effects comparable to currently used pharmaceutical drugs like Livolin.

Keywords: Albino rats, carbon tetrachloride, liver, potency, *Ziziphus mauritiana.*

INTRODUCTION

According to World Health Organisation (WHO) about 80 % of the population in some African and Asian countries relies on traditional medicine for their Primary Health Care needs (WHO, 2008). Traditional medicine, also referred to as indigenous medicine, is the sum total of the knowledge, skills, and practices based on the theories and beliefs of different cultures that are used in the maintenance of health and also in the prevention, diagnosis or treatment of diseases (WHO, 2008).

Humans have used medicinal plants throughout history to either cure or lessen symptoms from an illness, and continue to be in use because of their affordability, accessibility and perception of being free from adverse effects (Shai *et al*., 2008). In the earlier period of pharmaceuticals development, and even at this moment, pharmaceutical drugs are modelled after compounds found in medicinal plants (Mahesh and Satish, 2008).

Ziziphus mauritiana is a shrub belonging to family of *rhamnaceae* and is considered to be of importance in many traditional therapeutics in Africa and Asia (Gupta *et al*., 2012). *Ziziphus mauritiana* Lam in English it is called Lote, while in Hausa it is called *Magarya*. It is also called jujube tree or Indian jujube (Michel *et al*., 2002). Carbohydrates, proteins, mucilages and vitamins are abundantly found in *ziziphus* species (Clifford *et al*., 2002). The fruit (Ndhala *et al*; 2006) leaves (Dahiru and Obidoa; 2007) and seeds (Bhatia and Mishra; 2009) extracts have been shown to exhibit antioxidant activity, whereas bark (Pisha *et al*., 1995) is reported for cytotoxicity against different cancer cell lines. *Ziziphus mauritiana* fruit has been shown to possess hepato-curative action against liver damage in rats (Dahiru *et al*., 2010). The leaves are simple, shining green and rounded at both ends, highly variable in shape and size. The leaves' shape is alternate, ovate with depressed longitudinal veins at the base. It is about 2.5 to 3.2 cm long and 1.8 to 3.8 cm wide. It is dark green and glossy on upper phase and pale green on the lower side. *Ziziphus mauritiana* leaves contain 13-17% crude protein and 15 % fibre, and make an excellent fodder for livestock (Gupta *et al*., 2012). *Ziziphus mauritiana* is said to contain alkaloids, saponins, flavonoids, triterpenoid and phenolic compounds. *Ziziphus mauritiana* has antimicrobial, anti-inflammatory and antiulcer properties (Bhatia and Mishra 2009).

Ziziphus mauritiana has been used in traditional medicine in many countries including Nigeria, specifically the northern part of Nigeria. It is a recurrent ingredient in many local remedies. In many rural settings, many ailments, even those with no clear diagnosis or discernible causes are often empirically treated with the various components of the plants,

prepared in various formulations as single or mixed concoctions. It is often used for treatment of malaise, yellowness of eyes assumed to be from liver disease (Michel 2002), change of urine colour, (Dahiru *et al.*, 2005). Therefore, screening this plant for medicinal potentials on liver functions will be of great importance.

Increasing cost of established drugs, discovery of new side effects through pharmacovigilance and microbial resistance to antibiotics means that we are continuously being constrained as regards our therapeutic choices, and herbal sources could provide new opportunities for drugs discovery (WHO 2008).

This study was aimed at studying the effects of *Ziziphus mauritiana* aqueous leaf extract on Carbon Tetrachloride-induced liver damage in albino rats. This was achieved through evaluation of the effects of the aqueous leaf extract of *Ziziphus mauritiana* on the liver function indices of CCl_4-induced liver damage in rats, histological analysis on liver tissues of the rats and comparison of the effect of *Ziziphus mauritiana* with established hepatocurative drug (Livolin).

MATERIALS AND METHODS

Fresh leaves of *Ziziphus mauritiana* were collected in June, 2017 from Zogarawa village of Dawakin-kudu Local Government Area, Kano State, Nigeria. It was authenticated and assigned a voucher number of BUKHAN-0233 and kept in the herbarium of Botany unit, Biological Sciences Department, Faculty of Life Sciences, Bayero University, Kano, Nigeria.

The leaves collected were washed in clean water, then shade dried, after which they were pulverized to coarse powder using mixer grinder. One hundred grams (100 g) of the powdered leaves was macerated in 1500 ml distilled water in a flask, the content of the flask was then shaken and top covered with aluminium foil and kept for 24 hours. The extract was then obtained by filtration using Whatman No.1 filter paper and concentrated using vacuum evaporator. The concentration of the aqueous leaves extract was found to be 60 mg/ml.

REAGENTS

Reagents used are commercially prepared reagent kits for Alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline phosphatase (ALP), Total Bilirubin (TB) and Direct Bilirubin (DB), Total protein (TP), Albumin (ALB) and Malondialdehyde (MDA) obtained from Randox Laboratories, Antrim, UK, were used to assay the serum enzymes activity and other parameters concentrations.

ANIMALS AND DESIGN OF EXPERIMENT

A total of thirty-three (33) male albino rats (weighing between 120 g to 180 g) were purchased from the animal house of Biological Sciences Department, Bayero University, Kano. The rats were placed under standard laboratory conditions, and were fed with standard diet and water *ad libitum*. The rats were divided into six groups (1, II, III, IV, V and VI) and each group has six (6) rats.

Group 1 (Control rats): These control rats were administered with isotonic solution *per os* (0.5 cm^3 of saline/animal).

Group II (Test Control rats): Administered with CCl_4 only (120 mg/kg) to induce liver damage.

Groups III-V Administered with CCl_4 (120 mg/kg) to induce liver damage later administered with the *Z. mauritiana* aqueous leaf extract as follows:

Group III: Three rats were administered with aqueous leaf extract of *Z. mauritiana* (50 mg/kg) once daily for two weeks while the remaining three rats were administered with aqueous leaf extract of *Z. mauritiana* once daily for four weeks.

Group IV: Three rats were administered with aqueous leaf extract of *Z. mauritiana* (100 mg/kg) once daily for two weeks while the remaining three rats were administered with aqueous leaf extract of *Z. mauritiana* once daily for four weeks.

Group V: Three rats were administered with aqueous leaf extract of *Z. mauritiana* (150 mg/kg) once daily for two weeks while the remaining three rats were administered with aqueous leaf extract of *Z. mauritiana* once daily for four weeks.

Group VI: Three rats were administered with Livolin (31.2 mg/kg) for two weeks while the remaining three rats were administered with Livolin once daily for four weeks.

The rats in groups I and II were sacrificed 24 hours after inducement with $CC1_4$ and blood samples was collected to assess for liver damage in group II. Three rats each from Groups III, IV and V were sacrificed after two (2) weeks oral administration of aqueous leaves extract of *Ziziphus mauritiana,* while the remaining three rats were sacrificed after four (4) weeks oral administration of aqueous leaves extract of *Ziziphus mauritiana*. Similarly, three rats from group six were sacrificed after two (2) weeks oral administration of Livolin, while the remaining three rats were sacrificed after four (4) weeks oral administration of Livolin. The blood samples collected were allowed to clot, centrifuged and serum was separated for determination of AST, ALP, ALT activities, malondialdehyde, total and direct Bilirubin concentration, total protein and Albumin concentrations.

ESTIMATION OF BIOCHEMICAL PARAMETERS

Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were determined by the method of Reitman and Frankel (1957) which is based on transamination reaction. Alkaline phosphatase(ALP) was evaluated by the method of Rec (1972), serum total and direct bilirubin were assayed by the method of jendrassik and Grof (1938), serum total protein was determined by biuret method (Tiez,1995) and serum Albumin was assayed by the method of Grant (1987).

STATISTICAL ANALYSIS

The data was analysed with $p < 0.05$ considered statistically significant, and comparison between the groups were performed using one-way analysis of variance (ANOVA) using GraphPad software (2000). The data are given as the mean ± standard deviation.

RESULTS

The results of hepatocurative effects of aqueous leaf extract of *Zizuphus mauritiana* and the standard drug Livolin are

shown in tables 1 and 2 below. Results obtained after 24 hours administration of CCl_4 showed significant increases ($p < 0.05$) in serum levels of AST, ALT, ALP activities, DB, TB and malondialdehyde concentrations. There was a decline in serum concentrations of TP and Albumin, however these changes were not statistically significant ($p > 0.05$) (Table 1). The three groups (III, IV and V) administered with 50, 100 and 150 mg/kg aqueous leaves extract of *Zizuphus mauritiana* and Livolin administered to group VI once daily for two weeks respectively have mean serum activities of AST, ALT, ALP and concentration of DB, TB and Malondialdehyde significantly lower ($p < 0.05$) when compared to test control rats (group II), but still higher than the control group (table 1). Even though there was observed improvement in the level of TP and albumin with the various concentrations of the extract and Livolin, the observed difference has not reached level of statistical significance at the end of the second week ($p > 0.05$). The decreases in the mean serum activities of AST, ALT, ALP and concentration of TB, DB and Malondialdehyde were found to be dose dependent over the gradient of the extract concentration. However, the best performing extract (150 mg/kg) produced less improvement than Livolin.

After the fourth week of continuous administration of extract to group III, IV and V and Livolin to group VI (table 2), the activities of AST, ALT, ALP and the concentrations of TB, DB and Malondialdehyde which were found to have statistically significant ($p < 0.05$) dose dependent relationship were also found to have statistically significant ($p< 0.05$) time dependent relationship, with Livolin achieving marginally better result than the best performing extract (150 mg/kg extract).

DISCUSSION

Consistent with the Alhassan *et al.,* (2009), after 24 hours administration of CCl_4 there was significant increase ($p < 0.05$) in serum levels of AST, ALT, ALP, DB and TB. This indicated induction of acute liver injury in the test control group.

The three groups (III, IV, and V) administered with 50, 100 and 150 mg/kg aqueous leaf extract of *Zizuphus mauritiana* respectively and Livolin (group VI) administered once daily for two weeks had mean serum activities of AST, ALT, ALP, and concentrations of DB and TB significantly lower ($p < 0.05$) when compared to test control rats (group II), but still higher than the normal control group. Even though there was an observed improvement in the level of TP and albumin with the various concentrations of the extract and Livolin, the observed difference was not statistically significant ($p > 0.05$) at the end of the second week. The decrease in the mean serum activities of AST, ALT, ALP and concentrations of TB and DB was found to be dose-dependent over the gradient of the extract concentrations, with the higher doses showing significantly better ($p <0.05$) improvement in liver function based on analysis with ANOVA. However, the best performing extract (150 mg/kg) produced less improvement than the standard drug Livolin. A study by Alhassan *et al* (2012) showed that a plant (*Calitrophis procera)* with some phytochemical components (saponin and terpenoids) similar to those found in *Z.mauritiana* has hepatocurative effect against CCl_4-induced liver toxicity in rabbits. In addition to saponins and terpenoids, *Z.mauritiana* also has cardiac glycosides, phenols, flavonoids and tannins. The hepatocurative effect of both plants shows additional evidence that their common phytochemical components have inherent pharmacological potentials. This is further strengthened by the findings of Dahiru and Obidoa (2008) that *Z.maurtiana* has curative effect on liver damage induced by a different chemical, ethanol. They showed that treatment of chronic alcohol-induced hepatotoxicity for 2 weeks with aqueous leaf extract of *Ziziphus mauritiana* significantly ($p < 0.05$) lowered the levels of ALT, AST and TB compared to untreated group, with significant ($p<0.05$) increase in levels of antioxidant enzymes and non-antioxidant enzymes observed after treatment with *Ziziphus mauritiana* extract. They also showed that histopathological section of liver post-treatment with *Ziziphus mauritiana* extract enhanced quick recovery from alcohol-induced hepatotoxicity compared to untreated group. They further posited that curative potential of the aqueous extract of *Ziziphus mauritiana* leaf could be due to its ability to stimulate increased antioxidant activity and reduction in the level of hepatic lipid peroxidation. Similar histological changes were seen in this study, with improvement in liver architecture after treatment with extract for two and four weeks, with more improvement in higher doses and at at four weeks compared to lower doses at two weeks.

The dose and time-dependent nature of the curative effect of the extract where continuous administration and higher doses showed further statistically significant decreases in the activities of AST, ALT, ALP and the concentrations of TB and DB could be an additional point in support of potential effects of the extract as hepatocurative agent. The performance of the extract compared to Livolin, where Livolin achieved marginally better result than the highest dose (150 mg/kg) of the extract may suggest that further higher doses of the extract may achieve comparable results to standard treatment of Livolin. Livolin has earlier been investigated and found to be an effective hepatocurative agent in rats with liver damage (Olukiran *et al.,* 2014). Livolin is being used in hospitals worldwide in the management of liver diseases.

Further evidence in support of the potentials of *Z.mauritiana* in treatment of CCl_4 induced liver damage is the support provided by the histopathological assessment of the rats' liver in this study, with groups administered with the extract showing dose and time dependent improvements in liver architecture (i.e distinct hepatocytes arranged as thin plate) in a comparable manner to Livolin.

Table 1: Serum liver function indices of CCl_4-induced liver damaged after two weeks' oral administration of aqueous leaf extract of *Ziziphus mauritiana*

Group/treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (umol/L)	DB (umol/L)	TP (g/L)	ALB (g/L)	Malondialdehyde (mmol/L)
I								
No CCl_4 administered (Normal)	14.3±2.34	19.3±3.93	38.1±3.51	4.9±1.03	2.3±0.49	66.6±5.67	26.4±4.59	8.1±1.79
II								
Test control (CCl_4 induced but no extract)	86.2±11.92[a]	68.4±10.41[a]	87.3±12.12[a]	28.7±8.23[a]	13.9±4.32[a]	51.8±11.87	16.9±4.91	23.9±5.12[a]
III								
CCl_4 induced and administered with 50 mg/kg of extract	76.8±10.85[b]	52.7±7.13[c]	75.0±13..32[d]	23.0±6.67[e]	10.5±3.16[f]	48.4±13.36	19.2±3.15	15.1±4.92[g]
IV								
CCl_4 induced and administered With 100mg/kg of extract	65.9±12.47[b]	46.9±7.11[c]	69.6±12.17[d]	16.4±5.26[e]	9.6±2.28[f]	52.7±13.93	20.8±6.44	12.3±4.89[g]
V								
CCl_4 induced and administered with 150mg/kg of extract	63.8±11.12[b]	41.5±6.34[c]	63.5±10.43[d]	12.9±5.14[e]	8.4±2.11[f]	54.2±12.36	21.6±5.51	11.0±4.85[g]
VI								
Standard drug(Livolin 31.2mg/Kg)	51.1±10.32[b]	36.2±4.71[c]	46.0±7.98[d]	8.5±2.60[e]	5.5±1.05[f]	55.9±11.74	22.4±4.56	10.5±3.97[g]

Results are expressed as mean±SD, n=3

Values with superscript (a) are significantly different at $p < 0.05$ when compared with normal rats

Values in the same column with the same superscript(b,c,d,e,f and g) are siginifcantly different at $p < 0.05$ when compared with the test control

AST- aspartate amino transferase, ALP- Alanine amino transferase, ALP –Alkaline phosphatase, TB –Total bilirubin, DB- Direct bilirubin, TP –Total protein, ALB- Albumin

Table 2: Serum liver function indices of CCl_4 induced liver damaged after four weeks oral administration of aqueous leaf extract of *Ziziphus mauritiana*

Group/treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (umol/L)	DB (umol/L)	TP (g/L)	ALB (g/L)	Malondialdehyde (MDA)
I No CCl_4 administered (Normal)	14.3±2.34	19.3±3.93	38.1±3.51	4.9±1.03	2.3±0.49	66.6±5.67	26.4±4.59	8.1±1.79
II Test control (CCl_4 induced but no extract)	86.2±11.92^{a}	68.4±10.41^{a}	87.3±12.12^{a}	28.7±8.23^{a}	13.9±4.32^{a}	41.1±11.87^{a}	16.9±4.91	23.9±5.12^{a}
III CCl_4 induced and administered with 50mg/kg of extract	55.8±10.71^{h}	43.8±7.91^{i}	65.9±9.52^{j}	14.1±3.21^{k}	5.7±2.20^{m}	50.6±12.37	20.4±4.15	13.9±3.83^{n}
IV CCl_4 induced and administered with 100mg/kg of extract	41.1±9.38^{h}	38.5±7.36^{i}	44.7±10.32^{j}	9.1±3.15^{k}	3.9±1.99^{m}	51.3±11.87	20.5±5.44	11.1±3.27^{n}
V CCl_4 induced and administered with 150mg/kg of extract	33.8±8.73^{h}	31.4±3.97^{i}	34.3±7.81^{j}	6.9±2.29^{k}	2.7±0.81^{m}	58.8±11.06	23.5±4.98	10.7±3.41^{n}
VI Standard drug (Livolin 31.2mg/Kg)	31.1±7.43^{h}	25.7±3.87^{i}	28.0±6.42^{j}	6.3±1.89^{k}	2.5±0.65^{m}	65.4±6.71	26.1±6.01	9.5±3.57^{n}

Results are expressed as mean±SD, n=3

Values with superscript (a) are significantly different at $p < 0.05$ when compared with normal rats

Values in the same column with the same superscript (h,i, j, k, m and n) are significantly different at $p < 0.05$ when compared with the test control

AST- aspartate amino transferase, ALP- Alanine amino transferase, ALP –Alkaline phosphatase, TB –Total bilirubin, DB- Direct bilirubin, TP –Total protein, ALB- Albumin

HISTOPATHOLOGY OF LIVER

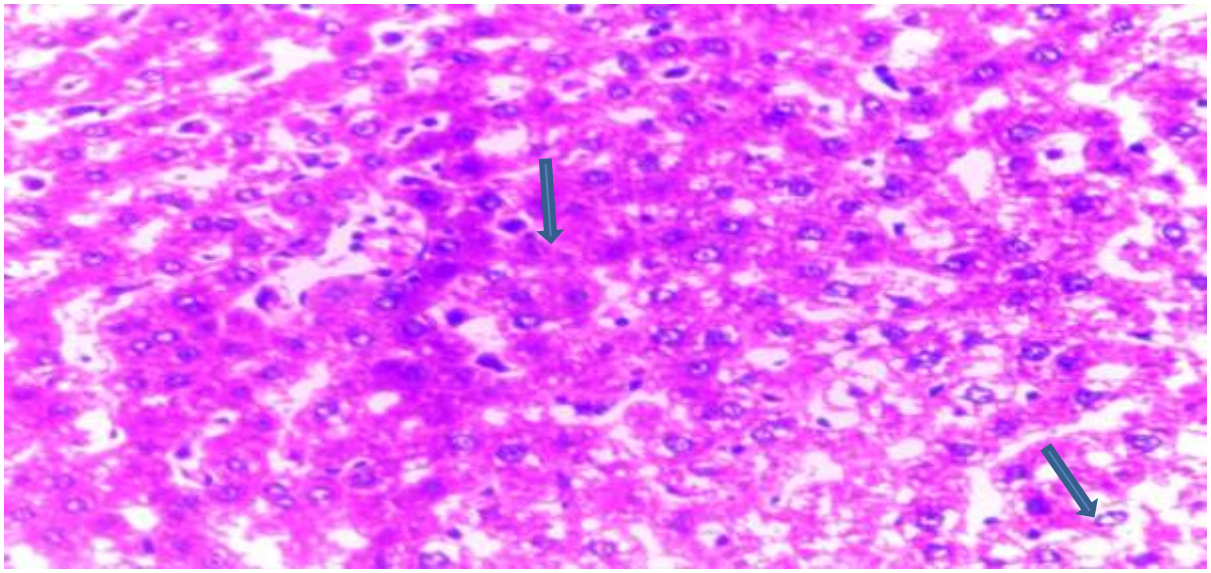

Plate 1: Normal liver (H&E stain, ×40)
Plate 1 shows normal liver architecture with normal hepatocyctes, portal triads and central veins all within normal limits.

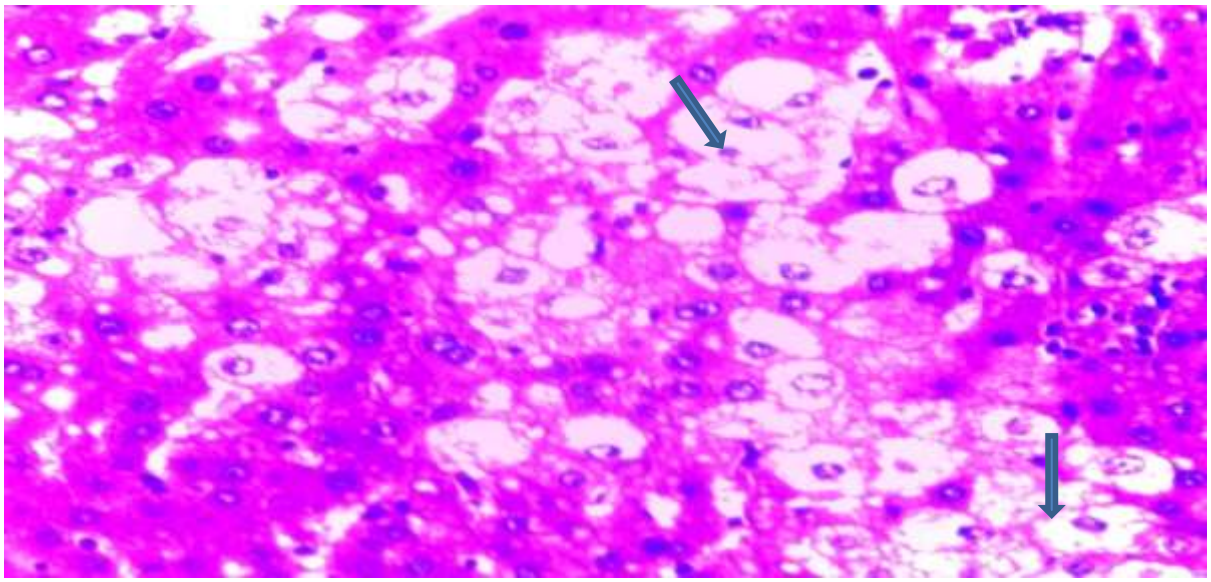

Plate 2: CCl_4-induced liver damage (H&E stain, ×40)
Plate 2 shows massive fatty changes with centri-lobular necrosis and inflammation due to damage caused by CCl_4.

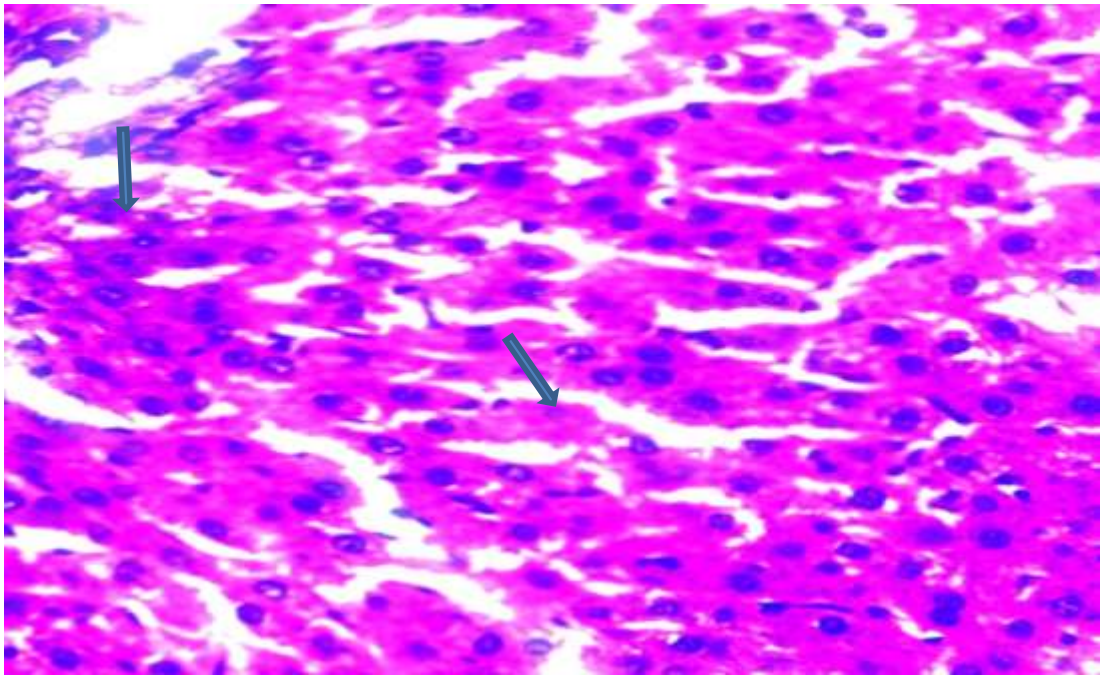

Plate 3: Liver 50mg, 2 weeks, (H&E stain, ×40)
Plate 3 shows moderate inflammation and fatty changes after 2 weeks administration of 50mg aqueous leaf extract of *Z. mauritiana.*

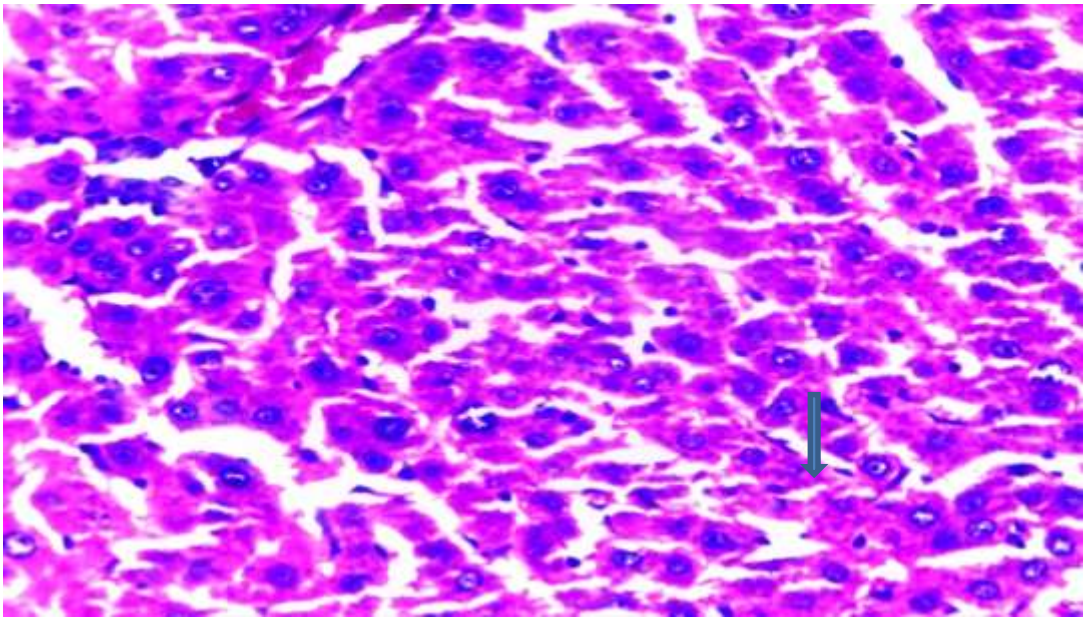

Plate 4: Liver 100mg 2 weeks (H&E stain, x40)
Plate 4 shows moderate inflammation and fatty changes after 2 weeks' administration of 100mg aqueous leaf extract of *Z. mauritiana.*

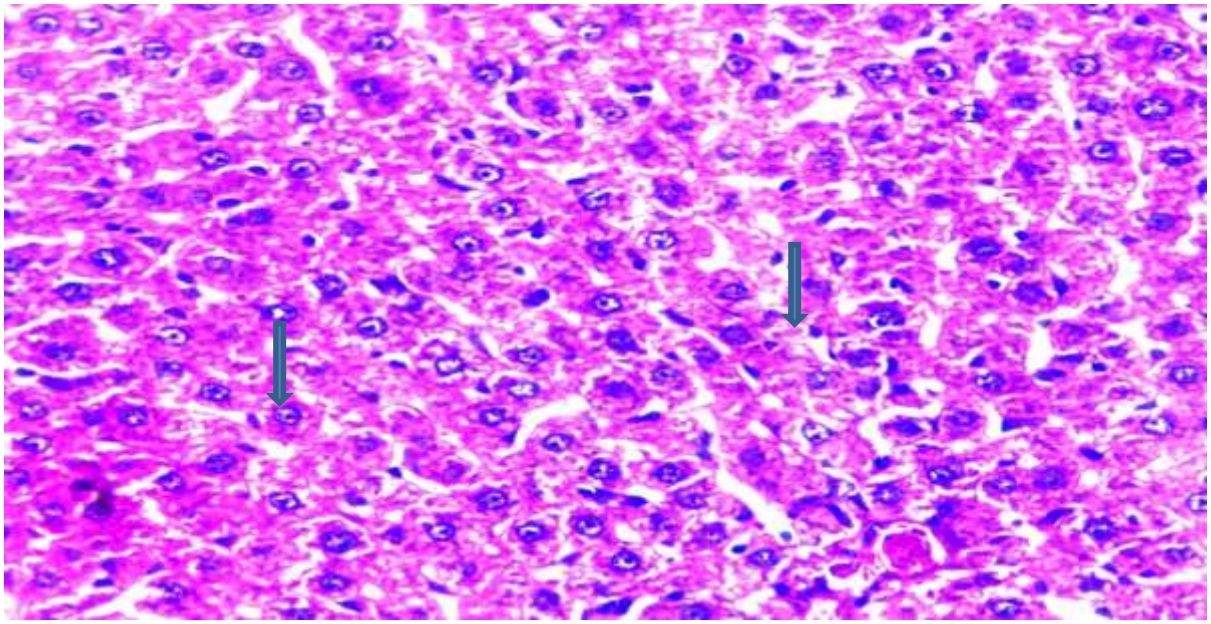

Plate 5: Liver 150mg -2 weeks (H&E stain, x40)
Plate 5 show histological features that have nearly normalised, and not remarkable different from controls after 2 weeks administration of 150mg aqueous leaf extract of *Z. mauritiana*

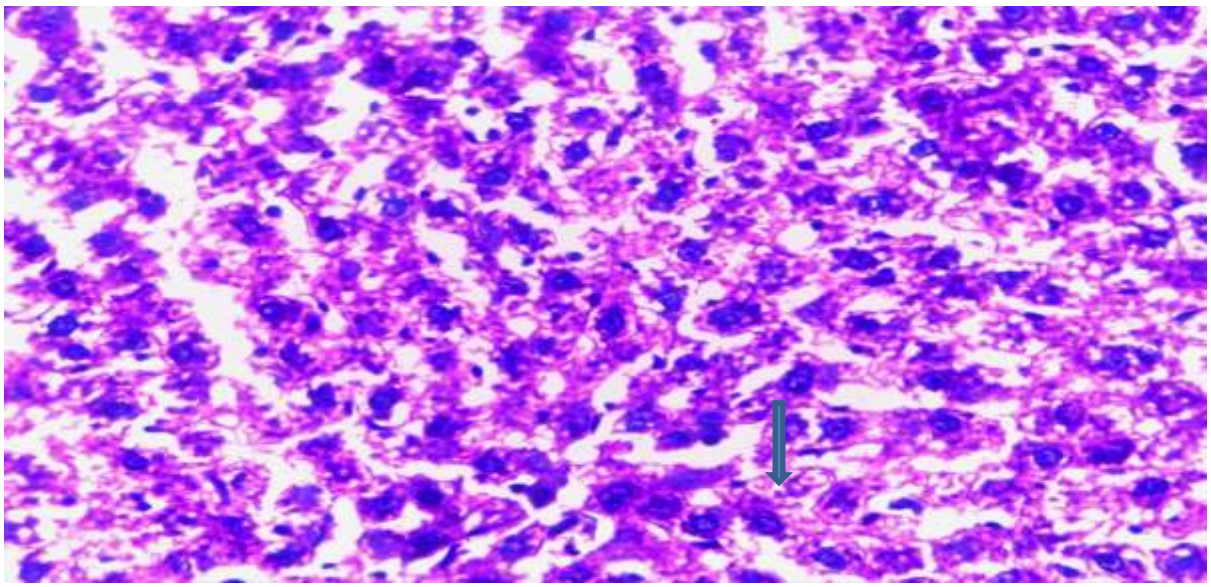

Plate 6: Livolin – 2 weeks, (H&E stain, x40)
Plate 6 shows histological features that have nearly normalised, and not remarkable different from controls after 2 weeks' administration of Livolin.

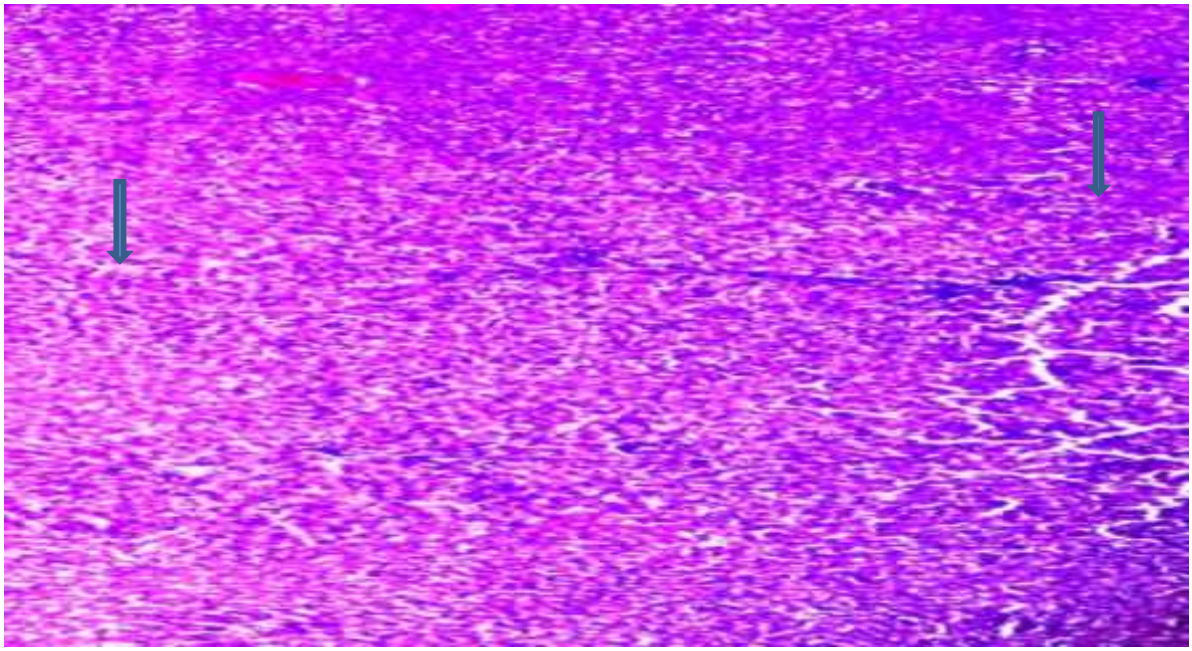

Plate 7: 50mg liver- 4 weeks, (H&E stain, x40)
Plate 7 shows mild inflammation and foci of fatty changes after 4 weeks administration of 50mg aqueous leaf extract of *Z. mauritiana*

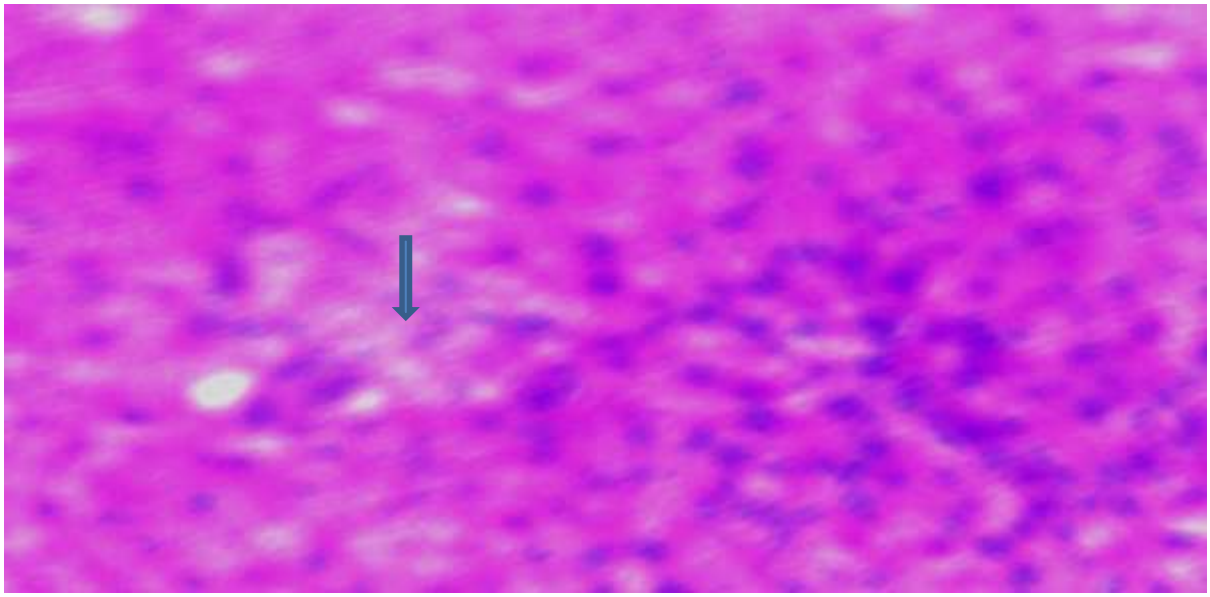

Plate 8: Liver 100mg -4 weeks, (x40)
Plate 8 shows normalised hapatocytes unremarkable different from control in terms of histological structure after 4 weeks administration of 100 mg aqueous leaf extract of *Z. mauritiana*.

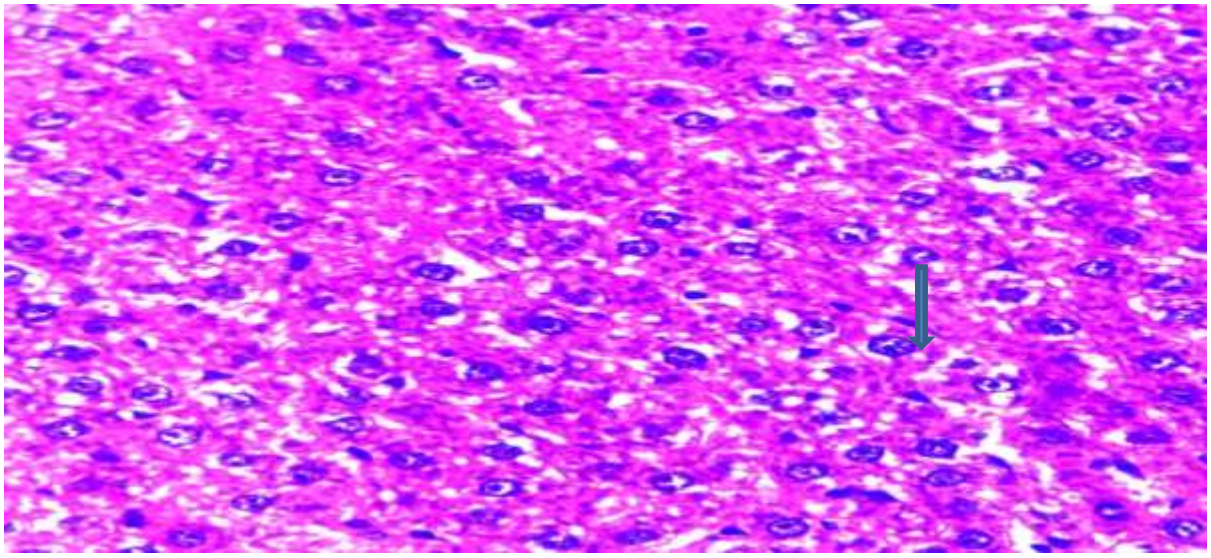

Plate 9: Liver 150mg- 4 weeks, (H&E stain, x40)
Plate 9 shows normalised hapatocytes unremarkable different from control in terms of histological structure after 4 weeks administration of 150 mg aqueous leaf extract of *Z. mauritiana*.

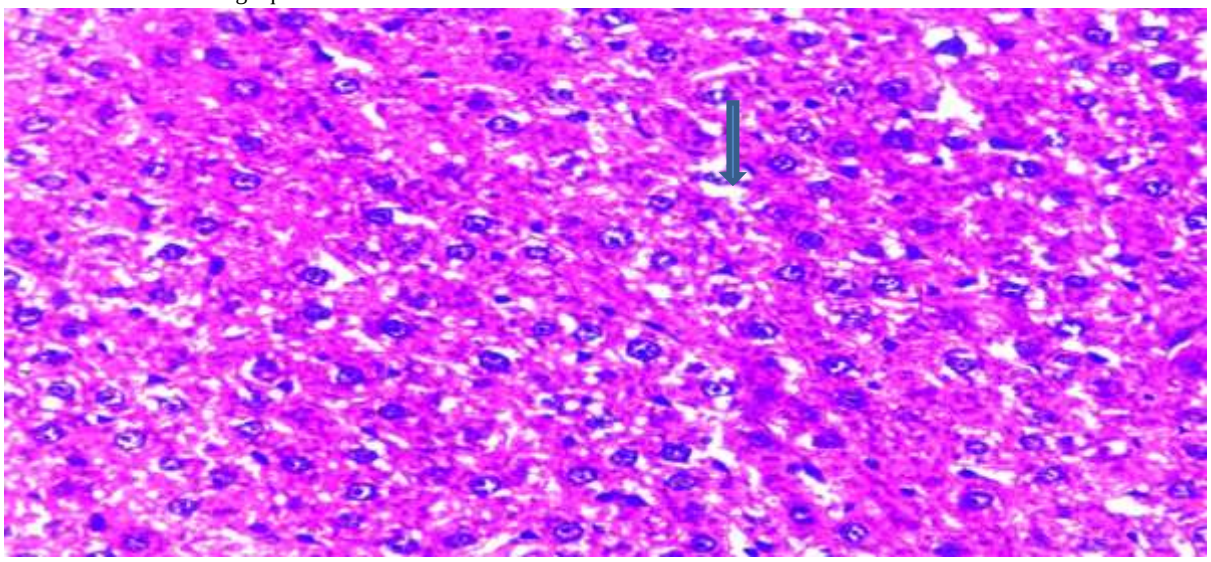

Plate **10**: Livolin - 4weeks (H&E stain, x40)
Plate 10 shows normalised hapatocytes unremarkable different from control in terms of histological structure after 4 weeks administration of Livolin.

CONCLUSION

The findings of this study shows that aqueous leaf extract of *Z. mauritiana* has phytochemical components that can potentially be useful in treating liver damage, with effects comparable to currently used pharmaceutical drugs like Livolin. In this study, time and dose dependent potency was found, and it is possible that additional dose of the extract will be more effective over a short period of time in speedily normalising both chemical and histopathological parameters of the liver damaged by CCl_4.

Further studies are needed to establish safety and effect of higher dose over shorter period of time, in order to see a faster hepato-curative effect can be obtained**.**

REFERENCES

Alhassan, A. J., Sule, M. S., Atiku, M. K., Wudil, A. M., Dangambo, M. A., Mashi, J. A., Zaitun, M. and Uba, G.K (2012). Effect of Calitropis Procera aqueous root extract against CCl_4 induced liver toxicity in rabbits. *Bayero Journal of Pure and Applied Sciences*, 5(1): Page 38 – 43.

Alhassan, A. J., Sule, M. S., Hassan, J. A., Baba, B. A. and Aliyu, M.D. (2009). Ideal Hepatotoxicity model using CCl_4. *Bayero Journal of Pure and Applied Sciences*. 2(2): 185-187.

Bhatia, A. and Mishra, T. (2009). Free radical scavenging and antioxidant potential of Ziziphus mauritiana (Lamk.) seed extract. *Journal of Complementary and Integrative Medicine*. 8(1): 42-46.

Clifford, S.C., Arndt, S.K., Popp, M. and Jones, H.G. (2002). Mucilages and polysaccharides in Ziziphus species (Rhamnaceae): localization, composition and physiological roles during drought-stress, *Journal of experimental Botany*, 2002, 53 (366),131-138.

Dahiru, D and Obidoa, O. (2008). Evaluation of the antioxidant effects of *Ziziphus mauritiana* Lam. Leaf extracts against chronic ethanol-induced hepatotoxicity in rat liver. *African Journal of Traditional, Complementary and Alternative Medicines*. 5 (1): 39 – 45

Dahiru, D and Obidoa, O. (2007). Pre-treatment of albino rats with aqueous leaf extract of *Ziziphus mauritiana* protects against alcohol-induced liver damage. *Tropical Journal of Pharmaceutical Research ;* 6 (2): 705-710.

Dahiru, D., Mamman, D.N and Wakawa, H.Y (2010). *Ziziphus mauritiana* Fruit Extract Inhibits Carbon Tetrachloride-induced Hepatotoxicity in Male Rats. *Pakistan Journal of Nutrition;* 9:Pp 990-993.

Dahiru, D., William, E.T and Nadro, M.S (2005). Protective effect of *Ziziphus mauritiana* leaf extract on carbon tetrachloride-induced liver injury. *African Journal of Biotechnology* Vol. 4 (10), Pp. 1177-1179.

Grant, G.H., (1987). Amino acids and Proteins; Fundamentals of Clinical Chemistry, Tietz N.W. Editor, 3rd Edition, WB Saunders Company Philadelphia USA, 328-329.

Gupta, M.K., Bhandari, A.K. and Singh, R.K. (2012). Pharmacognostical Evaluations Of the Leaves Of Ziziphus mauritiana. *International Journal of Pharmaceutical Sciences and Research* (2012), 3, Issue 03.

Jendrassik, L. and Grof, P. (1938).Colorimetric Method for Determination of Serum bilirubin *Biochem. Z*. 297: 81.

Mahesh, B. and Satish, S. (2008). Antimicrobial activity of some important medicinal Plant against plant and human pathogens. *World Journal of Agricultural Sciences.* 4(S):839-843.

Michel, A. (2002). Tree, Shrub and Liana of West African Zones. Margraf Publishers GMBH, Paris. P 440.

Ndhala, R., Mupure, C.H., Chitindingue, K., Benhura, M.A. and Muchuweti, M. (2006). Antioxidant potentials and degree of polymerization of six wild fruits. *Life Science Research Assays*. 1: 87–92.

Olukiran, O.S., Akomolafe, R.O., Bamitale, K.D., Ajayi, A.O., Okonji, R.E., Bejide, R.A (2014). Protective and curative effects of Livolin forte® on carbon tetrachloride-induced liver damage in Wistar rats. *Journal of Experimental Integrated Medicine*; 4(1): 57-65.

Pisha, E., Chai, H., Lee, I.S., Chagwedera, T.E., Farnsworth, N.R., Cordell, G.A., Beecher, C.W., Fong, H.H., Kinghorn, A.D. and Brown, D.M. (1995). Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. *Nature Medicine*. 1(10):1046–1051.

Rec, G.S. (1972). Colorimetric method for serum alkaline phosphatase determination. *Journal of Clinical Biochemestry.* 10(2):182.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetate and glutamic pyruvic transaminase. *American Journal of Clinical Pathology.* 28:56.

Shai, LJ., McGaw, L.J., Aderogba, M.A., Mdee, L.K. and Eloff, J.N. (2008). Triterpenoids with antifungal and antibacterial activity from Curtisia dentata (Burm.f) C.A. Sm. Leaves. *Journal of Ethnopharmacology*, 119: 238-241.

Tietz, N.W. (1995). Clinical Guide to Laboratory Tests. 3rd Edition W.B. Saunders Company, PA. Pp 518-519.

World Health Organisation (2008).Traditional medicine. Report, EB111/9, World Health Organization. Available at http://apps.who.int/gb/archive/pdf_files/WHA56/ea5618.pdf . [Accessed 12th February, 2017]