TOXIC EFFECT OF METHANOLIC LEAF EXTRACT OF *Vitex simplicifolia* ON LIVER AND KIDNEY FUNCTION IN WISTAR RATS

Salim, M.A.
Department of Human Physiology, Faculty of Basic Medical Sciences
Bayero University Kano, Nigeria

Abstract

Objective: This study evaluated the hepato-renal toxicological indices following 21 days administration of methanolic leaf extract of *Vitex simplicifolia* in Wistar rats.

Methods: Acute toxicity studies with very high concentrations of the crude extract was carried out followed by sub chronic toxicities studies involving administration of 250mg/kg, 500mg/kg and 1000mg/kg body weight of the methanolic extract to the experimental animals for 21 days. Liver and Kidney toxicological indices were evaluated from the serum as well as the tissues of the experimental animals after the 21 days period of administration.

Results: The result of acute toxicity studies indicate that this extract is well tolerated at doses as high as 5000mg/kg body weight. The results of sub-chronic toxicity studies indicate that there was a significant increase in the activities of ALT and ALP while AST activities was lower compared to the control. Unconjugated bilirubin levels were also significantly ($P<0.05$) higher in the test groups compared to the control. Similarly, the result of kidney toxicological indices showed that the levels of urea, Na^+, HCO_3^{2-} and Cl^- were significantly higher in the test animals compared to the control while K^+ and creatine levels showed no significant change in the extract administered groups compared to the control. Histopathology examination of the liver and kidneys showed mild hepatic damage at the highest dose (1000mg/kg body weight).

Conclusion: These observations shows that care should be taken when using the methanolic extract of *Vitex simplicifolia* as a phytoremedy against any ailments as high concentrations of the extract may induce moderate injury to the liver.

Keywords: *Vitex simplicifolia,* ALT, ALP, AST, Urea, Na^+, HCO_3^{2-} , Cl^-,

INTRODUCTION

Plants are known to be efficacious and most often could contain compounds that are potential drugs which would require further examinations .Interest in and the search for medicines from natural sources has served as a catalysts for exploring techniques of obtaining the required plants and probing their activities (Edeoga *et al*, 2005).A large proportion of such medicinal compounds have been discovered with the aid of ethnobotanical knowledge of their traditional uses. The rich knowledge base of countries like India and China in medicinal plants and health care has led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programs in the pursuit of discovering novel drugs (Krishnaraju *et al.*, 2005). Medicinal plants have been identified and used throughout human history. Plants components contain a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals.

At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total (Lai and Roy, 2004, Tapsell, *et al.*, 2006).

Chemical compounds in plants mediate their effects on the human system through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they function. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects (Lai and Roy, 2004, Tapsell, *et al.,* 2006).

Vitex simplicifolia (Verbenaceae) is a perennial shrub or small tree which grows to a height of aproximatively 8 m and is widely distributed from Egypt to Guinea. In BurkinaFaso, the plant is used to treat various internal or external diseases like skin diseases, dermatitis, bilharzia, migraines, fever, aches, amoebiasis, sore teeth, colic, infant tetanus (Nacoulma,1996). Investigations have also revealed that this plant is also used in the treatment of skin infections and wounds healing. In Burkina Faso, infectious diseases are the leading cause of infant (2.37%) and maternal (14.6%) mortality; therefore they constitute public health problems. The treatment of skin diseases dates back to ancient times, and many of their treatments were using medicinal plants. About 30% of traditional remedies are used to treat wounds and skin lesions, compared to only 1-3% of modern drugs (Mantle *et al.,* 2001). The healing process is an immune response that begins after injury and takes place in three stages: vascular and inflammatory stage, phase of tissue repair and phase of maturation. A drug having simultaneously the potential antioxidant and antimicrobial activities may be a good therapeutic agent to accelerate cicatrization and wound healing (Phillips *et al.,* 1991; Heike *et al.,* 1999). Aromatherapy is now considered to be another alternative way in healing people, and therapeutic values of aromatic plants lie in their volatile constituents such as monoterpenoids, sesquiterpenoids and phenolic compounds that produce a definite physiological action on the human system (Bruneton, 1993).It is locally called *Vitex* (English), *Dinya birri* (Hausa), *Ucha koro* (Igbo) and *Oori-nla* (Yoruba) (Burkill, 2000).

Several previous studies have established different parts of *Vitex simplicifolia* as a remedy against many ailments. In Nigeria, information available from the indigenous traditional healers indicates that, a decoction of the chopped stem barks and leaf of *Vitex Simplicifolia* is prepared and taken orally for treatment of diabetes and other disease conditions. The plant extracts have been used as medication for infertility, liver disease, anodyne, stiffness, hypertension, cancer, febrifuge, as tonic galactagogue to aid milk production in lactating mothers, sedative, digestive regulator and treatment of eye, kidney and as supplement for lack of vitamin A and B (Sofowora, 1993; Burkill, 2000). Despite the extensive use of different parts of this plant for managing various ailments, there has not been to our knowledge, an extensive review of its possible toxicity against any organ or the whole system. These studies therefore aim to bridge this gap by evaluating the toxicity indices of the liver and kidney following sub-chronic administration of methanolic extract of this plant.

Material and Methods

Plant and animal

The leaves of *Vitex simplicifolia* were collected from Bayero University Kano and were dried in shade at room temperature and grounded into powder. Wister rats were obtained from the department of Physiology animal house, Bayero University Kano; they were housed in colony cages at an ambient temperature and relative humidity. The animals had free access to standard palletized grower feed and drinking water.

Extract preparation

The powdered plant was dissolved in methanol overnight. It was filtered and the residue was discarded. The filtrate was evaporated to dryness using Vacuum evaporator. The dried plant residue was used to prepare different concentrations.

Experimental Design

A total of 33 white albino rats were used for the study. 13 rats were used for the acute toxicity study while 20 rats were used for the sub-chronic toxicity study. For the sub-chronic toxicity study, the 20 animals were divided into four groups of five rats each. Group 1 was the control fed only feed and water throughout the period of the experiment while groups 2, 3, and 4 were administered 250, 500 and 1000mg/kg body of the methanol extract respectively for 21 days. After the 21 days of administration, the animals were sacrificed, blood samples were collected in heparin bottles and the liver and kidney of the animals were removed and preserved in 9% formalin until histopathological analysis.

Determination of LD_{50}

The lethal dose (LD_{50}) was determined by the method of Lorke (1983).In the first phase, nine (9) Wistar rats were used. The nine animals were divided into three groups of three animals each. Each group were administered 10,100 and 1000mg/kg body weight of the extracts and then observed for 24 hours to monitor their behaviour and mortality. In the second phase two of the experiment, three animals were used; the animals were divided into three groups of one animal each. They were administered higher doses (1600, 2900 and 5000 mg/kg body weight) of the extracts and observed for behaviour as well as mortality. (Lorke, 1983). LD_{50} was calculated by the formula: $LD_{50} = \sqrt{(D_0 x D_{100})}$ where:

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produce mortality.

Liver and Kidney function test

Four enzymes indices of liver damage were assayed to determined liver toxicity. AST activity was determined by the method described by Karmen, (1955), ALP and ALT activities were determined by the methods of Reitman and Frankel (1957), while bilirubin levels were determined by the method of Sherloch (1951). Kidney function was evaluated by determining the levels of kidney function indices; urea, creatinine, sodium ion, potassium ion, chloride ion and bicarbonate ion using the methods described by Weatherburn (1967), Bartels and Bohmer (1972), Kraus and Madias, (2007), Henry (1974), White (1970) and Forrester et al., (1976) respectively.

Histopathological studies (Avwioro, 2010; Mitchell *et al.*, 2011)

The liver biopsies were fixed with 10% formal saline and then transferred to a cassette, a container designed to allow reagents to freely act on the tissue inside. This cassette was immersed in multiple baths of progressively more concentrated ethanol (to dehydrate the tissue with ascending grade of alcohol), cleared with toluene, infiltrated with molten paraffin wax. During this 12 to 16 hour process, paraffin will replace the water in the tissue, turning soft, moist tissues into a sample miscible with paraffin, a type of wax. This process is known as tissue processing. The processed tissue was then taken out of the cassette and set in a mold. Additional paraffin was added to create a paraffin block which is attached to the outside of the cassette. The process of embedding allows the sectioning of tissues into very thin (2 - 7 micrometer) sections using a microtome. The slices are thinner than the average cell, and are layered on a glass slide for staining. Tissue was dewax and hydrated, stained in Erich's haematoxylin for 15mins, rinsed in water, differentiated in 1% HCl and 70% alcohol for 1min, rinsed in water, counterstained with 1% eosin for 1min, rinsed in water again and finally dehydrated, cleared and mounted on microscope for examination.

Results

The result of phase I and II acute toxicity studies is presented in Table 1&2 below. In both phases no signs of toxicity or mortality were recorded after 24 hours of the administration.

Table 1: Phase I LD_{50}, of the methanolic leaf extract of *Vitex simplicifolia*

Group	No. of Animals	Doses (mg/Kg)	No. of Death
1	3	10	0
2	3	100	0
3	3	1000	0

Table 2: Phase II LD_{50} of the methanolic leaf extract of *Vitex simplicifolia*

Group	No. of Animals	Doses (mg/Kg)	No. of Death
1	3	1600	0
2	3	2900	0
3	3	5000	0

Table 3 below shows the activities of AST, ALT, ALP and Unconjugated billirubin in extract administered and control rats. ALP and ALT significantly (P<0.05) increased in the test groups (Groups II, III and IV) compared to the control. The activity of serum AST and serum concentration of unconjugated bilirubin between the test and control groups however, were similar (P<0.05). Similarly, the levels of Unconjugated billirubin were not significantly different from the control.

Table 3: Effect of oral administration of methanolic of leaf extract of *Vitex simplicifolia* on liver enzymes and unconjugated billirubin in Wistar rats.

GROUP	ALP/ (U/l)	AST/ (U/l)	ALT/ (U/l)	U.Bil (µmol/l)
1	200.33±7.84	84.667±4.70	39.00±3.05	1.148±0.325
2	354.0±67.95[a]	27.68±1.03[a]	33.00±1.73	0.633±0.064
3	939.00±0.00[a]	67.00 ±1.00[a]	36.00±1.00	0.720±0.110
4	1119.0±17.00[a]	26.86±0.44[a]	45.50±3.50	0.885±0.055

Grp 1: Normal control, Grps 2, 3 and 4 received 250, 500 and 1000mg/kg of extract, respectively. Values are presented as mean ± standard error of mean. [a] = significantly different (p<0.05) from the Normal control.

Table 4 shows the toxicological indices on the rats kidneys following administration of methanolic leaf extract of *Vitex simplicifolia* . Sodium, chloride, urea and carbonate levels of the test animals showed significant (P<0.05) increase compared to that of the control However, the concentration of potassium and creatine between the test and the control groups were similar (P< 0.05)

Table 4: Effect of oral administration of methanolic leaf extract of *Vitex simplicifolia* on urea, creatinine and electrolytes in Wister rats.

Groups	Urea (mg/dl)	Creatinine (µmol/l)	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	HCO_3^- (mEq/L)
1	25.72± 0.83	186.33±3.18	219.48±2.31	5.86 ±0.80	75.71±1.04	44.33±1.45
2	67.52±2.56[a]	118.67±23.68[a]	227.75±8.02	5.69±0.43	87.38±2.99	33.00±1.55[a]
3	112.38± 2.00[a]	185.00±1.00	221.98±0.00	5.70±0.10	78.150±2.00	36.00±2.00
4	85.57± 2.46[a]	149.00±2.00	249.33±5.91[a]	4.44±0.20[a]	89.38±2.24[a]	38.00±2.00[a]

Grp 1: Normal control, Grps 2, 3 and 4 received 250, 500 and 1000mg/kg of extract, respectively. Values are presented as mean ± standard error of mean. [a] = significantly different (p<0.05) from the Normal control.

Plates I and II below showed the result of liver histopathological examination of the control and group IV administered 1000mg/kg body weight of the methanolic extract of the plant. The liver architecture of the control group showed no pathological changes, while that of the test group show sinusoidal lymphocytosis and mild expansion.

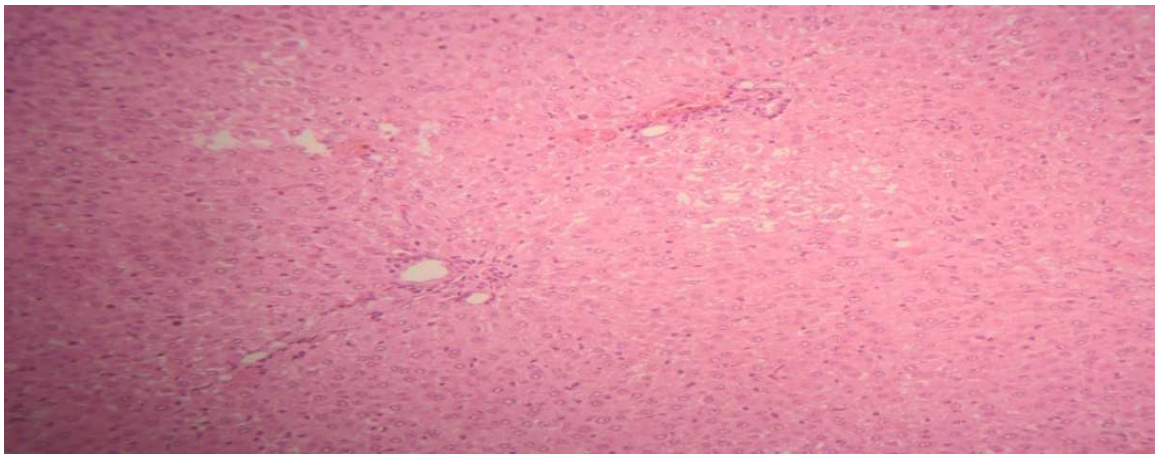

Plate 1: Stained cross section of liver of group I (control) albino rats administered no extract showing the portal tract area, with no pathological changes (normal). (H and E Stain (×250)

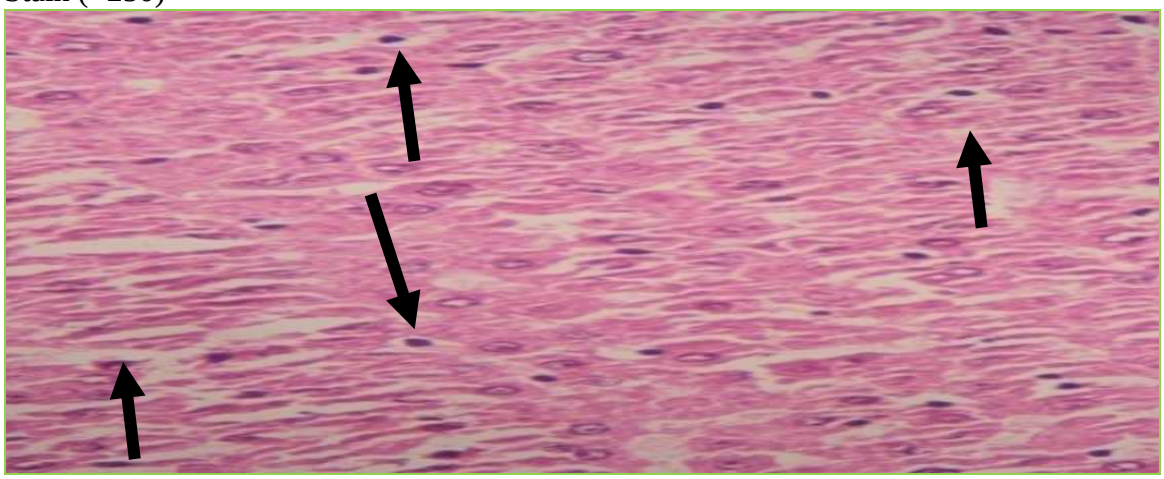

Plate II: photomicrograph of a section of liver from rats that were administered 1000 mg/kg methanolic extract of *Vitex simplicifolia* leaf extract using H and E stain and magnification of x250.This shows the portal tract area (black arrows), with mild sinusoidal lymphocytosis and mild expansion

Plates III and IV below showed the result of kidney histopathological examination of the control (Group I) and test group IV administered 1000mg/kg body weight of the methanolic extract of the plant. The kidney architecture when compared to the group I control showed no pathological changes in the 1000mg/kg body weight extract administered rats.

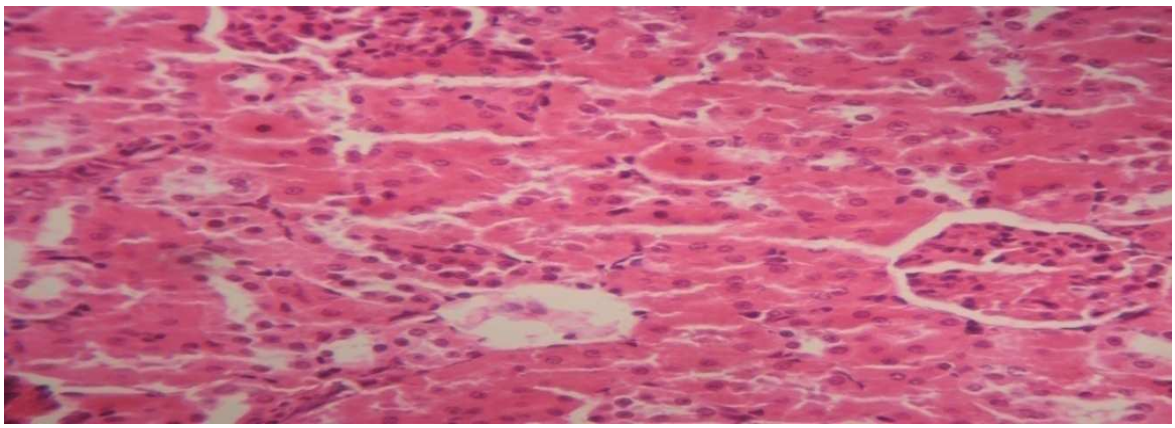

Plate III : Photomicrograph of a cross section of kidney of group I (control) wister rats administered no extract showing the portal tract area, with no pathological changes (normal). (H and E Stain (×250)

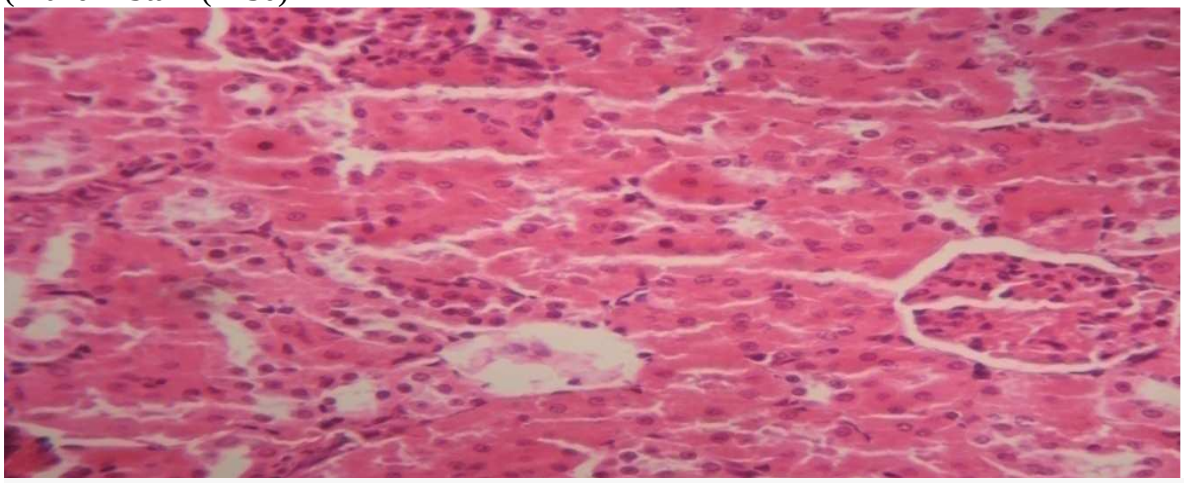

Plate IV: Photomicrograph of a section of kidney from wister rats that were administered 1000 mg/kg of methanol leaf extract of *Vitex simplicifolia* showing no pathological changes (normal) (H and E Stain (×250)

Discussion

The administration of methanol leaf extracts of *Vitex simplicifolia* at 250, 500 and 1000 mg/kg^{-1} doses for 21 days orally was observed to significantly increase ALT and ALP ($P>0.05$) and decrease significantly ($P>0.05$) AST and had no significant change on the level of Unconjugated billirubin. The elevation of levels of Alkaline Phosphatase (ALP) as observed in the present study may be an indication of either liver problem or bone disease, since the two main sources of ALP are liver and bone. ALT is a cystosolic enzyme more specific to the liver, so a rise only occurs with liver diseases (Amdar *et al*., 2008). Although high level of serum bilirubin is used as indices of liver function and bile excretion status (Abdurrahman *et al*, 2007), this study did not record appreciable differences in bilirubin levels between the test and the control groups.

The administrations of methanol extract of *Vitex simplicifolia* to Wistar rats showed no significant change in sodium, potassium, chloride but a significant increase in urea and creatinine levels and decrease in carbonate levels was recorded. The elevation of serum urea and creatinine observed in this study may have resulted from kidney damage from exposure to the extract.

It is an established fact that a wide variety of renal diseases with different permutation of glomerular, tubular, interstitial or vascular damage can cause an increase in serum urea and creatininine concentration (Roct *et al*., 1998). Histopathology result of the kidney (Plate IV) with mild distal tubular dame substantiate this observaion. Urea is a by product of protein metabolism that is excreted through the urine (Abdulrahman *et al*., 2007). Previous studies(Rabo *et al*., 1998 and Abdulrahman *et al*.,2006) on *Vitex donniana*; a related specie of *Vitex simplicifolia* reported similar observations which indicate that the Vitex family may contain some phytochemicals that could induce mild damage to the kidney.

Conclusion

This study evaluated hepato-renal toxicological indices following oral administration of aqueous leaf extract of *Vitex simplicifolia* to experimental animals. The recorded observations suggest that the plant is well tolerated up to a dose of 5000mg/kg body weight at acute level with tendency of hepato-renal toxicity as the dose and duration increases. Care should be exercised when using this plant as a phytoremedy against ailments especially with respect to the dose and duration of administration.

References

Abdulrahman, F.I. Onyeyili, P.A. Sanni, S and Ogugbuaja V.O (2007). Toxic Effect of Aqueous Root-Bark Extract of *Vitex doniana* on Liver and Kidney Functions. *International Journal of Biological Chemistry, 1: 184-195.*

Almdar T, Scharling H, Jensen J and Vestergaard H (2008). Higher prevalence of risk factors for type 2 diabetes mellitus and subsequent higher incidence in men. *European Journal of Internal Medicine*: 19(1):40-5.

Avwioro, O.G. (2010). Histochemistry and Tissue Pathology: Principles and Techniques 2nd edition. University Press Delta State University, Abraka Nigeria metabisulphite on the integrity of rat cellular system. Toxicology;81:173–179. Retrieved fromhttp://www.labtestsonline.org/understanding/analytes/creatinine/test

Bartels H and Bohmer M (1972) Serum creatinine determination without protein precipitation. *Clinical Chemistry Acta* 37: 193–197

Bruneton, J.,(1993). Pharmacognosy, phytochemistry, medicinal plants. 2nd Tech and Doc.Lavoisier Paris p915

Burkill HM (2000). Useful Plants of WestTropical Africa. 2nd ed. Vol. 5. Royal Botanic Garden Kew. pp 272-275

Edeoga,H.O,Okwu,O.E and Mbade(2005). Phytochemical constituents of some Nigerian Medicinal plants.Afr.J.Biotechno.,4:685- 688.

Forrester, R L Wataji, L J Silverman, D A and Pierre, K J (1975) Enzymatic method for for determination of CO2 in serum,*Clinical Chemistry*,22(2):243-5

Henry R.F. (1974). Clinical Chemistry Principles and Technics, 2nd Edition, Harper and Row, Hagerstein M.D. p20-23

Heike, S., Munz, B., Werner, S and Brauchle, M., (1999). Different types of ROS-scavenging enzymes are expressed during cutaneous wound repair. *Experimental Cell Research* 247:484-494

Karmen A. (1955). A note on the spectrometric assay of glutamic-oxalacetic transaminase in human blood serum. *Journal of Clinical Investigation*; 34:131–133

Kraut J A and Madias N E (2007) Serum Anion Gap: Its Uses and Limitations in Clinical Medicine, *Clinical Journal of American Society of Nephrology* 2: 162–174

Krishnaraju, A.V., Rao, TVN., Sundararajua, D., Vanisreeb, M., Tsayb, H.S and Subbarajua, G.V. (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (Artemia salina) lethality assay. *International Journal of Applied Science and Engineering* 2: 125-134.

Lai P.K. and Roy J (2004) "Antimicrobial and chemopreventive properties of herbs and spices". Curr. Med. Chem. **11** (11): 145160. doi:10.2174/0929867043365107. PMID 15180577.

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Arch. Toxicol.* 53:275-287.

Mantle, D., Gok ,M.A., Lennard T.W.J. (2001). Adverse and beneficial effects of plant extracts on skin and skin disorders. Adverse Drug Reactions and Toxicological Reviews 20(2): 103-89

Mitchell, S. Sheppard, R., Vinay, K., Abbas, A., Abul, K. and Fausto, Nelson. R. (2011). *Basic Pathology*. 8th edition. Chapter 11 Philadelphia: Saunders.

Nacoulma O, (1996). Medicinal plants and traditional medical practices in burkina faso. Ph.D thesis, University of Ouagadougo, Pp 328

Phillips, G.D., Whitehe, R.A., Kinghton, D.R. (1991). Initiation and pattern of angiogenesis in wound healing in the rat. *American Journal of Anatomy* 192:257-262

Rabo, J.S.(1998). Toxicity studies and trypano suppressive effects of stem-back extracts of Butyrospermun paradoxum in laboratory animals. PhD Thesis, University of Maiduguri, Maiduguri, Nigeria. p34-56

Roct, R.C, W.G. Walker and C.D. Jennings(1998).Nitrogen Metabolites and Renal Function.: Textbook of Clinical Chemistry. Tiez, N.W (ED), W.B. Saunders Company, Philadelphia, pp: 1254-1316

Reitman S., and Frankel S., Amer J. 1957; Clin Path. 28:56.

Sherlock,S (1951) Liver Disease. *Journal of Biochemistry*, 297:204-279

Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa.2ndEdn.,SpectrumBooks,Nigeria,ISBN13:9789782462190,pp142-144

Tapsell LC, Hemphill I and Cobiac L (2006) "Health benefits of herbs and spices: the past, the present, the future". Med. J. Aust. **185** (4 Suppl): S4–24. PMID 17022438.

Weatherburn MW (1967) Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry* 39: 971–4

White W.L. (1970). Chemistry for Technologist, 3rd Ed. The C.V Mosby Co., St. Louis p. 182