Phytochemical analysis of the Yoruba medicinal formulations- "Gbogbo nise" and its effect on some liver enzymes.

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Abstract

The Yoruba formulation called Gbogbo nise is one of the most widely consumed medicinal formulation in the rural areas of Nigeria. The aim of this study was to screen for the various phytochemicals in this medicinal formulation and the effects of these formulations on some liver enzymes of wistar albino rats. Sixty-four rats irrespective of sex were used in this study. They were divided into six groups (control and Groups A-E); with groups A-E split into three sub-groups for testing at week 2, 4 and 6. Each sub group contained four rats and was administered 2.9µl/Kg B.W of each medicinal formulation. Phytochemical screening indicated the presence of alkaloids, saponins, tannins, flavonoids and terpenoids. Biochemical examination of the serum of experimental groups after 2 weeks of administration, showed an initial reduction in activities of AST, ALT and ALP when compared with control groups. For the results at weeks 4 and 6, all five formulations resulted in significant increase in the level of these marker enzymes. Thus the prolong administration of these medicinal formulation at the dosage used in the study, results in rise in liver enzymes and may be indicative of hepatic damage.

Keywords: Yoruba, Gbogbo nise, phytochemicals,liver enzymes

1. Introduction

It is generally known that the consumption of a variety of local herbs and vegetables by man contributes significantly to the improvement of human health, in terms of prevention, and or cure of diseases because plants have long served as a useful and natural source of the therapeutic agents (Chevellier,1996). Moreover, traditional medicine is greatly relied upon especially by rural dwellers, for the treatment of various ailments: traditional doctors or healers are the dispensers of such formulations.

The formulations made from the leaves and/or bark of a mixture of plants have been reported to be used by many tribes for diarrhea, dysentery, sore throats, vomiting, stomach upsets, vertigo, malaria, typhoid fever, piles, body pains etc. The medicinal importances of these plants and plant derived formulations have been attributed to their phytochemical content. Thus phytochemical analysis of plants and plant derived formulations is predicated by the need for drug alternatives of plant origin, made imperative by the high cost of synthetic drugs. For example, L. owariensis leaves have been reported to contain various secondary plant metabolites of medicinal value including saponins, tannins, alkaloids and flavonoids (Nwogu et al., 2007 & 2008). These secondary plant metabolites extractable by various solvents exhibit varied biochemical and pharmacological actions in animals when ingested (Trease et al., 1996). Within the recent decade, a good number of medicinal plants and formulations have been reported to be employed in folk medicine in the treatment of Malaria, pile, typhoid fever, arthritis etc.,

The worldwide increasing demand for medicine from natural sources (Lapa, 1992) and the global Roll Back Malaria initiative that set up Medicines for Malaria Venture (MMV) to foster and accelerate research into innovative drugs with anti malarial properties (WHO, 2001) has motivated the search for plants with potential pharmacological and therapeutic uses. Plants or various parts of the plant have been used over the ages for therapeutic purposes (Lambo, 1979; Iwu, 1993; Sofowora, 1993).

Plants generally have varied chemical compositions (referred to as phytochemicals) depending upon species. A good number of plants are known to be of economic and medicinal value. Those that are of medicinal value are often used as herbal remedy for the restoration and maintenance of good health. Some herbs have been considered as drugs and therefore generally safe and effective (Treasure, 2000). Most herbs have been associated with broad actions on a number of physiological systems in concert unlike the pharmaceutical drugs which are usually designed to elicit a specific effect. Some researchers on medicinal plants are of the opinion that some herbal plants are usually oriented in the same general therapeutic direction and are complementary or synergistic, often non-specific, but very rarely adverse (Treasure, 2000; www.ibiblio.org).

2. Materials and methods

2.1 Formulations

Five different formulations where purchased from several local traditional medical dispensers peddling them at Omoko, Rumuchakara, Alakahia, Rumuosi and Rumuokoro communities in Rivers state. These formulations were those used mainly in treatment of malaria, fever, body and waist pains, typhoid, pile (Jedi-Jedi) and several miscellaneous illnesses as confirmed by several users in the rural population.

2.2 Phytochemical screenings

Screening for phytochemical weren carried out based on standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

2.3 Test for Tannins

About 20 ml of sample was placed in a test tube. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.4 Test for Saponins

10ml of the sample was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.5 Test for Flavonoids

Two methods were used to determine the presence of flavonoids in the sample (Sofowara, 1993; Harbrone, 1973). 5 ml of dilute ammonia solution were added to a portion of the sample followed by addition of concentrated H_2S0_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Few drops of 1% aluminium solution were added to a portion of each sample. A yellow colouration was observed indicating the presence of flavonoids.

2.6 Test for Terpenoids (Salkowski test):

5ml of each sample was mixed in 2ml of chloroform, and concentrated H_2S0_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

2.7 Test for Alkaloids

5ml of sample was warmed with 2% H_2SO_4 for two minutes. It was filtered and few drops of Drageneloff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

2.8 Animal materials

Sixty-four healthy wistar albino rats weighing between 125-175g were purchased from the University of Nigeria Enugu campus, animal house regardless of their sex. They were placed in cages and kept in a well aerated room at a temperature of 28-31ºC and humidity of 50-55%. They were then allowed to acclimatize to new housing conditions for twelve days prior to experimentation. They were feed normal feed (purchased from Top feed shop, Choba) and distilled water *Ad libitum*. The cages were cleaned of waste once daily. All animals were treated in a manner that complied with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1985).

2.9 Animal treatment

The rats were divided into six (6) groups (Group A-E and control) based on the number of formulations. Groups A-E was further sub-grouped into three (3) sub-groups each (based on duration of administration) with four (4) rats per sub-group, housed in griffin and George modular cage system. The control group was fed normal feed and distilled water *ad libitum*. Groups A-E were fed normal feed and distilled water *ad libitum* and also received the different medicinal formulation (labeled A-E) at 2.9µl/kg B.W (based on the estimated traditional human dosage of 200ml for an average man of 70kg). The experimental and control groups ran for forty-two days, while analysis was carried out every two weeks and weights measured also. At the end of week 4 of experimentation, administration of the formulation was discontinued while the experimentation continued for an extra 2 weeks to serve as a second control, to see the effect after withdrawal.

2.10 Sample collection

2.10.1 Blood

Each animal was sacrificed by anaesthetizing with chloroform first, then the jugular veins were slit with the aid of a surgical blade. Samples for enzyme analysis were collected in plane wash bottles.

2.10.2 Organs

Each animal was laid on a flat hard surface with the fore and hind limbs, pinned to the surface. Samples organs were collected after dissection of the underlying skin of the abdominal and thoracic cavities. The samples organs were collected by hand to prevent scaring of the liver tissues by other materials and weighed. Liver was stored in 10% formalin in preparation for histological examination. Samples were analyzed at the University of Port- Harcourt teaching hospital (U.P.T.H) chemical pathology Laboratory.

2.10.3 Liver enzyme analysis

In-vitro Determination of Alanine aminotransferase (ALT)

This was done by the method of Reitman & Frankel (1957). The reagent kit contained the following:

Content	Initial Concentration
R1 buffer	
L-Alanine	200mmol/L
α – Oxoglutatrate	2.0mmol/L
R2 2, 4-Dinitrophenylhydrazaine	2.0mmol/L

Principle

Alanine aminotransferase is measured by monitoring the contribution of pyruvate hydrazone formed with 2, 4 Dinitrophenylhydrazaine.

$$\alpha - \text{Oxoglutatrate} + \text{L- Alanine} \xrightarrow{\text{ALT}} \text{L- Glutamate} + \text{Pyruvate}$$

Procedure

This method is based on the method of Reitman S. and Frankel .J. (1957). Take 0.1ml of sample (serum) and 0.1ml of distilled water as blank. Add 0.5ml of R_1 (as mentioned above) to sample test-tube and also to blank. Mix, incubate for exactly 50minutes at 37ºC. Add 0.5ml of solution R_2 (as mentioned above) to sample and blank test-tubes. Mix and read absorbance of sample ($\mathbf{A}_{sample}$) against reagent blank after 5minutes at 546nm wavelength.

In-vitro Determination of Aspartate aminotransferase (AST)

The reagent kit contained the following:

Contents	Initial Concentration of solution
R_1 Buffer	
Phosphate Buffer	100mmol/L, pH 7.4
L- Aspartate	100mmol/L
α – Oxoglutarate	2mmol/L
R_2 2, 4-Dinitrophenylhydrazine	2mmol/L

Principle

Aspartate aminotransferase is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-Dinitrophenylhydrazine.

$$\alpha - \text{Oxoglutarate} + \text{L- Aspartate} \xrightarrow{\text{AST}} \text{L- Glutamate} + \text{Oxaloacetate}$$

Procedure

This is also based on the method of Reitman .S. and Frankel .J. (1957). Take 0.1ml of test sample (serum) and 0.1ml distilled water as blank, to each test-tube, add 0.5ml of reagent R_1. Mix in a water-bath for 30mins at 37ºC. Then, add 0.5ml of reagent 2 (R_2) solutions to both test tubes. Mix, allow standing for exactly 20mins at 20-25ºC. Add sodium hydroxide 5.0ml to both test tubes.

Mix and read the absorbance of sample ($\mathbf{A}_{sample}$) against reagent blank after 5mins at 546nm wavelength.

2.10.4 In-vitro determination of Alkaline Phosphatase activity

The reagent test kit contains the following:

Principle

This is based on the spectrophotometric measurement of inorganic phosphate or phenol liberated during the hydrolysis of p-Nitrophenyl phosphate.

Procedure

Take 0.05ml of test sample (serum) and add 1ml 0f reagents. Do same to 0.02ml of blank (distilled water). Incubate for 1-3mins at 37ºC. Add 2.5ml alkaline phosphatase colour developer at the same time and mix. Read absorbance at 590nm wavelength. Reading taken at time intervals.

2.11 Statistical analysis

The results are presented as mean ± standard deviation. The mean values of the various treatment groups were compared using SPSS version 17 for windows (SPSS Inc USA). The significant level during these tests was set at $p \leq 0.05$.

3. Results

The results of the study are shown in Tables 1-4 below, as well as figures 1-3. Preliminary screening of the formulations indicated the presence of alkaloid, tannins, flavonoids and phenols as illustrated in table 1.

Table 1. *Phytochemical profile of Formulations A-E.*

	Formulation A	Formulation B	Formulation C	Formulation D	Formulation E
Alkaloids	++	++	++	++	++
Flavonoids	+++	+++	++	+++	+++
Tanins	+++	+++	+++	++	+++
Saponins	+	++	++	++	++
Terpenoids	++	++	++	++	++
Key : +++ = highly present ++ = Moderately present + = Present - = Absent					

Table.2. Effect of formulations A-E on the mean serum AST activity (IU/L) at weeks 2, 4 and 6.

Formulation	Week 2	Week 4	Week 6
Control	120.00±1.63		
Group a	112.00±1.63a	122.00±1.41b	130.00±1.63a
Group b	100.25±2.5a	121.00±1.83b	129.00±0.82a
Group c	112.25±2.22a	122.00±1.41b	130.00±1.83a
Group d	115.00±0.82a	125.00±0.82a	132.00±0.82a
Group e	113.00±1.63a	130.00±1.63a	135.25±2.06a

Table. 3. Effect of formulations A-E on the mean serum AST activity (IU/L) at weeks 2, 4 and 6.

Formulation	Week 2	Week 4	Week 6
Control	30.00±0.82		
Group a	24.25±0.96a	31.00±0.82a	36.75±3.18a
Group b	23.25±0.96a	30.75±0.96a	42.00±4.83a
Group c	23.00±0.82a	29.25±0.96a	36.75±0.96a
Group d	22.50±1.29a	31.25±1.26a	38.25±0.96a
Group e	24.75±0.96a	32.75±0.96a	39.00±0.82a

Table.4. Effect of formulations A-E on the mean serum ALP activity (IU/L) at weeks 2, 4 and 6.

Formulation	Week 2	Week 4	Week 6
Control	320.00±1.00		
Group a	331.75±1.25a	356.25±0.96a	370.75±0.96a
Group b	326.25±1.70a	361.00±0.82a	371.50±1.29a
Group c	316.75±1.25b	342.75±2.21a	363.50±3.11b
Group d	319.00±0.82b	369.25±0.96a	380.25±0.50b
Group e	302.75±2.21b	353.50±3.11a	361.00±1.41a
Values are expressed as Mean ± Standard deviation. Superscript a indicates significant difference between experimental and control groups, b No a indicates significant difference between experimental and control groups.			

Fig.1. Effect of formulations A-E on the mean serum AST activity (IU/L) at weeks 2, 4 and 6.

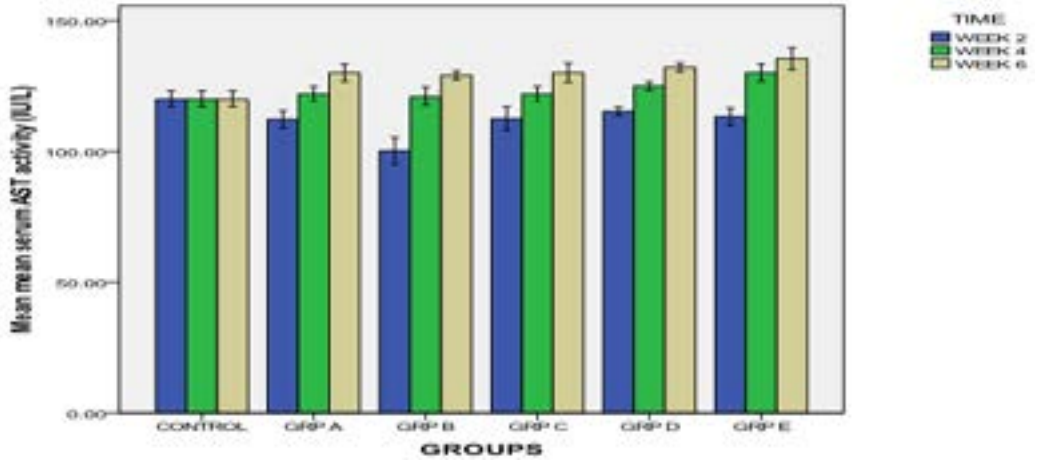


Fig . 2. Effect of formulations A-E on the mean serum ALT activity (IU/L) at weeks 2, 4 and 6.

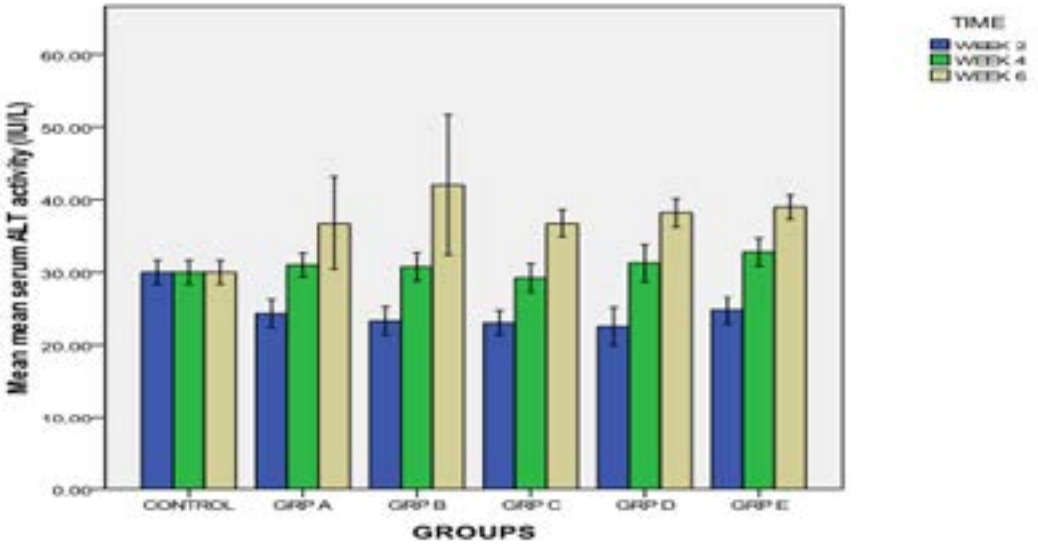
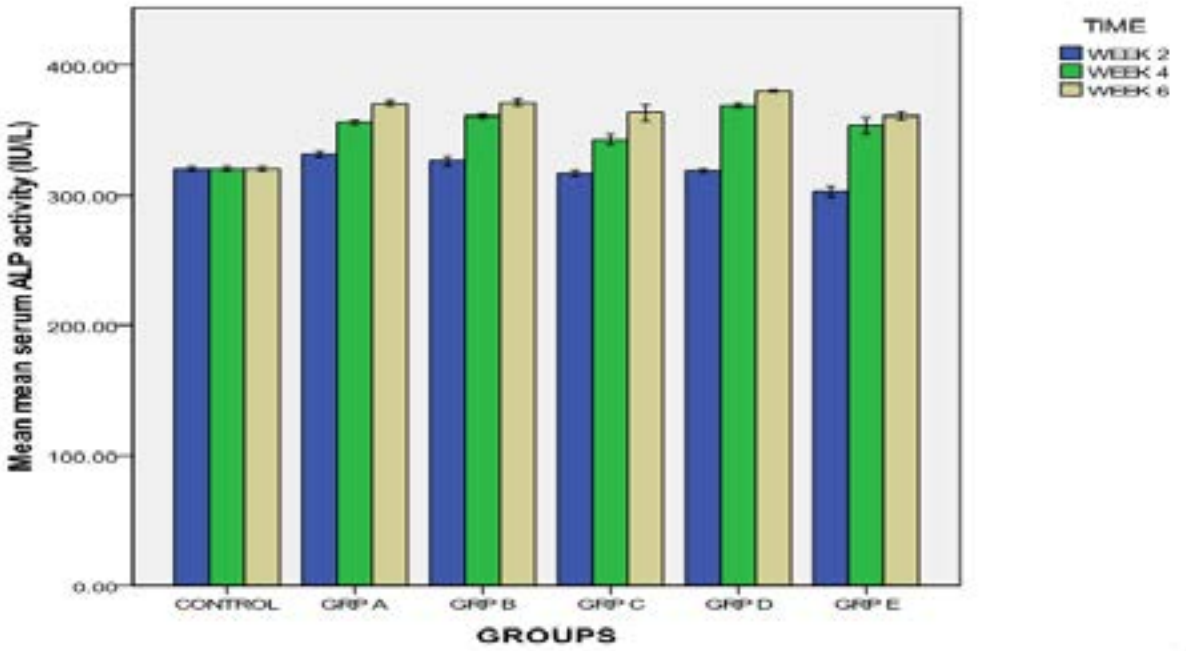


Fig.3. *Effect of formulations A-E on the mean serum ALP activity (IU/L) at weeks 2, 4 and 6.*



4. Discussion

These formulations are rich sources of various phytochemicals most abundant of these are the alkaloids, flavonoids and tannins (table 1). Various chemicals such as alkaloids, tannins, saponnins, cyanoglycosides, terpenoids, oleic and stearic acids which are naturally present in plants have been implicated in the conferment of antimicrobial activities on the plant containing them (Ingham, 1973 ;Osbourn, 1996) Abd El Rahman *et al*., 2003). The presence of some of these plant secondary metabolites in a significant amount in the formulation may have conferred antimicrobial physiological activity to the formulation.

Measurement of the activities of various enzymes in tissues and body fluids play a significant and well-known aid in disease investigation and diagnosis (Malomo, 2000). Tissue enzyme assay can also indicate tissue cellular damage long before structural damage is revealed by some other conventional techniques (Akanji, 1986).

The liver plays an important role in many metabolic processes; any disturbance in the liver would affect the normal level of measurable biochemical parameters in this organ. AST, ALT, and ALP are marker enzymes present in high concentrations in the liver, when liver cells are inflamed or damaged, these enzymes leak into the blood stream leading to a rise in the plasma level of these enzymes (David, 1985; Nkosi *et al.,* 2005). ALT is selectively a liver parenchymal enzyme than AST and a sensitive indicator of acute liver damage (Shah *et al.,* 2002). Thus elevation of these enzymes in our study indicates inflammation or damage of the liver cells.

After 2 weeks of administration of formulation, there was a reduction in the serum activity of the liver enzyme when comparing experimental groups with control group. It was observed that at week 4, the level of liver enzymes (AST, ALT and ALP) has increased which may be indicative of liver damage. At week 6 there was a significant increase in the serum concentration of liver enzymes when compared to the treatment groups at weeks 2 and 4 and also with the control groups.

ALT is a cytoplasmic enzyme found in very high concentration in the liver and an increase of this specific enzyme indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function (Aliyu *et al.,* 2006).

Alkaline phosphatase (ALP) is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum (Wright & Plummer, 1974), it is therefore an ectoenzyme of the plasma membrane (Shasjahan *et al.,* 2004). The corresponding increase in the rat serum ALP activity (Table 3 &4) confirmed that damage has been inflicted on the plasma membrane, which might lead to compromise of its integrity (Yakubu *et al.,* 2003). Such increase in alkaline phosphatase activity may lead to indiscriminate hydrolysis of phosphate esters in the organs and other cells requiring these essential molecules (Butterworth *et al.,* 1966). The observed effect proved irreversible at the dosage used in this study as the level of these liver enzymes still increased despite termination of formulation administration.

5. Conclusion

Humans believe that as long as a drug or supplement is of natural origin, it's the best. These medicinal formulations have proved to be a great source of phytochemicals. Despite the many beneficial effects of these phytochemicals, many of these phytochemicals normally function as toxins that protect the plants against insects and other damaging organisms. Plants evolved the ability to produce toxic substances and concentrate them in vulnerable regions (the skin, seeds and leaves) in order to dissuade insects and other organisms from eating and killing the plant.

Although this formulation also known as GboGbo nise was made from natural herbal products, it should be noted that despite its naturality, it still induced hepatotoxcity from week 4 at the dose administered in this study.

It could be clearly concluded that prolonged administration of the formulation- "Gbogbo nise" peddled in our communities at the dose used in this study tends to also toxic to the rats as time prolongs. The toxicity in rats indicates the presence of some toxic active compound which merit phytochemical isolation. The use of this formulation as a folkloric medicine should be with uttermost care.

6. References

1• Abd el Rahman HF, Skaug N and Whyatt A (2003) Volatile compounds in crude salvadora persica extracs. *Pharm. Biol.* 41, 392-404

2• Akanji MA (1986) A comparative biochemical study of the interaction of some trypanocides with rat tissue cellular system. Ph.d thesis, University of Ife, Ile-Ife.

3• Butterworth PJ and Moss DW (1966) The effect of urea on human kidney alkaline phosphatase. *Biochem. J.* 99, 9-10

4• Chevellier A (1996) The encylopedia of medical plant. London. Dorling kindersley ltd. (online). Http://www.chclibrary.org/plant.html

5• David LW and Vincent M (1985) Biochemistry in clinical practice. New york: worth publishers; test indicating hepatocellular damage. pp: 142

6• Harborne IB (1973) Phytochemical methods: a guide to modern techniques of plant analysis. 2nd edn, chapman and hall, NY, pp:88-185

7• Ingham JL (1973) Disease resistance in higher plants. The concept of pre-infectional and post- infectional resistance. *Phytopathol. Z.* 78, 314-335.

8• Iwu MM (1993) In: Handbook of african medicinal plants. Crc press, ll.c. USA. pp:1-13

9• Lambo JO (1979) The healing powers of herbs with special reference to obstetrics and gynaecology' african medicinal plants (ed sofowora e a). University of ife press ife, Nigeria. pp: 23

10• Nkosi CZ, Opoku AR and Terblanche SE (2005) Effect of pumpkin seed (*cucurbita pepo*) protein isolate on the activity levels of certain plasma enzymes in ccl4 induced liver injury in low protein fed rats. *Phytother. Res.*19, 341–345

11• Malomo SO (2000) Toxicological implications of ceftriaxone administration in rats. *Nig. J. Biochem. Mol. Biol.* 15, 33-38

12• Nwogu LA, Alisi CS, Ibegbulem CO and Igwe CU (2007) Phytochemical and antimicrobial activity of ethanolic extract of *landolphia owariensis* leaf. Afr. J. Biotechnol. 6(7),890-893

13• Nwogu LA, Igwe CU and Emejulu AA (2008) Effects of landolphia owariensis on the liver function profile and haemoglobin concentration of albino rats. Afr. J. Biochem. Res. 2(12), 240-242

14• Osbourn AE (1996) Preformed antimicrobial compounds and plant defense against fungal attack the plant cell, 8, 1821-1831

15• Reitman S and Frankel S (1957) Colorimetric GOT and GPT determination. *Am. J. Clin. Pathol.* 28,56-63

16• Trease GE and Evans WC (1996) Phenols and phenolic glycosides in: trease and evans pharmacognosy, 13th edn. Biliere tindall, London. pp: 832–833

17• Treasure J (2000) Medical herb (online) http://www.herbological.com

18• http://www.ibiblio.org 2000

19• Shah M, Patel P, Phadke M, Menon S, Francis M and Sane RT (2002) evaluation of the effect of aqueous extract from powder of root, stem, leaves and whole plant of *Phyllanthus debilis* against CCl_4 induced rat liver dysfunction. Indian drug. 39, 333-337

20• Shahjahan M, Sabitha KE, Mallika J and Shyamala-Devi CS (2004) Effect of *Solanum trilobatum* against carbon tetrachloride

induced hepatic damage in albino rats. *Indian J. Med. Res.* 120,194-198

21• Sofowora EA (1993) In: Medicinal plants and traditional medicine in Africa. (2nd ed), spectrum books limited. pp: 26-30

22• Wright PJ and Plummer DT (1974) The use of urinary enzyme measurement to detect renal damages caused by nephrotoxic compounds. *Biochem. Pharmacol.* 12, 65

23• Yakubu MT, Bilbis MT, Lawal M and Akanji MA (2003) Effect of repeated administration of *sildenafil citrate* on selected enzyme activities of liver and kidney of male albino rats. *Nig. J. Pure & Appl. Sci.* 18, 1395-1400