HEPATOTOXICITY OF CARBON TETRACHLORIDE: PROTECTIVE EFFECT OF *GONGRONEMA LATIFOLIUM*

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ABSTRACT
The protective effect of the ethanolic extract of *Gongronema latifolium* (GLE) on carbon tetrachloride (CCI_4) induced hepatic toxicity was studied. Liver enzymes studied included alanine aminotransferase (ALT), aspartate aminotraferase (AST), and alkaline phosphates (ALP). Hepatic injuries involved with possible necrosis which may have contributed to its possible pathogenesis was explored. Administration of toxicant only showed that the ALT level was significantly ($P<0.05$) increased to 345.83% when compared to control. Pretreatment with *Gongronema latifolium* extract (GLE) non-significantly ($P<0.05$) decreased to 13.08% when compared to those treated with toxicant only. Also under experimental conditions, increasing the concentration of *Gongronema latifoluim* extract (GLE) non-significantly ($P<0.05$) decreased dose-dependently the level of ALT to 18.20%. The AST level was non-significantly ($P<0.05$) increased to 41.55% on treatment with toxicant only. Pretreatment with GLE decreased the AST level non-significantly ($P<0.05$) to 25.76%. No evident increase or decrease in the level of ALP was observed. Treatments with toxicant showed liver cells filled with uniformly distributed dense small fat droplets, large nuclei, inflamed cells and evidence of necrosis and fibrosis. Pretreatment with 100mg/kg of the extract showed microvesicular fatty change with no evidence of inflammation, necrosis or fibrosis. The protective effect of the GLE was more pronounced in ALT and AST. However, the GLE has a strong modulatory effect against the hepatocellular damage induced by carbon tetrachloride.

Keywords: Hepatotoxicity, *G. latifolium,* modulatory effect, carbon tetrachloride.

INTRODUCTION

Man from his first awakening, has sought to combat and control disease and pain, with assistance, inspiration and guidance from its natural environment. From this time, several plant materials (including the roots, stems, barks, leaves, flower etc.) by instincts, intuition or trial and error were used to combat various ailments (Lambo, 1979) Plants were used according to their characteristic features and whether or not these plants have the needed medicinal effect was not of concern. For example, the use of many medicinal plants in Europe in 1400-1500 was based on the doctrine of signatures or similar developed by Paracelsus (1490-1514), a Swiss alchemist and physician. According to this doctrine, healing herbs have features made by God for identifying the plant with a specific disease or part of the body. And so, plants with heart-shaped leaves were used for treatment of heart disease, those with liver-shaped parts were prescribed for bilious diseases etc. (Sofowora, 1982). Apparently this did not take into consideration the active ingredients or species in these plants probably because man's selection of specific plant materials for the treatment of his ailments was based more on his ability to rationalize or on a knowledge of plant constituents (Ademuwagun, 1973).

This was primitive so to say, though still in practice and forms the concept of traditional medicine.

In recent times, with advent of western scientific methods most of these plants reported and considered medicinally important came under chemical scrutiny leading to the analysis and isolation of medicinally active principles or constituents and are tested and proven for their ability to exhibit certain proposed healing effect at certain concentrations, *in vivo* and *in vitro* before it can now be certified to have the desired healing/protective effect. This concept now forms the principles of modern or orthodox medicine (Sofowofa 1982).

Human beings are exposed on a daily basis to certain toxic chemicals and pathogens, which cause certain serious health problems. Certain chemicals and reagents that were thought to be health friendly have been proved to have serious adverse effects on health. Amongst these toxic substances is carbon tetrachloride.

Carbon tetrachloride or carbon tet as it is normally referred to, is a colourless liquid, non flammable, and is heavier than air. Consequently, it has been widely used as a fire extinguisher (The World Book Encyclopedia, 1992). Carbon tetrachloride was once widely used as a cleansing fluid in households and as solvent for oils, amongst other uses. The most important use of carbon tetrachloride is as an intermediate in the preparation of Freon (CCI_2F_2), which is used as a refrigerant and as a propellant in aerosol bombs (Price, 1980). Carbon

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tetrachloride is very toxic and because of this, most of its uses in households and industries have been suspended. The main routes of exposure of humans and animals to carbon tet includes inhalation, ingestion, and absorption. On entry into the body, carbon tetrachloride causes a lot of injury to the organs of the body (Reynolds *et al.*, 1984) including the lungs, heart, gastrointestinal tract, kidneys, CNS, and liver (ATSDR, 2003).

Of concern here is the adverse effect of carbon tetrachloride to the liver, having visualized the prominent functions of the liver for survival. According to Liu *et al.*, (1995), ingestion of carbon tetrachloride can lead to marked hepatotoxicity.

Hepatic diseases or injuries are modelly induced experimentally by administration of carbon tetrachloride, since it is known that carbon tetrachloride produces acute hepatocellular injury with centrilobular necrosis and steatosis (Recknagel, 1987). The effects of carbon tetrachloride include hepatic injury on biochemical characteristics such as increased lipid peroxidation (Recknagel, 1987) and the activities of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase, (GPT) and hepatobiliary excretion of xenobiotics have been widely studied. These hepatotoxic effects can be minimized or prevented or eliminated by certain active compounds serving as valuable antioxidants obtainable from natural plant resources (Akhtar and Ali, 1984).

Gongronema latifolium, a perennial climber crop, native of the humid tropic of south eastern Nigeria (Okafor, 1989), known locally among the Efik, Ibibio and Igbo speaking communities of Nigeria as utazi, could have some augmentary or protective effect against certain hepatocellular injury. Ugochukwu *et al.*, (2003) reported that the ethanolic extract of *G. latifolium* leaves possess antioxidant activity. Leaves of *G. latifolium* have protective role against diabetes, hypertension, stomach upsets and pains, malaria and typhoid fever. In view of these attributes since no evidence has been tendered as regards the remedial effects of the leaves of *G. latifolium* on liver malfunction, the burden at this work is reposed on the *in vivo* determination of the effects of ethanolic extract of the leaves of *G. latifolium* on some hepatocellular damage induced by CCl_4 in experimental animals.

MATERIALS AND METHODS

Plant material

Fresh *G. latifolium* leaves were obtained in Anua Offot in Uyo Local Government Area of Akwa Ibom State. The fresh leaves were authenticated at the Department of Botany, University of Uyo, Nigeria. The leaves were sun-dried and then homogenized into fine leaves particles, which weighed 205g. The leaves were extracted with 80% ethanol using Soxhlet apparatus. The extract obtained were evaporated/concentrated to about 1/10 of their original volumes at 80^{o}C using water bath evaporator. The concentrate was then weighed giving 37. 138g and preserved in sample bottles wrapped with aluminum foil, at 4^{o}C. The extract was dissolved in distilled water before use.

Experimental animals

20 male wistar rats with an average weight of 100g obtained from Biochemistry Department, University of Calabar were used. The animals were housed in the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria at temperature of 35-37^{o}C. Upon arrival the animals were acclimatized for at least 7 days and were maintained on a regular commercial rat feed and water on a daily basis.

Experimental design

Primarily the experiment was designed to study the *in vivo* effect of ethanolic extracts of *G. latifolium* on histopathological changes of hepatic lesions and some biomarkers indicating hepatocellular injury induced by carbon tetrachloride in male albino Wistar rats.

Hepatic injury or liver damage was induced by intraperitioneal injection of 1.2ml/kg of CCl_4. Control rats received only normal diet and water. Animals were then separated into five groups with four animals (based on their body weights) in each group.

Group A: (Control): Animals that received only normal diet and water.

Group B: Animals that were treated with 100mg/kg body weight of GLE twice daily for 14 days.

Group C: Animals that were treated with CCl_4 only.

Group D: Animals that were treated with 100mg/kg body weight of GLE for 13 days and 1.2ml/kg body weight of CCl_4 on the 14th day.

Group E: Animals that were treated with 200mg/kg body weight of GLE for 13 days and 1.2ml/kg body weight of CCl_4 on the 14th day.

After 14-days treatment, the rats were sacrificed six hours after the last treatment. Blood was collected from the heart with the use of 5ml sterile syringe individually for each animal, and transferred into plain sample bottles immediately (non-heparin anticoagulant bottles). Blood was left to clot for about 30 minutes and was then centrifuged at 4000g for 15 minutes to obtain the serum. The serum was removed using a Pasteur pipette into another set of tubes. After each analysis, the serum was

stored in a refrigerator at 4°C for subsequent analysis. Liver samples were surgically removed immediately and suspended in 10% formaldehyde for histologic evaluation.

Determination of alanine aminotransferase (ALT)
The activity of enzyme glutamic-pyruvate transaminase was analysed according to the method specified by Reitman and Frankel (1957) and, Schmidt and Schmidt (1963).

The principle involved measuring the concentration of pyruvate hydrazones formed with 2,4-dinitrophenyl-hydrazine.

Determination of aspartate aminotrasferase (AST)
The enzyme glutamic-oxaloacetate transaminase was analysed according to the method of Reitman and Frankel (1957) and Schmidt and Schmidt (1963)

The principle involved glutamic-oxaloacetate transa-minase measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4 – dinitrophenyl hydrazine.

Determination of alkaline phosphatase (ALP) activity
The method of King and King (1954) was used. Serum was incubated with disodium phenyl phosphate as substrate, buffered at pH10 for 15 minutes at 37°C. The hydrolytic products, phenol is condensed with 4-amino antipyrine and then oxidized with alkaline ferricyanide to give a complex, which is measured photometically at 510nm.

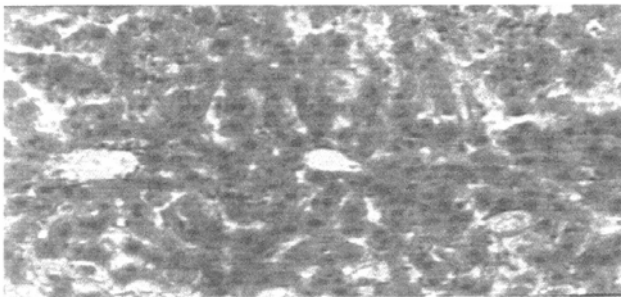
Histopathogical studies
10% formalin was freshly prepared and the right liver lobe of treated and control were fixed in 10% formalin for 48 hours and subsequently dehydrated in alcohol, cleared with xylem and embedded in paraffin wax. Sections of lobe at about 5µm were mounted on glass slides and stained with haematoxylin and eosin (Lillie, 1965).

STATISTICAL ANALYSIS

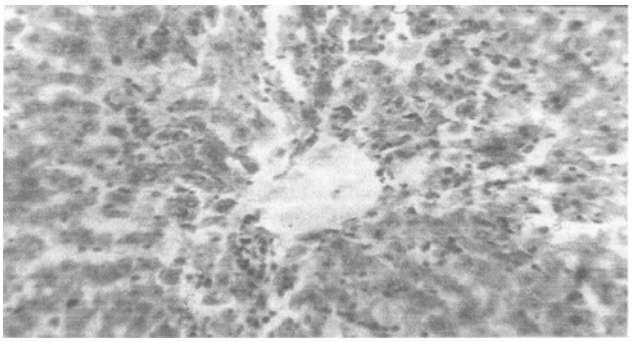
The results obtained were reported as mean (x) ± Standard Deviation (S.D) from four repeated determinations. The data obtained form this study was analysed statistically using student's t-test. Differences were considered to be statically significant at $P<0.05$.

RESULTS

The values are expressed as mean (x) ± SD for four animals in each group. The concentration of some liver enzymes in the serum were measured in rats undergoing treatment with the ethanolic extract of *Gongronema latifolium* leaves with and without induction of toxicity with carbon tetrachloride.



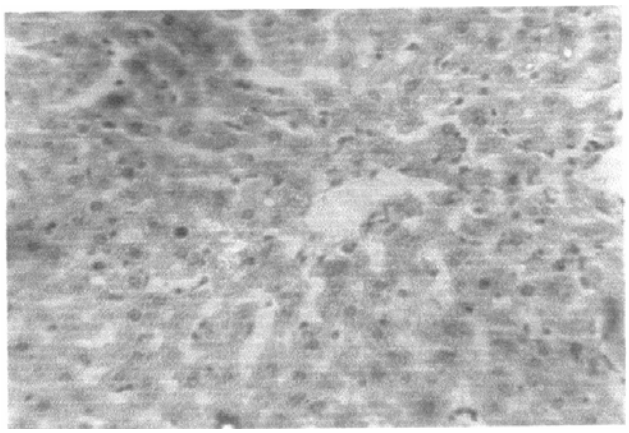
100mg/kg of Extract and Toxicant 1.2ml/kg
The Hepatocyte showed distinct microvacoules typical of fatty change. The nuclei are located centrally in the hepatocytes. Necrosis, fibrosis and inflammation are absent.



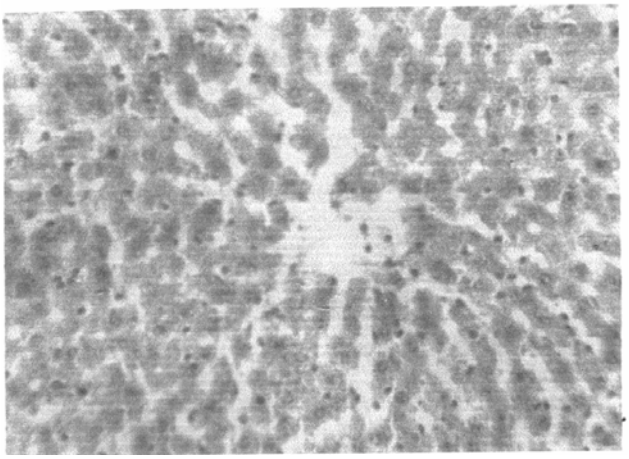
200mg/kg of Extract and Toxicant 1.2ml/kg
Terminal hepatic vein was shown surrounded by necrotic. The necrosis to the right of this vein is haemorrhagic.

Table 1. Hepatotoxicity of carbon tetrachloride: protective effect of *Gongronema latifolium* on liver enzyme activities

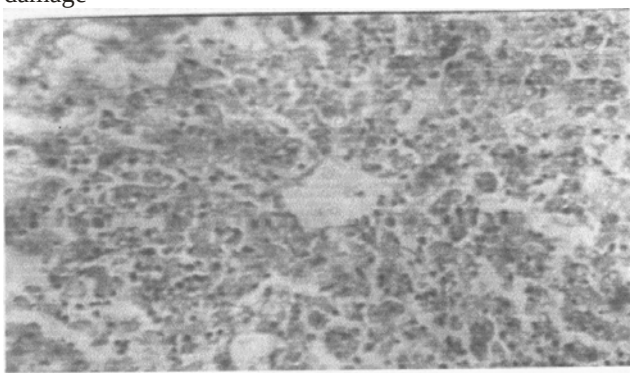
GROUP	ALT	AST	ALP
A: Control	6.00 ± 2.31	39.33 ± 2.89	31.38 ± 2.32
B: 100mg/kg GLE - Extract only	7.00 ± 2.00	10.00 ± 5.20	34.74 ± 0.00
C: Toxicant only	26.75 ± 14.30	55.67 ± 13.32	31.84 ± 3.29
D: Toxicant and 100mg/kg GLE Extract	23.25 ± 8.09	41.33 ± 5.51	32.89 ± 1.21
E: Toxicant and 200mg/kg Extract	19.67 ± 4.62	53.00 ± 8.49	29.89 ± 1.21



Control-normal liver cytoplasmic changes. No Inflammation of Cells



100mg/kg of extract only - normal liver cytoplasmic changes. no evidence of inflammation on liver cell damage



1.2ml/kg Toxicant only:
Liver cells are filled with uniformly distributed dense small fat droplets. The nuclei are large and cells inflamed. There is presence of necrosis and fibrosis.

DISCUSSION

As far as life is concerned subject of priority is health. But despite effort to maintain good health, man and animals alike still confront disease conditions which are due to exposure to physiopathological agents (Lambo, 1979). Such agents include microorganisms, noxious substances, etc in the environment. Though the body system is made in such a way that it tackles invading foreign substances in most cases, the body system is incapable to do so and needs to be protected, enhanced and activated (Murray *et al.*, 1990). This ability to activate the body defense mechanism, or to protect the body system have been found to be present in some nature vegetation/herbal sources (Morebise *et al.*, 2002; Okafor, 1989). And so it has become expedient to examine scientifically the protective effects of these herbal plants.

Gongronema latifolium has been phytochemically analysed and found to contain some bioactive compounds such as tannins, saponins, flavonoids etc, which have made it medicinally important (Morebise *et al.*, 2002; Akhta and Ali (1984). To examine medicinal importance and capacity *in vivo* certain parameters, were examined particularly the serum enzymes.

As indicated in the literature, serum enzymes in this care viz. alanine aminotransferase, (ALT), aspartate aminotransferase (ASL), are present in the hepatic and biliary cells (Jensen *et al.*, 2004). These enzymes are usually released from the hepatocytes and leak into circulation causing increase in their serum levels under hepatocellular injury or inflammation of the biliary tract cells resulting predominantly in an elevation of the alkaline phosphatase levels (Jensen *et al.*, 2004). Serum levels of these enzymes are particularly high in acute hepatocellular damage caused by drug toxicity and xenobiotics (Norman, 1998). The extent of the enzyme changes is related to the nature, closeness to toxic agent and duration of toxicity (Shi *et al.*, 2003; Song *et al.*, 2003; Bruckner *et al.*, 1984).

In an inflammatory condition, there is a leakage of cytoplasmic enzymes into circulation, hence ALT levels increased above that of AST. Thus, when there is gross cellular necrosis, as in carbon tetrachloride or paracetamol poisoning, the level of AST may rise higher than that of ALT (Recknagel, 1987). This is because ALT levels is increased in the serum solely due to conditions where cells of the liver have been inflamed or undergo cell death, and is specific for the liver cells (Jensen *et al.*, 2004) but the AST levels can be triggered in other conditions such as myocardial infarction apart from hepatocellular damage (Jensen *et al.*, 2004).

In the present study, there was a conformity in the levels of the enzymes with the available literature. The higher levels of AST observed may be attributed to the fact that carbon tetrachloride may have caused some injury to other organs, in addition to that caused to the liver. This now potentiate the release of AST into circulation over the ALT, hence the rise in the levels of AST over that of ALT. However, the increase in aminotrasferase are

generally produced by cellular necrosis (Bruckner *et al.*, 1986).

Carbon tetrachloride administration is observed to be associated with increased serum enzyme activities (Iga *et al.*, 1977; Wettstein *et al.*, 1990). Hence the elevated levels of the enzymes namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), generally, was attributed to injury caused to the hepatocytes by carbon tetrachloride which now affects the normal functions of the liver, since these enzymes are indisputably, markers of liver injury as stated earlier, they are localized in the cytoplasm under normal conditions and are released into the circulation under abnormal conditions (e.g. cellular damage) (Mourelle *et al.*, 1987, Romero *et al.*, 1999).

The increased levels of ALT and AST in rats treated with toxicant only was a clear indication of a kind of injury or the other, caused by carbon tetrachloride toxicity (Hsiao *et al*, 2003; Piyachaturawat *et al*, 1995). It is well documented from histological studies on the liver that necrosis in the centrilobular zone is a major cause of carbon tetrachloride induced acute liver injury (Shi *et al*, 2003). This hepatic necrosis in the rat involves a loss of mitochondrial functions (Brabec *et al.*, 1978). There is a possibility that apoptosis has a role in the development of carbon tetrachloride induced liver injury (Olatunde, 2000). Cell death is thought to take place by at least two distinct processes, apoptosis and necrosis (Prifchard and Butler, 1989). However both necrosis and apoptosis can also be caused by drugs and toxins (Shi *et al.*, 2003).

Some medicinal plants possess hepatoprotective effects. These effects are present because they contain some bioactive compounds (Shimkim and Anderson, 1963). The presence of saponins in a variety of herbal preparations administered to humans proved to be potent against cancer and hepatic cell proliferation (Lipkin, 1995). In a study by Hsiao (2003), PML (2,2,5,7,8 – pentamethyl-6-hydroxychromane), a derivative of alpha-tocopherol, dose-dependently (1-10mg/kg), ameliorated the increase in plasma AST and ALT levels caused by chronic repeated carbon tetrachloride induced hepatotoxicity *in vivo* in mice. In this present study, the GLE ameliorated the increase in serum AST and ALT levels caused by acute CCl_4 induced hepatotoxicity *in vivo*, through the active principle that actually initiated this effect is not known.

Reduced levels of ALT and AST in rats treated with GLE, from the result, could also be attributed to the ability of the GLE to prevent the metabolism of carbon tetrachloride into more toxic metabolite and minimized the production of free radicals and also boost the activities of the scavengers of free radicals (Chung *et al.*, 1999, Searle and Wilson, 1980), thus minimizing hepatocellular injury produced.

Absence of any concomitant increase of decrease on the ALP levels, under experimental conditions, was attributed to the fact that the single dose, intraperitioneal injection of the carbon tetrachloride at the pre-stated concentration/ dosage, may not have caused any significant ($P<0.05$) billiary tract obstruction or disease (Wornman, 1998) while causing acute hepatocellular injury (Recknagel, 1987).

REFERENCES

Ademuwagun ZA (1973). The Problem and prospects of legitimizing and integrating aspects of traditional health care system and method with modern medical therapy. The Igbo – ora experience. Traditional Medical Therapy – A Critical Appraisal, pp.1-23.

Agency for Toxic Substances and Disease Registry (ATSDR) (2003).

Akhtar MS and Ali MR (1984). Study of Antidiabetic effect of a compound medicinal plant prescription in normal and diabetic rabbits. *J. Pakistan. Med. Assoc.* **34**: 239-244.

Brabec MJ, Bradley Craig, Bernstein IA (1978). Studies on the antagonism by chloramphenicol of carbon tetrachloride induced damage: Examination of mitochlondrial protein synthesis. *Am. J. Toxicol. and Applied Pharmacol.*, **38**: 157-167.

Bruckner JV, Luthra R and Kyle GM (1984). Influence of time of exposure to carbon tetrachloride on toxic Liver injury. *Ann. Rev. Chronopharmacol.* **1**: 373-376.

Bruckner JV, Mackenzie WF and Muralidhara S (1986). Oral toxicity of carbon tetrachloride. *Acute, Fundam. Appl. Toxicol.*, **6**: 16-34.

Chung HS, Chong LC, Lee SK, Shamon LA, Breemen RBV, Mehta RG, Farnsworth NR, Pezzuto JN and Kinghorn AD (1999). Flavonoids constituents of chlorinzan diffused with potential cancer chemopreventive activity. *J. Agric. Food Chem.*, **47**: 35-41.

Hsiao G, Lin YH, Chou DS, Lin WC and Sheu JR (2003). The Protective effects of PMC against chronic carbon tetrachloride – induced hepatotoxicity *in vivo. J. Pharmacol.*, **4**: 9-10.

Iga T, Sugiyama Y, Yokota M, Tomono Y, Ansazu S and Hanano M (1977). Pharmacokinetic aspects of sulfobromophthalein transport in chronically carbon tetrachloride intoxicated rats. *Biochem. Pharmacol.*, **26**: 1867-1873.

Jensen JE, Stainberg SE, Freese P and Marino E (2004). Liver function tests. *J. Digest. Disord.*, **6**: 1-3.

King PR and King EJ (1954). Estimation of alkaline phosphatase. *Journal Clin. Path.*, **7**: 322-323.

Lambo JO (1979). The Healing powers of herbs with special reference to obstetrics and gynaecology. *In*:

Sofowora A. (ed). Conference on African Medicinal Plants. University of Ife Press, Ile-Ife, pp.23-30.

Lillie RD (1965). Nuclei, nucleic acid, general oversight stains. *In*: Histopathology Technique and Practical Histochemistry, 3rd edition, McGraw Hill Book Company, pp.142-179.

Lipkin R (1995). Secondary plant metabolitis. *Sci. News*, **14**: 8-9.

Lius SL, Espoti SO, Yao T, Diehl AM and Zern MA (1995). Vitamin E therapy of acute CCI_4- induced hepatic injury in mice as associated with inhibition of nuclear factor. *Kappa B Binding. J. Hepatol.*, **22**: 1474-1481.

Morebise O, Fatunso MA, Makinde JM, Olajide OA, Awe EO (2002). Anti-inflammatory property of leaves of *Gongronema latifolium. Phytother. Res.* **1**: 75-77.

Mourelle M, Ameziua JL and Hong E (1987). Effects of riprostil and colchicines on carbon tetrachloride acute liver damage in rats. Relationship with Plasma Membrane Lipids. *Prostaglandins*, **33**: 869-877.

Murray PR, Drew WL, Kobayashi GG and Thompson JH (1990). Medical Microbiology. Wolf Pub. Ltd., USA., pp.696-697.

Murray RK, Granner DK, Mayes PA and Rodwell VM (2000). Harper's Biochemistry, 25th Ed. McGraw Hill, USA, pp.868-869.

Norman JJ (1998). Common Laboratory Test in Liver Diseases. Columbia-Edu. Columbia.

Okafor JC (1989). Tropical plants in health care delivery (Guest Lecture Delivered on Pharmacology Society of Nigeria at the University of Nigeria Nsuka).

Olatunde F (2000). Mechanisms for the hepatoprotective action of Kolaviron. Studies on hepatic enzymes, microsomal lipids,and lipid peroxidation in carbon tetrachloride treated rats. *Pharmacol. Res*., **42**: 75-80.

Piyachaturawat P, Kingkaeohoi S and Toskulkao C (1995). Potentiation of carbon tetrachloride hepatotoxicity by piperine. *Drug Chem. Toxicol*., **18**: 333-344.

Price JE (1980). Carbon tetrachloride, Carborundum. *New Age Ecyclopedia*, **4**: 93-94.

Pritchard DJ and Butler WH (1989). Apoptosis; The mechanism of cell death in dimethyl-nitrosamine-induced hepatotoxicity. *J. Pathol*., **156**: 253-260.

Recknagel RO (1987). Carbon tetrachloride hepato-toxicity. *Pharmacol. Rev.*, **19**: 145-195.

Reitman S and Frankel S (1957). A Colourimetric Method for the determination of Serum glutamic-oxaloacetic and glutamic-pyruvic transaminase. *Am. J. Clin. Pathol.*, **28**: 56-61.

Reynolds ES, Treinen RJ, Farrish HH and Moslen MT (1984). Relationships between the pharmacokinetics of carbon tetrachloride conversion to carbon dioxide and chloroform and liver injury. *Arch. Toxicol.*, **7**(Suppl.): 303-306.

Romero S, Lasher SB, Suberviola LS and Genarruzabeitia E (1994). Protective effects of calcium channel blockers in carbon tetrachloride- induced liver toxicity. *Life Sci.*, **55**: 981-990.

Schmidt E and Schmidt FW (1963). Enzyme. *Biol. Clin.*, **3**: 1.

Searle AJ and Wilson RL (1980). Glutathione peroxide: Effect of superoxide, hydroxy and bromine free radicals on enzymes activity. *Int. J. Radiant Biol.*, **37**: 213-217.

Shi J, Asiaki K, Ikawa Y and Wake K (2003). Evidence of hepatocyte apoptosis in rat liver after the administration of carbontetrachloride. *J. Med. Res.* **4**: 1-8.

Shinkim MB and Anderson MN (1963). Acute toxicities of rotenone and mixed pyrethrins in mammals. *Proc. Soc. Exp. Biol. Med.*, **34**: 135-138.

Sofowora EA (1982). *Traditional Medicine Methods and Techniques.* John Wiley and Son Ltd., New York, pp.2626-2253.

Song I, Lee Y, Chung S and Shim C (2003). Multiple alteration of canalicular membrane transport activities in rat with CCl_4 induced hepatic injury. *J. Pharm. Tox.*, **8**: 506-571.

The World Book Encyclopedia (1992). World Book Inc., USA, **3**: 189-190.

Ugochukwu NH, Babady NE, Cobourne M and Gaset SR (2003). The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats *J. Bio. Sci.*, **28**: 1-5.

Wettstern M, Gerol W and Hausinger D (1990). Hypoxia and CCl_4-induced liver injury, but not acidosis, impair metabolism cysteinyl. *Hepatol*., **11**: 866-873.