Studies on the Hepatotoxicity Effects of Ethanolic Extract of *Cymbopogon Citrates* (Lemon Grass) on Wistar Albino Rats

[1]Adegbegi J. Ademuyiwa., [2]Onoagbe O. Iyere., [3]Usunnobu Usunnomena, [4]Anyawu O. Gabriel.

[1]Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Ondo State. Nigeria
[2]Department of Biochemistry, University of Benin, Benin City, Edo State, Nigeria
[3]Department of Biochemistry, Benson Idahosa University, Benin City, Edo State, Nigeria
[4]Department of Biochemistry, Bingham University, Karu, Nasarawa State, Nigeria

Abstract

This investigation was carried out to ascertain the efficacy and safety of ethanolic extract of *Cymbopogon citratus* using normal rats. In this context, the effect of graded doses of herbal therapy on the liver was tested. Medicinal plants have been proven to be effectual as hepatoprotective agents while many more are claimed to be hepatoprotective but lack any such scientific evidence to support such claims. Developing a satisfactory herbal therapy to treat liver diseases requires systematic investigation of safe dosages to prevent hepatotoxicity, stimulation of liver regeneration and choleretic activity. The scope of this work include the administration of dosages (200mg/kg, 500mg/kg, 1000mg/kg, 2000mg/kg, 4000mg/kg and 5000mg/kg) of ethanolic extract of *Cymbopogon citratus* to normal rats with control group. The result obtained showed significant changes ($p<0.05$) in the serum enzymes Alanine transaminase (ALT), Aspartate transaminase (AST), Alanine Phosphatase (ALP), total protein and albumin levels were observed at higher doses (2000mg/kg above) to be significantly ($p<0.05$) difference compared with the control as the lower doses showed no significant ($p<0.05$) differences in comparison with control. Ethanolic extract of *Cymbopogon citratus* did not induce anaemia or any other related disorders at lower doses (200mg/kg and 500mg/kg) administered. Histopathology showed no degenerative and necrotic changes in any of the organs assessed at lower dosages.

Keywords: Cymbopogon citrates, liver, hepatotoxicity, total protein, ethanolic extracts and Histopathology

Introduction

Medicinal plants have been acknowledged and extremely valued, and are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and minimal side effects (Brower, 1998).

The World Health Organization (WHO) defined traditional medicine (including herbal drugs) as therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Therefore, these plant drugs deserve detailed studies in the light of modern science. More than 500 vital functions have been identified with the liver and a person's nutritional level is not only determined by what he eats, but also by what the liver processes. Unfortunately, it is extremely difficult to detect early warning symptoms specific to liver metabolic imbalances and a person may suffer for a long time from a liver disorder without knowing it (Handa, 1991).

Hepatotoxicity and its mechanism

Liver plays a central role in transforming and clearing chemicals and is consequently susceptible to the toxicity induced from these agents. Chemicals that cause liver injury are termed hepatotoxins, and more than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which may be manifest by abnormal liver enzyme tests (Blazka *et al*., 1995). Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity (Trease and Evans, 1983).

In most instances, hepatic injury is initiated by the bioactivation of drugs to chemically reactive metabolites, which have the ability to interact with cellular macromolecules such as proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage, and oxidative stress (Blazka *et al.*, 1995). Activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also leads to oxidative stress (Figure 1). Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside liver. This promotes further liver damage. This impairment of cellular function can culminate in cell death and

possible liver failure (Lynch and Price, 2007). Hepatic cellular dysfunction and death also have the ability to initiate immunological reactions, including both innate and adaptive immune responses. Stress and damage to hepatocytes result in the release of signals that stimulate activation of other cells, particularly those of the innate immune system, including Kupffer cells (KC), natural killer (NK) cells, and NKT cells. These cells contribute to the progression of liver injury by producing proinflammatory mediators and secreting chemokines to further recruit inflammatory cells to the liver. It has been demonstrated that various inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-1β, produced during hepatic injury are involved in promoting tissue damage (Bourdi *et al.*, 2002). (Table 2) However, innate immune cells are also the main source of IL-10, IL-6, and certain prostaglandins, all of which have been shown to play a hepatoprotective role (Rubinstein *et al.*, 1986) (Figure 2). Thus, it is the delicate balance of inflammatory and hepatoprotective mediators produced after activation of the innate immune system that determines an individual's susceptibility and adaptation to hepatic injury.

In vitro studies

Fresh hepatocyte preparations may be exploited to study the anti- or hepatotoxic activity of drugs. Hepatocytes are treated and the effect of the plant drug is evaluated through the activities of transaminases released into the medium. An augmented activity of marker transaminases in the medium indicates liver damage (Kew, 2000).

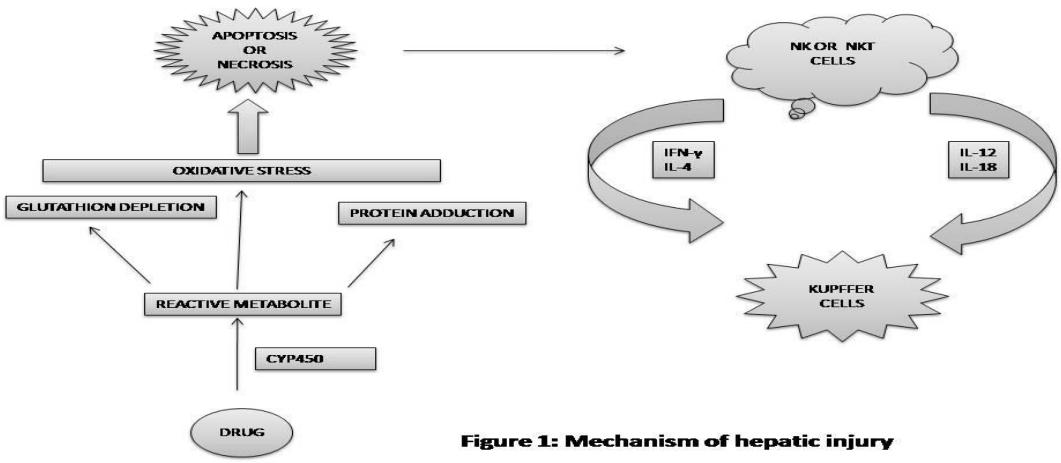


Figure 1: Mechanism of hepatic injury

Figure 2: Role of Hepatotoxic mediators and Hepatoprotective mediators

The administration of herbal preparations without any standard dosage, coupled with a scarcity of adequate scientific studies on their safety, has raised concerns regarding their toxicity. To determine the safety of drugs and plant products for human use, toxicological evaluations are carried out on various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dosage in humans. Hence the need to study the effect of *Cymbopogon citratus* for proper guidance on consumption and further therapeutic uses.

Cymbopogon citratus

Cymbopogon citratus commonly called lemon grass is an aromatic, perennial grass belonging to the family grimneae. It is a tropical plant, grown as an ornamental in many temperate areas with maximum a height of about 1.8m and its leaves 1.9cm wide covered with a whitish bloom. Like other members of the genus, *citratus* yields citral, a volatile oil with strong lemon fragrance. It is used in manufacture of perfumes, coloured soaps and synthesis of vitamin A. Folk medicine in certain parts of Nigeria use the essential oil as an insect repellant. In certain medications, it is used for mental illness (Ebomoyi, 1986). It is an antifungal, antitoxicant and deodorizing agent. In combination with other herbs, it has large use as cure for Malaria (Gbile, 1986). *Cymbopogon citratus* is a great interest due to its commercially valuable essential oils and widely used in food technology as well as in traditional medicine. Owing to the new attraction for natural products, it is important to develop a better understanding of their mode of biological action for new applications in human health, agriculture and the environment (Ferguson *et al*., 2003).

The purpose of this study, therefore, is to investigate the effects of graded dosages of ethanolic extract of *Cymbopogon citrates* on the liver of normal rats.

Materials and methods

Cymbopogan citratus was harvested and collected freshly from a native farms and authenticated in Environmental Biology Laboratory, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo.

Preparation of plant extract:

The fresh plant was washed, chopped into pieces and air-dried at room temperature. The dried plant part was milled into powder and weighed. The Plant powder was soaked in 90% absolute ethanol for 72 hours with intermittent shaking. Then, it was filtered through a muslin clothe and later Whatman No. 1 filter paper. The resulting filtrate was evaporated under reduced pressure using a rotary evaporator and there after freeze dried to get powder form ethanolic extract. The yield was stored in a refrigerator (4°C) till when needed (Onoagbe *et al.*, 1999).

Chemicals and Reagents

All chemicals were of an analytical grade and are supplied from sigma chemical co. USA. Distilled water was used in all biochemical assays.

Experimental animal

Male albino rats (Wistar strain) weighing between 109-170g, purchased from the central animal house of University of Ibadan were used for the study.

Acclimatization: 15 days prior to dosing.
Identification of animals: By cage number.
Diet: Pelleted feed
Water: Potable drinking water
Housing & Environment: 4 animals each in a group

Determination of the weight of animals

The weights of the animals were weighed using an electronic weighing balance every 7 days to verify and quantitate the change in weight over the period of administration.

Animal ethics

All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (USA). The ethic regulations have been followed in accordance with national and institutional guidelines for the protection of animals' welfare during experiments.

Experimental design

Group I: Normal control (distilled water)

Group II: 200mg/kg *Cymbopogon citratus*

Group III: 500mg/kg *Cymbopogon citratus*

Group IV: 1000mg/kg *Cymbopogon citratus*

Group V: 2000mg/kg *Cymbopogon citratus*

Group VI: 4000mg/kg *Cymbopogon citratus*

Group VII: 5000mg/kg *Cymbopogon citratus*

Method of administration

Oral administration of the extracts through the use of oral gavage.

Duration of treatment: 30 days

Chemicals and reagents preparation

All chemicals were if an analytical grade and are supplied from sigma chemical co. USA. Distilled water was used in all biochemical assays.

Blood Biochemistry

Blood samples were collected in glass tube from retro-orbital puncture to obtain haemolysis free clear serum for the analysis of ALT (Reitman and Frankel, 1957), AST (Reitman and Frankel, 1957), ALP, total protein (Lowry *et al.*, 1951) and albumin (Bacon, 1947)

Haematology

The method used as the impedance method for determining the WBC, RBC, and platelets data. The analysis cycle, the sample is aspirated, diluted and mixed before the determination for each parameter is performed.

Histopathology

Small pieces of tissues were collected in 10% formaldehyde solution for histopathological study. The pieces of the liver was soaked in formalin for 6 hrs, embedded in paraffin wax and the sections were made about 4-6µm in thickness. They were stained with hematoxylin and eosin and photographed (Luna, 1999).

Statistical analysis

The experimental results were expressed as the mean ± S.E.M. Statistical significance of difference in parameters amongst groups was determined by One way ANOVA followed by Duncan's multiple range test. $P<0.05$ was considered to be significant.

Results and discussion

Table 1: Effects of oral administration of ethanolic extract of *Cymbopogon citratus* on serum liver functions enzymes in normal wistar albino rats.

Group (mg/kg)	ALT (U/l)	AST (U/l)	ALP (U/l)	TotalProtein (g/dl)	Albumin (g/dl)
Control	45.00 ± 1.00^{b}	31.00 ± 2.00^{b}	277.53 ± 21.86^{b}	11.24 ± 2.14^{b}	3.44 ± 0.12^{a}
200	38.50 ± 2.50^{a}	33.50 ± 6.50^{b}	269.20 ± 6.90^{b}	12.25 ± 0.17^{b}	5.88 ± 0.21^{b}
500	49.00 ± 7.21^{b}	29.50 ± 4.35^{b}	254.35 ± 14.64^{b}	12.93 ± 0.28^{b}	3.63 ± 0.60^{a}
1000	43.00 ± 6.03^{a}	17.50 ± 3.51^{a}	341.70 ± 21.14^{c}	9.00 ± 0.18^{b}	6.72 ± 0.19^{b}
2000	47.50 ± 1.53^{b}	16.50 ± 1.53^{a}	259.5 ± 6.58^{a}	12.89 ± 0.18^{b}	6.28 ± 0.26^{b}
4000	54.00 ± 5.66^{c}	50.50 ± 5.66^{c}	380.75 ± 17.26^{c}	10.32 ± 1.52^{b}	5.54 ± 0.68^{b}

5000	69.00 ± 1.41[c]	41.33 ± 3.06[c]	431.28 ± 4.99[c]	13.35 ± 0.20[b]	6.24 ± 0.24[b]

Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different ($p < 0.05$)

From table I above, the serum ALT (Alanine aminotransferase) activity of the test group in normal rats was shown to decrease steadily after administration of the plant extract. The decrease was significant (*$p<0.05$) at 200mg/kg, 1000mg/kg and 4000mg/kg, as no significant *$p<0.05$) difference was observed in the groups treated with 500mg/kg and 2000mg/kg when compared to the control at day 30. The serum AST (Aspartate aminotransferase) activity of the test group was shown to decrease steadily after administration of the plant ethanolic extract. The decrease was significant ($p<0.05$) at all dosages except 200mg/kg where there is no significant different as compared with the control. The serum ALP (Alkaline phosphatase) activity of the test group was shown to increase steadily after administration of the ethanolic extract of the plant at all dosages when compared with the control. Ethanolic extract *Cymbopogon citratus* causes no changes in the serum total protein titer when compared with the controls.And the albumin levels was not significant at 500mg/kg when compare with control. This significant ($p<0.05$) increase in the albumin as compared with the control appears to be dose dependent.

TABLE II: Effects of oral administration of Ethanolic extracts of *Cymbopogon citratus* on Haematological parameters in normal rats.

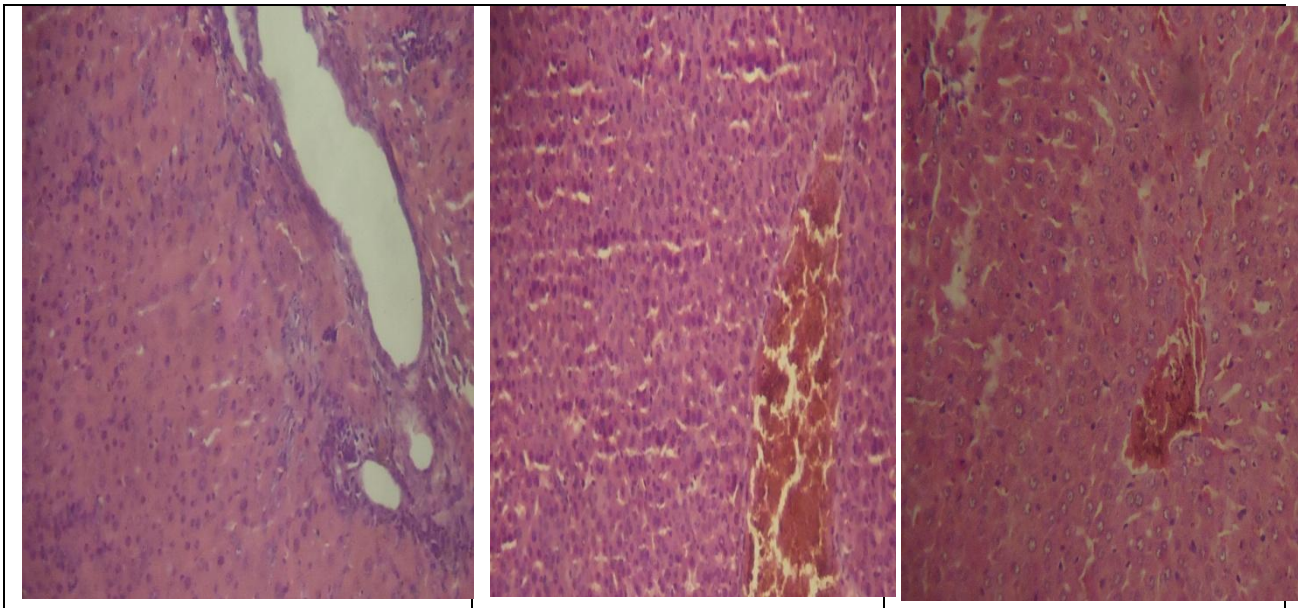
Group (mg/kg)	**PCV (%)**	**PLATELETS (cmm)/1 X 10^4**	**TWBC (10^{12}/mm^3) X 10^3**	**RBC (10^{12}/ml)**
Control	49.67 ± 3.86[b]	68.00 ± 0.12[b]	4.50 ± 0.54[a]	8.79 ± 0.70[b]
200	51.00± 2.65[b]	72.93 ± 0.14[c]	4.80 ± 1.11[a]	8.93 ± 0.11[b]
500	52.67 ± 1.53[ab]	62.00 ± 0.42[b]	6.70 ± 3.18[cd]	7.68 ± 0.54[a]
1000	47.30 ± 1.03[a]	53.20 ± 0.40[b]	6.30 ± 1.89[c]	7.11 ± 0.38[a]
2000	63.33 ± 0.58[c]	46.17 ± 0.15[a]	6.03 ± 2.76[bc]	10.17 ± 0.17[d]

4000	46.00 ± 4.00[a]	93.80 ± 0.15[d]	5.07± 1.08[ab]	6.26 ± 0.23[a]
5000	50.50 ± 0.71[b]	96.10 ± 0.50[d]	5.60 ± 2.26[b]	7.95 ± 0.11[a]

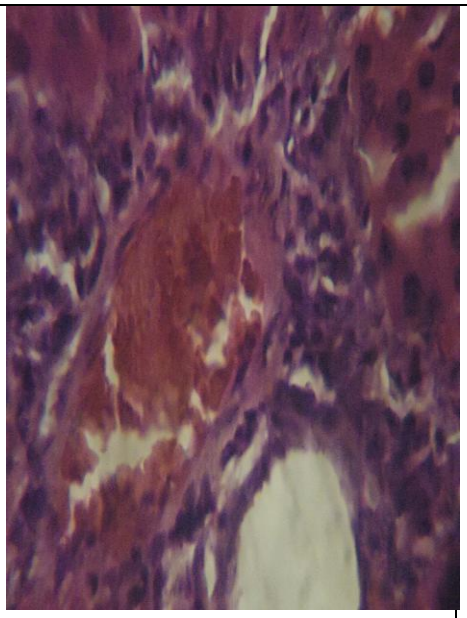
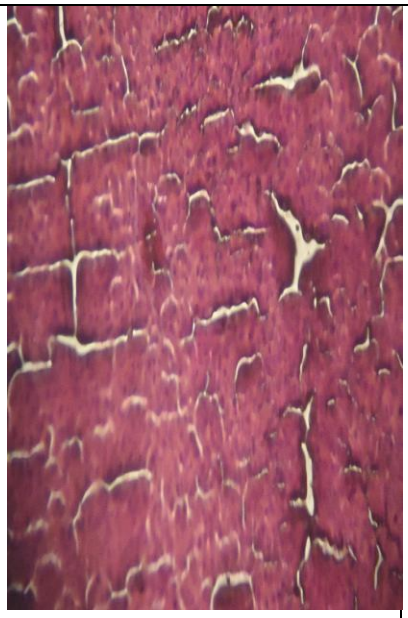
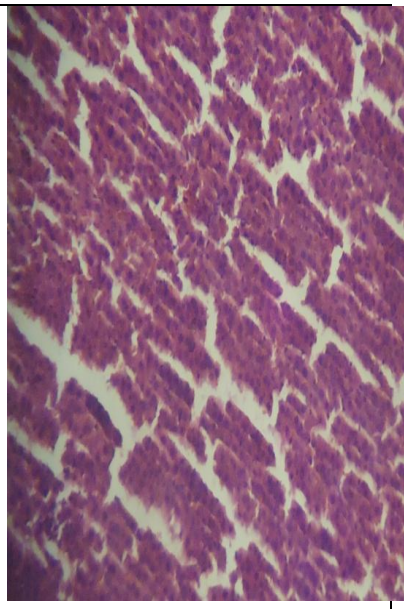
Values are expressed as means ± SEM of four independent experiments. Means in the same column not sharing the same letter(s) are significantly different ($p < 0.05$)

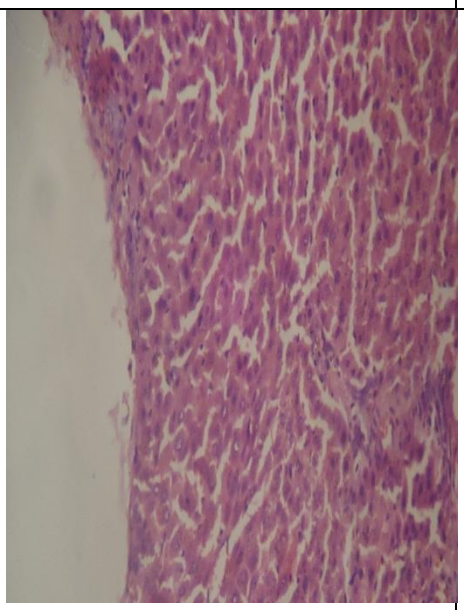
In table II, Haematological investigations revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; However, the increase or decrease ($p<0.05$) in the values obtained were significant was still within normal biological and laboratory limits or the effect was not dose dependent. In this haematological evaluation, marked decrease in RBC and PCV were observed in the ethanolic extract treated group. The decrease in RBC was an indication of changes in the rate of the RBCs production. In this context, the possibility that the extract does have the potential to stimulate erythropoietin release in the kidney was likely. A decrease was also noted in the platelets levels in extract in the group treated with 1000mg/kg and 2000mg/kg respectively.

PLATE 1: Showing the Histopathology of the liver of various groups



Group I: Control	**Group II: 200mg/kg.**	**Group III: 500mg/kg**

		
Group IV: 1000mg/kg	**Group V: 2000mg/kg**	**Group VI: 4000mg/kg**


Group VII: 5000mg/kg

From the plates above, group I represent the Control group of animal treated only with distilled water and the result showed no visible lesion. Meanwhile, similar result was obtained in groups III, V, IV and IIV (500mg/kg, 2000mg/kg, 4000mg/kg and 5000mg/kg) except the group (IV) treated with 1000mg/kg treated group where there was severe

periportal fibroplasia and cellular infiltration. Also a moderate congestion of the central veins was observed in the 200mg/kg extract treated group (II).

Discussion

The administration of herbal preparations without any standard dosage, coupled with a scarcity of adequate scientific studies on their safety, has raised concerns regarding their toxicity (Saad *et al.*, 2006). To determine the safety of drugs and plant products for human use, toxicological evaluations are carried out on various experimental animals to predict toxicity and to provide guidelines for selecting a 'safe' dosage in humans.

The liver is a vital organ present in vertebrates and some other animals. The liver can be injured by many chemicals and drugs (Leo and Arai, 1982). It is reported that more than 900 drugs have been implicated in causing liver injury (Friedman *et al.,* 2003) and it is the most common reason for a drug to be withdrawn from the market. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Friedman, 2006). Statins are now among the most frequently prescribed medications and are currently used by about million people worldwide. During hepatic damage, cellular enzyme like ALT, AST, ALP and serum bilirubin present in the liver cell, leak into the serum resulting to increase in concentration (Deb, 1998).

Normally, both AST and ALT assay indicates suspected liver damage that might be caused by the drug toxicity or any harmful chemicals compounds (Nelson *et al.*, 2005, Emeka *et al.*, 2011). Presently, normal or even slight decrease in both AST and ALT levels in rats were observed after plant extract treatment when compared with controls. In a study by Rosa *et al.,* (2009), result indicated some hepatic-protective properties of an orchid plant. It was demonstrated that ALT and AST pretreatment were significantly reduced in rats treated with different types of methanol extract of this plant and caused a significant reduction in parcetamol treated rats in a dose-related manner. These findings are similar to our finding which enforces the idea of the safe treatment usage of our extracts leading to confirm that treatment with *C. citratus* ethanolic extract at 200mg/kg and aqueous extract at 2000mg/kg did not altar the liver function by not elevating both ALT and AST levels, suggesting no plant hepatic-toxicity (Paris, 2004). Enzymes directly associated with the conversion of amino acids to ketoacids are ALT and AST (Parmar *et al.,* 1982). ALT and AST activities are used as the indicators of hepatocytes damage. In earlier

stage of livers damage, these cytoplasmic enzymes of hepatocytes penetrate the cells and enter the blood stream (Parmar *et al.*, 1982; Ramesh *et al.*, 2006). These enzymes have increased activities in diabetic rats which are due to hepatic damage.

The serum ALP (Alkaline phosphatase) activity of the test group was shown to increase steadily after administration of the ethanolic extract of the plant at all dosages when compared with the control. Carlson (1996) shows that at 500 mg/kg dose above, any plant extract can cause enzyme induction and this increased ALP may be due to hepatic insufficiency, cholestasis or obstruction of the bile ducts.

Ethanolic extract *Cymbopogon citratus* causes no changes in the serum total protein titer when compared with the control. The fact that proteins present several features as potentially interesting biomarkers of toxicity they might serve as peripheral indicators of toxic events in relatively inaccessible target organs **(**Bernard and Lauwerys, 1995). Protein titers stability after *C. citratus* extract administration demonstrate the fact that this plant did not exhibited any protein degradation leading to propose a non toxic effect at the levels primary organ, in this case the liver.

However, the increase or decrease ($p<0.05$) in the values obtained were significant was still within normal biological and laboratory limits or the effect was not dose dependent. In this haematological evaluation, marked decrease in RBC and PCV were observed in the ethanolic extract treated group and another decrease in RBC and WBC in the aqueous extract treated group as compared to the control. The decrease in RBC was an indication of changes in the rate of the RBCs production. In this context, the possibility that the extract does have the potential to stimulate erythropoietin release in the kidney was likely. A decrease was also noted in the platelets levels in both extracts in the group treated with 1000mg/kg and 2000mg/kg respectively. This is similar to results obtained with some other plants (Polenakovic and Sikole, 1996; Sanchez-Elsner *et al*., 2004). From this result, the extract did not significantly alter the calculated RBC indices which were indicative of its minimal effect on the size of RBC and in Hb weight per RBC. This implies that ethanolic extract of *Cymbopogon citratus* does not possess the potential to induce anaemia. Inflammatory process is characterized by the involvement of multiple inflammatory cells of the WBC (Kytridis and Manetas, 2006). WBC and indices relating to it such as lymphocytes usually show increase in activity in response to

toxic environment (Robins, 1974). In this study, WBC was not significantly altered while lymphocytes, the main effectors cells of the immune system (McKnight *et al.*, 1999) showed marginal increase thus suggesting that the extract only exerted minimal challenge on the immune system of the animals.

The histological examination is the golden standard for evaluating treatment related pathological changes in tissues and organs (OECD, 1995). The liver is the main target organ of acute toxicity where exposed to the foreign substances being absorbed in intestines and metabolized to other compounds which may or may not be hepatotoxic to the rats (Rhiouania *et al.*, 2008). In this study, the liver histology revealed evidence determines normal hepatocytes and did not cause any alteration to the structure of the liver cells between the controls and treated (plate 1). In contrast, the histological examination study conducted by Harizal *et al*. (2010) using *Mitragyna speciosa* extract revealed less severe morphological changes in liver of mice treated with extract at dose level 100 and 500 mg/kg.

Conclusion

In conclusion, according to the present study, ethanolic of *Cymbopogon citratus* (whole plant) did not induce any toxicity on the liver thereby making it to have a hepatoprotective properties. And further work is prescribed for other organ related toxicity studies on this plant.

Reference

Bacon F. Chow (1947). The determination of plasma or serum albumin by means of a precipitin reaction. *J. Biol. Chem*. 167:757-763.

Bernard A., Lauwerys R. (1995). Low-molecular-weight proteins as markers of organ toxicity with special reference to Clara cell protein. *Toxicology letter.* 77(1-3) 145-151.

Blazka M.E., Wilmer J.L., Holladay S.D., Wilson R.E., Luster M.I. (1995). Role of Pro-inflammatory cytokines in acetaminophen hepatotoxicity. *Toxicol Appl Pharmacol.* 133:43-52.

Bourdi M., Masubuchi Y., Reilly T.P. (2002). Protection against acetaminophen-induced liver injury and lethality by interleukin 10, role of inducible nitric oxide synthase. *Hepatology*. 35:289-298.

Brower V. (1998). Nutraceuticals, Nutritional Therapy, Phytonutrients, and Phytotherapy for Improvement of Human Health. *Nat Biotechnol.* 16:728–731.

Carlson G.P. (1996). Clinical chemistry tests in *Internal animal medicine,* edited by B.P. Smith. Boston: Mosby Publishers.

Deb A.C. (1998). Fundamental of Biochemistry. 7thEd. New Central Book Agency: Kolkata.

Ebomoyi O. (2008). Traditional Medicine in Mental health care. *The Journal of American Science;* 4(4):21-25.

Emeka E.J.I. and Funmilayo D.O. (2011). Hypoglycimic effects, biochemical and histological changes of *Spondias mombin linn* and *Perinari ployandra benth* seeds ethanolic extracts in alloxan induced rats. *Journal of pharm. Toxicol.* 6 (2) 1001-102.

Ferguson E.L., Gibson R.S., Opare-Obisan C., Osei-Opare F, Stephen A.M., Lehrfield J., Thompson L.U. (2003). The Zinc calcium copper, manganese, Non-starch, Polysaccharide and phylate content of sevety eight locally grown and prepared African. *Foods Fd Comp. Analy.* 6:87-89.

Friedman P.F. (2006). GI/Liver Secrets: with student consult Access. Saint Louis: C.V. Mosby.

Friedman S.E., Grendell J.H. McQuaid K.R. (2003). Current diagnosis and treatment in gastroenterology. New York: Lang Medical Books/McGraw-Hill. pp. 664–679.

Gbile (1986). Ethnobotany, Taxonomy and Conservation of Medicinal Plants. Ibadan University press, Ibadan p. 126 – 130

Handa S.S. (1991). Plants as drugs. *The Eastern Pharmacist.* 34:79-85.

Harizal S.N., Mansor S.M., Hasnan J., Tharakan J.K.J., Abdullah J. (2010). Acute toxicity study of the standardized methanolic extract of *Mitragyna speciosa Korth* in Rodent. *J. Ethnopharmacol*. *131*, 404-409.

Kew M.C. (2000). Serum aminotransferase concentration as evidence of hepato-cellular damage. *Lancet.* 355:591–592.

Kytridis V.P and Manetas Y. (2006). Maesophyll versus epidermal anthocyanins as potential *in vivo* antioxidants: evidence linking the putative antioxidant role to the proximity of oxy-radical source. *J Exp Bot.* 57: 2203-2210.

Leo M.A., Arai M. (1982). Hepatotoxicity of vitamin A and CCl_4. *Gastroenterology,* 82: 194-205.

Lowry O.H., Rosebrough N.J., Farr A.L. (1951). Protein measurement with the folin-phenol reagent. *J Biol Chem*. 193:265-275.

Luna L.G. (1999). Manual in histology and staining method. McGraw Hill: New York, p. 96.

Lynch T., Price A. (2007). The effect of cytochrome P-450 metabolism on drug response, interactions and adverse effects. *Amer Fam Physi*. 76:391-396.

Mc Knight D.C., Mills R.G., Bray J.J. and Crag P.A. (1999). Human Physiology. 4th Edition, Churchill Livingstone, 290-294.

Nelson D.L., Cox M.M. (2005). Principles of Biochemistry. 4th Edn. W.H. Freeman and Company, New York.

OECD (1995). *OECD Guideline for Testing of Chemicals. Repeated Dose 28-Day Oral Toxicity Study in Rodents*; Organisation for Economic Co-operation and Development: Paris, France.

Onoagbe I.O., Esekheigbe A. (1999). Studies on the anti-diabetic properties of *Uvaria Chamae* in streptozotocin-induced diabetic rabbits. *Biokemistri.;* 9: 79-84.

Ostapowicz G., Fontana R.J., Schiødt F.V. (2002). "Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States". *Ann. Intern. Med.* 137 (12): 947–54.

Paris L., Satheesh M.A. (2004). Antidiabetic activity of *Boerhavia diffusa* on hepatic key enzymes in experimental diabetes. *J. Ethnopharmacol*. 91: 109-113.

Polenakovic M. and Sikole J. (1996). Is erythropoietin a survival factor for red blood cells? *J Am Soc Nephrol.* 7: 1178-1182.

Ramesh B., Pugalendi K.V. (2006). Impact of Umbelliferone (7- hydroxycoumarin) on cancer patients? *Prostate Cancer Prostatic Dis*. 12: 78–82.

Reitman S. and Frankel S. (1957). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28:56-63.

Rhiouania H., El-Hilalya J., Israili Z.H., Lyoussia B. (2008). Acute and subchronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. *J. Ethnopharmacol. 118*, 378-386.

Robins S.L. (1974). Lymph nodes and spleen: Pathologic basis of disease. WB Saunders Co., Philadelphia, 1050.

Rosa M.P. Gutierrez R.V. (2009). Solis Hepatoprotective and Inhibition of Oxidative Stress in Liver of Prostechea michuacana. *Rec. Nat. Prod:* 3 (1) 46-51.

Rubinstein D., Roska A.K. and Lipsky P.E. (1986). Liver sinusoidal lining cells express class II major histocompatibility antigens but are poor stimulators of fresh allogeneic T-lymphocytes. *J Immunol.* 137:1803-1810.

Saad B., Azaizeh H., Abu-Hijleh G. and Said O. (2009). Safety of traditional Arab herbal medicine. *Pharmacol*. *3*, 354-358.

Sanchez-Elsner T., Ramirez J.R., Rodriguez-Sanz F., Varela E., Bernabew C. and Botella L.M. (2004). A cross talk between hypoxia and TGF-beta orchestrates erythropoietin gene regulation through SPI and SMADS. *J Mol Biol.* 36: 9-24.

Trease G.E. and Evans W.C. (1983). Pharmacognosy. London, Balliere Tindall Press; 1983:56-57.