TOXICITY STUDIES ON *ALCHORNEA CORDIFOLIA* LEAF EXTRACT IN MICE

C. Ansah, E. Oppong, E. Woode and M. Duwiejua
Department of Pharmacology,
Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

ABSTRACT
Alchornea cordifolia (Schum. and Thonn.) Müll. Arg (Euphorbiaceae), a widely used traditional medicinal agent was assessed for possible toxicity in mice. We investigated the effect of the ethanolic extract of Alchornea on blood cells and chemistry, histology, and relative weight of selected organs in mice. Administration of Alchornea cordifolia (250-2000 mg/kg, p.o daily) for two weeks did not affect significantly the relative organ weights, blood cells or renal function. Histology of liver and kidney at dose levels up to 1000 mg/kg was normal and similar to vehicle- treated controls. However, liver sections of mice treated with 2000 mg/kg Alchornea extract showed perivascular aggregates of lymphocytes, eosinophilia and pyknosis, evidence of hepatic damage. These results suggest that Alchornea cordifolia is relatively non-toxic but has the propensity to induce hepatic injury at high doses.

Keywords: *Alchornea toxicity, mice, blood cells, blood chemistry.*

INTRODUCTION

Alchornea cordifolia (Schum. and Thonn.) Müll. Arg. (Euphorbiaceace), known as christmas bush is found abundantly along the coast in the West Africa sub-region. Known traditionally as "Gyamma", *Alchornea* is used for a variety of diseases by traditional medical practitioners in Ghana. Powdered leaves of *Alchornea cordifolia* is used to treat diarrhoea, wounds, sores, cuts (Abbiw, 1990) and applied topically as an anti-inflammatory agent (Neuwinger, 2000). *Alchornea* is also reported to possess a multiplicity of biological effects. It is an antibacterial (Ajali, 2000; Igbeneghu *et al.*, 2007), spasmolytic (Ogungbamila and Samuelsson, 1990), anti-inflammatory (Manga *et al.*, 2004; Osadebe and Okoye, 2003), hepatoprotective (Olaleye *et al*., 2006), antidiarrhoeal (Agbor *et al.*, 2004), anti-oxidant (Olalye and Rocha, 2008) and antimicrobial (Ebi, 2001). These diverse pharmacological actions have been linked to several active principles isolated from the leaves and root bark of *Alchornea cordifolia*. For example, Quetin-Leclercq *et al.*, (2008) demonstrated the presence of anti-

inflammatory compounds in *Alchornea.* In addition, tannins, phenolic acids: gallic acid, ellagic acid, protocatechic acid (Banzouzi *et al.,* 2002; Lamikanra *et al.*, 1990; Ogungbamila and Samuelsson, 1990), flavonoids: quercetin, hyperin and guaijaverin (Ajali, 2000; Lamikanra *et al.,* 1990; Ogungbamila and Samuelsson, 1990) and an alkaloid: triisopentenylguanidine (Lamikanra *et al*., 1990) have been isolated from the leaves of *Alchornea cordifolia.*

In spite of the wide traditional use of *Alchornea crorditolia*, very little is known about its potential toxicity. We demonstrated recently in our laboratory that administration of ethanolic leaf extracts of *Alchornea* to rats resulted in increased neutrophil count and mild hepatotoxicity (unpublished). It is presently unknown if this effect occurs in other rodent species.

This study aimed at investigating the possible toxicity of *Alchornea* in mice since interspecies variation plays a key role in the toxicity of xenobiotics (Jemnitz *et al.,* 2008; Ratanasavanh *et al.,* 1996; Vassallo *et al.,* 2004).

MATERIALS AND METHODS

Preparation of *Alchornea cordifolia* extract

The fresh leaves of *Alchornea cordifolia* were obtained from the Kwame Nkrumah University of Science and Technology Botanical Gardens and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The leaves were sun-dried and then powdered with a hammer mill. Extraction of the powdered dried leaves was by cold maceration with 70% alcohol. The alcohol was evaporated with a rotary evaporator attached to a thermo chiller (Buchi 700, Recirculation chiller) at a temperature of 20 ^{0}C. The residue was freeze-dried to obtain a brown sample of the crude-extract subsequently referred to as the extract in this study.

Animals

ICR mice (20-30 g) were purchased from Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, and maintained in the Animal House of the Department of Pharmacology, Kwame Nkrumah University of Science of and Technology, Kumasi, Ghana. They were housed in stainless steel cages (34 x 47 x 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema, Ghana) and given water *ad libitum.* The animals were humanely handled throughout the experiment in accordance with internationally accepted principles for laboratory animal use and care (EEC directive of 1986: 86/609 EEC).

Subacute Toxicity

Male mice (20-30 g) were divided into five groups. (n=5). The groups received 250, 500, 1000, 2000 mg/kg of the extract in 2% tragacanth (p. o) daily for two weeks. The control group received tragacanth 2% only throughout the two week period. The animals were monitored closely for signs of toxicity. At the end of the two-week period, the mice were euthanized by cervical dislocation and blood was collected into tubes.

Blood analysis

Haematological analyses were performed on whole blood collected into tubes with EDTA. Haematocrit (HCT), platelets, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration(MCHC), white blood cell (WBC), Red blood cells (RBC), Lymphocytes (LYM), Granulocyte (GRAN), Mid cells (MID), Red cells distribution weight (RDW) and Platelets (PLT) were determined by an automatic analyzer (CELL DYN 1700, Abbott Diagnostics Division, Abbott Laboratories, Abbott Park, IL, USA).

Biochemical Analysis

Biochemical analysis were performed on serum obtained after centrifugation of whole blood (without anticoagulant) at 3000rpm for 5 min. Determination of total proteins, total bilirubin,

indirect bilirubin, albumin, globulin and A/G ratio were performed using an automatic analyzer (Random Access Chemistry System ATAC 8000, élan diagnostic laboratories, Brea, CA, USA). Levels of the liver enzymes alanine aminotransferase (ALT), aspartate transaminase, alkaline phosphatase (AP) and γ-glutamyl aminotransferase (GGT) were determined. Analysis for blood urea nitrogen (BUN), creatinine, sodium, potassium and chloride was also performed.

Organ weight determination

Vital organs including the liver, spleen, kidneys, heart, stomach, testes and lungs were quickly removed and weighed individually. Macroscopic appearances of the organs were observed and the relative weight of each organ was estimated.

Histopathological assessment

Livers and kidneys from treated and control animals were removed after dissection and immediately preserved in 10% formalin until processing. Tissues were dissected, placed into appropriate tissue preparation cassettes and processed with an auto processor (MICROM STP120, Spain) for embedding in paraffin. Sections of 7 μm were cut and stained with hematoxylin and eosin as previously described (Thompson, 1966).

Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Statistical analysis was performed by one-way ANOVA using Graph Pad Prism for Windows version 4.02 (Graph Pad software, San Diego, CA, USA), followed by Neuman Keuls test to evaluate significant differences between the groups. Differences were considered significant for $P < 0.05$.

RESULTS AND DISCUSSION

Historically, medicinal plants have been used for the treatment and prevention of a variety of diseases. Their beneficial effects support their continuing use by majority of people (Matthews *et al.*, 1999). The assumption that herbal medicines are safe as they are natural is disputed by well controlled clinical trials which confirmed the existence of adverse effects (Bagheri *et al.*, 1998; Drew and Myers, 1997).

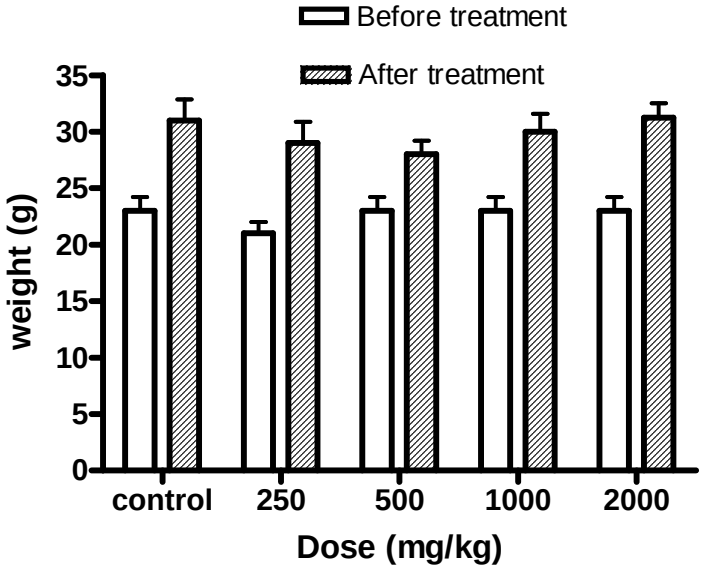

Figure 1: Weight of mice before and after 14-day treatment with *Alchornea cordifolia.* (n=5). Weights were taken before treatment at the indicated doses for a period of 14 days. Final body weights of the animals were then determined

In the present study, treated animals were observed closely over the period for signs of toxicity. We did not observe any abnormal behaviour, motor or neurological disorders. There was no diarrhoea or other signs of gastrointestinal disorders, wheezing or other symptoms of respiratory distress suggesting that our treatment did not affect the gastrointestinal or respiratory systems. There was no change in locomotor activity. No deaths were recorded throughout the study, food and water intake was also normal. Ordinarily, a loss of more than 10% of initial body weight in treated animals is an indication of adverse effects (Raza *et al.*, 2002; Teo *et al.*, 2002). We found no differences between the body-weight of treated and control animals (Figure 1). Our findings suggest that the extract did not provoke autonomic and/or central effects nor affected the general health of mice at the doses used since we found no evidence of signs of toxicity associated with these systems.

Table 1: Effect of *Alchornea cordifolia* on relative organ weights of mice treated for two weeks. Values are expressed as mean ± SEM (n=5) compared to the control by the Neuman Kuels test

Organ	Doses (mg/kg)				
	Control	250	500	1000	2000
HEART	0.47±0.04	0.49±0.06	0.47±0.02	0.40±0.04	0.38±0.03
LIVER	4.46±0.59	5.08±0.67	5.71±0.63	4.67±0.82	4.20±0.48
KIDNEY	1.20±0.01	1.21±0.09	1.23±0.09	1.09±0.13	1.07±0.06
SPLEEN	0.64±0.13	0.74±0.11	0.97±0.26	0.73±0.21	0.74±0.09
STOMACH	1.12±0.07	1.17±0.10	0.92±0.31	1.25±0.12	1.44±0.03
TESTIS	0.61±0.03	0.60±0.03	0.62±0.02	0.58±0.05	0.44±0.10
LUNGS	0.61±0.03	0.79±0.07	0.70±0.03	0.82±0.09	0.70±0.04

Table 2: Effect of *Alchornea cordifolia* on haematological parameters in mice treated for two weeks. Values are expressed as mean ± SEM (n= 5), compared to the control by Neuman Kuels test.

Parameter	Doses (mg/kg)				
	Control	250	500	1000	2000
WBC(K/μL)	5.10±0.63	6.83±0.70	6.80±1.07	8.40±1.08	9.00±2.55
LYM (%)	79.10±2.80	78.35±3.06	79.60±0.87	80.68±1.15	75.83±1.48
MID (%)	15.22±2.22	15.68±1.58	15.53±0.42	15.14±0.65	19.70±1.78
NEU (%)	5.68±1.40	5.98±2.09	4.98±1.03	4.18±0.66	4.47±0.73
RBC (M/μL)	6.81±0.14	5.55±0.51	6.34±0.39	5.97±0.31	6.66±0.14
HGB (g/dL)	11.44±0.30	10.05±0.67	11.28±0.92	10.2±0.69	10.83±0.09
HCT (%)	30.53±0.79	23.60±1.90	26.55±2.45	26.85±0.64	29.53±0.52
MCV(fl)	44.40±0.47	42.45±0.35	42.98±0.91	45.32±1.07	44.40±1.69
MCH(pg)	16.33±0.50	16.30±0.60	15.50±0.50	16.08±0.37	16.27±0.47
MCHC(g/dL)	36.47±0.59	38.25±0.95	36.77±0.29	35.63±0.53	36.70±0.36
RDW (%)	19.04±1.31	18.68±0.94	20.03±1.04	17.94±1.03	19.23±1.02

Organ swelling due to inflammation and hypertrophy could result in increased organ weight. In contrast, atrophy leads to reduction in organ weight. In our study, the relative weights of organs of treated animals did not differ significantly from control animals (Table 1), indicating that *Alchornea cordifolia* at the doses used did not produce damage in the form of organ swelling, atrophy or hypertrophy in the treated animals.

The haematopoietic system is an important index of physiological and pathological status in man and animals (Adeneye *et al.*, 2006) and a sensitive target for toxic compounds (Harper, 1973) since it is initially exposed to a high concentration of toxic agents. Results of the studies indicate that *Alchornea cordifolia* had no effect on the haematological parameters tested (Table 2) and therefore barring any species differences is unlikely to present toxicity to blood and its cellular elements.

Indicators of liver function, AST and ALT (Palmeiro *et al.*, 2003) are used as biomarkers for predicting possible toxicity to the liver (Rahman *et al.*, 2001). Damage to the liver parenchyma cells will induce leakage of these enzymes into serum leading to their elevation several fold (Wolf, 1999). These parameters did not change appreciably in the treated groups as compared to the control (Table 3), indicating that *Alchornea cordifolia* has no effect on these biochemical parameters and is therefore not a potential hepatotoxin.

The lack of elevation of hepatic transaminase levels observed in the present study would suggest that *Alchornea* had no adverse effect on the liver. However, initial and transient damage to the liver may not necessarily reflect in increases in the levels of liver enzymes. We assessed the liver sections of mice treated with *Alchornea* extract. Liver sections of mice were not different from control groups up to a dose of 1000mg/kg. However, in the 2000 mg/kg group, there were perivascular aggregates of lymphocytes (Figure 2) and some of the nuclei showed pyknosis with eosinophilic cytoplasm. Though this observation did not correlate with the biochemical findings, the degeneration observed in the histopathological evaluation is a clear sign of liver cell injury. In the present state, termination of the injuring agent will result in the regeneration of liver cells (Cotran *et al.*, 1996). These results suggest that

Table 3: Effect of *Alchornea cordifolia* on serum biochemistry of mice treated for two weeks. Values are expressed as mean ± SEM (n=5), compared to the control by the Neuman Kuels test.

Parameter	Doses (mg/kg)				
	Control	250	500	1000	2000
Total biIirubin µmol/L	28.04±6.48	26.51±5.94	32.49±5.46	32.49±10.59	28.22±3.59
Direct bilirubin µmol/L	10.26±2.65	13.68±4.58	11.97±2.29	14.54±4.76	13.25±4.15
Total protein g/L	82.60±3.17	75.50±5.49	73.20±4.91	84.25±2.10	84.25±2.06
Albumin g/L	47.00±4.63	48.50±7.53	44.20±3.29	54.00±2.83	50.50±2.90
Globulin g/L	3.56±0.54	2.70±0.55	2.90±0.34	3.03±0.47	3.38±0.40
Albumin/Globulin	1.56±0.40	2.18±0.76	1.60±0.19	2.03±0.48	1.58±0.28
Indirect Bilirubin µmol/L	17.78±4.64	12.83±4.85	20.52±5.03	17.98±5.85	14.98±1.89
AST U/L	46.40±2.87	47.75±2.14	42.80±2.67	45.00±4.24	39.25±1.25
ALT U/L	49.60±3.93	44.25±2.21	39.40±2.50	44.25±1.11	39.00±2.48
ALP U/L	118.40±4.79	118.40±4.79	104.40±2.75	116.00±7.10	108.00±5.82
GGT U/L	32.40±6.93	24.25±2.84	26.40±0.87	30.67±2.73	30.00±3.39

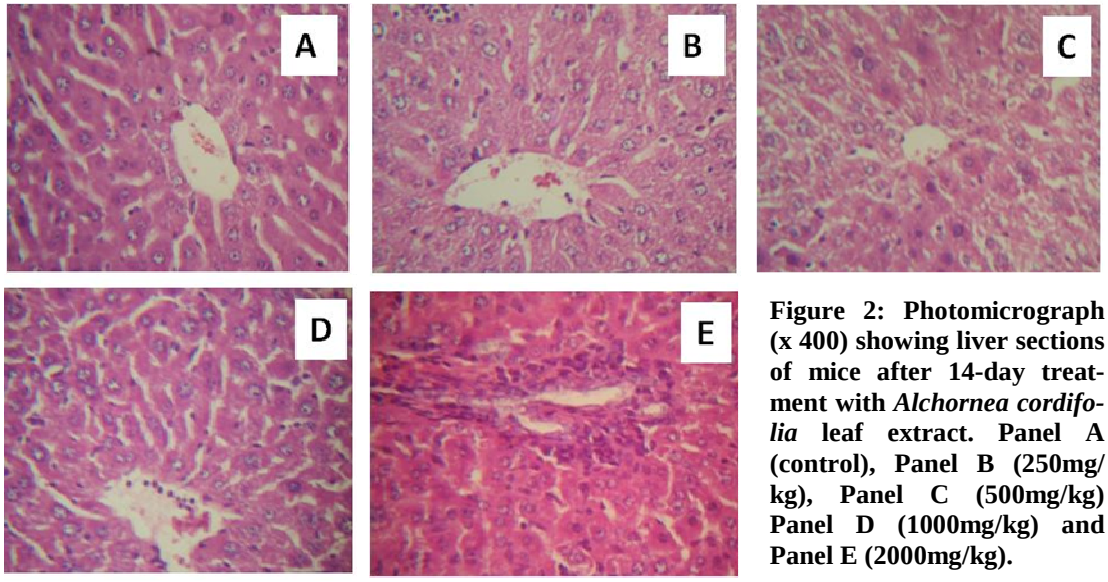

Figure 2: Photomicrograph (x 400) showing liver sections of mice after 14-day treatment with *Alchornea cordifolia* leaf extract. Panel A (control), Panel B (250mg/kg), Panel C (500mg/kg) Panel D (1000mg/kg) and Panel E (2000mg/kg).

Table 4: Effect of *Alchornea cordifolia* extract on renal function parameters of mice treated for two weeks. Values are expressed as mean ± SEM (n=5), compared to the control by the Neuman kuels test.

Parameter	Doses (mg/kg				
	Control	250	500	1000	2000
Sodium mmol/L	142.00±2.30	141.30±2.14	140.40±1.99	141.30±3.15	139.50±1.85
Potassium mmol/L	5.04±0.65	4.85±0.50	4.84±0.35	5.45±0.19	5.15±0.24
Chloride mmol/L	112.00±4.51	99.50±1.85	101.80±1.11	108.30±8.06	104.00±2.35
Blood urea nitrogen (BUN) mmol/L	3.46±0.60	4.73±1.14	3.28±0.40	4.30±0.58	4.50±0.39
Creatitine µmol/L	120.60±27.94	68.30±5.13	99.02±6.49	99.45±9.10	108.30±11.04
BUN /creatinine	8.80±2.922	16.75±3.12	8.40±1.33	10.00±1.30	10.50±1.32

high doses of *Alchornea* extract administered over a long period have the propensity to be hepatotoxic.

Biochemical studies on the renal system did not show any evidence of renal damage. Lack of significant changes in creatinine, BUN, sodium, potassium and chloride ions (Table 4) of treated mice in relation to the control groups suggests good renal function (Hilaly *et al.,* 2004) and confirms that *Alchornea* extract has no adverse effect on renal function. To confirm our observation, we examined photomicrographs prepared from kidney sections of treated and control animals. We found no alterations suggesting kidney damage in the groups treated with *Alchornea* extract (Figure 3).

Overall, our study has shown that apart from the potential hepatotoxicity of *Alchornea cordifolia* depicted by eosinophilia, lymphocyte aggregation and pyknosis of microscopic sections of the liver, it is generally non-toxic to mice at the doses used.

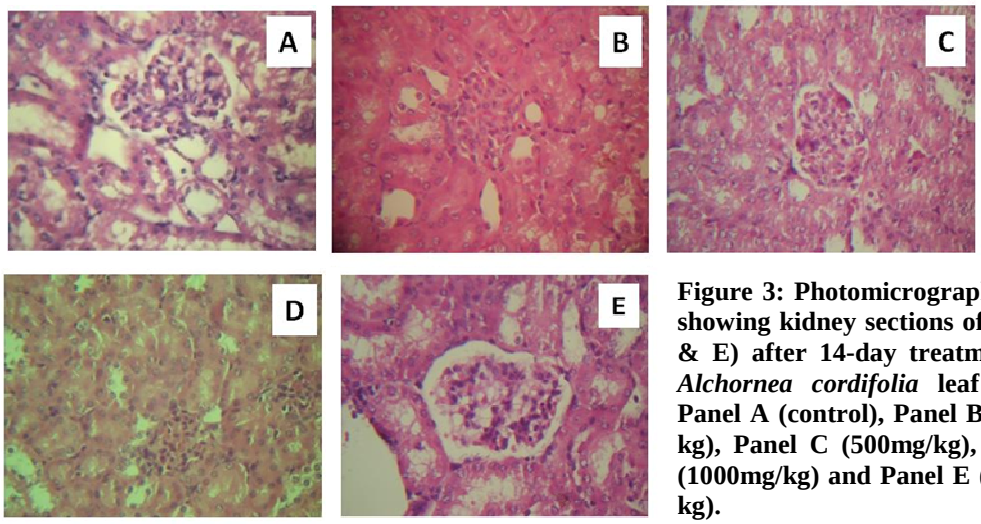

Figure 3: Photomicrograph (x 400) showing kidney sections of mice (H & E) after 14-day treatment with *Alchornea cordifolia* leaf extract. Panel A (control), Panel B (250mg/kg), Panel C (500mg/kg), Panel D (1000mg/kg) and Panel E (2000mg/kg).

CONCLUSION

The studies show that *Alchornea cordifolia* is relatively safe in mice at the doses used. Though this finding cannot be directly extrapolated to man, caution needs to be taken when high doses are administered over long periods as the extract has the potential to provoke liver damage.

ACKNOWLEDGEMENT

The authors are grateful to Dr. K. A. Boateng of the Pathology Department of the Komfo Anokye Teaching Hospital, Kumasi for assisting in the histopathological studies, Mr. Martey of the Pharmacology Department, Center for Scientific Research into Plant Medicine Mampong Akwapim for freeze drying the extract and Mr. Thomas Ansah for his technical support.

REFERENCES

Abbiw, D. (1990). Useful plants of Ghana. Intermediate Technology Publications and the Royal Botanic Garden, KEW. UK. pp 147.

Adeneye, A. A., Ajagbonna, O. P., Adeleke, T. I. and Bello, S. O. (2006). Preliminary toxicity and phytochemical studies of the stem bark aqueous extract of Musanga cecropiodes in rats. *Journal of Ethnopharmacology* 105: 374 -379.

Agbor, G. A., Leopold, T. and Jeanne, N. Y. (2004). The antidiarrhoeal activity of Alchornea cordifolia leaf extract. *Phytotherapy Research* 18**:** 873-876.

Ajali, U (2000) Antibacterial activity of Alchornea cordifolia stem bark. *Fitoterapia* 71: 436 -438.

Bagheri, H., Broué, P., Lacroix, I., Larrey, D., Olives , J. P., Vaysse, P., Ghisolfi, J. and Montastru, J. L. (1998). Fulminant hepatic failure after herbal medicine ingestion in children. *Térapie* 53: 77-83.

Banzouzi, J. T., Prado, R., Menan, H., Valentin, A., Roumestan, C., Mallie, M., P, Y. and Blache, Y (2002). In vitro antiplasmodial activity of extracts of Alchornea cordifolia and identification of an active constituent: ellagic acid *Journal of Ethnopharmacology* 82: 399-401.

Cotran, R. S., Kumar, V. and Robbins, S. L. (1996). Robbins Patologia Estruturale Funcional. Guanabara Koogan, Rio de Janeiro**.** pp 834-891.

Drew, A. and Myers, S. P. (1997). Safety issues in herbal medicine: implications for the health professions. *Medical Journal of Australia* 166: 538-541.

Ebi, G. C. (2001). Antimicrobial activities of Alchornea cordifolia. *Fitoterapia* 72: 69–72.

EEC (1986). Council Directive 86/609/EEC on the approximation of laws, regulations and administrative provisions of the member states regarding the protection of animals used for experimental and other scientific purposes. *Official Journal of the European Communities* L358, 1-29.

Harper, H. A. (1973) Review of Physiological Chemistry, 14th edition. *Lange Medical Publications, California*.

Hilaly, E. J., Israili, Z. H. and Lyoussi, B. (2004). Acute and chronic toxicological studies of Ajuga Iva in experimental animals. *Journal of Ethnopharmacology* 91: 43-50.

Igbeneghu, O. A., Iwalewa, E. O. and Lamikanra, A. (2007). A study of the In Vivo activity of the leaf extract of Alchornea cordifolia against multiply antibiotic resistant S. aureus isolate in mice. *Phytotherapy Research* 21**:** 67-71.

Jemnitz, K., Veres, Z., Monostory, K., Ko´bori, L. and Vereczkey, L. (2008). Interspecies differences in acetaminophen sensitivity of human, rat, and mouse primary hepatocytes. *Toxicology in Vitro* 22: 961-7.

Lamikanra, A., Ogundani, A. O. and Ogungbamila, F. O. (1990). Antibacterial constituents of Alchornea cordifolia leaves. *Phytotherapy Research* 4: 198-200.

Manga, H. M., Brkic, D., Marie, D. E. and Quetin -Leclercq, J. (2004). In vivo anti-inflammatory activity of Alchornea cordifolia (Schumach. & Thonn.) Mull. Arg. (Euphorbiaceae). *Journal of Ethnopharmacology* 92**:** 209-214.

Matthews, H. B., Lucier, G. W. and Fisher, K. D. (1999). Medicinal herbs in the United States: research needs. *Environmental Health Perspective* 107: 773-778.

Neuwinger, H. D. (2000). African Traditional Medicine—A Dictionary of Plant Use and Applications. Medpharm, Stuttgart, Germany. pp 29-30.

Ogungbamila, F. O. and Samuelsson, G. (1990). Smooth muscle relaxing flavonoids from Alchornea cordifolia. *Acta Pharmaceutica Nordica* 2: 421-422.

Olaleye, M. T., Adegboye, O. O. and Akindahunsi, A. A. (2006). Alchornea cordifolia extract protects wistar albino rats against acetaminophen-induced liver damage. *African Journal of Biotechnology* 5: 2439-2445.

Olalye, M. T. and Rocha, J. B. (2008). Acetaminophen-induced liver damage in mice: Effects of some medicinal plants on the oxidative defense system. *Experimental and Toxicologic Pathology* 59**:** 319-327.

Osadebe, P. O. and Okoye, F. B. (2003). Anti-inflammatory effects of crude methanolic extract and fractions of Alchornea cordifolia leaves. *Journal of Ethnopharmacology* 89**:** 19-24.

Palmeiro, N. M. S., Almeida, C. E., Ghedini, P. C., Goulart, L. S., Pereira, M. C. F., Huber, S., Silva, J. E. P. and Lopes, S. (2003). Oral subchronic toxicity of aqueous crude extract of Plantago australis leaves. *Journal of Ethnopharmacology* 88: 15-18.

Quetin-Leclercq, J., Mavar-Manga, H., Haddad, M., Pieters, L., Baccelli, C. and Penge, A. (2008). Anti-inflammatory compounds from leaves and root bark of Alchornea cordifolia (Schumach.&Thonn.)M¨ull. Arg. *Journal of Ethnopharmacology* 1: 25-29.

Rahman, M. F., Siddiqui, M. K. and Jamil, K. (2001). Effects ofVepacide (Azadirachta indica) on aspartate and alanine aminotransferase profiles in a sub chronic study with rats. *Journal of Human and Experimental Toxicology* 20: 243-249.

Ratanasavanh, D., Lamiable, D., Biour., M, Gue´de`s, Y., Gersberg, M., Leutenegger, E. and Riche´, C. (1996). Metabolism and toxicity of coumarin on cultured human, rat, mouse and rabbit hepatocytes. *Fundamental and Clinical Pharmacology* 10: 504-510.

Raza, M., Al-Shabanah, O. A., El-Hadiyah, T. M. and Al-Majed, A. A. (2002). Effect of prolonged vigabatrin treatment on hematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. *Scientia Pharmaceutica* 70: 135-145.

Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A. and Khetani, V. (2002). A 90-day oral gavage toxicity study of d-methylphenidate and d,l-methylphenidate in Sprague Dawley rats. *Toxicology* 179: 183-196.

Thompson, S. W. (1966). Selected histological and histopathological methods. *Charles C. Thomas, Springfield, IL, USA.*

Vassallo, J. D., Hicks, S. M., Daston, G. P. and Lehman-McKeeman, L. D. (2004). Metabolic detoxification determines species differences in coumarin- induced hepatotoxicity. *Toxicological Science* 80: 249-257.

Wolf, P. L. (1999). Biochemical diagnosis of liver diseases. *Indian Journal of Clinical Biochemistry* 14: 59-90.