ACUTE AND SUB-ACUTE TOXICITY PROFILE OF THE AQUEOUS STEM BARK EXTRACT OF *ENANTIA CHLORANTHA* OLIVER (*ANNONACEAE*) IN LABORATORY ANIMALS

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Summary

Enantia chlorantha Oliver (*Annonaceae*), widely used in Cameroonian folk medicine, has been previously shown to possess anti-ulcer, anti- trypanosomial and anti-*Helicobacter* properties. Other investigations have revealed the anti-malarial, antiviral, antibacterial and anti-hepatotoxic properties of this plant. In the present study the acute and sub-acute toxicity profiles of the aqueous stem-bark extract was evaluated. Swiss mice were administered single oral doses of 1000, 3000 and 5000 mg/kg and monitored for death and growth impairment for seven days (acute toxicity). In sub-acute toxicity, experimental rats, received daily doses of 250, 500, and 1000 mg/kg for 42 consecutive days and the toxic effects of the extract were assessed using biochemical and haematological parameters as well as the study of histological sections of vital body organs (heart, lungs, liver, kidneys, spleen, gonads and stomachs). No death or growth impairment was noticed in the mice in acute toxicity test. At 1000 mg/kg in the sub-acute toxicity study, the rats presented histopathological signs in the liver, lungs and kidneys, as well as significant ($P < 0.05$) increases in values of ALT, AST and platelet counts. The rest of the organs studied showed no signs of pathology. The extract of *E. chlorantha,* is not toxic in acute intake up to 5000 mg/kg, but can cause lung, hepatic and kidney disorders following medium-to-long term use at doses greater than 500 mg/kg.

Keywords: ***Enantia chlorantha*, toxicity, rats**

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Introduction

Enantia chlorantha Oliver (*Annonaceae*), also known as the African yellow wood, is a dense forest tree found in Cameroon, Nigeria and Gabon. In the southern forest zone of Cameroon, it is used for the traditional treatment of stomach problems, jaundice, urinary tract infections, malaria, tuberculosis, hepatitis and some forms of ulcer (1,2). The stem-bark of this plant features among the common medicinal plants sold in local markets in Cameroon (3). Phytochemical studies of the stem bark of *E. chloranta* have resulted in the isolation of berberine and protoberberine alkaloids possessing antimalarial (2,4), antibacterial (5,6), trypanosomicidal (7), anti HIV (8) and anti-hepatotoxic (9) properties. A phytomedicine named Hapasor (LABOTHERA, Cameroon), used for the treatment of viral hepatitis A,B,C,D,E since 1989 has been developed from a combination of palmatine (65%), jatrorrhozine (20%) and columbamine (15%) bases (10,12). Tan and co-workers (12,13) have demonstrated the cytoprotective and ulcer healing actions of the protobrberine alkaloid (7.8.-dihydro-8-hydroxypalmatine) obtained from the bark of *E. chlorantha*, as well as its *in vitro* and *in vivo* anti *Helicobacter* actions.

Due to these well-known activities of the chemically isolated principles of the plant, the bark is routinely sold in the open markets and concoctions prepared from it are widely used both by rural and urban communities in Cameroon for the management of various disease conditions. This unregulated use is practised in total ignorance of any possible toxic effects. In the present study the acute and sub-acute toxicity profile of the aqueous stem bark extract of *E. chlorantha* was evaluated using mice and rats, in order to obtain useful biochemical and histological backing for the safe use of the extract.

Material and methods

Plant materials

The stem-bark of *E. chlorantha* (Cameroon National Herbarium voucher specimen N° 25918/SRFCAM) harvested in Ambam (South Province of Cameroon) was sun dried till constant weight, and ground to powder consistency. A 10 % (w/v) mixture of the plant powder and distilled water was boiled by simmering for 15 to 20 minutes. When cooled to room temperature, the preparation was sieved through four-layers cotton fabric gauze. The filtrate was allowed to stand for 90 to 120 minutes after which the supernatant was filtered through Whatman filter paper N°1. The decoction obtained was evaporated at 40°C till total dryness using a convection air oven. The dry solid material obtained (yield: 4.6 % w/w) was used immediately or stored at 4°C.

Animals

Inbred Swiss mice (18 and 31 grams) were used for the acute toxicity test and inbred young albino (wistar) rats (60 and 125 grams) were used for the sub-acute toxicity test. The animals were maintained on a 12-hour light/dark cycle with water *ad libitum*, and fed with laboratory baked food made of maize (50%), soybeans (25%) and wheat flour (25%), supplemented with table salt, palm oil, fish and bone powder. Prior authorization for the use of laboratory animals was obtained from the Cameroon National Ethics Committee (Reg. N° FWA-IRB00001954).

Acute toxicity test

The test was carried out following the methods described by CDER (1996) and 25WHO (1992) (26,27); Mice were deprived of food for 24 hours prior to extract administration and allocated (n=10; 5 males and 5 females) into four extract treatment groups (0, 1000, 3000 and 5000 mg/kg). Extract-treated mice received the product by intragastric means while control animals were given the vehicle (distilled water). The animals were observed continuously for 3 hours for behavioural changes (dizziness, motion, reaction to contact and noise, aggressiveness and reaction to food supply). Thereafter, food intake was resumed and their weights were taken daily for seven consecutive days.

Sub-acute toxicity study

Sub-acute toxicity study was carried out following WHO (1992) (27) and OECD 407 (1995) (28) guidelines. Rats deprived of food for 24 hours were divided into four groups of 10 animals each (5 males, and 5 females) corresponding to extract doses of 0, 250, 500 and 1000 mg/kg body weight. Prior to the 1st extract administration, the fasting blood glucose of each rat was measured using a drop of blood obtained from the tail. The animals were administered the plant extract by intragastric route once daily for 42 consecutive days. The control group (0 mg/kg) received distilled water. Food intake was measured daily, while body weights were recorded twice a week and the volume of extract administered was adjusted accordingly. On day 43 the fasting blood glucose was measured again and the animals were ether-anaesthetised and blood was collected by cardiac puncture for the determination of biochemical and haematological parameters. The heart, lungs, liver, kidneys, gonads, spleen and the stomach were removed for histological analyses. The biochemical parameters evaluated included urea, creatinine, alanine aminotranferase (ALT), aspartate aminotransferase (AST), total protein, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride (using commercial kit obtained from Randox Laboratories, UK), and glucose (using Gluco*Plus*^{TM} blood glucose meter, Canada). Haematological parameters were measured using an automated haematological machine (Cell-Dyn^{TM}, Abbot, US); these included the red blood cell count (RBC), White blood cell count (WBC), Lymphocyte percentage (Lymp %), Granulocyte Percentage (Gran %), Platelet count (Plt), Haemoglobin level (Hb), Heamatocrit level (HCT), Mean Cell Volume (MCV), Mean Cell Haemoglobin level (MCH) and Mean Cell Haemoglobin Concentration (MCHC).

Statistical analyses

The mean changes in body weight, the daily food intake, the organ weight relative to body weight, biochemical and haematological parameters were statistically analysed and significant differences within groups were calculated using the one way ANOVA test and the values of $P \leq 0.05$ were considered statistically significant. The LSD (Least Significant Difference) test was used to compare means where P was significant. The results presented are expressed as Mean $\pm$ SEM (standard error of the mean).

Results

Acute toxicity

The administration of single doses of extract (1000, 3000, 5000 mg/kg) caused no remarkable behavioural changes in the treated mice (Dizziness, reaction to food supply and reaction to contact and noise). However, mobility and aggressiveness was reduced in all the extract-treated animals compared with the control group within the first 3 hours. No deaths were recorded during the seven-day observation period, but extract-treated

mice showed a 10.73% to 15.12% increase in body weight compared to 5.20% for the controls (Table 1).

Table 1. Effect of the stem bark aqueous extract of *Enantia chlorantha* on body weight change in mice (acute study) and in rats (sub acute study) and on food intake in rats (sub acute study).

Percentage change in weight				Food intake (g/kg/day)	
Mice		Rats		Rats	
Extract (mg/kg)	Mean± SEM	Extract (mg/kg)	Mean± SEM	Extract (mg/kg)	Mean±SEM
0	5.2 ± 1.1[a]	0	67.7 ± 0.0[a]	0	141.1 ± 6.8[a]
1000	12.9 ± 1.3[a]	250	67.4 ± 7.6[a]	250	141.7 ± 6.0 [a]
3000	15.1 ± 2.4[a]	500	70.1 ± 6.5[a]	500	147.1 ± 9.7[a]
5000	10.7 ± 2.6[a]	1000	59.3 ± 4.4[a]	1000	145.7 ± 9.3[a]

SEM, Standard error of the mean

[a] values with same superscript are not significantly different, $p \leq 0.050$

Sub-acute toxicity

Mean food intake during the 6-week treatment period ranged from 130.77 to 154.20 (g/kg/day) with no significant differences between groups. Similarly, percentage live weight change varied between 50.33% and 89.58%, with no treatment- or sex-related variation ($P \geq 0.05$) (Table 1).

The effects of the extract of *E chlorantha* on blood biochemical parameters are presented in Table 2. At the dose of 1000 mg/kg, mean ALT values were significantly higher (69.49 UI/L) compared to those of the control group (45.40 UI/L). Mean AST values for the 500 and 1000 mg/kg doses (102.07 and 155.74 UI/L, respectively) were also significantly higher compared to the control (76.87 UI/L) and 250 mg/kg dose (76.87 UI/L) values. Triglyceride values for the 1000 mg/kg group were significantly higher and LDL cholesterol values were lower for the same group compared with the groups given 500, 250 and 0 mg/kg of extract. There was no significant difference in the mean values of urea, creatinine, total cholesterol, glucose and total proteins (Table 2). During the experimental period, there were no treatment-related effects on the haematological parameters studied except for the platelet count values which were significantly low in the groups treated with 500 (6.24 x 10^5/mm^3) and 1000 (6.15 x 10^5/mm^3) mg/kg of extract compared with the control group (6.91 x 10^5/mm^3) (Table 3).

The relative heart and liver weights were significantly high for the 500 mg/kg doses compared to the control values, and lung weights were equally higher for the 1000 mg/kg dose. Kidney, gonad, spleen and stomach relative weights showed no treatment-related abnormalilties. (Table 4). Histological sections of the vital organs revealed abnormal tissue features in some rats treated with the 1000 mg/kg dose. These included: steatosis of the liver (2/5 males, 1/5 females), interstitial oedema of the liver (3/5 males), mild inflammatory infiltration of the liver (2/5 males), vein congestion of the liver (2/5 males, 2/5 females), pulmonary emphysema (2/5 females), interstitial oedema of the heart (1/5 males, 1/5 females) and vein congestion in the kidney (2/5 males, 1/5 females) (Figure 1A to 1K). No histological abnormalities were observed in organs from rats given 500 and 250 mg/kg of extract.

Table 2. Effect of the stem-bark aqueous extract of *Enantia chlorantha* on rat blood biochemical parameters (values expressed as Mean ± SEM).

	Treatment dose			
Parameter	**0 mg/kg**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
ALT (UI/L)	$45{,}40 \pm 6.81^{a}$	53.60 ± 6.49^{a}	52.96 ± 4.81^{a}	69.49 ± 7.78^{b}
AST (UI/L)	76.87 ± 6.19^{a}	87.47 ± 9.18^{a}	102.07 ± 6.89^{b}	155.74 ± 9.18^{c}
Urea (mg/dl)	29.87 ± 4.07^{a}	29.07 ± 3.19^{a}	32.59 ± 7.09^{a}	30.40 ± 2.26^{a}
Creatinine	0.84 ± 0.08^{a}	0.70 ± 0.05^{a}	0.72 ± 0.06^{a}	0.77 ± 0.06^{a}
Total Proteine (mg/dl)	65.06 ± 2.42^{a}	57.57 ± 2.17^{a}	63.57 ± 2.61^{a}	62.32 ± 2.27^{a}
Triglyceride (mg/dl)	43.40 ± 6.60^{a}	45.70 ± 6.29^{a}	47.11 ± 9.34^{ab}	65.40 ± 4.32^{b}
Total Cholesterol (mg/dl)	125.03 ± 11.44^{a}	111.52 ± 15.21^{a}	137.45 ± 17.01^{a}	87.02 ± 14.59^{a}
HDL Cholesterol (mg/dl)	82.31 ± 4.05^{a}	76.41 ± 3.78^{a}	89.74 ± 3.41^{a}	82.31 ± 4.93^{a}
LDL Cholesterol (mg/dl)	44.98 ± 6.8^{a}	57.69 ± 6.56^{a}	52.55 ± 7.52^{a}	24.74 ± 3.79^{b}
Glucose (g/l) (pre-treatment)	0.72 ± 0.08^{a}	0.75 ± 0.04^{a}	0.69 ± 0.04^{a}	0.88 ± 0.04^{a}
Glucose (g/l) (post-treatment)	0.75 ± 0.03^{a}	0.73 ± 0.05^{a}	0.86 ± 0.04^{a}	0.81 ± 0.04^{a}
Values with different superscripts ($^{a, b, c}$) are significantly different, $P \leq 0.05$.				

Table 3. Effect of the stem bark aqueous extract of *Enantia chlorantha* on haematological parameters in rats (values expressed as Mean ± SEM.

	Treatment dose			
Parameter	**0 mg/kg**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
WBC (x 10^3) /mm^3	7.99 ± 0.79^{a}	7.58 ± 0.74^{a}	7.84 ± 0.71^{a}	7.98 ± 0.43^{a}
RBC (x 10^6) /mm^3	6.31 ± 0.24^{a}	6.65 ± 0.28^{a}	6.41 ± 0.27^{a}	6.74 ± 0.23^{a}
Platelet (x 10^5)/mm^3	6.91 ± 0.23^{a}	6.70 ± 0.18^{ab}	6.24 ± 0.14^{b}	6.15 ± 0.23^{b}
Hb (g/100ml)	12.49 ± 0.36^{a}	12.69 ± 0.54^{a}	12.82 ± 0.52^{a}	13.38 ± 0.21^{a}
HCT (%)	34.49 ± 1.40^{a}	38.67 ± 2.02^{a}	38.27 ± 1.63^{a}	39.30 ± 1.27^{a}
MCV (fl)	58.43 ± 1.20^{a}	59.78 ± 1.51^{a}	58.36 ± 2.59^{a}	54.70 ± 1.03^{a}
% Granulocytes	17.07 ± 2.08^{a}	12.12 ± 2.48^{a}	16.92 ± 2.53^{a}	21.79 ± 7.17^{a}
% Monocytes	10.88 ± 1.35^{a}	10.46 ± 0.48^{a}	8.61 ± 0.44^{a}	8.81 ± 0.39^{a}
% Lymphocytes	72.05 ± 2.55^{a}	75.83 ± 2.19^{a}	74.47 ± 2.37^{a}	69.40 ± 7.14^{a}
MCHC (g/l)	363.60 ± 4.68^{a}	334.50 ± 18.49^{a}	337.56 ± 13.11^{a}	342.70 ± 9.66^{a}
MCH (pg)	19.87 ± 0.30^{a}	19.12 ± 0.43^{a}	20.04 ± 0.45^{a}	19.98 ± 0.46^{a}

Values with different superscript ($^{a, b}$) for each parameter are significantly different, $P \leq 0.05$.

Table 4. Effect of the stem bark aqueous extract of *Enantia chlorantha* on rat organ weights (values expressed as **%** organ weights relative to body weight (Mean ± SEM)

	Treatment dose			
Organ	**0 mg/kg**	**250 mg/kg**	**500 mg/kg**	**1000 mg/kg**
Heart	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}
Lung	0.9 ± 0.1^{a}	0.9 ± 0.1^{ab}	0.9 ± 0.1^{ab}	1.1 ± 0.1^{b}
Liver	4.0 ± 0.2^{a}	4.0 ± 0.3^{a}	5.4 ± 0.4^{b}	5.7 ± 0.4^{b}
Spleen	0.4 ± 0.0^{a}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}
Stomach	1.0 ± 0.1^{a}	0.9 ± 0.1^{a}	0.9 ± 0.1^{a}	1.3 ± 0.4^{a}
Right gonad	0.4 ± 0.1^{a}	0.5 ± 0.2^{a}	0.5 ± 0.2^{a}	0.6 ± 0.2^{a}
Left gonad	0.4 ± 0.1^{a}	0.5 ± 0.2^{a}	0.5 ± 0.2^{a}	0.6 ± 0.2^{a}
Right kidney	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.5 ± 0.0^{a}
Left kidney	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}	0.4 ± 0.0^{a}

Values with different superscript ($^{a, b}$) for each organ are significantly different, $P \leq 0.05$.

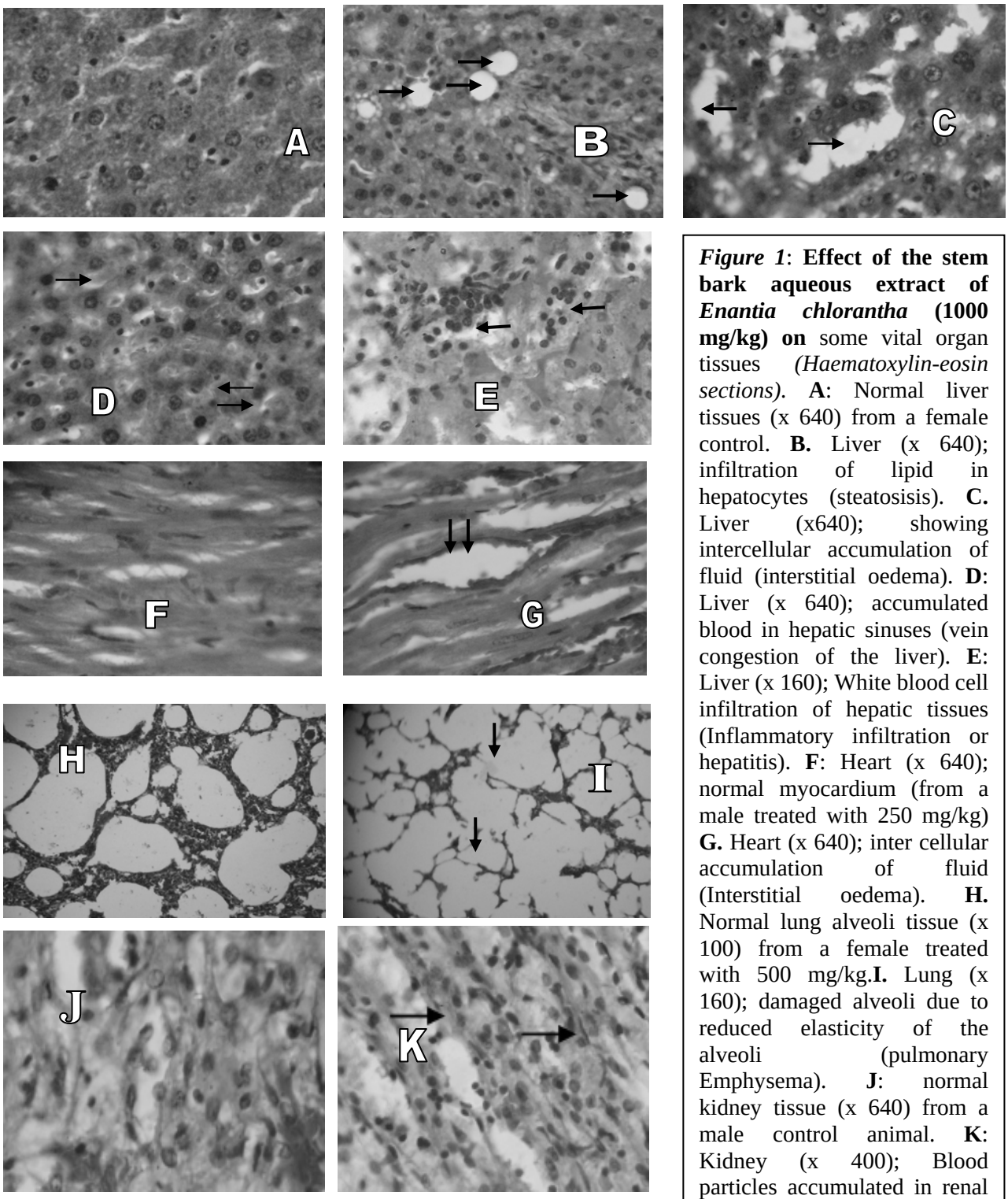

Figure 1: **Effect of the stem bark aqueous extract of *Enantia chlorantha* (1000 mg/kg) on** some vital organ tissues *(Haematoxylin-eosin sections)*. **A**: Normal liver tissues (x 640) from a female control. **B.** Liver (x 640); infiltration of lipid in hepatocytes (steatosisis). **C.** Liver (x640); showing intercellular accumulation of fluid (interstitial oedema). **D**: Liver (x 640); accumulated blood in hepatic sinuses (vein congestion of the liver). **E**: Liver (x 160); White blood cell infiltration of hepatic tissues (Inflammatory infiltration or hepatitis). **F**: Heart (x 640); normal myocardium (from a male treated with 250 mg/kg) **G.** Heart (x 640); inter cellular accumulation of fluid (Interstitial oedema). **H.** Normal lung alveoli tissue (x 100) from a female treated with 500 mg/kg.**I.** Lung (x 160); damaged alveoli due to reduced elasticity of the alveoli (pulmonary Emphysema). **J**: normal kidney tissue (x 640) from a male control animal. **K**: Kidney (x 400); Blood particles accumulated in renal tissues (vein congestion of the kidney).

Discussion

The need for the evaluation of the toxicity profile of *E. chlorantha* water extract has been prompted by its rampant use by the population for various treatment interventions given the proven pharmacological actions of some of the active principles as well as the prohibitive cost of the commercialized antiviral product, Hepasor, compared with the prevailing financial capacity of the local population.

Local herb dealers recommend that about 1000 g of fresh bark be boiled in 3 litres of water. It is estimated that the final extract solution has a concentration of 8.3 mg/ml. When 250 to 300 ml of the extract solution are taken three times per day, this corresponds approximately to an intake of 107 mg/kg of the extract by a 70 kg human adult. From the acute toxicity test, the calculated LD_{50} value was above 5000 mg/kg, indicating (14) that the extract is poorly toxic in acute usage. Extract doses lower than or equal to 500 mg/kg did not show any biochemical, histological or haematological signs of toxicity, indicating that the traditionally recommended dose (107 mg/kg) could be very safe. However, sub acute toxicity results indicate that continuous medium-term use of the extract at doses higher than 500 mg/kg can lead to pathological incidents involving the heart, lung and liver. The elevated transaminase levels (AST & ALT) at 500 to 1000 mg/kg are blood biochemical signs of deep-seated pathological conditions. AST is normally widely distributed in cardiac, skeletal muscle, liver, and kidney tissues, and small quantities are also found in brain, pancreatic and lung tissues (15). Thus pathological conditions involving various tissue injuries are usually accompanied by elevated serum AST levels. On the contrary, ALT, a mainly liver-based enzyme, is present in only minute quantities in other major organs, and elevated serum levels constitute a specific detector of hepatocellular injury (16-18). During hepatocellular damage, serum ALT and AST levels usually rise in the same proportion. In the present experiment, 1.5- and 2-fold increases in serum ALT and AST levels, respectively, were obtained in response to the continuous intake of 1000 mg/kg of *E. chlorantha* extract (Table 2). At tissue level, the toxicity signs were expressed macroscopically by an increase in liver weight, and histologically by fatty infiltration of hepatocytes (steatosis), intercellular hepatic oedema, hepatic vein congestion and mild inflammatory leucocytic infiltration. (Figure 1A to 1D)

Toxic agents can produce liver injury either by direct physico-chemmical destruction of the hepatocytes causing focal necrosis and/or steatosis, or by indirect hepatocyte damage through interference with specific biochemical pathways resulting in dispersed necrosis and/or steatosis (19). The histological presentation of damage by *E. chlorantha* extract is therefore suggestive of an indirect toxic effect. Steatosis, almost considered a “normal” degenerative process, is usually of minimal or no short- or long-term danger, and may occur singly and either herald or accompany many other pathologies. It can be transient or permanent.

Lipids exit the liver in the form of low density lipoprotein (LDL) synthesised from triglycerides. However, defective synthesis of the apoprotein moiety of the VLDL or a faulty assembly of triglyceride to form VLDL can lead to hepatocyte infiltration by lipids and consequent impairment of lipid homeostasis (19,20). This may explain why raised serum levels of triglyceride and low levels of LDL–cholesterol were obtained in animals treated with 1000 mg/kg of *E. chlorantha* extract (Table 2). This situation can be responsible for the observed interstitial oedema and inflammatory infiltration (hepatitis). Inflammatory infiltration can be mild or severe depending on the causative agent, and

could be transient of complicated. Similar disturbances in intra/extra cellular electrolyte balance in cardiac tissue would be responsible for the interstitial oedema observed in the heart tissue of rats given 1000 mg/kg of extract (Figure 1B and 1F). The absence of focal necrosis or chronic active hepatitis suggest that the observed liver injury may have been due a generalised over taxation of the liver in its detoxification role (15,21) since, in addition, the other biochemical parameters of liver function (total protein, total cholesterol and fasting blood glucose) remained normal for the 1000 mg/kg rats compared with the controls.

Hepatic and renal vein congestion were also observed in rats given 1000 mg/kg of extract (Fig 1C & 1J). Vein congestion in an organ usually results from a back pressure within the vein leading to blood cell accumulation. Vein congestion causes leakage of fluid into the hepatic and renal parenchyma and consequently interstitial oedema. This may have the short-term effect of vascular rupture with consequent haemorrhage, or atrophy/necrosis of hepatocytes. The effect can be transient or permanent, depending on the causative factor. Veno-occlusive disease can be caused by substances that cause vein injury or interfere with blood flow in the venules (19,21). Serum creatinine, urea, and total proteins remained normal (Table 2), and no histological signs of injury were observed in the kidneys of rats given the lower doses of extract.

Repeated insult by a toxic agent can result in chronic respiratory disease (pulmonary emphysema) in which alveoli develop thickened inelastic epithelium (22). Air intake then causes alveolar breakage leading to diffuse alveolar diseases. The histopathological condition observed at the dose of 1000 mg/kg of *E. chlorantha* extract was manifested by a collapse of the alveolar structure leading to the fusion of neighbouring alveolar sacs (Fig. I). This usually causes a decrease in respiratory volume and function (hypoxemia, hypercapnia). The damage to the air sacs is irreversible and results in permanent "holes" in the tissues of the lower lungs, early signs of which include shortness of breath, chest pain, cough and fatigue.

Haematological parameters studied appeared normal except for the significantly low platelet counts (thrombocytopenia) observed with the groups treated with 1000 and 500 mg/kg of extract. Thrombocytopenia is indicative of an impairment of haemostatic function and herbal remedies are cited among the causes of drug-induced thrombocytopenia (17). Drug-induced immune thrombocytopenia, usually known to result from the adsorption of drug-antibody complexes to platelet membranes, causes massive destruction of platelets by the spleen. However, the condition is usually reversible after withdrawal of the offending drug (23).

The aqueous extract of *E. chlorantha* is well known for its anti-ulcer properties. We have also demonstrated its *in vivo* anti-*Helicobacter* action at the dose of 100 mg/kg (unpubished data), as well as the cytoprotective actions (100 mg/kg), the chronic ulcer healing actions (80 mg/kg) and the in vivo anti *Helicobacter* action (50 mg/kg) of the protoberberine alkaloid 7.8.-dihydro-8-hydroxypalmatine obtained from the bark extract (24). The commercialized alkaloid combination (Hepasor) is used to treat viral hepatitis due to its ability to stimulate blood circulation, normalise liver function and inhibit viral proliferation, and no toxicity signs have been reported in blood, hepatic, renal and nervous tissue at therapeutic doses. However, the present toxicity study shows that the bark aqueous extract, although non toxic after sub acute intake up to 500 mg/kg, can be toxic at higher doses. These results sharply contrast those of Agbaje and Onabanjo (1994) (25) who recorded no fatality or significant damage to body organs when mice were given 5mg-3000 g/kg of *E. chlorantha* aqueous extract to drink ad lib for 5 weeks. Given the multiple ethnopharmacological applications of *E. chlorantha* extract which

exploit its anti-hepatotoxic, antiviral, antimalarial, antibacterial and antiulcer properties, the present toxicity results constitute safety information that can be useful in the formulation of local phytomedicine products using the crude bark extract. The strict respect of safe dosage by local users needs to be emphasised. Chronic toxicity evaluation and teratogenic studies need to be carried out.

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