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Original Research Article

Evaluation of Chronic Toxicity of *Newbouldia laevis* Leaf Extract in Rats

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ABSTRACT

Newbouldia laevis is a medicinal plant widely used as herbal remedy in Africa. Chronic toxicity of the leaves of the plant has not been evaluated. The study evaluated the chronic toxicity of *N. laevis* leaf extract (CEE) in Wistar rats. Rats were treated orally with CEE (300 and 600 mg/kg body weight) for 180 days. The weight, relative organ weight, biochemical and hematological parameters were determined, and the kidney, liver, heart, and lung were examined for histological changes. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, and $p<0.05$ was considered statistically significant. Results showed that oral administration of CEE (300 and 600 mg/kg body weight) for 180 days caused a significant reduction ($P<0.05$) in percentage weight gain and relative organ weight compared to control. *Newbouldia laevis* extract also caused significant ($p<0.05$) increase in serum level of creatinine, urea, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphate compared to control. It caused a significant decrease in red blood cells, packed cell volume, hemoglobin, and an increase in the level of white blood cells. There were inflammation and congested vessels in the structures of the kidney, liver, heart, and lung in the treated rats compared to control. Withdrawal of CEE for 28 days after 180-day treatment resulted in slight amelioration of the adverse effects. Findings from this study suggest that prolonged use of hydroalcoholic preparation of *Newbouldia laevis* may cause toxic effects in vital organs of the body. Therefore, the plant should be used with caution.

Keywords: chronic toxicity, *Newbouldia laevis*, rats, extract

Introduction

The use of complementary and alternative therapy is on the increase. More and more people in developed nations are using herbal products as medications.[1, 2] Herbal therapy is even more widespread in developing nations around the world.[3] Increase in the use of alternative therapies has been attributed to the growing public concern about the accessibility and affordability of mainstream orthodox medicine. It is also believed that most of these alternative/complement ary therapies are safe since they are 'natural', and have been around for a long time.[4, 5] In addition, it is believed that modern medicine has failed some patients, while alternative therapies offer them hope.[6] Herbal therapy has remained a major part of the alternative/compleme ntary medicine currently gaining ground all over the world.

Herbal remedies are plant-based. About 25% of present pharmaceutical preparations contain at least one active ingredient extracted from plant sources.[7] Moreover, some of the modern drugs were originally derived from plants, including digitalis (foxglove), aspirin (willow) and paclitaxel (Pacific yew).

In the case of orthodox drugs, the active ingredient is isolated from the plant, chemically standardized, subjected to critical clinical assessment and then replaced with a synthetic analogue. In contrast, the herbalist uses mixtures of diverse herbal ingredients of varying potency. However, if the potency of an herbal formulation is unknown, it is difficult to know what dose to prescribe to get the desired effect without causing problems of toxicity.[8]

Every drug with therapeutic benefits must therefore be subjected to preclinical and clinical evaluation before it is approved for use. Preclinical investigations usually precede clinical trials in steps leading to drug discovery or further investigations on those already developed.[9] Such studies involve the use of laboratory animals such as mice, rats, rabbits and dogs. Preclinical studies include toxicity tests to determine the level of safety of the substance. Acute toxicity test is usually the first to be carried out. It is an assessment of the risk of exposing animals to a substance for a short time.[10] In the test, the LD_{50} of the substance, as well as other observable adverse effects is determined after a single dose. The LD_{50} of a drug provides a preliminary indication of the potential of the substance for toxicity, but there is a vast range of possible adverse effects that do not necessarily bear a predictable relationship to the LD_{50}. In addition, the LD_{50} of a drug may be substantially different from species to species, even after differences in mass or surface area are taken into account.[11] Most adverse effects are dose dependent, occurring with increasing frequency and severity as doses of a drug and duration of use are increased. This means that toxicity can be delayed until after long time exposure to a drug. For example, carcinogenic effects of chemicals may not manifest until 10-30 years after exposure.[12] Moreover, occurrence of pathological conditions like cancer may, in some instances, reflect the accumulated toxic influences on nucleic acids that have been exerted by many different mutagenic compounds

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encountered over several decades.[13] This makes it very important for chronic toxicity test to be carried out on substances intended for repeated use. In a chronic toxicity study, the test compound is administered for major part of the animal's life span, and this should be more than 90 days. For rodents, it is usually considered to be six months in duration.[14, 15] Since medicinal plants are widely consumed for therapeutic purposes, their safety should be assessed by subjecting them to these toxicity tests. *Newbouldia laevis* (P. Beauv) is an angiosperm which belongs to the family of *Bignoniaceae*. It grows in the savannah and dense forests of many African countries. It is commonly referred to as 'Fertility Tree'. In many African countries, it is used to treat diseases like diabetes, convulsions, infertility and bacterial infections.[16] Despite widespread and prolonged uses of *Newbouldia laevis*, reports on its chronic toxicity are not available in the literature. This study evaluated the chronic toxicity of the leaf extract of the plant in rats.

Materials and Methods

Preparation of Plant Extract

Newbouldia laevis leaves were collected in the month of July 2020, from Onibueja area in Osogbo, Nigeria. Identification of the plant was done at Forest Research Institute of Nigeria (FRIN) where voucher specimen (FHI 107753) was deposited. Dry samples of *N. laevis* leaves were pulverized using a grinding machine. The pulverized sample (800 g) was extracted in 2 L of 80% ethanol using a Soxhlet apparatus. Concentration of the resulting hydroalcoholic extract was done by rotary evaporator (Heidolph-Rotacool, Germany) at a temperature of 40^{o}C. The extract (CEE) was kept in a refrigerator. CEE was reconstituted in distilled water during the study.

Ethical Consideration

The Protocols and procedures employed in the study were as outlined in the "Guide for the Care and Use of Laboratory Animals" published by the National Research Council,[17] and also as approved by the Laboratory Animal Use Committee of Pharmacology Department, Ladoke Akintola University of Technology, Nigeria (Ethical Approval Number: PT21/004).

Acute Toxicity

This study was carried out in two phases, according to a modified method of Lorke.[18]

Phase 1

Nine rats were grouped into three sets of three rats. Each group was given 10, 100, or 1000 mg/kg b.w. of CEE orally. The rats were placed under observation for 24 hours to monitor their behavior and mortality.

Phase 2

Another three rats were assigned to three groups of one rat. Each rat was administered 1600, 2900, or 5000 mg/kg b.w. of CEE, and subsequently observed for 24 hours for signs of toxicity and mortality. Then the LD_{50} was calculated by the formula:

$LD_{50} = \sqrt{(D_0 \, x \, D_{100})}$

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

Experimental Design

Forty-eight rats were assigned into four major groups (I, II, III, and IV) of 12 rats each. Each of these groups was divided into two subgroups A and B (IA and IB, IIA and IIB, IIIA and IIIB, IVA and IVB) of 6 rats each. Rats in each subgroup were treated per oral daily for 180 days as follows:

IA and IB: 150 mg/kg b.w. CEE; IIA and IIB: 300 mg/kg b.w. CEE; IIIA and IIIB: 600 mg/kg b.w. CEE; IVA and IVB: 10 ml/kg b.w. distilled water. Animals in the subgroups IA, IIA, IIIA, and IVA were sacrificed after the 180-day treatment, while rats in subgroups IB, IIB, IIIB, and IVB were not sacrificed until 28 days later (for reversibility study). After they were sacrificed, blood samples and organs (liver, kidney, heart, and lung) were collected and prepared for hematological, biochemical, and histopathological analyses.

Measurement of Body Weight and Relative Organ Weight

Weights of the rats were measured before and after treatment. Harvested organs (kidney, liver, lung, and heart) were weighed, and relative organ weight was expressed as g/100 g body weight.

Haematological and Biochemical Analyses

Haematological analysis was done by an automated analyzer, Sysmex XE-2100 (Sysmex Corporation, USA). Biochemical parameters were analyzed by standard diagnostic test kits (Randox Laboratories, UK) on Automated Clinical System (Sychron Clinical System®, model: CX5 PRO; Beckman Coulter Inc., Galway, Ireland).[19]

Histopathological Study

Organs fixed in 10% formalin were prepared on slides as previously described.[20] Tissue sections (5 µm thick) were cut by a microtome. The sections were incubated with H & E and then examined under a photomicroscope (Model N-400ME (CEL-TECH Diagnostics, Hamburg, Germany). Photomicrographs were taken and interpreted.

Statistical Analysis

Data obtained were expressed as mean ± standard error of mean (SEM). Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's *Post Hoc* test. A level of $P < 0.05$ was taken as significant.

Results and Discussion

The LD_{50} of *N. laevis* leaf extract was more than 5000 mg/kg body weight which agrees with previous studies.[21,22] This is an indication that the toxicity of CEE in rats is low after short time exposure. However, acute toxicity may not accurately reflect how toxic a substance is when used for long duration.[23] The type of adverse response generated by a substance may differ significantly with increase in exposure time.[24] This underscores the need for chronic toxicity study. Chronic toxicity may be assessed in much the same way as acute toxicity, but the animals are exposed to the substance for much longer duration and more biological parameters are measured. Chronic effects are more related to the level of a substance accumulated in the organism or even in specific tissue after many months or even years of repeated ingestion.[25]

The results obtained from toxicity tests showed that the acute toxicity of CEE is low, but chronic administration may result in deleterious effects. Compared with the control, significant decrease ($p<0.05$) in body weight was observed in the group treated with 600 mg/kg body weight of CEE. Percentage decrease in body weight of rats was 69.90 ± 12.20 following the 180-day treatment. Administration of CEE (150 and 300 mg/kg body weight) also resulted in decreased body weight, although the decrease was not significant (Table 1). There was significant increase ($p<0.05$) in the relative weight of heart, kidney, liver, and lung of rats treated with the extract compared to the control. Relative weight of heart increased from 1.33 ± 0.71 to 1.91 ± 0.20 and 2.11 ± 0.11 with 300 and 600 mg/kg b.w respectively. The lung increased from 0.98 ± 0.20 to 1.52 ± 0.02 and 1.78 ± 0.27 with 300 and 600 mg/kg b.w. respectively (Table 2). Relative weight of kidney increased from 1.10 ± 0.27 to 1.85 ± 0.10 and 1.98 ± 0.02 in the groups treated with 300 and 600 mg/kg b.w respectively.

Table 1: Body weight of rats treated with CEE for 180 days

Group	1st day (g)	180th day (g)	Δ180th day (%)
IA	118.61 ± 15.20	226.90 ± 20.61	91.30 ± 17.10
IIA	112.64 ± 8.33	220.41 ± 29.65	95.68 ± 15.02
IIIA	124.32 ± 18.26	211.22 ± 25.82	69.90 ± 12.20*
IVA	106.54 ± 17.41	212.60 ± 24.73	99.55 ± 14.81

Group I = 150 mg/kg b.w; Group II = 300 mg/kg b.w; Group III = 600 mg/kg b.w; Group IV = 10 ml/kg distilled water. Each value represents mean ± SEM (n = 6); *$p < 0.05$ compared with control. Δ180th day = % change in weight after treatment for 180 days

The liver also increased from 3.12 ± 0.30 to 4.93 ± 0.21 with 600 mg/kg b.w. In the reversibility study, although the relative weight of organs increased in the treated rats, the changes were not significant compared with the control (Tables 3).

The decreased weight of rats observed when treated with CEE is likely due to impairment of carbohydrate and lipid metabolism. The extract might also have enhanced the synthesis of 5-hydroxytryptophan (5-HT) production, leading to reduced appetite.[26] Significant increase in relative weight of heart, lung, kidney, and liver indicates that these organs were susceptible to the adverse effects of CEE at the doses administered and the duration of treatment. The results of the reversibility studies suggest that the change in weight and relative organ weight caused by CEE was ameliorated following its withdrawal. The liver and the kidney are especially vulnerable to toxins. The hepatic portal venous system first delivers substances absorbed from the gastrointestinal tract to the liver. This organ contains many catabolic enzymes and is thus capable of metabolizing practically any type of exogenous compound, usually to a less toxic form. However, metabolites may be even more toxic than their molecular precursors. In fact, some toxins, such as amatoxins and microcystins, are relatively selective hepatotoxins because they are able to gain easy access into the hepatic cells by means of a special solute transport system normally used for reabsorption of bile salts.[27]

The kidney is also relatively selective to certain toxins, particularly those that enter the renal tubule by glomerular filtration but are not readily reabsorbed. This causes them to be concentrated in the nephron and urine, enhancing their ability to damage renal cells. In this study, the concentrations of urea and creatinine were significantly increased (p<0.05) in rats treated with CEE at 300 and 600 mg/kg body weight compared with control. Following the withdrawal of the extract for 28 days, the difference in urea level between treatment and control group was not significant (p>0.05). Although the creatinine level of treated group dropped after the withdrawal of CEE for 28 days, there was significant difference (p<0.05) between the treatment group and the control (Figures 1 and 2).

Creatinine is a waste product from the muscle breakdown of a compound called creatine, which is excreted by the kidney.[28, 29] It is measured in blood, urine or both, to evaluate kidney function. Kidney disease is also associated with reduced urea excretion and consequent rise in blood concentration. Urea is the principal nitrogenous waste product of metabolism and is generated from protein breakdown. It is eliminated from the body almost exclusively by the kidneys in urine.[30]

Repeated administration of 300 and 600 mg/kg b.w. CEE for 180 days caused significant increase (p<0.05) in the serum levels of AST, ALT, ALP, total protein, and albumin in treated rats compared to control (Table 4). Levels of these parameters were not significantly different (p>0.05) from the control following the withdrawal of CEE for 28 days as shown in Tables 5. Significant changes in the level of these biochemical parameters suggest that CEE caused hepatic and renal dysfunction, as well as other tissue damage in the rats. These results were corroborated by the findings from histopathological examinations of these organs.

Treatment of rats with 300 and 600 mg/kg b.w. CEE caused significant (p<0.05) decrease in red blood cell count (RBC), packed cell volume (PCV), and hemoglobin level (Hb) compared to control. MCV and MCH were also significantly reduced at the end of the 180-day treatment. There was a significant increase in white blood cell count (WBC) in the CEE-treated group compared to the control (Table 6). All other hematological indices were not significantly altered. In the reversibility study, RBC and PCV values in the CEE-treated rats were significantly different (p<0.05) from those of the control group as shown in Table 7.

Table 2: Relative organ weight of rats treated with CEE for 180 days (g/100g b.w)

Group	Liver	Kidney	Heart	Lung	Testes
IA	3.62 ± 1.02	1.09 ± 0.31	1.14 ± 0.62	1.10 ± 0.31	1.34 ± 0.31
IIA	4.64 ± 1.21*	1.99 ± 0.11*	1.91 ± 0.20*	1.52 ± 0.02*	1.41 ± 0.22
IIIA	4.95 ± 0.11*	1.95 ± 0.04*	2.11 ± 0.11*	1.78 ± 0.27 *	1.25 ± 0.30
IVA	3.16 ± 0.23	1.10 ± 0.27	1.33 ± 0.71	0.98 ± 0.20	1.30 ± 0.40

Group IA= 150 mg/kg b.w; Group IIA = 300 mg/kg b.w; Group IIIA = 600 mg/kg b.w; Group IVA = 10 ml/kg distilled water. Each value represents mean±SEM (n = 6); *p<0.05 compared with control

Table 3: Relative organ weight of rats in reversibility study (g/100 g b.w)

Group	Liver	Kidney	Heart	Lung	Testes
IB	2.99 ± 0.61	0.63 ± 0.01	1.66 ± 0.02	1.24 ± 0.14	1.31 ± 0.11
IIB	2.93 ± 0.33	0.98 ± 0.02	1.75 ± 0.04	1.30 ± 0.11	1.52 ± 0.15
IIIB	3.95 ± 0.26	1.12 ± 0.04	1.84 ± 0.07	1.18 ± 0.15	1.21 ± 0.20
IVB	3.02 ± 0.30	0.81 ± 0.02	1.41 ± 0.04	0.83 ± 0.03	1.42 ± 0.24

Group IB= 150 mg/kg b.w; Group IIB = 300 mg/kg b.w; Group IIIB = 600 mg/kg b.w; Group IVB = 10 ml/kg distilled water. Rats were left for 28 days after the 180-day treatment before they were sacrificed. Each value represents mean±SEM (n = 6); *p < 0.05 compared with control,

Table 4: Effects of CEE on serum levels of AST, ALT, ALP, total protein, and albumin

Group	IA	IIA	IIIA	IVA
ALT (U/L)	89.54 ± 14.20	102.10 ± 13.43	129.68 ± 12.82 *	94.73 ± 11.60
AST (U/L)	262.06 ± 18.66	307.61 ± 21.98*	320.92 ± 19.64*	234.51 ± 18.38
ALP (U/L)	169.86 ± 14.20	166.36 ± 18.42	206.33 ± 21.93*	149.11 ± 15.64
Total Protein (mg/L)	78.75 ± 12.70	76.37 ± 10.05	56.30 ± 14.52*	84.41 ± 12.20
Albumin (mg/L)	38.85 ± 5.77	33.80 ± 7.61	29.05 ± 7.26*	46.56 ± 11.30

Group IA = 150 mg/Kg b.w; Group IIA = 300 mg/Kg b.w; Group IIIA = 600 mg/Kg b.w; Group IVA = 10 ml/kg distilled water. Each value represents mean ± SEM (n = 6); *p < 0.05 compared with control

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Table 5: Effects of CEE on serum level of AST, ALT, ALP, total protein, and albumin in reversibility study

Group	IB	IIB	IIIB	IVB
ALT (U/L)	86.71 ± 12.23	101.63 ± 11.50	109.24 ± 13.13	88.47 ± 10.32
AST (U/L)	243.21 ± 16.74	256.32 ± 18.55	261.90 ± 15.51	243.08 ± 18.16
ALP (U/L)	124.85 ± 17.60	135.20 ± 18.41	144.52 ± 14.53	133.65 ± 13.28
Total Protein (mg/L)	72.74 ± 12.32	70.65 ± 13.68	73.62 ± 19.43*	86.44 ± 14.72
Albumin (mg/L)	36.76 ± 4.08	40.82 ± 8.20	36.30 ± 8.64	44.732 ± 9.12

Group IB= 150 mg/kg b.w; Group IIB = 300 mg/kg b.w; Group IIIB = 600 mg/kg b.w; Group IVB = 10 ml/kg distilled water. Rats were left for 28 days after the 180-day treatment before they were sacrificed. Each value represents mean ± SEM (n = 6); *p<0.05 compared with control

Table 6: Hematological parameter in rats treated with CEE for 180 days

Group	IA	IIA	IIIA	IVA
RBC(x10^6/L)	8.28 ± 0.21	6.52 ± 0.43*	6.32 ± 0.11*	10.85 ± 1.04
Hb (g/dL)	14.42 ± 3.20	10.51 ± 3.20	8.86 ± 2.72*	13.60 ± 2.41
PCV (%)	43.54 ± 6.85	38.11 ± 5.03	30.60 ± 5.10*	44.74 ± 4.88
MCV (fL)	55.73 ± 4.61	42.08 ± 6.82	40.64 ± 4.50*	53.99 ± 7.56
MCH (pg)	18.14 ± 2.00	16.75 ± 3.51	13.83 ± 2.78*	19.52 ± 2.06
MCHC (mg/L)	32.62 ± 3.87	33.81 ± 2.20	32.57 ± 3.31	34.06 ± 2.44
Platelet (x10^3/L)	542.51 ± 29.40	589.73 ± 24.22	602.64 ± 29.77	593.91 ± 31.20
WBC (x10^3/L)	16.00 ± 2.87	14.89 ± 1.54	12.32 ± 2.90*	18.68 ± 3 51
Lymphocytes (%)	67.59 ± 5.01	62.08 ± 4.66	58.98 ± 6.70	69.02 ± 5.22
Monocytes (%)	14.61 ± 2.51	13.77 ± 1.21	14.80 ± 2.32	14.00 ± 2.82
Neutrophils (%)	27.20 ± 2.03	22.51 ± 3.64	28.77 ± 4.52	25.57 ± 4.80
Basophils (%)	3.60 ± 0.60	2.91 ± 0.41	3.24 ± 0.50	3.44 ± 0.38
Eosinophils (%)	1.02 ± 0.02	0.96 ± 0.01	1.01 ± 0.02	1.11 ± 0.02

Group IA = 150 mg/kg b.w; Group IIA = 300 mg/kg b.w; Group IIIA = 600 mg/kg b.w; Group IVA = 10 ml/kg distilled water. Each value represents mean ± SEM (n = 6); *p<0.05 compared with control

Table 7: Hematological parameter in rats treated for 180 days in reversibility study

Group	IB	IIB	IIIB	IVB
RBC(x10^6/L)	9.20 ± 1.31	7.82 ± 1.23	6.10 ± 1.41	9.82 ± 1.30
Hb (g/dL)	15.52 ± 1.20	13.67 ± 2.10	12.46 ± 1.22	13.72 ± 2.22
PCV (%)	44.14 ± 5.52	45.63 ± 4.13	32.41 ± 3.10*	44.91 ± 5.63
MCV (fL)	56.33 ± 4.11	55.41 ± 5.52	56.08 ± 4.16	53.55 ± 6.16
MCH (pg)	17.14 ± 2.30	16.65 ± 2.51	18.13 ± 2.08	17.70 ± 1.12
MCHC (mg/L)	32.00 ± 2.10	33.11 ± 3.60	32.97 ± 2.62	34.02 ± 2.74
Platelet (x10^3/L)	527.11 ± 23.40	587.22 ± 32.41	572.64 ± 27.70	582.73 ± 32.50
WBC (x10^3/L)	15.60 ± 2.27	16.81 ± 1.34	18.50 ± 2.20	15.98 ± 2.66
Lymphocytes (%)	63.50 ± 4.82	62.71 ± 7.74	62.68 ± 6.40	68.52 ± 6.31
Monocytes (%)	14.71 ± 2.21	13.42 ± 2.21	14.51 ± 1.82	14.90 ± 2.42
Neutrophils (%)	28.84 ± 2.50	24.33 ± 3.34	28.40 ± 3.22	25.53 ± 3.16
Basophils (%)	3.31 ± 0.12	2.78 ± 0.14	3.23 ± 0.47	3.70 ± 0.23
Eosinophils (%)	1.31 ± 0.02	0.89 ± 0.04	1.23 ± 0.02	1.31 ± 0.01

Group IB = 150 mg/kg b.w; Group IIB = 300 mg/kg b.w; Group IIIB = 600 mg/kg b.w; Group IVB = 10 ml/kg distilled water. Rats were left for 28 days after the 180-day treatment before they were sacrificed. Each value represents mean ± SEM (n = 6); *p<0.05 compared with control

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Reduction in RBC, PCV, and Hb indicates that CEE induced anemia in the rats. Generally, the causes of anemia can be categorized as blood loss, increased blood destruction and decreased blood production.[31] Treatment of rats with CEE might have led to the development of anemia through one or more of these ways. In addition, anemia is usually associated with kidney damage. A major reason for this is the loss of peritubular fibroblasts within the renal cortex that synthesize erythropoietin (EPO). Impairment of EPO production in the kidney leads to low concentrations within the blood for the concomitant hemoglobin concentration.[32] (Panjeta *et al*., 2017). Other causes of anemia include absolute or functional iron deficiency, folic acid and vitamin B12 deficiencies, chronic inflammation, and reduced red cell survival.[33] Change in body weight is also an important symptom of anemia, not only with regard to dietary intake, but because it suggests the presence of malabsorption or an underlying wasting disease of infectious, metabolic or neoplastic origin.[34] Significant loss in weight of rats treated with CEE indicates that its toxicity is high at the doses and duration of treatment. White blood cell count (WBC) in the CEE-treated rats is significantly higher than in the control. This suggests infection, inflammation, and/or tissue damage in the rats following prolonged use of the extract.[35]

Administration of CEE at 300 and 600 mg/kg b.w for 180 days caused changes in structural architectures of liver, kidney, lung and heart of the treated rats. Kidney section of the control rats showed renal tissue with normal architecture. The tubules, afferent and efferent arterioles, and stroma appear normal. Renal tissues of rats treated with CEE appeared distorted with slightly larger renal cells. There were obliterated areas and few red blood cells seen within the renal glomeruli. Section also shows hyanilization, areas of hemorrhage, congested vessels and the basement membrane appears thickened. Following the withdrawal of CEE for 28 days, sections from the treated rats still showed some distorted renal parenchyma with proliferation of tubules, forming compact structures. However, there are signs of regeneration as most of the glomeruli appeared normal, and there were no inflammatory cells seen (Figure 3).

Liver sections of control rats showed normal hepatic cells with well-preserved cytoplasm. The central vein appeared normal and well brought out. Sections from the CEE-treated rats showed hemorrhaging of the central vein, vessel dilation, and congestion. Inflamed hepatocytes, edema in hepatic parenchyma and degeneration of the hepatocytes were also seen. In the reversibility studies, liver sections of CEE-treated rats appeared to have background tissue damage with signs of regeneration. Hepatic cells were seen as near normal and the central vein was well brought out (Figure 4).

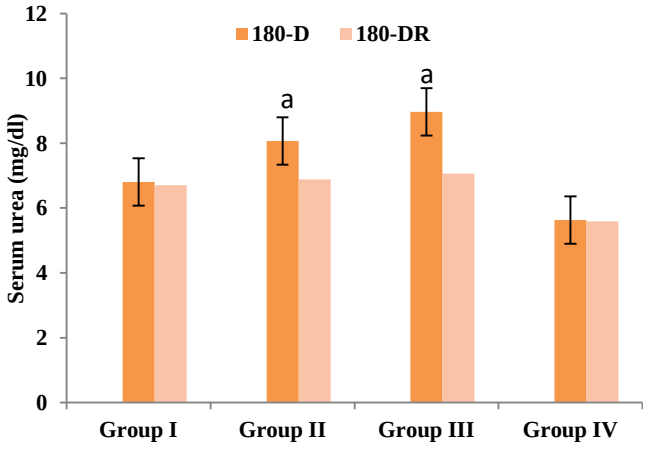

Figure 1: Serum urea in rats following treatment with CEE for 180 days.
Group I = 150 mg/g b.w; Group II = 300 mg/kg b.w; Group III = 600 mg/kg b.w; Group IV = 10 ml/kg distilled water (n = 6); $^{a}p < 0.05$ compared with control. 180-D = group of rats treated for 180 days and sacrificed immediately; 180-DR = group of rats treated for 180 days and sacrificed 28 days later for reversibility study.

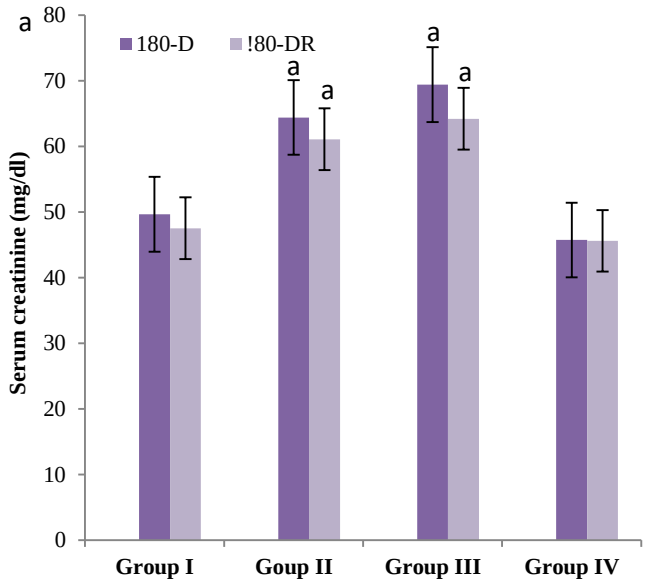

Figure 2: Serum creatinine in rats following treatment with CEE for 180 days.
Group I = 150 mg/g b.w; Group II = 300 mg/kg b.w; Group III = 600 mg/kg b.w; Group IV = 10 ml/kg distilled water (n = 6); $^{a}p < 0.05$ compared with control. 180-D = group of rats treated for 180 days and sacrificed immediately; 180-DR = group of rats treated for 180 days and sacrificed 28 days later for reversibility study.

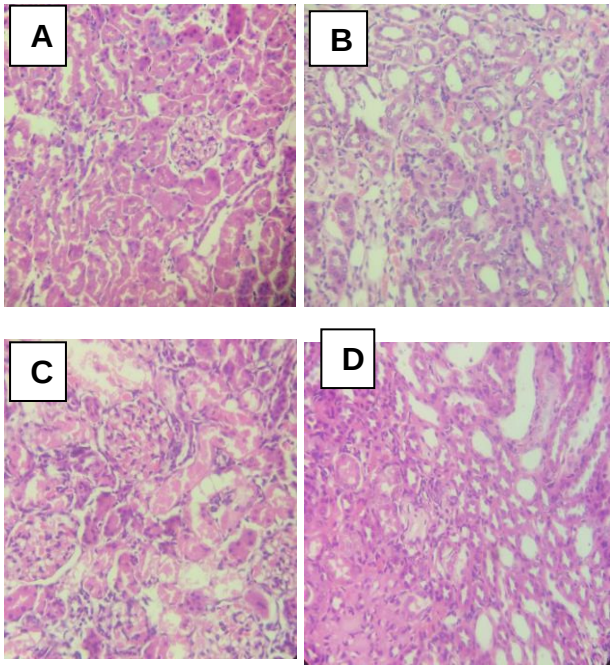

Figure 3: Kidney sections of rats treated with CEE for 180 days.
(A) Kidney section of rats treated with distilled water; (B) Kidney section of rats treated with 300 mg/kg b.w; (C) Kidney section of rats treated with 600 mg/kg b.w; (D) Kidney section of rats in the reversibility study. (x 300 magnification). Section from the control showed renal tissue with normal architecture. The tubules, afferent and efferent arterioles, and stroma appear normal. Renal tissues of rats treated with CEE appeared distorted with slightly larger renal cells. Sections also show hyanilization, areas of hemorrhage, congested vessels and the basement membrane appears thickened. In section D, there are signs of regeneration as most of the glomeruli appeared normal, and no inflammatory cells are seen.

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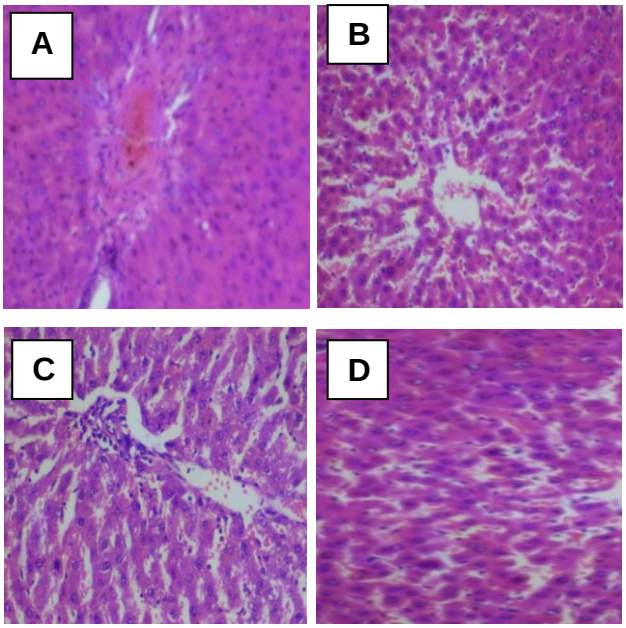

Figure 4: Liver sections of rats treated with CEE for 180 days. (A) Liver section of rats treated with distilled water; (B) Liver section of rats treated with 300 mg/kg b.w; (C) Liver section of rats treated with 600 mg/kg b.w; (D) Liver section of rats in the reversibility study. (x 300 magnification). Liver sections of control rats showed normal hepatic cells with well-preserved cytoplasm. The central vein appeared normal and well brought out. Sections from the CEE-treated rats showed hemorrhaging of the central vein, vessel dilation, and congestion. Inflamed hepatocytes, edema in hepatic parenchyma and degeneration of the hepatocytes were also observed. In the reversibility study, liver section appeared to have background tissue damage and signs of regeneration. Hepatic cells were seen as near normal.

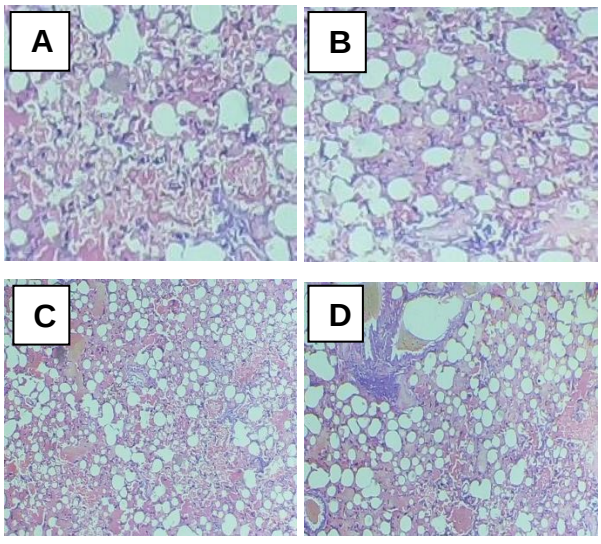

Figure 5: Lung sections of rats treated with CEE for 180 days. (A) Lung section of rats treated with distilled water; (B) Lung section of rats treated with 300 mg/kg b.w; (C) Lung section of rats treated with 600 mg/kg b.w; (D) Lung section of rats in the reversibility study. (x 300 magnification). Section of lung from control rats show normal lung parenchyma and alveoli. Sections from CEE-treated rats show appreciable infiltration of the intestitium by inflammatory cells. There was also extravasations of red blood cells into the interstitium and congestion of some dilated alveolar spaces. Section from reversibility study show hyperplasia of the alveolar septae but there were signs of tissue regeneration.

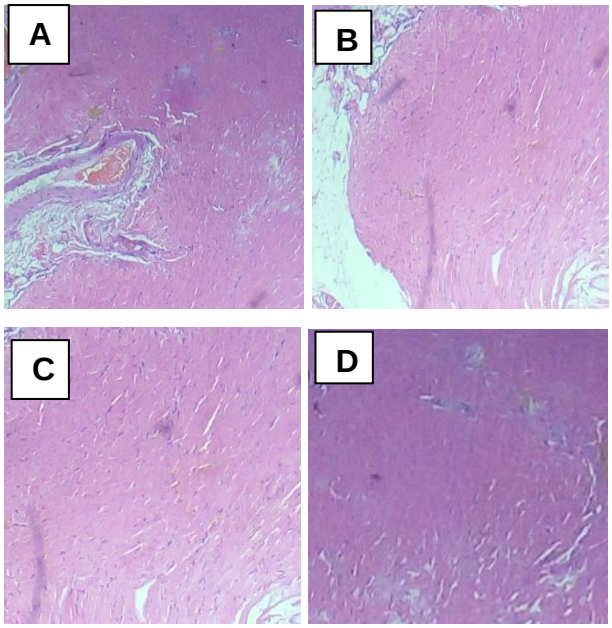

Figure 6: Heart sections of rats treated with CEE for 180 days. (A) Heart section of rats treated with distilled water; (B) Heart section of rats treated with 300 mg/kg b.w; (C) Heart section of rats treated with 600 mg/kg b.w; (D) Heart section of rats in the reversibility study. (x 300 magnification). Sections from the heart of control rats show normal fascicles and cardiac myocytes. Sections from the heart of rats treated with CEE show infiltration of inflammatory cells around cardiac myocytes, but there were no significant histological changes in the heart of rats in the reversibility study.

As shown in Figure 5, sections of lung from control rats showed normal lung parenchyma and alveoli. Following repeated administration of CEE, lung section show appreciable infiltration of the intestitium by inflammatory cells. There was also extravasations of red blood cells into the interstitium and congestion of some dilated alveolar spaces. Few congested thick and thin walled vascular channels were also seen. Section of lung of rats in the reversibility studies showed hyperplasia of the alveolar septae but there were signs of tissue regeneration. Sections from the heart of control rats show normal fascicles and cardiac myocytes. Sections from the heart of CEE-treated rats showed infiltration of inflammatory cells around cardiac myocytes, but there were no significant histological distortions in the heart of treated rats after CEE was withdrawn for 28 days (Figure 6). Distortion of structural architecture of the kidney, liver, lung, and heart is further evidence that CEE produced toxic effects in the rats.

Conclusion

The results suggest that chronic use of *Newbouldia laevis* leaves may be toxic to vital organs in the body, including the kidney, liver, heart and lung. However, the adverse effects are reversible when the extract is withdrawn. Therefore, caution should be exercised when it is used for therapeutic purposes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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