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Original Research Article

Evaluation of The Toxic Effects of Ethanolic Leaf Extract of *Tapinanthus cordifolius* in Mice and Rats

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ABSTRACT

Tapinanthus cordifolius (TC), a hemiparasitic plant in the Loranthaceae family and the *Tapinanthus* genus, possesses numerous medicinal properties. In order to establish its medicinal quality and toxicity profile for appropriate application in traditional medicine. This study was designed to determine the safety profile of *Tapinanthus cordifolius*. Oral acute and sub-chronic toxicity studies of the ethanolic extract of *Tapinanthus cordifolius* were evaluated. The OECD (No. 423) limit test was followed to determine the LD_{50} in Swiss mice, while for the sub-chronic toxicity in Wistar rats, the OECD 407 guideline was followed. Acute toxicity studies in rats revealed no mortality or observable toxicity symptoms at doses of 2000, 5000 mg/kg for a 14-day observation period. The 28-day toxicity tests of the extract showed no significant changes in body weight, food and water intake, urine output and faecal output. Haematological parameters, kidney function indices, and liver function parameters showed no significant effect after treatment with *Tapinanthus cordifolius*. Histological examination of various organs showed no changes in the normal architecture of the cells in the tissues of the organs. Thus, based on the results, *Tapinanthus cordifolius* is considered relatively safe when administered orally at the dose tested.

Keywords: *Tapinanthus cordifolius*, safety profile, Acute toxicity, sub-chronic toxicity

Introduction

Tapinanthus cordifolius is an African mistletoe in the Loranthaceae family and the *Tapinanthus* genus.[1,2] Mistletoe refers to woody shoot parasites found in a variety of plant groups, including the Loranthaceae and Viscaceae families.[1] The Hausa, Yoruba, and Ibo speakers in Nigeria refer to it as "kaucì", "ewe afomo," and "ohumagana" respectively.[1] It is an obligatory hemi-parasitic evergreen tropical plant that grows on a wide range of trees.[1] In West Africa, mistletoe can be found on a variety of indigenous trees as well as several economically important tree crops, such as shea butter, neem, cocoa, and rubber. Mistletoe is prevalent in South-western Nigeria, especially on tree crops such as cocoa, kola, coffee, bush mango, and others. Mistletoe can also be found on citrus trees such as oranges and guava trees. It grows as a parasitic plant on the branches and trunks of trees, sending out haustoria that pierce the tree to absorb nutrients. Mistletoe, like other plants, can also grow on its own and make its sustenance through photosynthesis.[3]

Mistletoe is commonly utilized in practically every culture to treat various conditions such as hypertension, cancer, and diabetes, or as a diuretic agent.[1,2] In Nigeria, mistletoe is used to treat a variety of human and animal problems, including stomach aches, diarrhoea, dysentery, wounds, and cancer. Ruminants and local fowls enjoy it with no documented digestive problems.[3,4]

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Mistletoe has been shown to have hypoglycaemic effects, due to its ability to lower blood glucose levels and to regulate weight loss in people with diabetes.[5,6] Mistletoe has also been used to treat conditions such as epilepsy, male and female infertility, menopausal syndrome, and rheumatism.[6] The existence of bioactive chemicals, the composition of which varies depending on the host plant, has been related to the health benefits of African mistletoes.[7] As a result, they are suitable as botanical sources of therapeutic agents for the treatment of a variety of ailments.

The use of medicinal plants as a source of bioactive compounds has been an effective strategy for solving global health problems due to their numerous beneficial effects.[8] However, several scientific studies have suggested that medicinal plants and their compounds should be used with caution. Overdosing, prolonged use, contaminations, adverse effects of bioactive chemicals, interactions, and allergies are some of the hazardous effects that might occur after treatment.[9,10] As a result, it is necessary to investigate their chemical profiles and establish their toxicological potentials to maximize therapeutic results while minimizing potential adverse effects.

The Phytochemical and toxicity studies of some mistletoes have been reported, however, there are little or no reports on the toxicity of *Tapinanthus cordifolius*. Jemilat *et al*. investigated the profile of basic secondary metabolites in many Nigerian Loranthaceae, including *Tapinanthus cordifolius* from *Citrus auranthifolia*, *Cassia sp*, *Syzygium eucalyptoide*, and *Ficus sp*.[11] They reported the occurrence of chemical variations in the same species found on different hosts and advised caution when using Loranthaceae.[12] Chemical changes caused by different host species may also result in differences in the toxicological effects of the plants. As a result, the acute and sub-chronic toxicological profiles of ethanolic *Tapinanthus cordifolius* extract were examined in this study.

Materials and Methods

Plant collection and preparation of extracts

Fresh plants of *Tapinanthus cordifolius* were collected in January 2022, from *Citrus sinensis* (Orange) tree at Nibo in Awka South, Anambra State, Nigeria. The plant was identified and authenticated at the National Institute for Pharmaceutical and Research Development (NIPRD), Idu, Abuja, Nigeria. Identification was done by a taxonomist, Mr. Lateef A. Akeem, with the specimen deposited in the institute's herbarium. Voucher reference number NIPRD/H/7203 was assigned to *Tapinanthus cordifolius*. The plant was carefully cleaned, and air-dried at room temperature (25°C) for four weeks; afterward, it was pounded into a fine powder using a mortar and pestle. *Tapinanthus cordifolius* powder (2.8kg) was macerated in 22 L of ethanol (85%) for 72 hours, four times consecutively. The extract was filtered using muslin cloth and concentrated at 78°C using a rotary evaporator, and the percentage yield was calculated. The extracts were stored in an airtight glass container and kept inside the refrigerator.[13]

Experimental Animals

Wistar rats (130–148 g) and Swiss mice (23–30 g) were procured from the Animal House Facility of the National Institute of Pharmaceutical and Research Development (NIPRD) in Abuja, Nigeria. Before commencing the research, the animals were allowed two weeks to acclimatize. They were fed standard animal feed and given free access to water. The National Health Research Council's recommendations for the care and use of laboratory animals were followed. For the use of animals, an ethical approval with the number (NIPRD/05:03:05-24) was granted by the Institutional Animal Care and Ethics (ACE) Committee of NIPRD.

Oral Acute toxicity test

Acute toxicity assessment was done according to Organization for Economic Cooperation and Development (OECD) guidelines 423.[14] A total of 12 mice were weighed, randomized, and placed in 3 groups of 3 mice each. They were left to acclimatize to their environment for 2 weeks. The animals were left to fast overnight, and then a toxicity test was carried out using a control and two test groups as follows: Group 1: Distilled Water 10 ml/kg; Group 2: *Tapinanthus cordifolius* extract (2000 mg/kg); Group 3: *Tapinanthus cordifolius* extract (5000 mg/kg). The animals were observed closely for behavioral changes including hyperactivity, aggression, passivity, stereotype and signs of toxicity such as changes in respiration, abdominal constriction, diarrhoea, sedation, tail erection, tremors, convulsion, coma, and death. Animals were observed closely for the first 30 minutes after the extract was administered and at different time intervals for 24 hours, with more focus on the first 4 hours, then daily for 14 days. On the 15th day, the animals were euthanized using ketamine/xylazine (60 mg/kg and 6 mg/kg respectively). The internal organs were isolated and placed on a filter paper to blot out excess blood. The organs were observed and weighed. The relative organ weight was then calculated using the formula:

$$Relative\ organ\ weight = \frac{weight\ of\ organ\ (g)}{weight\ of\ the\ animal\ on\ the\ day\ of\ sacrifice\ (g)} \times 100$$

Afterward, histopathology assessments were carried out on the organs. Plasma was also collected for biochemical and hematological assessments.

Sub-chronic toxicity assessments in rats

Sub-chronic toxicity assessment was done according to the Organization for Economic Cooperation and Development (OECD) guidelines 407 (OECD, 2008). Before the treatment commenced, the rats were placed in metabolic cages (Techniplast metabolic cage systems for rats) for a seven-day acclimatization period. The doses used for the toxicity test were estimated from the highest dose of the acute toxicity study. Rats of both sexes were grouped into three groups: one control and the treatment groups. The extract was prepared fresh every day and a single dose was administered using a gavage while the control group received water. Behavioral changes were observed, water intake, faecal output, urine output, and food intake daily. The rats were also weighed once every week. At the end of the experiment, the rats were euthanized using ketamine/xylazine (60 mg/kg and 6 mg/kg respectively) and then sacrificed. The blood sample was collected by cardiac puncture into plain bottles for biochemical assessment and EDTA bottles for haematological analysis. Organs were harvested for histological assessment. The organs were observed weighed, and preserved for histological studies. The relative organ weight was calculated. Biochemical analysis: Kidney function test, lipid profile, liver function test and haematological analysis were done.

Biochemical assay

Biochemical evaluations including liver and kidney functions and lipid profile were carried out on blood samples. The blood plasma was collected by centrifuging at 3000 rpm for 5 min. The Randox test kits (Randox Laboratories Limited, Crumlin, County Antrim, BT294QY, United Kingdom) following the manual's instruction. The following parameters were measured: total protein, bilirubin, urea, creatinine, albumin, high-density lipoprotein (HDL) cholesterol, aspartate transaminase (ALT), alanine transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and gamma-glutamyl transferase (GGT). A spectrophotometer was used to conduct the measurements in order to produce precise and quantitative data (model T80, UV/visible, double beam, U.K).[15]

Haematological assays

A computerised haematology analyzer (2800 Haematology Auto-Analyzer) was used to perform a haematological examination on the blood samples. White blood cell (WBC), red blood cell (RBC), haemoglobin concentration (Hg), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were among the 14 parameters that were assessed.

Histopathological examination

Following a thorough histological examination, the kidney and liver samples from each rat were promptly preserved in 10%v/v formalin in normal saline and dehydrated using increasing isopropyl alcohol concentrations (80-100%) for a period of seven days. The paraffin-embedded organs were cut into paraffin slices using a Leica rotary microtome (Bright B5143 Huntington, England) that was 5 m thick. The usual steps of deparaffinization, hydration, staining, rinsing, and clearing in xylene were then followed by the routine staining of the sections with hematoxylin and eosin (H&E). A Leica DM750 Camera Microscope (X 400) was used to take photomicrographs while slides were being examined under a light microscope, this was done at 100x magnification.[16,17] If > 5% of the hepatocytes on a hematoxylin and eosin(H&E) stained slide had a fatty liver, it was determined by the presence of macro or microvesicular fat and a measurement of adipocyte size.[18]

Statistical analysis

The data were presented as mean ± standard error mean (SEM). The data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA). The statistics carried out included the one-way analysis of variance (ANOVA) and Duncan's post-hoc test was used to determine significant differences, which was considered at $p < 0.05$.

Results and Discussion

The pharmacological activities of medicinal plants are mostly attributable to bioactive phytochemicals synthesized in plant tissues as primary and secondary metabolites.[19] Plants produce these non-nutritive bioactive chemicals to defend themselves against pathogenic microbes, dangerous insects, and severe environmental changes. Not only do phytochemicals protect plants, but they can also protect humans and animals from certain diseases.[20] Hence, medicinal plants have been a major source of drugs for the treatment of a variety of diseases.[21,22] To serve as a guide, for its use in traditional preparations and as a source of new drugs, the safety profile of *Tapinanthus cordifolius* extract was evaluated.

Acute Toxicity Evaluation of Ethanol Extracts of TC

The ethanolic extract of *Tapinanthus cordifolius* extract was investigated for its safety and potential negative effects. An oral acute toxicity test was conducted on *Tapinanthus cordifolius* extract to determine the potential short-term adverse effect that might occur during the use of the extract or due to accidental or deliberate oral exposure to a high dose of the extract. The toxicity result revealed that there was no fatality or apparent symptom of toxicity in the mice orally treated with 2000 mg/kg and 5000 mg/kg for the first 24 hours and the following 14 days of observation. There was also no significant ($p<0.05$) change observed in the body weight of the animals (Table 1). The relative organ weight also showed no significant change when compared to the control group (Table 2).

Sub-chronic toxicity test

Sub-chronic studies are designed to find possible side effects of medications or test agents after they have been taken frequently for a given period. Throughout the 28-day administration period of *Tapinanthus cordifolius* extract, no appreciable alterations in the test animals' body weight were noted. The food and water consumption, as well as urine and faeces excretion, did not alter, indicating that *Tapinanthus cordifolius* extract did not affect the uptake and absorption of nutrients from meals.[23,24] There was a similar behavioral pattern observed between the control and treatment groups. There was a continuous increase in weight in all treatment groups though no observable significant difference on Days 7, 14, 21 and 28. There was no significant change in the water and food intake, faecal and urine output of the treatment groups when compared to the control groups as shown in Table 4.

Table 1: Effect of *Tapinanthus cordifolius* extract on bodyweight of mice after acute oral administration

Treatment	Dose	BW(Kg)		
		Day 0	Day 7	Day 14
Control	10ml/kg	0.038 ± 0.002	0.041 ± 0.004	0.043 ± 0.004
TC	2000	0.032 ± 0.006	0.034 ± 0.007	0.035 ± 0.003
TC	5000	0.035 ± 0.005	0.037 ± 0.003	0.038 ± 0.003

Data are represented as Mean ± SEM; Two-way ANOVA, no significant difference treatment vs. control group $p<0.05$. TC= *Tapinanthus cordifolius* extract (mg/kg)

Table 2: Effect of *Tapinanthus cordifolius* Extract on relative organ weight of mice after acute oral administration

Organs	Control	2000 mg/kg TC	5000 mg/kg TC
Heart(g)	0.53 ± 0.06	0.46 ± 0.11	0.45 ± 0.14
Liver(g)	4.77 ± 0.62	5.33 ± 0.71	5.30 ± 0.52
Pancreas(g)	0.28 ± 0.06	0.27 ± 0.03	0.29 ± 0.06
L. kidney(g)	0.64 ± 0.04	0.72 ± 0.05	0.77 ± 0.09
R. kidney(g)	0.67 ± 0.05	0.67 ± 0.05	0.67 ± 0.07
Ovary(g)	0.93 ± 0.06	0.87 ± 0.10	0.92 ± 0.30
Brain(g)	1.44 ± 0.11	1.20 ± 0.09	1.18 ± 0.09
Spleen(g)	0.95 ± 0.32	0.93 ± 0.19	0.90 ± 0.16
Lung(g)	0.87 ± 0.08	0.77 ± 0.05	0.82 ± 0.02
Stomach(g)	1.44 ± 0.31	1.64 ± 0.36	1.68 ± 0.35

Data are represented as Mean ± SEM; Two-way ANOVA, no significant difference treatment vs. control group $p<0.05$.

Effects of Tapinanthus cordifolius extract on the Haematological parameters

Treatment of mice and rats with *Tapinanthus cordifolius* extract did not show any significant difference in the haematology parameters compared to the control, as shown in Tables 3 and 7. The extract appears not to cause any damage to the blood cells of the mice and rats as shown by the result of the haematological parameters

Effects of Tapinanthus cordifolius Extract on Some Biochemical parameters

The treatment of mice with the *Tapinanthus cordifolius* extract did not cause any significant difference in most liver function indices. The serum levels of the albumin, protein, total bilirubin, and direct bilirubin were comparable to the control values (Table 6). The activity of the liver enzymes (alanine transaminase, aspartate transaminase, gamma-glutamyltransferase and lactate dehydrogenase) were not significantly different from the control values. However, there was an increase in the alkaline phosphatase activity when compared to the control value. The renal function parameters including urea, creatinine and uric acid of the treated rats were not significantly different when compared with the control. For the electrolytes, the concentrations of the electrolytes were not significantly different from those of the control. Similarly, there was no significant difference in the lipid profile comprising cholesterol, triglycerides, and lipoproteins when compared with the control (Table 6). The extract also caused no significant changes to any of the kidney function indices, and except for alkaline phosphatase, there was no significant change in the liver function parameters tested. For the electrolytes, sodium, chloride and phosphate levels were significantly reduced while calcium level was significantly increased.

Effects of Tapinanthus cordifolius Extract on Relative Organ Weight

The relative organ weights of mice and rats treated with *Tapinanthus cordifolius* extract are shown in Tables 2 and 5 respectively. When the control was compared to the treatment group, there was no significant change observed. There were no observable lesions, swellings, or congestion signs observed.

Organ weight variations are frequently evaluated in toxicological research and are an important marker of chemically-induced changes in biological systems. Depending on the goals of the investigation, other organs may be of interest. However, the potential toxic effect could have been counteracted by the antioxidant action of some of the bioactive compounds present in the extract as there was no observable damage to the organ as revealed by the histological examinations.[25] There were no discernible differences between the *Tapinanthus cordifolius* extract-treated rats in this study of their relative organ weights and the control group after 28 days of study. This implies that the phytochemical

constituents of *Tapinanthus cordifolius* extract might not significantly interfere with regular physiological functions that could result in internal organ toxicological consequences.

Effect of Tapinanthus cordifolius extract on the histological examination of organs

Macroscopic examinations of the organs showed no abnormality in the normal features and colour of the heart, liver, pancreas, small intestine, kidney, ovary/testes, brain, spleen, lung and stomach of animals treated with 2000mg/kg. The cellular structures of the treated groups were normal without noticeable pathological changes compared to the control (Table 8). However, the histological investigation of the organs including lungs, kidneys, and liver revealed no apparent congestion, lesions, necrosis, or edema. Because of their sensitivity to foreign substances and the ability to provide insight into the mechanism of toxicity, these organs are highly relevant in toxicity research[26]. Changes in membrane permeability and cell death caused by a chemical's harmful effects could raise the serum levels of these enzymes. Increased levels of liver-specific enzymes like ALP, as observed in this study, indicate a change in the functional integrity of hepatocyte cell membranes and the possibility of liver lesions.[27,28]

Table 3: Haematological parameters of mice after a 14-day treatment with *Tapinanthus cordifolius* extract

Parameter	Control	2000 mg/kg TC	5000 mg/kg TC
WBC($x10^9$/L)	3.27 ± 0.24	4.03 ± 0.13	3.78 ± 0.51
LYM($x10^9$/L)	3.42 ± 0.84	3.47 ± 0.98	3.20 ± 0.76
MXD	0.51 ± 0.12	0.50 ± 0.13	0.50 ± 0.21
NEUT($x10^9$/L)	0.18 ± 0.04	0.17 ± 0.02	0.23 ± 0.19
RBC ($x10^{12}$/L)	3.67 ± 0.43	4.33 ± 0.78	4.78 ± 0.66
HGB(g/L)	135.67 ± 8.84	150.75 ± 15.74	143.30 ± 1.76
HCT%	17.23 ± 1.55	20.51 ± 3.62	22 ± .83 ± 0.57
MCV(fL)	46.87 ± 1.10	44.43 ± 1.45	45.05 ± 1.05
MCH(Pg)	37.66 ± 2.26	34.46 ± 1.87	35.99 ± 1.73
MCHC	797.00 ± 28.62	777.50 ± 69.71	778.30 ± 52.22
PLT (10^9/L)	480.67 ± 33.45	469.50 ± 32.53	421.80 ± 29.62
MPV (fL)	8.12 ± 1.50	5.19 ± 0.16	7.83 ± 0.96
PDW(Fl)	14.80 ± 0.00	14.86 ± 0.05	15.03 ± 0.32
PCT	0.41 ± 0.13	0.23 ± 0.05	0.32 ± 0.07

Data are represented as Mean ± SEM (3). WBC, LYM, MXD, NEUT, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, MPV, PDW, PCT represent white blood count, lymphocytes, mixed cell percentage, neutrophil, red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean platelet volume, platelet distribution width, and plateletcrit respectively. TC=*Tapinanthus cordifolius* extract

Table 4: Effect of *Tapinanthus cordifolius* Extract on body weight, water and food intake, faecal and urine output of rats

Parameter	D_0	D_7	D_{14}	D_{21}	D_{28}
		Vehicle 10 ml/kg			
Body weight (kg)	163.00 ± 11.79	189.66 ± 5.69	202.66 ± 3.28	209.00 ± 5.19	219.00 ± 4.72
Food consumption (g)	_	13.33 ± 0.33	13.00 ± 0.57	14.66 ± 0.66	16.00 ± 0.57
Water intake (mL)	_	17.00 ± 1.08	20.00 ± 1.19	16.66 ± 0.88	19.33 ± 1.17
Faecal Output (g)	_	9.00 ± 0.57	9.33 ± 0.33	10.66 ± 0.66	12.00 ± 1.15
Urine Output (mL)	_	2.75 ± 0.12	1.22 ± 0.14	0.98 ± 0.091	0.75 ± 0.07
		TCE 800 mg/kg			
Body weight (kg)	157.34 ± 7.44	172.45 ± 6.21	190.64 ± 11.7	196.47 ± 9.00	204.61 ± 7.64
Food consumption (g)	_	10.55 ± 0.74	12.87 ± 0.76	11.33 ± 0.41	12.63 ± 0.99
Water intake (mL)	_	22.66 ± 1.18	20.33 ± 0.88	19.00 ± 0.57	21.33 ± 0.82
Faecal Output (g)	_	7.03 ± 0.09	7.42 ± 1.51	7.36 ± 0.17	7.81 ± 0.83
Urine Output (mL)	_	1.51 ± 0.80	1.16 ± 0.31	0.86 ± 1.04	0.51 ± 0.45
		TCE 1600 mg/kg			
Body weight (kg)	145.33 ± 9.67	167 ± 10.30	173 ± 12.50	182.66 ± 11.42	194.66 ± 10.42
Food consumption (g)	_	11.33 ± 0.66	11.66 ± 1.33	12.00 ± 1.15	12.33 ± 0.66
Water intake (mL)	_	18.65 ± 1.33	19.05 ± 0.45	19.85 ± 1.06	20..49 ± 1.09
Faecal Output (g)	_	7.66 ± 0.66	7.33 ± 1.20	8.00 ± 1.52	8.66 ± 0.88
Urine Output (mL)	_	1.53 ± 0.11	1.369 ± 0.11	0.915 ± 0.10	1.146 ± 0.17

Table 5: Effect of *Tapinanthus cordifolius* Extract on relative organ weight of rats after 28-day oral treatment

Organs	Control	800 mg/kg TC	1600 mg/kg TC
Heart	0.34 ± 0.02	0.43 ± 0.03	0.36 ± 0.02
Liver	2.83 ± 0.13	3.12 ± 0.43	2.93 ± 0.21
Pancreas	0.29 ± 0.03	0.25 ± 0.06	0.28 ± 0.04
Small Intestine	0.14 ± 0.04	0.22 ± 0.04	0.12 ± 0.01
Kidney	0.32 ± 0.01	0.33 ± 0.01	0.34 ± 0.02
Ovary/Testes	1.29 ± 0.05	0.55 ± 0.10	1.33 ± 0.07
Brain	0.76 ± 0.08	0.83 ± 0.13	0.90 ± 0.06
Spleen	0.20 ± 0.02	0.35 ± 0.08	0.35 ± 0.08
Lung	0.58 ± 0.06	1.08 ± 0.15	1.08 ± 0.15
Stomach	0.77 ± 0.04	1.09 ± 0.05	1.09 ± 0.05

TC=*Tapinanthus cordifolius* extract

Table 6: Serum biochemical parameters of rats after 28-day oral treatment with *Tapinanthus cordifolius* Extract

Parameters	Control	800 mg/kg TC	1600 mg/kg TC
T-Protein (g/dL)	7.44 ± 0.32	8.00 ± 0.56	7.36 ± 0.41
Albumin (g/dL)	4.48 ± 0.04	3.85 ± 0.96	4.33 ± 0.27
Globulin (g/dL)	2.96 ± 0.28	4.15 ± 0.48**	3.04 ± 0.25*
T-bil (mg/dL)	18.56 ± 0.77	19.67 ± 1.03	18.38 ± 1.06
D-bil (mg/dL)	20.91 ± 1.13	25.18 ± 4.36**	19.84 ± 1.64
Urea (mg/dL)	53.25 ± 0.51	54.66 ± 3.55	57.06 ± 1.10*
Creatinine(umol/L)	1.78 ± 0.13	1.33 ± 0.13**	1.57 ± 0.26*
Uric-acid (mg/dL)	4.74 ± 0.49	4.58 ± 0.35	4.47 ± 0.28
T-Glucose	56.97 ± 6.33	55.12 ± 7.01	77.34 ± 7.75*
T-chol (mg/dL)	92.60 ± 3.82	92.86 ± 4.77	90.28 ± 6.34
Trig (mg/dL)	70.26 ± 2.08	67.11 ± 3.24	91.63 ± 9.02*
HDL-C (mg/dL)	63.84 ± 9.94	57.57 ± 6.75	59.09 ± 3.06
LDL-C (mg/dL)	28.76 ± 10.74	35.29 ± 2.67*	15.75 ± 23.67**
Sodium (mmol/L)	127.34 ± 1.09	126.96 ± 1.98	128.20 ± 1.25
Potassium(mmol/L)	5.96 ± 0.42	4.91 ± 0.57	6.13 ± 0.33
Chloride (mmol/L)	83.68 ± 0.71	81.12 ± 0.87	80.03 ± 0.17
Bicarb (mmol/L)	42.55 ± 2.67	54.27 ± 8.30*	43.06 ± 0.73
Calcium(mg/dL)	7.69 ± 0.15	7.75 ± 0.79	8.22 ± 0.14
Phosph(mmol/L)	15.63 ± 1.66	18.84 ± 1.21*	17.69 ± 0.68
ALP(U/L)	89.94 ± 16.88	98.53 ± 8.04	116.62 ± 1.11**
ALT(U/L)	15.62 ± 2.48	15.30 ± 6.22	17.19 ± 2.08**
AST(U/L)	61.05 ± 12.70	78.25 ± 6.77*	71.93 ± 11.75
LDH(U/L)	204.06 ± 27.81	198.10 ± 32.83**	215.52 ± 11.16
GGT(U/L)	9.63 ± 2.75	8.25 ± 2.38	11.01 ± 4.96

HDL=High density lipoprotein, LDL=Low density lipoprotein, Alkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Lactate dehydrogenase (LDH), Gamma-glutamyl transferase (GGT), Total bilirubin (T-Bil), Direct bilirubin (D-Bil). Data represented as Value ± SEM (n=6). TC=*Tapinanthus cordifolius* extract

In this investigation, *Tapinanthus cordifolius* extract had no discernible detrimental impact on the architecture of cells or tissues. This implies that there may not be any compounds in the extract that could have a major impact on the cell's development and function.[29]

Conclusion

The toxicity profile of the ethanol extract of *Tapinanthus cordifolius* was evaluated in order to establish its medicinal quality and safety for proper application in traditional medicine. There was no mortality or apparent toxicity symptom in the rats exposed to different doses of *Tapinanthus cordifolius*. The extract had little or no effects on haematological, biochemical and histological parameters of the rats.

Therefore, *Tapinanthus cordifolius* extract can be said to be relatively safe.

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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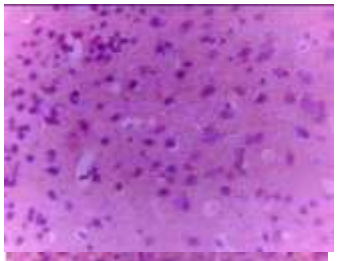
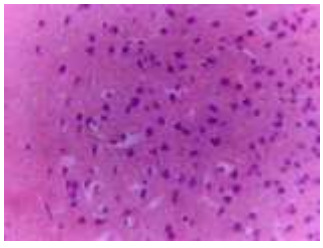
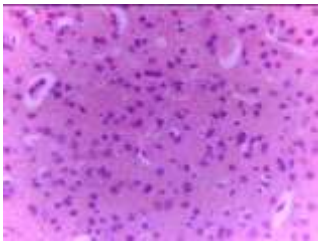
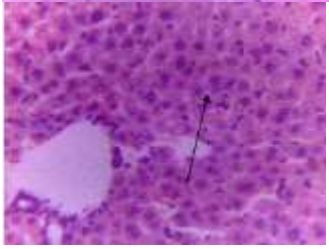
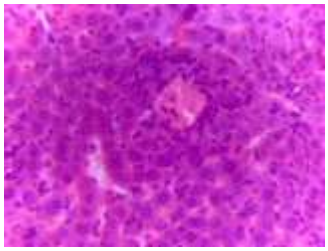
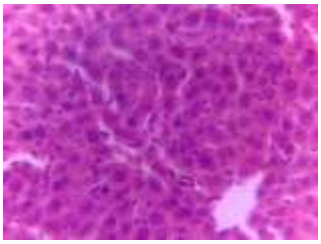
Table 7: Haematological of Rats after 28-day Treatment with *Tapinanthus cordifolius* extract

Parameter	Control	TC 800 mg/kg	TC 1600 mg/kg
WBC($x10^9$/L)	4.25 ± 0.31	5.57 ± 0.70	3.27 ± 0.73
LYM($x10^9$/L)	1.68 ± 0.58	2.21 ± 0.71	1.66 ± 0.22
MXD (%)	1.11 ± 0.42	1.10 ± 0.28	0.79 ± 0.19
NEUT($x10^9$/L)	1.01 ± 0.30	2.26 ± 0.73	0.82 ± 0.12
RBC($x10^{12}$/L)	7.95 ± 0.40	7.83 ± 0.26	6.86 ± 0.22
HGB(g/L)	147.33 ± 2.40	152.33 ± 4.19	124.33 ± 6.06
HCT (%)	38.00 ± 1.69	36.44 ± 1.56	32.28 ± 1.98
MCV (fL)	47.80 ± 0.40	46.53 ± 1.29	46.97 ± 1.51
MCH(Pg)	18.61 ± 0.69	19.49 ± 1.83	18.15 ± 0.37
MCHC (g/dl)	388.67 ± 13.25	417.33 ± 17.39	386.67 ± 14.70
PLT (10^9/L)	691.00 ± 32.43	426.33 ± 34.11*	600.67 ± 32.34
MPV (fL)	7.28 ± 0.05	7.09 ± 0.11	7.48 ± 0.47
PDW (fL)	19.36 ± 4.16	19.42 ± 4.13	19.16 ± 4.26
PCT (ng/mL)	0.49 ± 0.04	0.49 ± 0.02	0.43 ± 0.04

Data are represented as Mean ± SEM. Values annotated with (*) vary significantly from the cluster of control rats when P-value is less than 0.05. WBC, LYM, MXD, NEUT, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, MPV, PDW, PCT represent white blood count, lymphocytes, mixed cell percentage, neutrophil, red blood cell count, haemoglobin, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet, mean platelet volume, platelet distribution width and plateletcrit respectively. TC =*Tapinanthus cordifolius* extract

Table 8: Cellular architecture of rat's tissue after 28-day oral treatment of *Tapinanthus cordifolius* extract

Organ	Normal Control	800 mg/kg TC	1600 mg/kg TC
Brain			
Liver			

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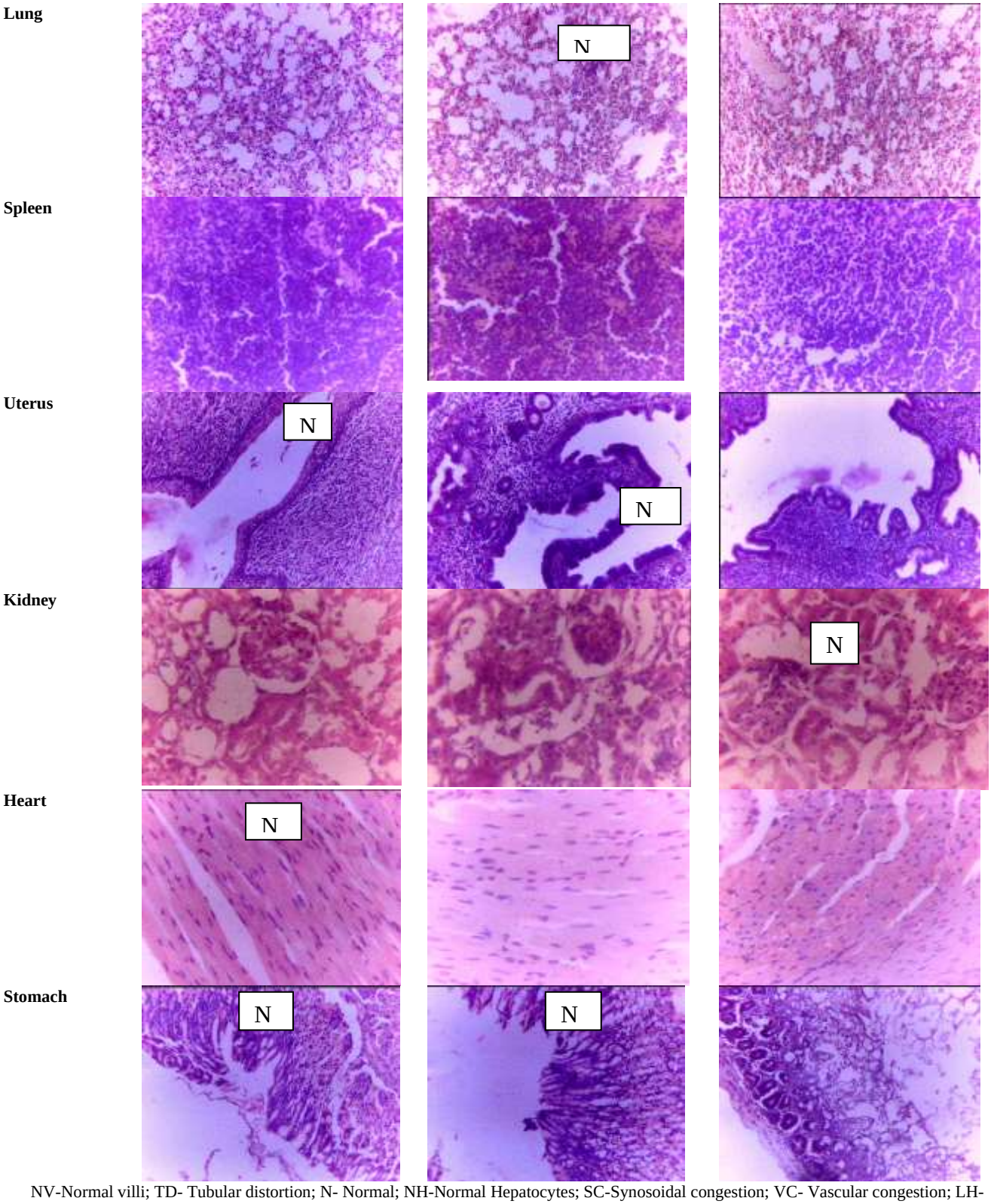

NV-Normal villi; TD- Tubular distortion; N- Normal; NH-Normal Hepatocytes; SC-Synosoidal congestion; VC- Vascular congestion; LH-hyperplasia of inflammatory cells

References

1. Adesina SK, Illoh HC, Johnny II, Jacobs IE. African mistletoes (Loranthaceae); ethnopharmacology, chemistry and medicinal values: an update. Afr J Tradit Complement Altern Med. 2013; 10(4):161-170.
2. Chike-Ekwughe A, Adegboyega AE, Johnson TO, Adebayo AH, Ogunlana OO. *In vitro* and *in silico* inhibitory validation of *Tapinanthus cordifolius* leaf extract on alpha-glucosidase in the management of type 2 diabetes. J Biomol Struct Dyn. DOI: 10.1080/07391102.2023.2212791.
3. Ogunmefun OT, Fasola TR, Saba AB, Oridupa OA. The ethnobotanical, phytochemical and mineral analyses of *Phragmanthera incana* (Klotzsch), a species of mistletoe growing on three plant hosts in South-Western Nigeria. Int J Biomed Sci. 2013; 9:37-44.
4. Jadhav N, Patil CR, Chaudhari KB, Wagh JP, Surana SJ, Jadhav RB. Diuretic and natriuretic activity of two mistletoe species in rats. Pharmacognosy Res. 2010; 2(1):50-57.
5. Saleh, I., Maigandi, S.A., Hudu, M.I., Abubakar, M.I. and Shehu, A.U. Uses and chemical composition of Misletoe (*Viscum album*) obtained from different species. Dutse J. Agric. Food Sec. 2015; *2*: 8-12.
6. Egbewande OO, Jimoh AA, Ibitoye EB, Olorede BR. Utilization of African mistletoe (*Tapinanthus bangwensis*) leaf meal by broiler chickens. Pakistan J Nutr. 2011; 10:19-22.
7. Obatomi DK, Bikomo EO, Temple VJ. Anti-diabetic properties of the African mistletoe in streptozotocin-induced diabetic rats. J Ethnopharmacol. 1994; 43:13-17.
8. Amarachi CE, Olamilekan AN, Adamu A, Humphery AA, Olujoke OO. Comparative Phytochemical Contents and Antioxidant Activities of *Tapinanthus cordifolius* and *Irvingia wombolu* Leaf Extracts. In: Isibor PO, Akinduti P, Oranusi SU, Popoola JO, editors. Biotechnological Approaches to Sustainable Development Goals. Springer, Cham. https://doi.org/10.1007/978-3-031-33370-5_15.
9. Oyeniran OH, Ademiluyi AO, Oboh G. African mistletoe (*Tapinanthus bangwensis* Lor.) infestation improves the phenolic constituents, antioxidative and antidiabetic effects of almond (*Terminalia catappa* Linn.) host leaf in sucrose-rich diet-induced diabetic-like phenotypes in fruit fly. Food Front. 2021; 2:77-90.
10. Evbuomwan SA, Omotosho OE, Akinola OO. A Mini Review on Some Known Medicinal Uses of *Tridax procumbens*. Trop J Nat Prod Res. 2023;7(8):3573-84.
11. Ibrahim JA, Egharevba HO, Iliya I, Tarfa F, Ayodele AE. Chemical profiles as chemotaxonomic tools for Loranthaceae in Nigeria. Afri J Plan Sci. 2014;8(7):343-52.
12. SE, Okhale, G. E. Ugbabe, P. O. Oladosu, Ib-rahim JA, H. O. Egharevba, O. F. Kunle, E. P. Elisha, A. J. Chibuike, and U. O. Ettah. Chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea* L (Rubiaceae) collected from North Central. Int J Bioassays. 2018; 5:5630-5637.
13. Chike-Ekwughe A, Adegboyega AE, Johnson TO, Adebayo AH, Ogunlana OO. *In vitro* and *in-silico* inhibitory validation of *Tapinanthus cordifolius* leaf extract on alpha-amylase in the management of type 2 diabetes. Inf in Med Unl. 2023; 36:101148.
14. OECD. OECD Guidelines for the Testing of Chemicals. OECD. 2001; 1-14.
15. Egbuji JV, Ejere VC, Ugwu GC, Ugwu CL. Effects of Aqueous Leaf Extracts of *Loranthus micranthus* Linn. on Biochemical Profile of Wistar Rats Infected with *Trypanosoma brucei* brucei. Tro J Nat Prod Res. 2023; 1:7(6).
16. Zakerkish M, Jenabi M, Zaeemzadeh N, Hemmati AA, Neisi N. The effect of *Iranian propolis* on glucose metabolism, lipid profile, insulin resistance, renal function and inflammatory biomarkers in patients with type 2 diabetes mellitus: A randomized double-blind clinical trial. Sci Rep. 2019; 9(1):1-11.
17. Asuquo GO, Udonkang MI, Oboma YI, Bassey IE, Beshe BS, Okechi OO, Owo DU, Effiong GS. *Gongronema latifolium* Ethanol Crude Leaf Extract Ameliorates Organ Injuries Via Anti-Inflammatory and Anti-Diabetic Mechanisms in Streptozotocin-Induced Diabetic Rats. Trop J Nat Prod Res. 2023;7(8):3823-8.
18. Sharif A, Ashraf M, Javeed A, Anjum AA, Akhtar MF, Akhtar B, Saleem A. Oxidative stress responses in Wistar rats on subacute exposure to pharmaceutical waste water. Environ Sci Pollut Res. 2016;23(23):24158-65.
19. Haggag MW, Abou El Ella SM, Abouziena HF. Phytochemical analysis, antifungal, antimicrobial activities and application of *Eichhornia crassipes* against some plant pathogens. *Planta Daninha*. 2017; 35: p. e17159560.
20. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. Nucleic Aci Res. 2018; 46(W1): W257-63.
21. Johnson TO, Istifanus G, Kutshik RJ. *In vitro* and *in vivo* analysis of the anti-plasmodial activity of ethanol extract of *Phyllanthus nivosus* W. Bull leaf. J Parasit Dis. 2020; 44:166-173.
22. Miras-Moreno B, Sabater-Jara AB, Pedreño MA, Almagro L. Bioactivity of phytosterols and their production in plant *in vitro* cultures. J Agric Food Chem. 2016; 28;64(38):7049-58.
23. Kritchevsky D, Chen SC. Phytosterols-health benefits and potential concerns: A review. Nutr Res. 2005; 25:413-428.
24. Ntie-Kang F. An *in silico* evaluation of the ADMET profile of the Streptome DB database. Spri plus. 2013; 2:1-11.
25. Bello I, Bakkouri AS, Tabana YM, Al-Hindi B, Al-Mansoub MA, Mahmud R, Asmawi MZ. Acute and sub-acute toxicity evaluation of the methanolic extract of *Alstonia scholaris* stem bark. Med Sci. 2016; 4(1):4.
26. Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicol. 2008; 245(3):194-205.
27. Ranawat L, Bhatt J, Patel J. Hepatoprotective activity of ethanolic extracts of bark of *Zanthoxylum armatum* DC in CCl4 induced hepatic damage in rats. J Ethnopharmacol. 2010; 127:777-780.
28. Balogun FO, Tshabalala NT, Ashafa AOT. Antidiabetic medicinal plants used by the Basotho tribe of Eastern Free State: a review. J Diab Res. 2016; 34.
29. Adigwe OP, John-Africa LB, Adzu B, Ajoku GA, Danraka AM, Ibrahim JA. Evaluation of the toxic effects of the aqueous extract of Niprineem tea in mice and rats. Int J Biol Chem Sci. 2021; 15(5), 1979-1990.