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Full Length Research Article

Subacute Toxicity Studies on Aqueous Stem Extract of *Cissus Populnea* in *Wistar* Albino Rats

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ABSTRACT
Cissus populnea is a plant whose medicinal benefits have been well but there is a dearth of studies on subacute toxicity of any part of the plant. The aim of the study is to ascertain the subacute toxicity of the aqueous stem extract of *C. populnea* in Wistar albino rats. Forty animals (20 males and 20 females) grouped into 8 groups (n=5) (4 males, 4 females) were used. The animals were treated with daily oral doses (125mg/kg, 250mg/kg, and 500mg/kg) of the extract for 28 days, while the control groups received distilled water. The animals were weighed at 7-day intervals. After the test period, the animals were anaesthetised. Blood samples were taken for haematological and biochemical analysis, the organs were harvested and weighed. There was no significant ($p>0.05$) difference between the weight of the control groups and all the treated groups. Treatment with the various doses of the extract did not cause any significant ($p>0.05$) changes in the relative organ weight, biochemical parameters, red blood cell count, haemoglobin, and white blood cell count of the experimental animals. Oral administration of the aqueous stem extract of *C. populnea* in the doses used in this study caused no observable toxic effect on the experimental animals.

Keywords: *Cissus populnea*, Biochemical parameters, Haematological parameters, Subacute toxicity, Wistar albino rats

INTRODUCTION
The use of plant parts as medicine is becoming more popular and acceptable in various parts of the world. The increased patronage and acceptability have been attributed to supposed safety, availability as well as affordability (Hosseinzadeh *et al*., 2015). *Cissus populnea*, a liane from the family Vitaceae (Amplidaceae) is a plant with various uses in ethnomedicine and food condiment. In Nigeria, this plant is known as *Okoho* by the Idoma and Igala tribes, *Dafara* or *Lututuwa* by the Hausas *Orogboro* or *Afato* by the Yorubas (Olooto *et al.* 2022). Apart from being used as a soup condiment, the stem is also used to manage erectile dysfunction and infertility among men in southwest Nigeria. Ojekale *et al*. (2015) demonstrated the spermatogenic properties of stem bark extract in *Wistar* rats. Furthermore, Osibote *et al.* (2010) in their study reported that essential oils from the stem of the plant can be used to correct male infertility due to bacterial infection. The aqueous extract of the stem has also been found to alleviate hyperglycemia in diabetic rats (Aondoaseer *et al*., 2021). Aletan *et al.* (2022) detected the presence of various bioactive constituents in the stem of the plant. Notwithstanding the widespread medicinal use of *Cissus populnea* stem extract, there is no documented evidence to the best of our knowledge of acute and sub-acute toxicity effects due to the use of any part of the plant. This study was designed to evaluate the toxic side effects of *Cissus populnea* aqueous stem extract by oral administration in *wistar* albino rats.

MATERIALS AND METHODS
Plant Material Collection
Stem parts of *C. populnea* bearing the leaves were purchased from Mushin market in the Mushin Local Government Area of Lagos State, Nigeria. The plant was identified by a taxonomist at Forest Herbarium, Forest Research Institute Ibadan with Voucher number FHI 1133642.

Pre-Extraction Treatment
The stem was washed and cut into pieces spread on brown paper and allowed to air dry for 3 weeks. The dried pieces were then crushed using a local mortar to a coarse powder.

Preparation of the Extracts
The coarse powdered sample was used for extraction. A quantity of 500 g of the sample was soaked in 5 litres of distilled water and allowed to stand for 72 hours at room temperature with intermittent shaking. The extract was filtered with Whatman filter paper (No. 1), the filtrate was then concentrated to dryness in a water bath at 70 ºC; then labeled and stored until use for administration.

Preliminary Phytochemical Screening
The plant extract was subjected to phytochemical screening analysis using the methods of Sowofora (1993)

Experimental Animals
The animals were obtained from the animal house, Department of Pharmacognosy, University of Lagos, Lagos State, Nigeria. The animals were acclimatized for 7days before the experiment, during which they were fed with pelletized animal feed and clean water. The NIH Guide for the care and use of laboratory animal (National Institute of Health, 2011) was strictly followed. The research was approved by the NOUN Research and Ethical Committee (ETC/2023/NOUN/08/001).

Acute Toxicity Study
In accordance with the Organization for Economic Cooperation and Development (OECD) guidelines 425 (OECD, 2002), an acute toxicity study was carried out

using Swiss albino mice aged between 8 and 10 weeks old (21-24 g). The animals were grouped (n=3) into three groups for the first phase of the experiment then another three groups for the second phase of the experiment. Before administration, the animals were fasted between 3 to 4 hours, then 1 to 2hrs after administration. During the first phase, the groups were administered 100, 300, and 500 mg/kg of the extract respectively via oral gavage (Lorke,1983). Signs of toxicity such as salivation, lethargy, diarrhea, tremors, convulsions, and mortality were observed for 24 hours with special attention given to the first 4 hrs, with no death recorded. This was followed by administration of the extract at 1000, 2000, 5000 mg/kg respectively to the next three groups of mice and equally observed, and daily for 14 days for any signs of delayed toxicity.

Sub-Acute Oral Toxicity Study

A total of 40 W*istar* albino rats (20 males and 20 females) were used for the sub-acute toxicity study. The animals were weighed and grouped into 8 (4 males and 4 females) groups of 5 animals per group. Three groups from each sex (Groups 2,3 and 4) were given daily doses of the plant extract (125, 250, and 500 mg/kg), respectively in 1 ml of distilled water while the control group (Group 1) for each sex was given 1ml distilled water for 28 days. These doses were chosen based on pharmacologically active doses that were effective in the spermatogenic effect (Ojekale *et al*., 2015) and antidiabetic effect (Aondoaseer *et al*.,2021) of the extract. The animals were weighed at the end of each week for the duration of the study. At the end of the 28-day treatment period, the animals were deprived of feed but had free access to drinking water for 24 hours before being anaesthetized under inhaled chloroform. Blood samples were collected through cardiac puncture, 1 ml in ethylene diamine tetraacetic acid (EDTA), and 5 ml plain sterile tubes (without anticoagulants) for haematological and biochemical investigations respectively. The organs were harvested, physically observed, and weighed. The relative weight of the organs was calculated using the relationship:

Relative organ weight $=$ $(weight\ of\ organ(g)/weight\ of\ animal\ (g)) \times 100$

Haematological Investigations

The White Blood Cell (WBC) count, Lymphocyte count, Granulocyte count, Haemoglobin (HB), Haematocrit (HCT) Red Blood Cell (RBC) count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW) and Platelet count were determined using automated haematology analyser (Mindray BC 3200). The analyzer uses the electrical impedance method to determine the count and size distribution of RBC, WBC, and PLT; and uses the colorimetric method to determine HGB. Other parameters were calculated by the analyzer based on the data got. A quantity of 9 µl of whole blood sample was aspirated directly into the analyzer and diluted with 1.41 ml of diluent. The procedures employed for the determination were in accordance with the manufacturer's instructions.

Biochemical Investigation

The blood samples collected in the tubes without anticoagulants were used for the biochemical investigations. The samples were left to coagulate for 30 minutes and then centrifuged at 3000 rpm for 10 minutes to get clear sera. The sera were carefully collected into sterile tubes and frozen at -20 ºC until required. The activities of serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT), total protein, albumin, total bilirubin, total cholesterol, high density lipoprotein (HDL) -cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, urea, sodium, potassium, and chloride, using an automated machine (Erba Mannheim XL-200) in accordance with the manufacturer's instructions.

Statistical Analysis

The results from the investigations were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to determine the differences between groups. Tukey's multiple comparison test was used where significant differences existed. Differences in means were considered significant at $P < 0.05$.

RESULTS

Phytochemical Screening

The phytochemical screening revealed the presence of alkaloids, tannins, phenols, phlobatanins, cardiac glycosides, saponins, and flavonoids,

Acute Toxicity Studies

The results of the acute toxicity studies showed no alterations in the skin and fur, eyes and mucus membrane, respiratory rate, heart rate, salivation, perspiration, urinary incontinence, or defecation; likewise, no drowsiness, change in gait, tremors and convulsion were noticed in the animals administered up to a dose of 200 0mg/kg body weight. No mortality was recorded even up to a dose of 5000mg/kg body weight. Fourteen days after the acute toxicity study no death was recorded among the experimental animals.

Sub-acute Toxicity Studies

Effect of Plant Extract on Body Weight of Experimental Animals

Figure1 shows the weight of the experimental animals taken at weekly intervals during the 28-day study. For the control male group, the weight ranged between an average initial weight of 171.40 ± 12.14 g to an average final weight of 173.60 ± 15.36 g. The weight for the low dose male group ranged between an initial 170.20 ± 13.25 g and a final weight 173.80 ± 14.63 g.

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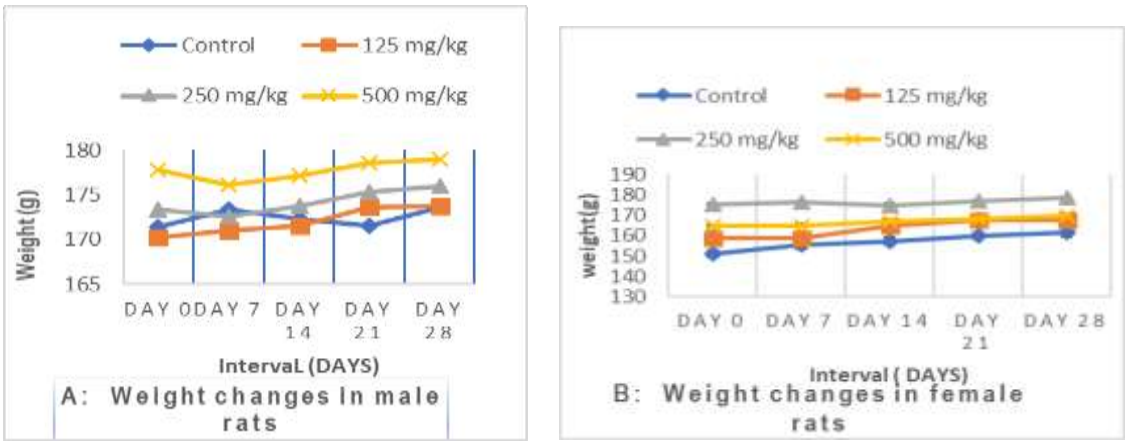

Figure 1: Changes in body weight of *Wistar* albino rats due to oral administration of aqueous stem extract of *C. populnea*
a : Weight changes in males rats **b:** Weight changes in females rats

For the females, the weights for the control group ranged between an initial weight of 151.20 ± 8.11 g and a final weight of 161.40 ± 5.94 g. The low dose female group had an average initial weight of 158.80 ± 13.38 g and an average final weight of 167.60 ±15.43. There were no significant ($p>0.05$) changes in the weight of the experimental animals due to the oral administration of the various doses of the extract for the duration of the study.

Table 1 shows the changes in the relative weight of some vital organs in the experimental animals due to the oral administration of the aqueous stem extract of *C. populnea*. The relative weight of the liver from the male animals ranged between 4.32 ± 0.56 % for the control group and 5.04 ± 0.40 % for the medium (250 mg/kg) dose group. In the female animals, the relative liver weight was between 4.24 ± 0.09 % and 5.06 ± 0.32 % for the control and medium (250 mg/kg) dose group respectively. The relative weight of the testes for the males ranged between 2.10 ± 0.12 % for the low (125 mg/kg) dose group and 2.30 ± 0.14 % for the control group. In the females, the ovaries showed a relative weight of between 0.35 ± 0.05 % for the medium (250 mg/kg) dose group to 0.22 ± 0.04 % for the high (500 mg/kg) dose group. Oral administration of the various doses of the aqueous stem extract of *C. populnea* caused no statistically significant ($p>0.05$) changes in the relative organ weights of either the male or the female experimental animals.

Table 2 shows changes in haematological parameters due to oral administration of aqueous stem extract of *C. populnea*. The White Blood Cell (WBC) count ranged between (5.23 ± 0.58 $x10^3$/µL to 8.88 ±1.49) x 10^3/µL for the medium (250 mg/kg) and low (125 mg/kg) dose groups respectively in the males while for the female group, the range was between (5.38 ± 0.30 and 8.38 ± 1.12) $x10^3$/µL for the control and medium dose (250 mg/kg) groups respectively. The haemoglobin levels for the male animals ranged between (15.94 ± 0.91 and 17.08 ± 0.41) $x10^6$/µL for the low dose and control groups respectively. In the female animals, however, the values ranged between (15.05 ±1.90 and 16.68 ± 0.44) $x10^6$/µL for the medium dose and low dose groups respectively. Except for the Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH), all the parameters studied showed no significant ($p > 0.05$) changes in the male animals due to the oral administration of the various doses of the aqueous stem extract of *C. populnea* used in this study. In the female animals, however, no significant ($p > 0.05$) changes were observed in the hematological parameters due to the administration of the extract for the duration of the study.

Table 1: Changes in relative organ weights (%) due to administration of aqueous stem extract of *C. populnea*

ORGAN	GENDER	CONTROL	125 mg/kg	250 mg/kg	500 mg/kg
Liver	Male	4.32 ± 0.56	4.38 ± 0.39	5.04 ± 0.40	4.48 ± 0.61
	Female	4.24 ± 0.09	4.70 ± 0.46	5.06 ± 0.32	4.62 ±0.35
Kidney	Male	0.86 ± 0.11	0.92 ± 0.13	1.00 ± 0.10	0.95 ± 0.33
	Female	0.78 ± 0.04	1.08 ± 0.23	1.08 ± 0.10	0.96 ± 0.09
Heart	Male	0.42 ± 0.08	0.44 ± 0.11	0.48 ± 0.04	0.53 ± 0.05
	Female	0.44 ± 0.09	0.50 ± 0.07	0.58 ± 0.05	0.44 ± 0.05
Lungs	Male	1.32 ± 0.22	1.34 ± 0.19	1.52 ± 0.26	1.43 ± 0.15
	Female	0.80 ± 0.09	1.12 ± 0.37	1.33 ± 0.33	1.64 ± 0.53
Testes	Male	2.30 ± 0.14	2.10 ± 0.12	2.28 ± 0.26	2.23 ± 0.26
Ovaries	Female	0.26 ± 0.11	0.26 ± 0.11	0.35 ± 0.05	0.22 ± 0.04

Values represent mean ± standard deviation (n = 5)

Table 2: Changes in hematological parameters of *Wistar* albino rats due to oral administration of aqueous stem extract of *C. populnea*

PARAMETERS	GENDER	CONTROL	125 mg/kg	250 mg/kg	500 mg/kg
WBC (x10^3/µL)	Male	6.54 ± 1.16	8.88 ±1.49	5.23 ± 0.58	7.78 ±1.49
	Female	5.38 ± 0.30	7.72 ±1.22	8.38 ± 1.12	7.15 ±1.79
Lymphocytes (x10^3/µL)	Male	2.48 ± 0.51	3.10 ± 0.83	2.10 ± 0.16	2.54 ± 0.27
	Female	1.92 ± 0.20	3.24 ± 0.75	3.33 ± 0.83	3.24 ± 1.39
Granulocytes (x10^3/µL)	Male	2.74 ± 0.44	3.90 ± 1.51	2.20 ± 0.42	3.64 ±1.09
	Female	2.38 ± 0.18	2.96 ± 0.17	2.90 ± 0.37	3.22 ± 1.55
Haemoglobin (g/100ml)	Male	17.08 ± 0.41	15.94 ± 0.91	16.15 ± 0.59	16.20 ± 0.70
	Female	16.68 ±0.35	16.68 ± 0.44	15.05 ±1.90	15.16 ± 0.91
RBC (x10^6/µL)	Male	9.51 ± 0.21	9.43 ± 0.43	9.51 ± 0.40	9.45 ± 0.36
	Female	9.44 ± 0.16	9.15± 0.37	8.41 ± 0.95	8.57± 0.45
HCT (%)	Male	51.98 ± 1.57	48.54 ± 3.28	48.45 ± 1.71	48.82 ±2.23
	Female	51.04 ± 0.37	49.80 ±1.72	46.80 ±3.79	47.20 ± 2.36
MCV (fl)	Male	55.32 ± 1.88	51.50 ± 1.74*	51.00 ± 1.27*	51.72 ± 0.95*
	Female	54.14 ± 0.76	54.50 ± 1.52	57.73 ± 4.04	54.32 ±2.21
MCH (pg) picogram	Male	18.08 ± 0.37	16.86 ± 0.27*	16.98 ± 0.45*	17.10 ±0.23*
	Female	17.62 ± 0.48	18.20 ±0.34	18.38 ± 0.48	17.66 ± 0.59
MCHC (g/dl)	Male	32.82 ± 0.89	32.82 ± 0.94	33.28 ± 0.40	33.54 ± 0.90
	Female	32.64 ± 0.69	33.44 ±0.48	32.03 ± 1.72	32.58 ± 0.71
RDW (%)	Male	15.92 ± 0.66	15.66 ±0.65	15.70 ± 0.63	15.46 ± 0.53
	Female	15.56 ± 0.21	15.68 ± 0.19	15.65 ± 0.13	15.56 ± 0.24
Platelet Count (x10^9 /L)	Male	650.0 ±152.6	747.4 ±245.80	847.0±132.40	750.8±128.10
	Female	741.40 ±69.89	701.8±45.16	813.50±75.67	775.2±103.70

Values represent mean ± standard deviation (n = 5) * indicates a significant difference at ($p < 0.05$) from the control.

Table 3 shows the changes in liver function parameters due to the oral administration of aqueous stem extract of *C. populnea.* The serum activities of alanine aminotransferase (ALT) in the male animals ranged between 57.16 ± 8.95 IU/L for the control group and 72.73 ± 9.22 IU/L for low dose group. For the female rats the serum activities of ALT ranged between (51.63 ± 5.49 and 61.55 ±11.38) IU/L for the control group and high dose group respectively. There were no statistically significant ($p > 0.05$) differences in the activities of ALT in either the male or the female rats due to oral administration of the extract. Likewise, oral administration of the aqueous stem extract of *C. populnea* did not cause any significant ($p > 0.05$) changes in the activities of the other liver enzymes studied in the experimental animals. Similarly, the total bilirubin levels of the experimental animals did not experience any statistically significant ($p > 0.05$) alterations due to the administration of the extract. The total protein and albumin did not show any significant alteration due to the administration of the extract for the duration of the study.

Table 4 shows the changes in the lipid profile of the experimental animals due to the oral administration of aqueous stem extract of *C. populnea* to the experimental animals. There was no significant alteration in the lipid profile of the experimental animals due to the administration of aqueous stem extract of *C. populnea*. The total cholesterol level for the male animals ranged from 2.28 ± 0.13 mmol/L for the control group and 1.86 ± 0.26 mmol/L for the animals administered 500 mg/kg of the extract (the high dose). In the female animals, the total cholesterol levels ranged between 2.00 ± 0.16 mmol/L for the low (125 mg/kg) dose group and 1.72 ±0.23 mmol/L for the control group. Oral administration of aqueous stem extract of C. populnea did not cause any significant ($p>0.05$) change in the lipid profile of the experimental animals.

The urea levels for the male animals ranged between 7.49 ± 1.64 mmol/L for the medium (250 mg/kg) dose group and 9.36 ± 1.00 mmol/L for the control group. In the female animals, the values were between (5.81 ± 0.74 and 7.61 ± 1.99) mmol/L. Likewise, the serum electrolytes studied, sodium, potassium, and chloride ions, did not experience any statistically significant alterations due to the administration of the extract for the duration of the study high dose group. Details are presented on Table 5 group. Oral administration of the aqueous extract of the *C. populnea* stem did not cause any statistically significant ($p >0.05$) change in the kidney function parameters of the experimental animals.

Table 3: Changes in liver function parameters due to oral administration of aqueous stem extract of *C. populnea*

PARAMETERS	GENDER	CONTROL	125 mg/kg	250 mg/kg	500 mg/kg
ALT (IU/L)	Male	57.16 ± 8.95	72.73 ± 9.22	70.70 ± 12.59	66.43 ± 8.00
	Female	51.63 ± 5.49	52.00 ±7.58	53.00 ± 9.70	61.55 ±11.38
AST (IU/L)	Male	161.90 ± 14.20	158.6 ± 1.00	150.70 ± 8.27	149.40 ± 14.24
	Female	120.80 ± 8.19	120.5 ± 22.48	129.10± 17.53	136.10 ± 1.77
ALP (IU/L)	Male	258.86 ± 39.49	267.52 ± 58.73	231.28 ± 82.78	224.16 ± 87.03
	Female	204.28 ± 34.48	178.62 ±87.47	167.18 ± 34.90	164.04 ± 12.74
Total Bilirubin (µmol/L)	Male	2.48 ± 0.21	2.68 ± 0.98	2.24 ± 0.17	2.41 ± 0.29
	Female	2.23 ± 0.19	2.21 ± 0.43	3.16 ± 0.97	2.52 ± 0.61
Albumin (g/L)	Male	30.98 ± 0.88	29.06 ± 4.39	28.68 ± 2.87	29.23 ± 2.46
	Female	32.12 ± 1.63	32.72 ± 2.43	29.40 ± 2.87	29.72 ± 1.76
Total Protein (g/L)	Male	83.60 ± 2.88	84.96 ± 11.61	90.62 ± 3.90	89.35 ± 5.19
	Female	85.66 ± 4.02	86.28 ±3.69	84.75 ±5.60	85.16 ± 3.69

Values represent mean ± standard deviation(n=5)
ALT- Alanine transaminase; AST - Aspartate transferase; ALP-Alkaline Phosphatase

Table 4: Changes in lipid profile due to oral administration od aqueous stem extract of *C. populnea*

PARAMETERS	GENDER	CONTROL	125 mg/kg	250 mg/kg	500 mg/kg
Triglyceride (mmol/L)	Male	0.46 ± 0.31	0.51 ± 0.13	0.50 ± 0.11	0.41 ± 0.10
	Female	0.44 ±0.08	0.52 ± 0.12	0.55 ±0.11	0.44 ± 0.19
Total Chol (mmol/L)	Male	2.28 ± 0.13	1.97 ± 0.37	2.25 ± 0.56	1.86 ± 0.26
	Female	1.72 ± 0.23	2.00 ± 0.16	1.97 ± 0.20	1.88 ± 0.42
HDL-Chol (mmol/L)	Male	1.58 ± 0.19	1.24 ± 0.26	1.41 ± 0.34	1.23 ± 0.10
	Female	1.26 ± 0.10	1.42 ± 0.16	1.30 ± 0.20	1.29 ± 0.22
LDL-Chol (mmol/L)	Male	0.51 ± 0.19	0.46 ± 0.16	0.65 ± 0.28	0.61 ± 0.08
	Female	0.25 ± 0.13	0.35 ± 0.07	0.29 ± 0.14	0.39 ± 0.22

Values represent mean ± standard deviation (n = 5)

Table 5: Changes in kidney function parameters due to oral administration of aqueous stem extract of *C. populnea*

PARAMETERS	GENDER	CONTROL	125 mg/kg	250 mg/kg	500 mg/kg
Urea (mmol/L)	Male	9.78 ± 1.14	8.84 ± 1.35	7.31 ± 1.52	7.46 ± 1.05
	Female	8.59 ± 2.18	4.96 ± 0.94	6.64 ± 1.56	7.51 ±0.98
Sodium	Male	152.20 ± 2.85	153.00 ± 4.19	151.10 ± 3.25	154.00 ± 3.96
	Female	146.9 ± 0.95	148.6 ± 5.67	143.1± 2.64	145.5 ± 4.37
Potassium	Male	10.75 ± 1.27	10.95 ± 1.62	10.73 ± 0.60	9.64 ± 0.67
	Female	9.20 ± 1.01	8.80 ± 1.04	12.42 ± 2.37	9.72 ± 0.63
Chloride	Male	116.90 ± 1.74	118.5 ± 4.80	115.80 ± 2.55	118.70 ± 2.94
	Female	113.0 ± 1.80	113.1± 2.51	110.2 ± 2.77	111.9 ±2.29

Values represent mean ± standard deviation (n = 5)

DISCUSSION

The use of plant parts as medicine is as old as mankind, in the quest for cure man has sometimes met serious complications in the use of plant medicine. These unfortunate incidences have however not affected the use of herbal medicine significantly. Lee *et al.* (2019) reported that 70 to 80% of Africa's emerging urban and rural population, for instance, rely on traditional herbal medicine for health intervention. Consequently, in other to protect mankind from the dangers of herbal medicine when they exist, there is a need to carry out acute and sub-acute toxicity studies on such plant parts. By ascertaining the effect of the aqueous stem extract of *C. populnea* on the weight of the experimental animals and their organ weights, haematological as well as biochemical parameters following repeated administration of the various doses for the duration of the study, we intend to establish its safety and provide recommendation on the safe use of the extract for medicinal purpose.

The absence of mortality at an oral dose of up to 5000 mg/kg in acute studies provides a preliminary indication of the potential safety of the aqueous stem extract of *C. populnea*. However, there was a need for further studies leading to the 28-day sub-acute toxicity study using doses (125 mg, 250 mg and 500 mg) within pharmacologic active doses which have been shown spermatogenic (Ojekale *et al*., 2015) and antidiabetic (Aondoaseer *et al*., 2021) in rats.

According to Piao *et al.* (2013), organ weight is one of the most sensitive drug toxicity indicators and usually occurs before morphological changes. In this study, the relative weight of the organs studied in both the female and male animals did not show any changes due to the

administration of the extracts. Therefore, the organs did not show any observable signs of toxicity due to the administration of the extract. The administration of the extract also did not cause any effect on the weight of the experimental animals

According to Arika *et al.* (2016) and Seibel *et al*. (2021) haematological parameters provide substantial information about the state of an animal. Hence, the haematological parameters of the experimental animals were studied in the sub-acute studies. The administration of the various doses (125 mg/kg,250 mg/kg and 500 mg/kg) of the extract used in this study caused decreases in the Mean Corpuscular Volume (MCV) which were not dose-dependent in the male animals. MCV indicates the average volume of the red blood cell. According to Ashafa *et al.* (2011), MCV, MCH and MCHC relate to individual red blood cells while HCT, RBC and haemoglobin are associated with the total population of the red blood cells. In the present study, although there was a significant decrease in both the MCV and MCH in the experimental male animals, these values were still within the acceptable range for normal rats (Filho *et al*., 2018). Furthermore, neither the red blood cell (RBC) count, the haemoglobin level nor the hematocrit level was affected by the oral administration of various doses (125 mg/kg,250 mg/kg and 500 mg/kg) of the extract used in this study. Hence, no noticeable effect on the red blood cell production process was observed due to the oral administration of the various doses of the extract to either male or female animals for the duration of the study. Likewise, the total white blood cell (WBC) count and the various types of WBCs examined in this study were not affected by the oral administration of aqueous stem extract of *C. populnea*. WBCs are cells of the immune system which help to protect the body against infectious diseases and foreign invaders. The two major forms of disorders associated with WBC are proliferative disorders-causing excessive numbers, and leukopenias-causing insufficient numbers (Kumar *et al*., 2017). An excess WBC usually indicates infection or inflammation or certain blood cancers or bone marrow disorders whereas a low WBC count can signal that an injury or condition is destroying cells faster than the body is making. Therefore, it can be assumed that the oral administration of the various doses (125 mg/kg,250 mg/kg and 500 mg/kg) of aqueous stem extract of *C. populnea* used in this study caused no issues to the immune system of the experimental animals that could cause an increase or decrease in the WBC count.

The serum activities of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) in the liver were determined in the experimental animals. The significance of the liver cannot be overemphasized. The responsibility of the liver includes primary detoxification of several metabolites, protein synthesis as well as the production of digestive enzymes (Iluz-Freundlich *et al.,* 2020). Usually in evaluating the state of the liver, the serum activities of liver enzymes which include ALT, AST and ALP as well as total bilirubin level, total protein and albumin among parameters have been used and the elevation pattern can help organise a differential diagnosis (Ribeiro *et al*., 2019). In this study, none of these liver function parameters showed any statistically significant ($p > 0.05$) changes either in the male or the female animals due to the oral administration of the various doses (125 mg/kg,250 mg/kg, and 500 mg/kg) used for the study. Therefore, no noticeable effect was observed in the liver function parameters due to the administration of the various doses (125 mg/kg,250 mg/kg and 500 mg/kg) of the extract for the duration of the study. The lipid profile of the experimental animals showed no significant changes due to the oral administration of any dose of the extract during the duration of the study. Therefore, oral administration of the aqueous extract of *C. populnea* in the doses (125 mg/kg, 250 mg/kg, and 500 mg/kg) used for this study had no noticeable effect on the lipid profile of both the male and the female experimental animals for the duration this study.

The kidneys play a vital role in the excretion of waste products and toxins such as urea and also the regulation of electrolyte concentration among other things. Urea is a nitrogen-containing compound formed in the liver as the end product of protein metabolism and the urea cycle. About 85% of the urea is eliminated through the kidneys (Gounden *et al*., 2023). Serum urea levels increase in conditions where clearance decreases (in acute and chronic renal failure/impairment). Increase in urea is usually noticed earlier in renal disease (Gounden *et al*., 2023). There was however no increase in serum urea due to the oral administration of the extract, thus it can be safely presumed that the kidneys of the experimental animals were not adversely affected. Similarly, the levels of the electrolytes studied were not adversely affected by the oral administration of the extract during the duration of the study.

CONCLUSION

The results of this study suggest that the aqueous stem extract of *C. populnea* in the doses used in this study (125 mg/kg,250 mg/kg, and 500 mg/kg) for the duration of this study did not possess any toxic effect that could compromise its use as medicine. The acute toxicity study did not result in the death of the experimental animals. Furthermore, the 28 -day sub-acute toxicity study did not lead to any observable effect on the relative organ weights. The haematological parameters were not affected by the prolonged oral administration of the various doses (125 mg/kg,250 mg/kg, and 500 mg/kg) of the extract. None of the biochemical parameters studied showed any effect of the doses used on the liver or the kidney of either the male or the female experimental animals. The results obtained in this study serve to improve the confidence in the medicinal use of the aqueous stem extract of *Cissus populnea* at the therapeutic doses used for this study. Nevertheless, there

is still a need for further studies on its teratogenicity, mutagenicity as well as its carcinogenic potential.

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