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Multicellular Effects from Aqueous Stem Bark Extract of *Cadaba farinosa Forsk* on Selective Internal Organs of Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author SMG conceive, experimented, and wrote the manuscript. Author AAB analyzed the data. Author OGA designed the experiment, supervised, and revised the manuscript. Author AAM analyzed the data and reviewed the manuscript. Author MU participated in the experiment and revised the manuscript. Authors EEA, ESB and EMH participated in the experiment and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Cadaba farinosa Forsk belongs to the family Cappariraceae used in the treatments of snakebite, breast cancer, and retained placenta in African traditional medicine. However, information about the plant toxicity profile and risk awareness are largely unknown.

Aim: To determine the histopathological, biochemical, and hematological effects from aqueous stem bark extract of *Cadaba farinosa Forsk* on selective internal organs of Wistar rats.

Methods: Twenty (20) Wistar rats were randomly divided into five (5) groups of four rats each. The extract was administered by oral gavage in doses of 100, 200, 300, and 400 mg/kg body weight for 28 days. Blood samples were collected for hematological and biochemical analyses, while the liver, kidney, spleen, lung, brain, and stomach were harvested and processed histopathologically using standard methods.

Results: Oral administration of the aqueous stem bark extract from *Cadaba farinosa Forsk* caused a significant increase in hematocrit (P=0.044) and hemoglobin (P=0.046). However, the white blood cell was significantly (P=0.011) reduced at doses of 300 and 400 mg/kg compared to control. The serum albumin of treated animals was also significantly (P=0.017) reduced in a dose-dependent manner compared to control. In addition, histopathological sections of treated animals showed pulmonary hemorrhage, stomach edema, vacuolation in the spleen, and necrosed hepatocytes.

Conclusion: Oral administration of the aqueous stem bark extract from *Cadaba farinosa Forsk* for 28 days is toxic to the liver, spleen, stomach, and lung. Reducing the therapeutic dose and period of exposure may minimize the deleterious effects of plant drugs.

Keywords: Cadaba farinosa forks; leukocytopenia; fatty changes; hypoalbuminemia; liver cell damage; apoptotic changes.

1. INTRODUCTION

Since ancient times, people in different parts of the world have developed ways to maintain health as well as prevent and treat diseases [1]. Ethnomedicinal studies documented that about 80% of the population in developing countries depend on medicinal plants for their healthcare needs [2]. The limited accessibility and affordability of modern healthcare in most developing countries have necessitated the extensive use of plant [3,4]. However, the toxicity study for most medicinal plants are unevaluated [5,6].

Cadaba farinosa Forsk (C. farinosa) belongs to the family Cappariraceae (capparaceae), a slender, evergreen woody shrub growing up to 5 meters tall and rarely trees with 45 genera and about 600 species found throughout the world, mostly in tropical and subtropical regions [7]. The leaf extracts are used for the treatments of diabetes [8], snakebite [9], haemorrhage [10], parasitic worms [8], Mastitis, and breast cancer [11]. The flowers are used as stimulant, purgative, anthelmintic, and antiphlogistic agents [7]. The stem bark is reported to be antimicrobial [12], anti-ulcerative [13] and hepatoprotective [7]. The roots are used for retained placenta [11], breast cancer [14] and female infertility [12]. The plant posses secondary metabolites such as flavonoid, alkaloid [8], amino acid, saponin, steroid [12], tannin, phenol, and diterpenes [15], spermidine alkaloid new Cadabicine, L-Stachydrine, 3hydroxystachydrine [10], spermidine alkaloids, capparisine, cadibicilone, β-Dihydroferulic acid [16]. and ά. Other phytochemicals include isoorientin, quercetin, hydroxybenzoic acid, vanillic acid, syringic acid, and 2-hydroxy-4-methoxy benzoic acid [17]. These secondary metabolites may explain the anti-oxidative, anti-diabetic, anti-inflammatory, anti-tumor, and antimicrobial effects [8,14,18].

In Northern Nigeria, *C. farinosa* sprouts are used to spice foods [19]. The leaves are scorched with cereals to make pudding (cake) called Farsa, Tigiraganda, or Balambo [20]. The chocolatecolored cake (Farsa) appears common in the markets for consumption [19] without considering the body's physiological state. In Maiduguri [21] and Jigawa States [22], the decoctions are documented to be treatments for breast and skin cancers. "One of the interviewed respondents claimed that there is no type of cancer the plant cannot cure and he was ready to bet the authors in case they doubted his submission" [8]. However, information about the plant toxicity profile and risk awareness are largely unknown. This study aimed to determine the multicellular effects from aqueous stem bark extract of *C. farinosa Forsk* on selective internal organs of Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Material

The fresh plant was collected from The Faculty of Pharmaceutical Sciences and authenticated by Dr. H.E Mshelia, Department of Pharmacognosy, Usmanu Danfodiyo University Sokoto, Nigeria. A voucher specimen (Ref. No. PCG/UDUS/CAPP/0002) was deposited for future reference.

2.2 Preparation of Extracts

The aqueous extract was prepared using the standard procedure as outlined by Gamde et al. [23] to yield 10.67% with few modifications. Plant remains from the aqueous extract were resuspended in methanol and allowed to stay for 24 hours. Both the aqueous and methanol extracts were stored in air-tight containers in a refrigerator below 10°C. The suspensions of aqueous and methanol extracts for phytochemical screening tests were prepared by using distilled water as the solvent and for the oral administration of the aqueous extract to the experimental animals.

2.3 Determination of Aqueous Extract Yield

The percentage yield of the aqueous extract (dry weight) was calculated from the equation:

Percentage yield $(g/g) = W_1 / W_2 \times 100$.

Where W_1 signifies the dry extract weight (22.4 g) following solvent evaporation of the aqueous extract and W_2 signifies the powder weight of plant material (210 g) before maceration.

2.4 Phytochemical Tests

Both the aqueous and methanol stem bark extracts of Cadaba farinosa were characterized

by color reactions indicating the presence or absence of alkaloids, glycosides, tannins, saponins, terpenoids, flavonoids, saponins, cardiac glycosides, anthraquinones following standard methods [12,15].

2.5 Animals Used

Twenty Wistar rats of both sexes weighing 160±10g were procured from The Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto Nigeria. Animals were fed with standard chow and water ad libitum. Animals were maintained in standard environmental conditions of temperature (25±2°C), relative humidity, and 12 hours of dark/light cycle before and during the experiment. All experimental procedures were approved by the Animal Ethics Committee, Usmanu Danfodiyo University Sokoto Nigeria.

2.6 Experiment Design

The experiment followed the 'Guide for Care and Use of Laboratory Animals of Laboratory Animal Centre at the Animal House, Usmanu Danfodiyo University Sokoto Nigeria. Animals were randomly divided into five groups (n = 4). The first group received distilled water (control) and the treated groups received a new formulation of C. farinosa extract administered orally at doses equivalent to 100, 200, 300, and 400 mg/kg body weight extracts. Under partial anesthesia using chloroform, blood (about 8 mL) was withdrawn by cardiac puncture and the target organs were excised from rats 24 h post-dosing for analysis.

2.7 Sample Collection

Within 24 hours of the last dose being administered, all animals were weighed and anesthetized with chloroform. Animal blood samples were collected by cardiac puncture. The blood samples for hematological analysis were collected in EDTA bottles and we used plain sample bottles for biochemical analysis following standard procedures. The fresh stomach, brain, kidney, spleen, liver, and lung tissue samples were excised from the animals, weighed, washed, and fixed with 10% formal saline for histopathological studies.

2.7.1 Haematological assessment

Blood samples were collected into tubes containing ethylene diamine tetraacetic acid (EDTA) for the determination of red blood cell (RBC), total leukocyte count (WBC), platelet count (PLT), hematocrit (HCT), and hemoglobin (Hgb) concentration using an autoanalyzer (Sysmex XK-21N, USA).

2.7.2 Biochemical assessment

Blood samples collected for the liver and kidney function tests were allowed to clot, spun, and analyzed for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), bilirubin (BIL), and electrolytes; potassium (k^+), sodium (Na⁺), and chloride (Cl⁻) using an auto-analyzer (Raytochemray 120, Germany).

2.7.3 Histopathological assessment

Tissue sections measuring 3 mm thick were processed by the paraffin wax method and the paraffin sections were cut at 3 μ m using Rotary Microtome (Surgcare Microtome, Model 335A

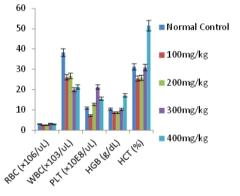


Fig. 1. Effects of extract on some haematological parameter. Analysed by ANOVA and Bonferroni post hoc. Significant at **P*≤ 0.05

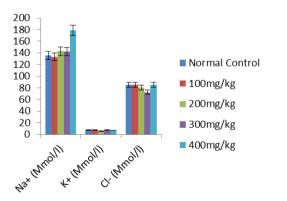


Fig. 3. Effects of extract on electrolytes. Analysed by ANOVA and Bonferroni post hoc. Significant at $*P \le 0.05$

USA) and stained with hematoxylin and eosin (H&E) for the demonstration of general tissue structures.

2.8 Statistical Data Analysis

All statistical values were expressed as Mean \pm SD and analyzed using the one-way ANOVA (SPSS, 23.0 version, Chicago, IL, USA) followed by the Bonferroni post hoc test. A statistically significant difference between experimental groups was accepted at *P*≤0.05.

3. RESULTS

3.1 Phytochemical Analysis

The phytochemical tests for both aqueous and methanolic stem bark extracts of *C. farinosa* showed the presence of carbohydrate, phenol, flavonoid, tannin, saponin, protein, diterpene, cardiac glycoside, and triterpene (Table 1).

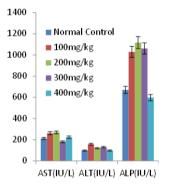


Fig. 2. Effects of extract on serum aminotransferases. Analysed by ANOVA and Bonferroni post hoc. Significant at $*P \le 0.05$

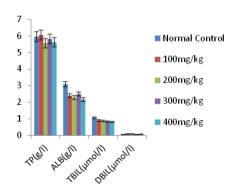


Fig. 4. Effects of extract on liver functions. Analysed by ANOVA and Bonferroni post hoc. Significant at **P*≤ 0.05

Selected Phytochemical	Test Methodology	Observation	Aqueous Inference	Methanol Inference	
Flavonoids	Shinoda's test	The intermediate orange color formed	present	present	
	Ferric chloride test	A Blue-black color was formed	present	present	
Phenols	FeCl3 reagent	A Blue-black color was formed	present	present	
Carbohydrates	Molisch test	Golden interphase was formed	present	present	
	Fehling's solution	The orange color was formed	present	present	
Alkaloids	Mayer reagents	The brown precipitate was formed	present	present	
	Wagner's reagents	The brown precipitate was formed	present	present	
	Dragendorff's reagent	Red precipitate was formed	present	absent	
Protein/amino acid	Million's test	The yellow color was formed	present	present	
Saponins	frothing test	Frothing formed remains	present	present	
Diterpenes	Copper acetate test	An Emerald green color was formed	present	present	
Tannins	FeCl3 reagent	A Blue-black color was formed	present	present	
	Lead sub- acetate test	The conspicuous precipitate was formed	absent	present	
Cardiac glycosides	Keller-Killiani's test	Brown interphase was formed	present	absent	
Triterpenoids	Salkwaski's test	The golden yellow color was formed	present	present	

Table 1. Phytochemical Analysis of *C. farinosa* aqueous and methanolic stem bark extracts

Table 2. Effect of C. farinosa extract on the hematological parameter

Parameter	Unit	Control	100 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg	P-value
Platelet	109/L	1081.7±297.3	721.0±103.00	1270.3±327.52	2128.7±841.69	1551.7±106.49	0.232
Haematocrit	%	31.233±0.284	25.500±4.957	25.800±5.100	30.900±0.000	51.500±10.300*	0.044
Haemoglobin	g/dL	10.400±0.100	8.600±1.700	8.600±1.700	10.300±0.000	17.167±3.433*	0.046
Red blood cells	109/L	3.003±0.467	2.473±0.353	2.473±0.353	3.180±0.000	2.827±0.353	0.529
White blood cells	109/L	38.297±6.020	26.100±0.907	26.800±1.000	19.933±2.239*	21.300±1.234*	0.011

Statistical data are expressed as Mean ± SD and analyzed by ANOVA followed by Bonferroni Post Hoc Test. A statistically significant difference was accepted at *P≤ 0.05

Parameters	Units	Control	100 mg/kg	200 mg/kg	300 mg/kg	400 mg/kg	P-value
Albumin	g/L	3.100±0.28	2.400±0.12 [*]	2.300±0.00 [*]	2.500±0.10 [*]	2.167±0.15 [*]	0.017
Total protein	g/L	5.967±0.6	6.067±0.18	5.567±0.04	5.833±0.66	5.633±0.47	0.941
Total bilirubin	µmol/L	1.067±0.06	0.913±0.09	0.877±0.19	0.837±0.08	0.817±0.04	0.544
Dir. Bilirubin	µmol/L	0.067±0.00	0.090±0.02	0.097±0.01	0.067±0.01	0.083±0.01	0.203
ALP	UI/L	670.6±109.4	1029.0±92.31	1117.3±196.5	1060.7±153.7	597.6±99.9	0.064
ALT	UI/L	97.6±8.41	158.3±17.0	119.6±23.0	129.3±19.3	197.6±5.2	0.113
AST	UI/L	12.6±21.07	261.3±17.9	269.0±42.1	180.6±38.5	223.6±22.0	0.285
Chloride (Cl-)	mmol/L	84.667±1.453	85.000±1.732	80.667±5.238	72.33±5.239	84.67±3.283	0.162
Sodium (Na⁺)	mmol/L	135.67±1.453	133.00±0.577	142.67±16.384	142.33±15.026	178.67±4.910	0.058
Potassium (K ⁺)	mmol/L	7.2667±0.376	7.6000±0.322	5.5333±1.656	7.2667±0.607	7.000±0.116	0.462

Table 3. Effect of *C. farinosa aqueous* stem bark extract on the biochemical parameter

Statistical data are expressed as Mean ± SD and analyzed by ANOVA followed by Bonferroni Post Hoc Test. A statistically significant difference was accepted at P≤ 0.05

3.2 Physical Observation and Mortality

No obvious signs of toxicity and mortality were observed in the treated animals as compared to the normal control.

3.3 Effect of Extract on the Haematological Parameter

The effect of the extract on hematological Parameters is presented in Table 2. Oral administration of aqueous stem bark extract of *C. farinosa* for 28 days caused a significant (P<0.05) increase in hemoglobin (P=0.046) and hematocrit (P=0.044), while white blood cell count (leukocyte) significantly (P<0.05, P=0.011)

reduced at 300 and 400 mg/kg. No significant differences were observed in the platelet and red blood cell count of treated animals.

3.4 Effect of Extract on the Biochemical Parameter

The effect of the extract on the biochemical parameter is presented in Table 3. Oral administration of aqueous stem bark extract of *C. farinosa* on the biochemical parameter for 28 days significantly (P<0.05, P=0.017) reduced serum albumin compared to control. Other biochemical changes were not statistically different from the control.

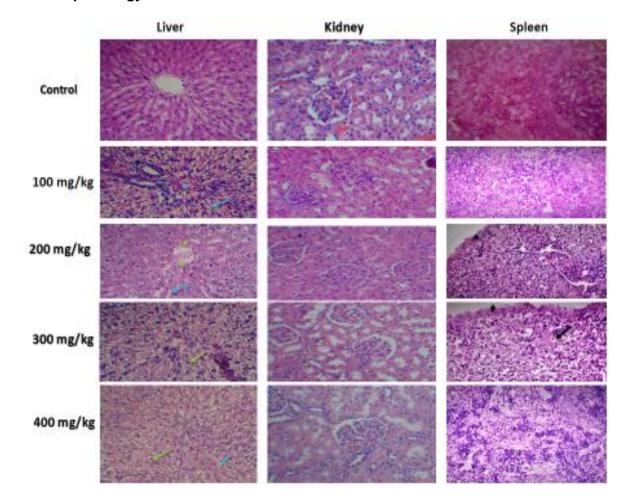


Fig. 5. The representative liver sections of animals administered extract of *C. farinosa* showed morphological characteristics of vacuolated degeneration of hepatocytes (green arrow), piecemeal necrosis (green arrowhead), inflammatory cells (blue arrowhead), and fatty changes (blue arrow). The spleen showed apoptotic changes (black arrow) and thickened capsule (black arrowhead). No histopathological changes were observed in the kidney parenchyma. (H&E. X 400)

3.5 Histopathology Effect

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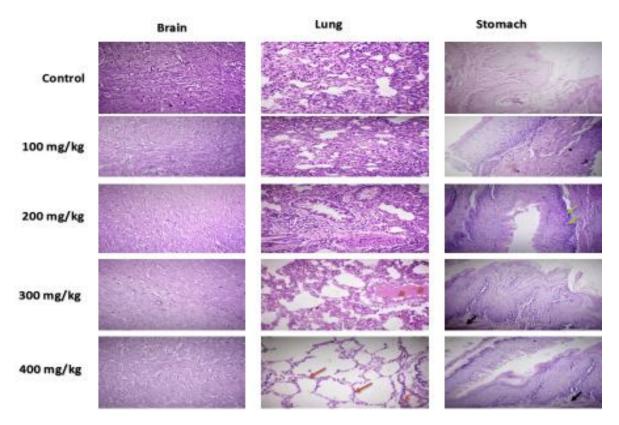


Fig. 6. The representative sections of the lung of animals administered with extract of *C. farinosa* showed expanded alveoli septae (red arrow) and pulmonary hemorrhage (red arrowhead). The stomach showed areas of interstitial edema (black arrow) and hyperplastic cells (green arrow). No histopathological changes were observed in the brain parenchyma (H&E. X 400)

4. DISCUSSION

The principal aim of evaluating the safety of any medicinal plant is to identify the nature and significance of the adverse effect and to establish the exposure level at which the effect is observed [24]. Based on our result, chronic oral administration of the aqueous stem bark extract from C. farinosa Forsk caused statistically significant hematological changes (P<0.05). The hemoglobin and hematocrit values in the treated animals were significantly elevated (Fig. 1). The elevated hemoglobin and hematocrit values might be beneficial to sickle cell patients with reduced haemoglobin [25]. The identified (Table. 1) antioxidant properties of the extract such as flavonoid preserved the heme iron of blood in its ferrous state. This trend is in agreement with the previous reports [25,26] where the extract increases the oxygen-carrying capacity of red blood cells. However, our data showed that the extract significantly reduced the white blood cell count (P<0.05, Table. 2) compared to the control. The white blood cell is

one of the most important immune components against invading pathogens [27]. Some studies have associated the reduced white blood cell count with the effects of toxicants in the extract and to stress [28]. Our finding is in agreement with previous reports [25,29]. This implied that prolonged oral administration of aqueous stem bark extract of *C. farinosa* induced leukocytopenia.

In the biochemical result, oral administration of aqueous stem bark extract of C. farinosa caused a statistically significant decrease in serum albumin (P<0.05, Table 3). However, serum aminotransferases (Fig. 2), electrolytes (Fig. 3), and other metabolic functions (Fig. 4) were not statistically different compared to the control. aminotransferases may show Serum low sensitivity in chronic liver damage where there is significant degeneration of the hepatocytes [30,31]. Moreover, the reduced serum albumin deduced that the extract of C. farinosa induced hypoalbuminemia in animals (Table. 3). Hypoalbuminemia is a sensitive indicator for liver

dysfunction [32,33]. furthermore, the histopathological sections of the liver showed morphological characteristics of cell death which models hepatocyte apoptosis (Fig. 5). The significance of apoptosis is appreciated to be the main mode of cell death in liver diseases [34].

The functional integrity of the kidney is to maintain the body's homeostatic functions through the excretion of metabolic waste products and in the regulation of intracellular fluid volume, electrolyte compositions, and acid-base balance [35]. The electrolyte result showed that the extract of *C. farinosa* has no statistically significant effect on the kidney (Table. 3). This biochemical result was further supported by the normal histological sections of the kidney (Fig. 5).

Further histopathological studies showed morphological characteristics of apoptosis in the spleen (Fig. 5). A similar trend to this model of apoptosis was caused by the chronic consumption of Chili pepper [36]. Apoptosis is a mode of cell death characterized by specific morphological and biochemical features [34]. For the reason that spleen is the body's filter against foreign substances from the circulatory system, some neutrophils wander from the peripheral blood into the splenic parenchyma to be contributing to the humoral immunological responses [37]. This may perhaps explain the neutrophils in the spleen (Fig. 5). This trend is in agreement with the number of effete erythrocytes in the hematological parameters (Table. 2).

In addition, extract induced interstitial hemorrhage in the lungs of treated animals compared to control (Fig. 6). A similar finding was documented from the aerial parts of Caralluma dalzielii [38]. Furthermore, druginduced gastrointestinal injury is a common medical problem [39]. We studied animals treated with Cadaba farinosa extract and model the characteristic features of stomach edema with mild inflammatory cell presentation (Fig. 6). The representative sections of stomach edema were further justified by the reduced serum albumin (hypoalbuminemia) in all treated animals (Table. 3). However, the extract did not cause any pathological changes in the brain cells (Fig. 6). This trend is also in agreement with the report of Kaid et al. [6], where oral administration of Goniothalamus extract had no remarkable pathology on the brain.

5. CONCLUSION

Oral administration of the aqueous stem bark extract of *Cadaba farinosa Forsk* for 28 days is toxic to the liver, spleen, stomach, and lung. Reducing the therapeutic dose and period of exposure may minimize the deleterious effects.

CONSENT

Consent was obtained from all authors.

ETHICAL APPROVAL

Approved by the Animal Ethics Committee, Usmanu Danfodiyo University Sokoto Nigeria.

DATA AVAILABILITY

Data are available from the corresponding author upon request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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