

Original Research Article

Histopathological and Biochemical effects of aqueous leaf extract of *Cadaba farinosa* on the liver of adult Wistar Rats

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ABSTRACT

Background: Plants are important source of chemical substances with therapeutic effects. Although, the promising potentials for good number of medicinal plants are being established, there exists in developing countries where people resort to herbal plants without proper awareness of the associated risks particularly in event of excessive or chronic use. Hence, the need to evaluate the histological and biochemical effects of aqueous leaf extract of *Cadaba farinosa* used traditionally for treatments of gastrointestinal parasites, cancer and diabetes in North-Eastern Nigeria. To evaluate the histological and biochemical effects of aqueous leaf extract of *Cadaba farinosa* on liver of adult Wistar rats.

Methods: Twelve adult Wistar rats of both sexes were used and divided into four groups of three rats each. Group 1 served as control. Aqueous leaf extract were orally administered for 28 days at doses of 100, 200 and 300mg/kg respectively. Biochemical and histological analysis were performed.

Results: This study showed significantly elevated levels of aspartate transaminase, alkaline phosphatase and alanine transaminase in animals treated with *Cadaba farinosa* (especially the highest dose 300mg/kg) compared to negative control. Elevated liver enzymes were corroborated by histopathological changes of liver exhibiting ballooning degenerations and steatohepatitis.

Conclusions: *Cadaba farinosa* causes hepatic injury. Hence, further work needs to be done to ascertain whether reducing the dose of *Cadaba farinosa* would ameliorate this effect. Authors speculate that injury to multiple organelles including fat droplets and endoplasmic reticulum contribute to this characteristic finding.

Keywords: Ballooning degenerations, *Cadaba farinosa*, Liver enzymes, Steatohepatitis

INTRODUCTION

Medicinal plants are important source of chemical substances with therapeutic effects.¹ The promising potentials for good number of herbal products are clearly established.² However, there exists in developing countries where people often resorts to herbal medicinal plants without proper awareness or information on the

associated risks, particularly in the event of excessive or chronic use.³ In most developing countries, many unregistered and poorly regulated herbal products are sold freely with little or no restraint. The consequence of this is an inadequate knowledge of their mode of action, potential adverse reactions, contraindications, and interactions with existing orthodox pharmaceuticals and functional foods.⁴ One of the major plants used for

treatments of gastrointestinal parasites, cancers and diabetes is *Cadaba farinosa* Forsk.⁵ The local names of *Cadaba farinosa* in Arabic, Fula and Hausa are Suraya, Baggahi and Bagayi respectively.⁶ The plant is native to Angola, Cameroon, Democratic Republic of Congo, Egypt, Ethiopia, India, Kenya, Niger, Senegal, Somalia and Nigeria inclusive.⁷ In folklore medicine, leaf extract of *Cadaba farinosa* are used for treatments of cancer and diabetes in the North-Eastern parts of Nigeria.⁸ The root, stem and leaf are used for treatments of female infertility, syphilis infections with other physiological and pharmacological applications including potency against liver damage.⁷ However, safety is a major issue with the use of herbal therapies.⁹ It has become essential to furnish the general public including healthcare professionals with adequate information to facilitate better understanding of the risks associated with use of the plant.

METHODS

Plant Collection and Identification

Identifiable parts of *Cadaba farinosa* was collected in January 2019 from the Faculty of Pharmaceutical Science, Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. The plant identification and authentication was duly made by a plant taxonomist in the Department of Pharmacognosy and Ethno-medicine with voucher number PCG/UDUS/CAPP/0002.

Plant Extraction

Plant leaves were collected and shade dried for fourteen days to ensure complete dryness. The dried materials were pulverized in clean mortar and pestle into coarse powder and stored in a polythene bag for safety till time of use. 100g of the powder was macerated in 300mL of water at room temperature for 24 hours and the solution was filtered with Whatman's filter paper to obtain particle free solution. Filtrates were evaporated to dryness at 45°C in water bath as described by Majekodunmi.¹⁰

Phytochemical Screening

Phytochemical analysis was performed on the crude extracts using the standard methods of for the qualitative screening of phytochemicals of interest.¹¹⁻¹³ The phytochemicals that were screened are: alkaloids, flavonoids, saponins, tannins, cardiac glycosides, proteins, carbohydrates, phenols, steroids, terpenoids, reducing sugar and diterpenes.

Animal Source and Handling

Prior authorization for the use of laboratory animals in the experimental study, ethical clearance (Reg. No: PTAC/Cf/OT/004-18) was obtained from the Ethical Committee for the use of laboratory animals, Department of Pharmacology, Usmanu Danfodiyo University Sokoto Nigeria. A total of twelve adult Wistar rats weighing 180-

200g were used in the experiment. The rats were obtained from Animal House, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University Sokoto. The rats were kept in cages, supplied with clean drinking water and fed ad libitum with standard commercial feed.

Experimental Design

Twelve adult Wistar rats were used and divided into four groups of three rats each. Group 1 served as negative control. Aqueous extracts of plant was administered to study groups (2, 3 and 4) at different doses of 100, 200 and 300 mg/kg respectively. Biochemical parameters such as total protein, albumin, total and conjugated bilirubin, AST, ALT, and AP were determined after 28 days (sub-chronic) using standard techniques. Histology of liver was examined 28 days after administration of the extracts at the highest dose of 300mg/kg.

Laboratory Examination

At the end of intervention, animals were sacrificed using chloroform. Blood was culled by cardiac puncture using 10ml sterile plastic syringes and needles from each subject aseptically; about 2.5ml EDTA anticoagulant bottle for hematological analysis and the rest blood into dry bottle which was allowed to clot at room temperature. Serum was collected into clean dry container after centrifugation at 3,000 RPM for 5 minutes and stored at -20°C prior to liver function tests analysis.

The liver was excised through abdominal incision and fixed in 10% formal saline for 24 hours, processed and stained by H&E.¹⁴

Tissue preparation

Blood was culled by cardiac puncture using 10ml sterile plastic syringes and needles from each subject aseptically; about 2.5ml into Ethylene Diamine Tetra Acetic Acid (EDTA) and the rest into dry bottle and allowed to clot at room temperature. The serum was collected into clean dry container after centrifugation of the blood at 3,000rpm for 5 minutes and stored at -20°C prior to analysis. Microscopic tissue slides were processed using the standard procedures described.¹⁵ Paraffin sections of 4-5µm were stained with hematoxylin and eosin.¹⁴

Statistical Analysis

The data were validated using Microsoft excel version 13 and exported it into SPSS version 23.0 (Chicago IL) for windows; for statistical analysis. All the data was expressed as Mean±standard deviation (SD). Kolmogor of seminerof method was applied for all groups to find out if the data was nonparametric. Since all the data was parametric, all the comparisons among groups were carried out using two way Analysis of Variance (ANOVA) followed by Bonferroni post hoc test.

RESULTS

In our findings, phytochemical analysis qualitatively performed on the aqueous leaf extract of *Cadaba farinosa* showed alkaloids, flavonoids, saponins, tannins, cardiac glycosides, proteins, carbohydrates, phenols, steroids, terpenoids and diterpenes (Table 1).

The liver enzymatic activity of alanine (ALT) and aspartate (AST) aminotransferases and alkaline phosphatase (AP) were significantly elevated in all experimental animals treated with the plant extract especially at the highest concentration of 300mg/kg where P=0.001 (Table 2, 3).

Table 1: Qualitative Phytochemical Screening of Aqueous Leaf Extract of *Cadaba farinosa*.

Phytochemicals	Status
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Cardiac glycosides	+
Proteins	+
Carbohydrates	+
Phenols	+
Steroids	+
Terpenoids	+
Diterpenes	+

Key = + present

Table 2: Effect of Aqueous Leaf Extract of *Cadaba farinosa* on some Biochemical Parameters of adult Wistar rats (n=3).

Factors	TB	DB	TP	ALB	AST	ALT	AP
Control	0.79±0.11	0.16±0.04	6.63±0.15	33.27±50.95	92.00±0.00	260.00±10.00	194.00±7.21
Group1	0.46±0.04*	0.34±0.48	6.80±0.26	2.73±0.23	129.67±6.66*	248.67±2.31	526.33±11.85
Group2	0.71±0.22	0.11±0.01	7.17±0.84	3.23±0.71	118.67±2.89	214.67±15.89*	816.67±423.23*
Group3	0.97±0.21	0.06±0.04	6.97±0.68	2.83±0.15	144.00±21.66*	214.00±1.00*	875.67±64.38*

Data were presented as mean ± SD. Analysed by ANOVA followed by Kolmogor of Seminerof method and Bonferroni Post Hoc Test applied, (n=3). Asterisks values are statistically significant. Considering P-values (F-teats 19.364, P = 0.001) Pillai's Trace.

Table 3: Multivariate Analysis Effects of Aqueous Extraction of *Cadaba farinosa* on Liver Function Parameters of Adult Wistar Rats.

Dependent Variable	Sum of Squares	df	Mean square	F	Sig.
TB	0.393	3	0.131	4.88	0.032
DB	0.13	3	0.043	0.746	0.555
TP	0.469	3	0.156	0.496	0.695
ALB	2070.67	3	690.223	1.063	0.417
AST	4351.583	3	1450.528	11.122	0.003
ALT	4993.333	3	1664.444	18.563	0.001
AP	879477.667	3	293159.222	6.392	0.016

The negative control liver shows normal histomorphological features of radiating chords of hepatocytes, bile duct and central vein (Figure 1).

Liver Histology

Liver section treated with 100mg/kg of extract shows infiltrating inflammatory cells and hepatocytes exhibiting nuclear enlargements, H&E. Mag. X 100 (Figure 2).

Liver treated with 200mg of extract shows Hepatocytes exhibiting ballooning degeneration with steatosis and necrosis, H&E. Mag. X 100 (Figure 3, 4).

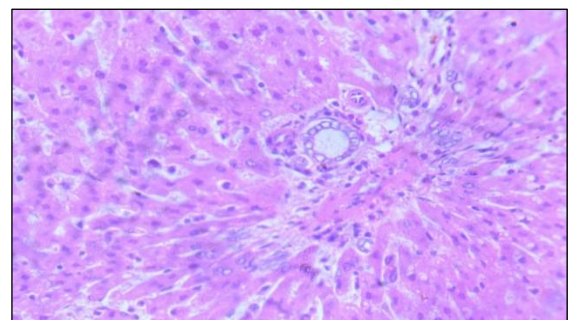


Figure 1: Normal liver section shows central vein, bile duct, hepatic artery and radiating chords of hepatocytes. H&E. Mag. X 100.

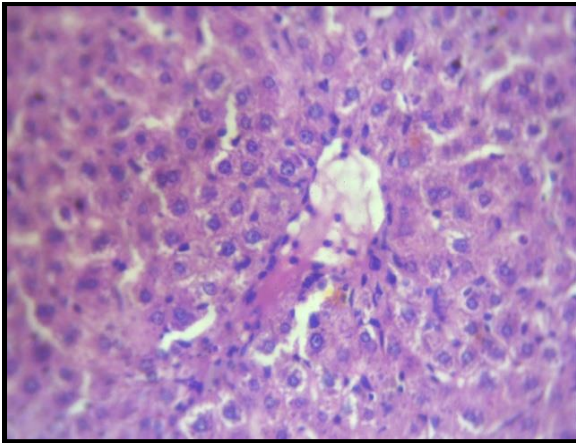


Figure 2: Liver section treated with 100mg of extract shows infiltrating inflammatory cells and hepatocytes exhibiting nuclear enlargements. H&E. Mag. X 100.

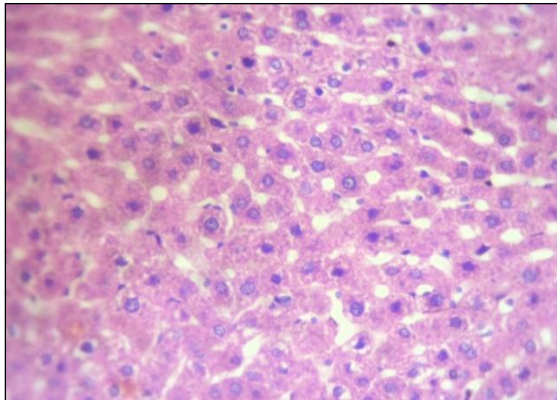


Figure 3: Liver section treated with 200mg of extract shows Hepatocytes exhibiting ballooning degeneration with steatosis and necrosis. H&E. Mag. X 100.

The ASC was more in males bilaterally than in females. The difference was statistically significant on right side ($p = 0.00$). The ASS was more in females than in males on right and vice versa on the left side.

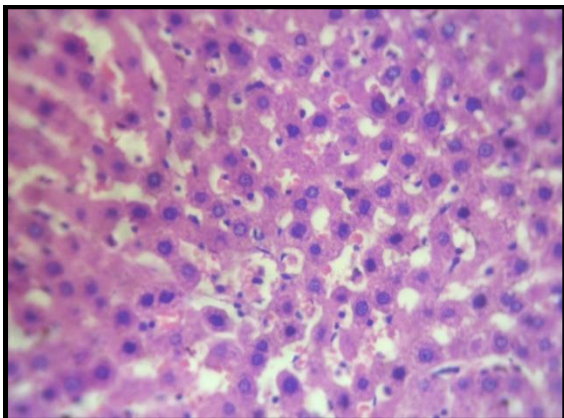


Figure 4: Liver section treated with 300mg of extract shows Hepatocytes exhibiting ballooning degeneration with steatosis and necrosis. H&E. Mag. X 100.

DISCUSSION

The liver performs different kinds of biochemical, synthetic and excretory functions in the body.¹⁶ In this study, we found that aqueous leaf extracts of *Cadaba farinosa* has effects on the liver. The injured hepatocyte releases AST, ALP and ALT into the bloodstream.

The classical laboratory findings of hepatotoxicity are elevation in AST, ALP and ALT.¹⁷ Therefore, liver enzymes levels in serum and the liver histology were examined as indicators of hepatocellular damage.¹⁸ In our findings, the enzymatic activity of alanine (ALT) and aspartate (AST) aminotransferases and alkaline phosphatase (ALP) were significantly elevated in all experimental animals treated with 300mg/kg (highest concentration where $P=0.001$) compared to the negative control group (Table 2). Alanine transaminase (ALT) and alkaline phosphatase (ALP) were significantly increased in animals treated with 200mg/kg, while only aspartate transaminase (AST) was elevated at 100mg/kg. It implies that the effects of *Cadaba farinosa* is dosage dependent since enzymes concentrations were expressed according to the dose administered (100, 200 and 300mg) compared to controls. Moreover, many studies involving the experimental induction of liver injury with several drugs and chemicals have reported an upsurge in the levels of these markers of liver toxicity.¹⁷ ALP is a membrane-bound enzyme that is usually used as a marker for the integrity of the plasma membrane and endoplasmic reticulum and an increase in ALP activities of the serum implies membrane damage to the tissues. Ukwenya, 2019 reported that the increase in the level of ALP may be as a result of stress imposed on the tissue by the drug, which might have resulted in the loss of the enzyme molecule through exudation into extracellular fluid. It was also inferred that, in a bid to offset this stress, the tissue may increase the de novo synthesis of ALP, thus accounting for the upsurge in ALP activities in this tissue.¹⁷ This finding was in agreement with the report that liver enzymes were more elevated in animals treated with higher dose compared to the control.¹⁹ This could be due to either the severe liver injuries caused with increased dose of extract administered or due to the fact that the release of liver enzymes (AST and AP) is in higher concentration from different tissues apart from the liver such as kidneys, heart or pancreas.²⁰ Similarly, a report on the biochemical and histopathological profiling of Wistar rat treated with *Brassica napus* (where P value is 0.001).²¹ When liver tissue is damaged, additional AST, AP and ALT are released into the bloodstream and raise the serum enzymes level. As a result, the amount of AST, AP and ALT in the blood is directly associated with the amount of tissue damage.²¹ Ordinarily, liver cell damage caused by chemical substances is characterized and assessed by the level of plasma enzymes.²²

The histologic findings revealed hepatocytes exhibiting ballooning degeneration, steatosis and tissue necrosis which corroborated the hepatotoxicity of the plant at

different concentrations (100, 200, and 300mg) that was expressed by the increased liver enzymes (AST, ALT, and AP). The insult on the liver cells following the administration of antioxidant vitamins during caffeinated and non-caffeinated paracetamol was in agreement with this study.²³ Hepatocellular ballooning with steatohepatitis are integral pathological changes in the diagnosis of nonalcoholic steatohepatitis (NASH) that is defined by cellular enlargement 1.5-2 times the normal hepatocyte diameter with rarefied cytoplasm.²⁴ ²⁵.Therefore, NASH a major distinguishing feature which corroborated the elevated liver enzymes, indicating a greater risk of disease progression in the liver.¹⁶

CONCLUSION

This study indicates that aqueous leaf extract of *Cadaba farinosa* causes hepatic injury. We speculate that injury to multiple organelles including fat droplets and endoplasmic reticulum contribute to this characteristic finding. Hence, further work needs to be done to ascertain whether reducing the dose of *Cadaba farinosa* would ameliorate this effect.

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