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Acute and sub-acute toxicity studies of Methanol stem bark extract of *Cadaba farinosa* (Forssk) in wistar rats.

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Abstract
The study focused on evaluating the toxicity profile of the methanol stem bark extract of *Cadaba farinose* (Forssk), considering possibilities of adverse effects arising from continual use of herbal plants especially in the management of long-term conditions. *Cadaba farinosa* is a slender shrub plant widely used traditionally in the treatment of various diseases such as diabetes mellitus, inflammations, rheumatic pains and various infections including skin and intestine both in Africa and India. The acute toxicity study and sub-acute toxicity studies were carried out in Wistar rats using Lorke's Method (1983) and the OECD Guidelines 425. For the sub-acute toxicity studies, twenty rats weighing between 120 and 170g were randomly grouped into 4 groups of 5 rats each. Group 1 (control) received 10ml/kg of distilled water and the others received doses of 40, 200 and 1000mg/kg *p.o* of extract for 28 days. The acute toxicity revealed no mortality or behavioural signs of toxicity in both phases up to 5000mg/kg. The haematological indices revealed a significant (p<0.05) increase in the WBC count, MCV and PLT of the groups that received 1000mg/kg of the extract after treatment for 28 days. The liver function test showed a significant increase (p<0.05) in AST and ALT. Renal indices showed a significant decrease in creatinine and a significant increase (p<0.05) in plasma Na^+, K^+ and Cl^- ion concentration across the groups. Histopathological section of the spleen revealed follicular hyperplasia. The results suggest that the plant could be slightly toxic to the liver and the spleen on long-term use.

Keywords: *Acute and sub-acute toxicity, Cadaba farinose, medicinal plant, diabetes, haematological parameters, aspartate transaminase.*

Introduction
Plants have been utilized by man for its food and medicinal values for as long as man existed on earth. Worldwide cultures have responded to the need and challenges of having to manage various ailments based on their folklores, beliefs or cultures they imbibed or inherited. Plants are common features in different ethnic groups as a major source for remedy to their health needs and challenges. Plants still remains an important source for treatment globally. Majority of the populace especially in rural areas still depend on plant for their health needs in spite of considerable breakthrough in modern medicine (Ekor, 2014; WHO 2004). Chemical constituents present in plants have been used widely and are responsible for the different effects produced by these plants in preventing and treating diseases or to promote health and general well-being (Ekor, 2014; Ibegbu *et al.*, 2012). *Cadaba farinosa* (CF) is a slender shrub with a strongly furrowed skin rarely straight with a yellowish grey bark. Leaves are numerous and small, leaf blade is elliptic to obovate 4-40×3-36mm. Petiole are up to 3-4mm long (El-Kamali and Bosch, 2012; Fici *et al.*, 2008). Flowers are yellowish green in racemes with farinosa on axis 0.8-4.5cm long. The interior of the fruit is orange-red when mature, the seeds are the size of a millet grain, comma shaped, shinning, dark-brown and arranged in a single layer within the fruit (Halilu *et al.*, 2021; Amber,1990).

The plant is known to be widely used in the treatment of various ailments such as diabetes mellitus, inflammation, intestinal worm, pains such as rheumatism and constipation (Nadkarni, 2002; Amber, 1990; Burkil, 1985). The leave extracts have been used to for various domesticated purposes such as inducing and stimulating milk production nursing mothers in Burkina Faso (Lompo-Ouedrago, 2003) and as food. A mixture of the crushed leaves of CF and millet flour is used to treat cough (Orwa *et al.*, 2009). A major concern in the use of traditional medicinal plants is usually their safety. Some of these plants are consumed for long periods especially in the management of certain chronic diseases. Hence, there are possibilities of exposing users to toxic levels of potentially toxic plants due to repeated use. Considering the probable long-term usage of this plant in the treatment of certain ailments like diabetes. This study seeks to evaluate the acute and subacute toxicity profile of the methanol stem bark extract of *Cadaba farinosa* in Wistar albino rats.

Materials and Methods

Plant Collection and Preparation of Extract

The stem bark of *Cadaba farinosa* was collected in January 2019, beside the medicinal garden of Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto by Mallam Ibrahim Dan Ahmadu (Faculty herbalist). The plant was identified and authenticated in the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Sokoto State Nigeria by Mshelia, H.E. A herbarium specimen was prepared and assigned voucher number (PCG/UDUS/CAPP/0002). The herbarium specimen was deposited at the department for reference purposes.

Fresh stem bark of *Cadaba farinosa* was harvested was shade dried for 14 days. The dried bark plant material was pulverized to powder using wooden pestle and mortar. Five hundred grams (500 g) of the dried powder was subjected to cold maceration for 48/72 hours with methanol as the solvent for extraction, the mixture was allowed to stand for 5 hours and shaken periodically. Whatman filter paper (size 1.5) was used for filtration of the mixture and the extract was concentrated on water bath at 40°C. The dried extract transferred into an airtight container and labelled for storage.

Experimental Animals

Adult Wistar rats of both sexes weighing between (120g and 170g) were used for this study. Animals were obtained from the animal house facility of Ahmadu Bello University Zaria, Kaduna State Nigeria. The rats were housed under controlled conditions in the experimental animal handling facility of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. The experimental animal room had 12-hour light /12-hour dark scheduled and maintained at a temperature of 23±3°C throughout the study. Animals were fed with commercially available rat pelleted diet (livestock feed) and were allowed access to water *ad libitum* throughout the period of the experiment. The experimental protocols were approved by the institutional animal care and use committee, Department of Pharmacology and Toxicology, Usmanu Danfodiyo University Sokoto. Animals were certified fit for the experiment before the commencement of the study. Animal beddings were changed on alternate days and the animals were sacrificed in a humane manner at the end of the experiment by cervical dislocation.

Preliminary Phytochemical Tests

The plant extract was subjected to standard phytochemical methods and qualitative analysis according to protocols described Sofowora (2008) and Evans (2009). The presence of alkaloid, flavonoids, sterol, triterpenes, anthraquinone, saponins and cardiac glycoside were tested for.

Acute Toxicity Study

The oral acute toxicity was studied using the method described by Lorke (1983). The study was conducted in two phases. In the first phase, nine animals of both sexes were randomly distributed into three groups of three animals each. Groups 1, 2 and 3 received 10, 100 and 1000mg/kg of the extract and were observed for 24 hours for any signs of toxicity and mortality. In the second phase four animals were used, one animal per group and were administered with

1200, 1600, 2900 and 5000 mg/kg doses of extract respectively. The LD_{50} was determined by calculating the geometric mean of the highest dose that survived and the lowest dose that killed the rat.

Sub-Acute Toxicity Study

Twenty Wistar albino rats previously fasted for 24hours were divided into four groups each containing five animals respectively. Each group received treatment as follows:

Group1: (control): received 10mg/kg/day of distilled water
Group 2: received 40mg/kg of the extract
Group 3: received 200mg/kg of the extract
Group 4: received 100mg/kg of the extract

Administration of extract was done orally once daily for 28 days. The rats had access to food and water throughout the period of the experiment. Animals were observed daily for general symptoms of toxicity and mortality. The weight of the animals was taken every week.

On the 29th day, the animals were sacrificed under light chloroform anaesthesia. Blood samples were collected by cardiac puncture for biochemical and haematological analysis. The heart, liver, kidney and the spleen were excised and fixed for histopathological analysis.

Biochemical Parameters Analysis

Portion of the blood sample collected was stored in plane tubes for biochemical analysis. Liver function tests, electrolytes, urea and creatinine parameters were carried out in Specialist Hospital Sokoto.

Haematological Analysis

Portion of the collected blood stored in the EDTA tubes were used to carry out the haematological analysis in the Department of Haematology, Usmanu Danfodiyo University Teaching Hospital (UDUTH). The following analysis were carried out; White Blood Cell Count (WBC) and differentials, Haematocrit (HCT), Haemoglobin (Hb), Red Blood Cell Count (RBC), Mean Corpuscular volume and Platelet count.

Histopathological Assessment

The tissues (heart, liver, kidney and spleen) which were fixed were dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax melting at 60^0C. It was then cut into session of 4-5 um thick, stained with haematoxylin eosin and observed under the microscope at a magnification power of 100X.

Statistical Analysis

All results are expressed as the mean ±S.E.M. Differences between groups were determined by one way analysis of variance (ANOVA) using statistical package for social sciences (SPSS, version 20.0) software for windows. Post-hoc test for inter-groups was applied, significance was considered at $p<0.05$.

Results

Preliminary Phytochemical Test

The phytochemical screening revealed the presence of carbohydrates, saponins, phenol, cardiac glycosides, alkaloid and triterpenes, while flavonoids, tannins and anthraquinones were absent.

Acute Toxicity Results

After the oral administration of the highest dose of 5000 mg/kg in the second phase, there was no mortality. There were also no signs of behavioural toxicity such as increase in motor activity, tremor, rolling, loss of righting reflex, sedation, colic convulsion, tonic extension and muscle spasm observed in the animals.

Acute toxicity studies

There was no mortality observed in the first and second phase of acute toxicity study. This is depicted in Table 1.

Table 1: Result of oral acute toxicity studies of methanol stem-bark extract of *Cadaba farinosa* in rats.

Dose (mg/kg) PO	Number of Rats Dead/used	% Mortality
	PHASE I	
10	0/3	0
100	0/3	0
1000	0/3	0
	PHASE II	
1600	0/1	0
2900	0/1	0
5000	0/1	0

Effect of Methanol stem-bark Extract of *Cadaba farinosa* (Forssk) on haematological indices following 28 days' sub-acute oral treatment in Wistar rats

After twenty-eight days' oral administration of methanol stem-bark extract of *Cadaba farinosa*, there was a significant ($p < 0.05$) increased in white blood cell count (WBC) and at a dose of 1000mg/kg extract there was a significant increase in platelets. (Figure 1).

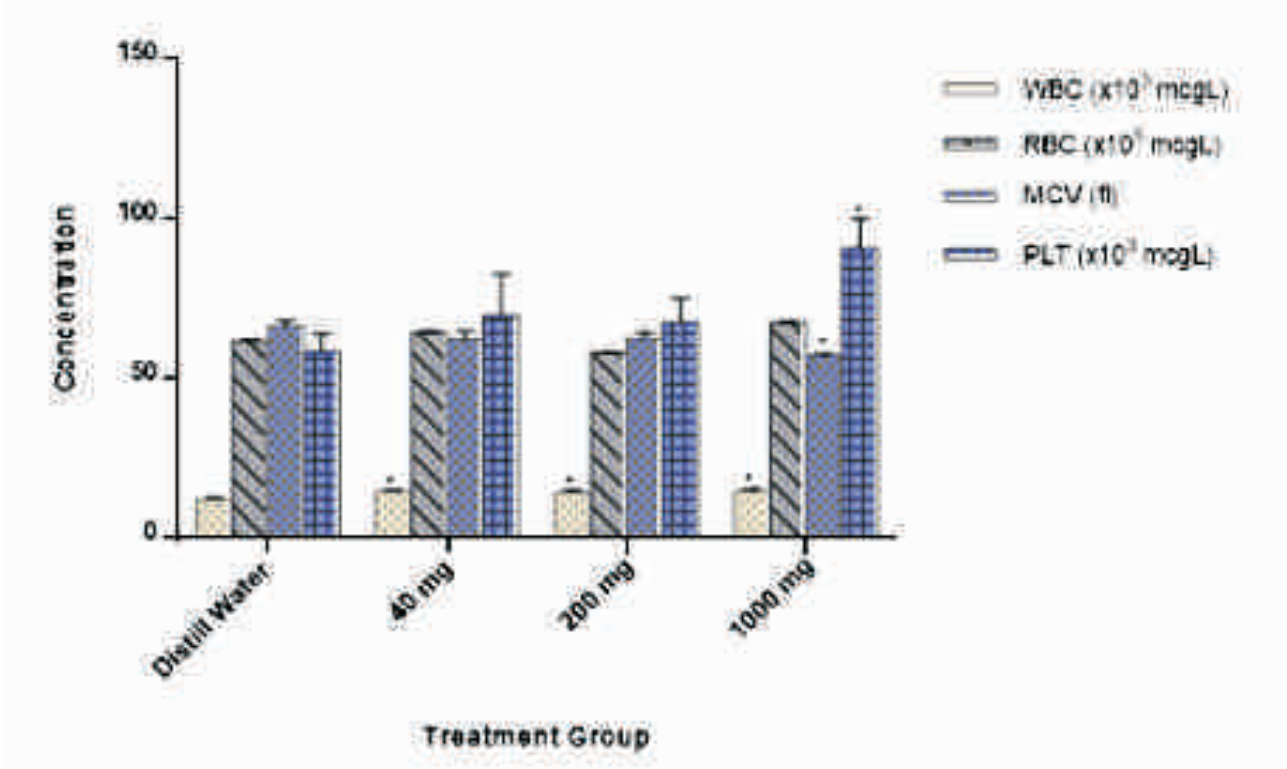

Figure 1: Effect of Methanol stem-bark extract of *Cadaba farinose* hematologic parameters
*Statistical significance $p < 0.05$ using One-Way Anova (Dunnet post-hoc test) WBC = White Blood Cells, RBC = Red Blood Cells, MCV = Mean Cells Volume, PLT = Platelet.

Effect of Methanol stem-bark Extract of *Cadaba farinosa* (Forssk) on Renal Function Indices following 28 days' sub-chronic oral treatment in wistar rats

There was a significant ($p< 0.05$) decrease in creatinine and sodium ion, while at the dose of 1000 mg/kg extract a significant increase in serum chloride was observed. (Table 2).

Table 2: Effect of Methanol stem-bark Extract of *Cadaba farinosa* (Forssk) on renal function indices following 28 days sub-chronic oral treatment in Wistar rats

Treatment/ Dose (mg/kg)	Urea (mmol/L)	Creatinine (mg/dl)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3 (mmol/L)
Distilled water	5.92±0.18	1.32±0.08	141.00±0.70	6.56±0.18	108.80±1.15	25.00±0.70
40	5.74±0.26	1.04±0.50*	156.80±0.66*	5.80±0.13	107.80±1.35	25.60±0.92
200	6.40±0.10	1.12±0.05	158.60±1.02*	6.34±0.35	106.60±1.36	25.20±0.58
1000	5.66±0.05	1.04±0.09*	163.20±1.24*	5.90±0.04	119.80±2.10*	25.80±0.58

Data expressed as Mean ± SEM, SEM = Standard Error of Mean n= 6, *p <0.05, Na^+ = Sodium ion, K^+ = Potassium ion, Cl^- = Chloride ion, HCO_3 = Bicarbonate. Dunnet Post-hoc test.

Effect of methanol stem-bark extract of *Cadaba farinosa* (Forssk) on liver function Indices following 28 days' sub-chronic oral treatment in Wistar rats

There was significant ($p< 0.05$) increase in liver enzyme specifically, aspartate transaminase (AST) and Alanine transaminase (ALT) across all doses of the extract used. Figure 2.

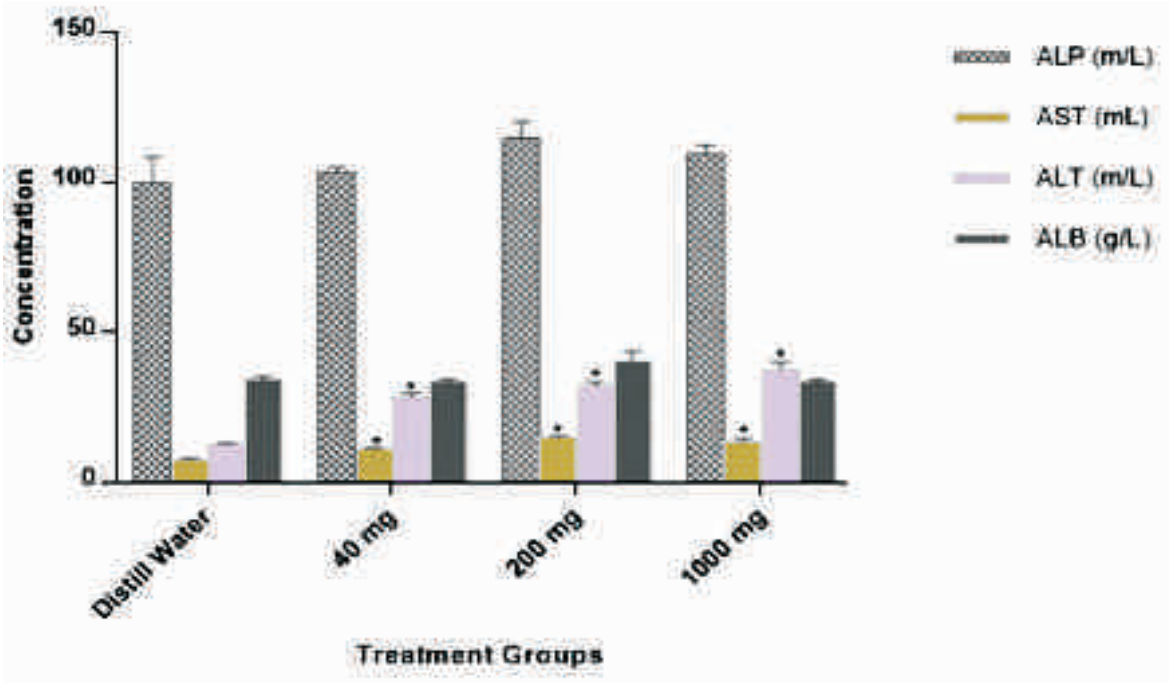

Figure 2: Effect of Methanol stem-bark extract of *Cadaba farinosa* on liver function parameters

* Statistical significance p <0.05 using One-Way Anova (Dunnet post-hoc test) ALP = alkaline phosphatase, AST = aspartase transaminase, ALT= alanine transaminase, ALB = albumin.

Effect of methanol stem-bark Extract of *Cadaba farinosa* (Forssk) histology of the liver, kidney and spleen following 28 days' sub-chronic oral treatment in Wistar rats

The histology of the liver sections of rats following 28 days' oral administration of methanol stem-bark of *Cadaba farinosa* showed normal portal triad, central vein and hepatocytes in both the control and extract treated groups. The histology of the kidney also showed normal glomeruli, tubules and interstitium in both the control and extract treated groups. While the histology of the spleen showed few pathological features such as follicular hyperplasia, congestion and oedema. (black arrow) in the extract treated groups (Plate 1).

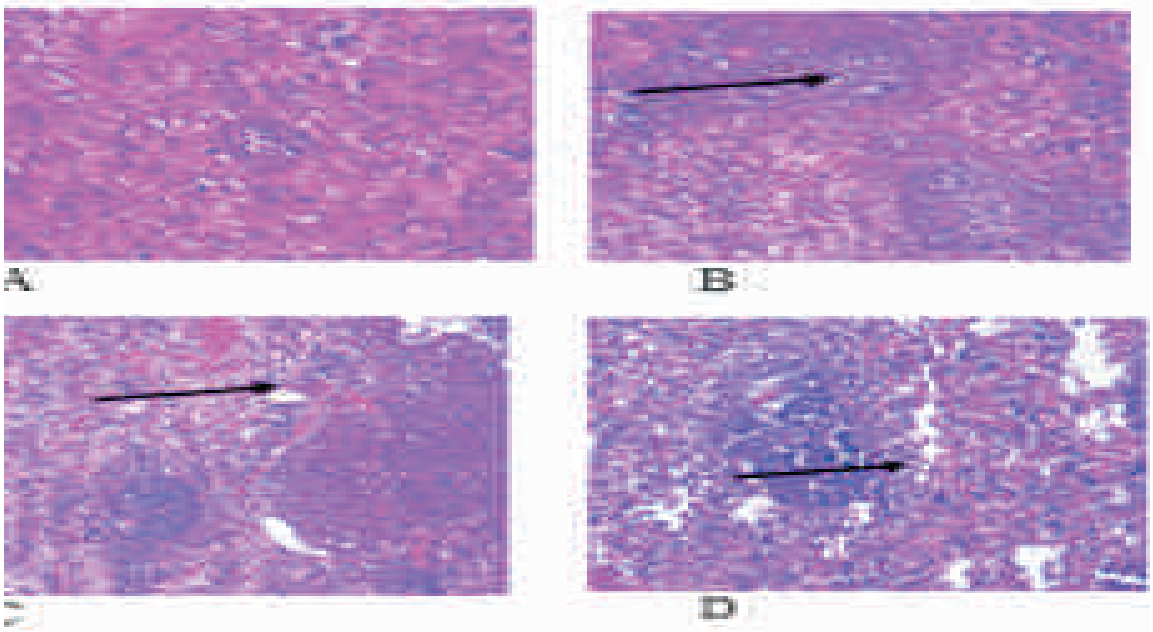

Plate 1: Photomicrograph of a section of rat spleen treated with methanol stem-bark extract of *Cadaba farinosa* following 28 days' oral administration

(H and E, 100x) showing follicular hyperplasia, congestion and oedema. (black arrow). (A) Distilled water treated group while B, C and D are 40, 200 and 1000mg/kg extract treated groups.

Discussion

Herbal plants have become an important component of healthcare for people not only rural Africa but the world over. It is important in medicinal plant use to establish the nature and the extent of toxic effect of their constituents (Ibrahim *etal*., 2016). Some of the potent constituents of these herbal plants may not always end up in the development of pharmaceuticals, however, their effect on the body cannot be ignored, considering the fact that plants are edible and are ingested for various reasons. Repeated exposures of doses of extracts of medicinal plants may reveal its toxic effects on vital organs of the body. Haemopioetic parameters are very sensitive to toxic substances and are important markers for evaluating the physiologic and pathological effect of toxins in animals.

This study revealed some phytochemical constituents similar to those previously reported (Gamde *et al*., 2019; Ezekiel and Kadam, 2015; Umesh *et al*., 2010; Aber, 1990). However, flavonoids and tannins were absent from this study. This could be attributed to the part of the plant used, while this study utilised methanol extract of the stem bark of *Cadaba farinose* previous studies used the aqueous leaf extract of the plant. The level of toxicity of herbal plants may sometimes be proportional to the duration of the use. The median lethal dose (LD_{50}) calculated suggest that this plant is relatively safe, this finding is in agreement with the findings by Umesh *et al*. (2010).

Repeated exposure of rats to this extract over 28 days revealed no physical toxic behaviours. The hematologic indices showed a significant

increase in the white blood cell (WBC) count while other parameters were within normal limit. This increase in WBC count could be due to certain phytochemical constituents in the extract that could trigger the production of WBC as defence mechanism against foreign materials. This comparable to the findings of Alkali *et al*., (2019), which reported an increase in WBC after 28 days but no significant toxic effect of extract on some organs analysed.

The liver function test showed a significant increase in aspartate transaminase (AST) and alanine transaminase (ALT). Our finding is consistent with previous reports (Gamde *et al*., 2019; Abubakar *et al*., 2015). However, histological reports showed follicular hyperplasia, congestion and oedema in the spleen tissues and not the liver. The increase in AST and ALT might be as a result of possible inflammatory effect of extract on the liver. However, congestion and oedema in the spleen tissues suggest a slight toxic effect on the spleen, which may be amplified if the extract is administered for much longer periods than 28 days or at much higher doses. This hypothesis however will require further studies to confirm it.

Conclusion

The acute and sub-acute toxicity profile of *Cadaba farinosa* was assessed. Results shows that the methanol stem bark extract of the plant is relatively safe in short term and moderately dose usage. However, *Cadaba farinosa* could be slightly toxic to the liver and the spleen on long-term usage.

Conflict of Interest Declaration: The authors declare that they have no conflict of interest.

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