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Haematological and Lipid Studies of Methanol Stem Bark Extract of *Detarium microcarpum* in Rats

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ABSTRACT: Detarium microcarpum (DM) also known as Tallow tree or Sweet dattock trees are used as medicinal plant in northern Nigeria for the management of disease and ailments. In this study, the stem bark extract (methanol) of D. microcarpum was evaluated for phytochemical constituents, haematological parameters, serum lipid and its effect on body weight of the rats. The preliminary phytochemical studies were carried out using standard protocols. The oral median LD50 of the stem bark extract was calculated to be 471.2 mg/kg body weight in rats. The haematological studies showed significant ($p<0.05$) increase in mean corpuscular volume (MCV) at doses of 70 and 140 mg/kg and also a significant ($p<0.05$) elevation of lymphocytes at all the doses tested. Serum lipid profile indicated significant ($p<0.05$) decrease in total cholesterol and low-density lipoprotein (LDL-C) at 70 and 140 mg/kg. The extract at dose of 140 mg/kg significantly ($p<0.05$) decreases the mean body weight of the rats after the third and fourth week of oral administration.

Keywords: *Detarium microcarpum,* Phytochemicals, Haematological Parameters, Secondary Metabolites, Lipid profile

Introduction

Today, about half of the global population lacks complete coverage of basic health services, and for many, herbal and traditional products derived from medicinal herbs remain of crucial importance [1]. Medicinal plants motivated many pharmaceuticals industries due to their usage in both therapeutics and immune enhancers. The medicinal plants have varying unique roles such as anticancer, sedatives, analgesics, anti-inflammatory, cardiac diseases etc. [2]. Therefore, exploring and understanding plant chemicals and their properties will stress their contribution in the modern-day drug discoveries.

Detarium microcarpum Guill and Perr, commonly known as Tallow or Sweet dattock tree belong to a legume family *Caesalpiniaceae.* Although, the tree is underexploited, it is naturally grown in both the dry and humid forests of Central and West African regions. *D. microcarpum* fruit is edible and its bark, roots and leaves are used as herbal medicines [3, 4]. The stem bark extract is used for the treatment of diarrhoea including dysentery; amoebiasis, gonorrhoea rheumatism and other infections [5]. *D. microcarpum was* used in the treatment of inflammations and pains [4], and in management of typhoid fever [6].

The present study was to evaluate effect of methanol stem bark extract of *Detarium microcarpum* for phytochemical, haematological parameters and serum lipid in Wistar albino rats.

Materials and Methods

Plant Materials

The plant material was collected at Tsolonbashi village of Jigawa State, Nigeria. The plant was identified and authenticated in the Herbarium with specimen voucher number 0071.

Experimental Animals

Wistar rats (100-130g) of either sexes were obtained from the Department of Pharmacology, Bayero University Kano. The animals were allowed free access to standard feed and water *ad libitum*. Experiments were carried out according to NIH Publication No. 80- 23.

Chemicals and Reagents

Methanol was purchased from Sigma–Aldrich (Steinheim, *Germany*) and Normal saline from Unique pharmaceuticals. All other reagents used were of analytical grade.

Preparation of Extract

As described in the previous studies [4], powdered plant material was soaked in 70% methanol for seven days. The sample was filtered using Whatman filter paper and subjected to dryness on water bath. The extract obtained was kept in a desiccator until further use.

Phytochemical analysis

Phytochemical analysis for terpenoids, alkaloids, saponin, tannin, anthraquinone, volatile oil, cardiac glycoside, reducing sugars, phlabotannins and flavonoids were conducted based on the standard procedures described by Trease and Evans [7].

Toxicity Studies

Acute toxicity study

Median Lethal Dose (LD50) Determination

The LD_{50} of the extract was determined using Lorke's (1983) method [8]. Animals were deprived of food for 12-16 hours prior to administration of extract. The study was carried out in two phases. In phase one, three groups of three Wistar rats per group were used. The extract was administered orally in geometrical increasing doses (10, 100 and 1000 mg/kg). The treated animals were observed for four hours post administration and subsequently for 24 hours for signs of toxicity including death. In the second phase specific doses of (140, 225, 370 and 600 mg/kg) were administered based on the result of the first phase. The LD_{50} value was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

Sub-chronic Toxicity Studies

The study was conducted in accordance with WHO (1993) guidelines [9]. Twenty-four rats of either sexes were deprived of food for 12 hours, and divided in to four groups of six rats each. Group one served as control and received normal saline, while rats in groups 2, 3 and 4 were given graded dose of extract (35, 70 and 140 mg/kg) respectively for 28 days using oral gavage tube. The rats were allowed access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality. The body weight of individual rat in each group was taken at weekly intervals for 4 days using digital weighing balance. Rats were sacrificed on the 29^{th} day of the experiment, blood samples were collected for haematological and lipid profile studies.

Results

Body weight result for phase I and phase II median lethal dose in Rats

The dose of oral median lethal dose 10, 100 and 1000 mg/kg were used in the first phase of the oral median lethal dose and mortality was recorded in all the three rats used for 1,000 mg/kg, in the second phase mortality was recorded in the rat used for 600 mg/kg.

Table 1: Drug administration for phase I and phase II and median lethal dose in rats

Drug Administration	Phase I			Phase II			
Oral dose (mg/kg)	10	100	1000	140	225	370	600
No. of animals that died	0	0	3	0	0	0	1
No. of animals used	3	3	3	1	1	1	1

LD_{50} is calculated as geometric mean on the doses for which 0/1 and 1/1 were found. In the phase II for oral median lethal dose is as follows:

370 mg/kg body weight 0/1
600 mg/kg body weight 1/1

$$= \sqrt{370\ x\ 600}$$

LD_{50} = 471.2 mg/kg.

By taking the 30% of 470 is 141, so we used 140, 70 and 35 mg/kg as our doses for the experiment.

Phytochemical constituents of the methanol stem bark extract of *Detarium microcarpum*

The phytochemical analysis showed the presence of terpenoids, alkaloids, saponins, tannin, volatile oil, cardiac glycoside, reducing sugars, phlabotannins and flavonoids while anthraquinone is not detected.

Table 2: Phytochemical constituents of the methanol stem bark extract of *D. microcarpum*

Phytochemicals	Qualitative Assessment
Steroids	+
Alkaloids	+
Reducing sugars	+
Phlabotanins	+
Tannins	+
Saponins	+
Flavonoids	+
Terpenoids	+
Cardiac glycosides	+
Volatile oils	+
Anthraquinones	–

+ = Detected
– = Not detected

Effect of methanol stem bark extract of *D. microcarpum* on body weight after 28 days daily oral administration in rats

The extract at doses of 35 and 70 mg/kg body weight produced no significant changes in body weight of rats during 28 days daily oral administration when compared with the normal control. However, at the dose of 140 mg/kg significant ($p<0.05$) decrease in mean body weight of the rats was observed after the third (100.33±6.7) and the fourth (99.83±6.8) weeks of oral daily administration, when compared with the normal control (Table 3).

Table 3: Effect of methanol stem bark extract of *D. microcarpum* on body weight after 28 days daily oral administration in rats

Treatment	Day 1	Week 1	Week 2	Week 3	Week 4
Control	117.82 ± 6.2	115.50 ± 7.5	120.17 ± 7.6	124.82 ± 6.9	126.01 ± 7.6
D.M. (35 mg/kg)	103.03 ± 3.1	103.67 ± 3.0	118.33 ± 2.9	125.83 ± 3.2	116.62 ± 2.9
D.M. (70 mg/kg)	108.67 ± 8.3	119.67 ± 9.8	118.33 ± 2.9	115.83 ± 9.2	116.62 ± 9.1
D.M. (140 mg/kg)	105.33 ± 8.4	103.83 ± 8.3	104.50 ± 7.5	100.33 ± 6.7[a]	99.83 ± 6.8[a]

Values are expressed as the mean ± SEM (n=6). Values with superscript indicate significant difference between the control and treatment groups at $p < 0.05$.
D.M. = Extract of *Detarium microcarpum*.

Effect of methanol stem bark extract of *D. microcarpum* on haematological Parameters after 28 days daily oral administration in rats

The extract at all doses tested (35, 70 and 140 mg/kg) showed no significant differences in white blood cells (WBC), RBC, HGB, HCT, MCH, MCHC and PLT, when compared with normal control. However, the extract at doses of 70 and 140 mg/kg body weight significantly ($p<0.05$) increased MCV when compared with normal control, the extract also significantly ($p<0.05$) increased lymphocyte count at all doses tested (35, 70 and 140 mg/kg) when compared with normal control after 28 days daily oral treatment (Table 4).

Table 4: Effect of methanol stem bark extract of *D. microcarpum* on haematological indices after 28 days oral daily administration in rats

Haematological Parameters	Control	Treatments (mg/kg)		
		D.M. (35)	D.M. (70)	D.M. (140)
WBC ($x10^3$/µl)	20.25 ± 2.04	19.85 ± 1.23	17.92 ± 1.75	16.22 ± 1.39
RBC ($x10^6$/µl)	6.43 ± 0.49	6.79 ± 0.17	6.34 ± 0.15	6.88 ± 0.19
Hb (g/dL)	11.65 ± 0.26	12.12 ± 0.18	11.88 ± 0.19	11.75 ± 0.18
HCT (%)	33.97 ± 0.27	37.17 ± 0.94	38.18 ± 0.48	38.48 ± 0.47
MCV (fl)	52.22 ± 1.14	54.97 ± 1.52	55.27 ± 0.93[a]	58.65 ± 0.64[b]
MCH (pg)	18.19 ± 0.89	17.95 ± 0.37	17.37 ± 0.28	17.70 ± 0.36
MCHC (g/dL)	36.05 ± 2.28	35.20 ± 1.22	32.78 ± 0.70	31.92 ± 0.45
PLT ($x10^3$/µl)	309.0 ± 20.20	332.50 ± 15.41	348.50 ± 14.42	357.80 ± 9.74
LYM (%)	53.25 ± 2.81	61.53 ± 1.10[a]	60.98 ± 0.99[a]	65.60 ± 1.33[b]

Values are expressed as Mean ± SEM (n=6); a and b indicate significant difference between normal control and treatment groups in the same column at $p< 0.05$ and $p < 0.01$ respectively.
MCV= Mean corpuscular volume, LYM =Lymphocytes, WBC =White blood cell, RBC= Red blood cell, HGB= Haemoglobin, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration, PLT= Platelets, D.M = Extract of *Detarium microcarpum.*

Effect of methanol stem bark extract of *D. microcarpum* on lipid profile after 28 days daily oral administration in rats

The effect of oral administration of extract for 28 days on lipid profile showed significant ($p<0.05$) decrease in total cholesterol (3.53±0.12) and low density lipoprotein cholesterol (1.38±0.11) when compared with control (4.65±0.92) and (1.97±0.41) respectively, while triglycerides and high density lipoprotein cholesterol did not show any significant differences (Table 5).

Table 5: Effects of methanol stem bark extract of *D. microcarpum* on serum lipid profile of rats after 28 days daily treatment

Parameters	Control	Treatments (mg/kg)		
		D.M. (35)	D.M. (70)	D.M. (140)
T. CHOL	4.65 ± 0.92	4.89 ± 0.21	3.54 ± 0.12[a]	3.31 ± 0.09[b]
TRIG	3.43 ± 0.22	3.39 ± 0.14	3.43 ± 0.32	3.46 ± 0.54
HDL-C	1.89 ± 0.06	1.95 ± 0.03	1.96 ± 0.04	1.83 ± 0.04
LDL-C	1.97 ± 0.41	1.52 ± 0.14	1.38 ± 0.11[a]	1.21 ± 0.47[b]

Values are expressed as Mean ± SEM (n=6); a and b indicate significant difference between normal control and treatment groups in the same column at $p< 0.05$ and $p < 0.01$ respectively.
HDL-C= High density lipoprotein cholesterol, LDL-C= Low density lipoprotein cholesterol, TRIG= Triglycerides, T. CHOL= Total Cholesterol, D.M = Extract of *Detarium microcarpum.*

Discussion

This study revealed that the methanol stem bark extract of *D. microcarpum* contained alkaloids, flavonoids, saponins, tannins, cardiac glycoside, phlabotanins and volatile oils. Secondary metabolites interact directly with both the cell receptors, membranes and nucleic acids due to the nature of their functional groups and chemical structures [11] . The presence of these bioactive ingredients is responsible for various pharmacological activities. Therapeutic effect such as Analgesic and anti-inflammation of flavonoids, steroids and tannins of *Detarium microcarpum* have been reported [4, 12].

The oral median lethal dose of the extract was found to be 471.2 mg/kg in rats. Based on LD_{50} value and according to classification of Ouédraogoa [13], the chemical labeling and classification of acute systemic toxicity from World Health Organization [14], the plant could be assigned as a class 5 drug and then, recognized as low toxic product. Acute toxicity studies are usually carried out to determine the dose that will cause death or serious toxic manifestations when administered singly or severally at few doses in order to establish dose that should be used in subsequent studies [15].

Decrease in the body weight has been used as an indicator of adverse effects of drugs and chemicals [16, 17]. The reduction in the body weight of rats treated with 140 mg/kg at 3rd and 4th weeks may have been due to direct toxicity of the extract at high dose used. Loss of appetite occur as a result of stress or physiological adaptation to a drug's intake may leads to the reduction of caloric intake which reduces the body weight [13].

Analysis of blood parameters is relevant to risk evaluation and changes in haematological system have a high predictive value for human toxicity [18]. Lymphocytes are mediators of the specific immune response against pathogens [19]. The mean corpuscular volume MCV is an index that measures the average volume of erythrocytes [20]. MCV increases in macrolytic anaemia and decreases in microcytic anaemia. The Increase in MCV observed in this research may be due to over production of haematopoietic regulatory elements such as erythropoietin [21].

The result of daily oral administration of methanol stem bark extract of *D. microcarpum* for 28 days on serum lipids showed significant decreased total cholesterol and LDL-C levels, and reduction in LDL-C level may be associated with impaired lipolysis [22]. Changes in the level of major lipid profiles

such as triglyceride, total cholesterol, Low density lipoprotein cholesterol (LDL-C), High density lipoprotein cholesterol (HDL-C), could provide useful information on the predisposition of the heart of the animals to arthrosclerosis and it is associated coronary heart disease (Yakubu *et al*., 2008). Disease conditions like nephrotic syndrome and hypothyroidism increases total cholesterol [15] and low density lipoprotein cholesterol (LDL-C) while infection and inflammation may decrease total cholesterol [15] and high density lipoprotein cholesterol (HDL-C), and increase triglycerides [23].

Conclusion

The methanol stem bark extract of *D. microcarpum* has an oral LD_{50} of above 5000 mg/kg, and the presence of some phytochemical constituents which demonstrate the relationship between the haematological parameters, serum lipids and the body weight which is associated with the plant activities. This partly justifies the claim for the traditional use of the plant in the management of diseases and ailments.

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