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Safety Evaluations of the Aqueous Extract of *Acacia karroo* Stem Bark in Rats and Mice

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Abstract: The aqueous extract from the shoot of *Acacia karroo* was evaluated for its acute toxicity by the oral route in mice and for the sub acute effect on haematological, biochemical and histological parameters in rats. In the acute toxicity test, *A. karroo* extract caused death in animals that received 1600 and 3200 mg/kg doses. Oral treatments in rats with this extract at 800 mg/kg did not cause any significant change in the red blood cell count (RBC), packed cell volume (PCV), haemoglobin concentration (HB), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), white blood cells and its differentials. It, however, caused a significance decrease in the levels of platelets. In the biochemical parameters, the extract caused a significant decrease in the levels of total protein, albumin, globulin, aspartate amino transferase (AST), alanine amino transferase (ALT), total and unconjugated bilirubin. Changes were also noted in the body weights but no significant changes were observed in the levels of some electrolytes (sodium, potassium and chloride). Clinico-pathologically, starry hair coat, respiratory distress and mortality were recorded. Lung with multiple abscess, kidney and liver with mild congestion were also observed histopathologically. The study concluded that caution must be exercised in the use of the plant for medicinal purposes.

Keywords: *Acacia karroo;* haematology; histopathology; serum chemistry; rats; mice.

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1. Introduction

Acacia karroo Hayne (Fabaceae) or sweet thorn is one of South Africa's most beautiful and useful trees. It may be found from the Western Cape through to Zambia and Angola [1, 2]. *A. karroo* occurs in all major terrestrial habitats, from deserts (where it may be a little as 3-4 meters tall and has large thorns and high tannin concentrations in its leaves) to coastal forests (where it may be 40 meters tall and have small thorns and invest little in chemical defenses). It is a particularly good fodder tree; stock and game feed on the leaves, flowers and pods. It is also used for the enrichment of the range for livestock, restoration of soil fertility in bush fallows, intercropping with grain crops and provision of woodlots for fuel-wood or gum. The gum is an important rural food source [1, 3].

Phytochemical screenings showed that *A. karroo* is very rich in proanthocyanidin and flavonols [2, 4]. Natural flavonoids are known for their significant scavenging properties on oxygen radicals *in vivo* and *in vitro*, affecting various steps in the arachidonate cascade via cyclo-oxygenase or lipoxygenase [5]. In addition to these important effects, they have membrane-stabilizing properties and also affect some processes of intermediary metabolism [6, 7].

The sweet thorn has many medicinal uses ranging from wound poultices to eye treatments and cold remedies. The bark, leaves and gum are usually used. It is also used to treat cattle which have tulip poisoning [3].

The study therefore seeks to assess *Acacia karroo* for safety or toxic effects using haematology, serum chemistry and histopathological changes as indices of toxicosis.

2. Materials and Methods

2.1 Plant collection and extract preparation

The barks of *A. karroo* were collected in July 2006 in the Eastern Cape Province of South Africa. The area falls within the latitudes 30°00-34° 15´S and longitudes 22° 45´-30° 15´E. It is bounded by the sea in the east and the drier Karoo (semi-desert vegetation) in the west [8]. These areas consist of villages which are generally classified as rural and poor. The plants were identified Sam Boltina by their vernacular names and later validated at the Department of Botany, University of Fort Hare and voucher specimens (Aded Med 2007/1-10) were deposited in the Griffen Herbarium of the University.

The barks were air dried at room temperature to get constant weights. The dried plant materials were later ground to powder. Each ground plant material (250 g) was shaken separately in distillated water for 48 h on an orbital shaker at room temperature of 24°C. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was concentrated to dryness under reduced pressure at 40ºC using a rotary evaporator. The thick solution was lyophilized using freeze drying system for biological investigations. The extract yield was 8.5%.

2.2 Animals

The animals used in this study were 30 male Swiss albino mice (weighing between 22 and 34 g) and 10 female Wistar rats (111-166 g). They were maintained at the Experimental Animal House of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare. They were kept in rat cages and fed on commercial rat cubes (EPOL Feeds, South Africa Ltd.) and allowed

free access to clean fresh water in bottles *ad libitum*. All experimental protocols were in compliance with University of Fort Hare Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

2.3 Acute toxicity study

The acute toxicity of *A. karroo* aqueous extract was determined according to the method of Sawadogo et al. [9]. Mice fasted for 16 h were randomly divided into 5 groups of six per group. Graded doses of the extract (400, 800, 1600 and 3200 mg/kg p.o.) corresponding to groups B, C, D and E were separately administered to the mice in each of the 'test' groups by means of bulbed steel needle. The control group representing group A was treated with orally administered distillated water (3 ml/kg p.o.) only. All the animals were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period of time was recorded.

Table 1: Acute toxicity study in mice after 48 h of administration of aqueous extract of *A. karroo*. (n=6).

Group	Dose (mg/kg)	T/D *	Period of signs observation	Signs of toxicity observed
A	2ml/kg (distillated water)	6/0	48 h	-
B	400	6/0	48 h	Dullness in the first 2 hours of extract administration, thereafter normalcy restored.
C	800	6/0	48 h	Dullness in the first 3 hours of extract administration, thereafter normalcy restored.
D	1600	6/1	48 h	Mortality, dullness in the first 5 hours of extract administration, thereafter normalcy restored.
E	3200	6/2	48 h	Mortality, dullness after the first 5 hours of extract administration, thereafter normalcy restored.

*T/D: number of mice treated/number of deaths.

2.4 Sub-acute toxicity study

Using a modified method of Cruz et al. [10], the rats were divided at random into an experimental and a control group with five animals in each group. While the control group received distillated water, the experimental group received aqueous extract (800mg/kg), administered orally by means of bulbed steel needle for 14 days. All the animals were weighed on the first and 14^{th} day of the experiment.

2.5 Collection of blood and serum samples

Paired blood samples were collected by cervical decapitation from diethyl ether anaesthetized rats into heparinised bottles for haematological studies; and clean non-heparinised bottles and allowed to clot. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

2.6 Determination of haematological and serum biochemical parameters

The haematological and serum biochemical parameters were determined using Beckman DXC 600 (USA) for serum chemistry and Advia 2120 (Bayer, Germany) for haematology. Erythrocytes indices (MCV, MCHC and MCH) were determined from values obtained from RBC count, haemoglobin concentration and PCV values [11].

Table 2. Effects of the graded doses of the aqueous extracts of *A. karroo* on haematological parameters of Wistar rats. X±S.D.

Parameters	Control (n=5)	800 mg/kg (n=2)
PCV (%)	43.6 ± 2.7	41.5±12
Hb (g/L)	14.3±0.8	13.6±3.0
RBC ($X10^{12}$g/L)	7.8±0.3	7.3±1.6
MCV (fl)	55.4± 2.2	56.9±2.8
MCH (pg)	18.2±0.9	18.7±0.1
MCHC (%)	32.9±1.4	32.8±1.6
WBC ($X10^9$/L)	6.9±1.1	8.3±3.5
Lymphocytes ($x10^9$/L)	4.1±0.6	4.5±2.2
Neutrophils ($x10^9$/L)	0.5±0.5	1.2±0.6
Monocytes ($x10^9$/L)	1.7±0.5	1.6±1.5
Eosinophils ($x10^9$/L)	0.1± 0.01	0.1±0.1
Large unstained cells ($x10^9$/L)	0.5±0.3	0.8±0.3f
Basophils ($x10^9$/L)	0.02±0.01	0.03±0.0
Platelets	1049.2 ± 62.4	$917.0±53.7^b$

Superscripted items are significantly different from control at $P<0.05$.

2.7 Histopathology

The liver, kidney, heart, spleen, and testes of all the animals were fixed in 10% buffered formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections 5-µ thick were cut, stained with haematoxylin and eosin and examined under the light microscope.

2.8 Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M.). Where applicable, the data were subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Duncan's Multiple Range test using the Statistical Analysis System (SAS, 1999) program. P values at 5% were regarded as significant.

Table 3. Effects of the graded doses of *A. karroo* on the serum biochemical parameters of rats. X±S.D.

Parameters	Control (n=5)	800mg/kg (n=2)
Total Protein (g/dL)	55.2±1.9	38.0±1.4[a]
Albumin (g/dL)	17.6±1.1	10.0±1.4[a]
Globulin (g/dL)	37.6±1.3	28.0±2.8[a]
ALT (U/L)	127.8±13.8	77.0 ± 2.8[a]
AST (U/L)	176.8 ± 14.5	161.0 ± 1.4[a]
ALP (U/L)	293.8±26.8	285.0±4.2
GGT (U/L)	3.6 ± 0.5	8.0 ± 1.4[a]
Total bilirubin (mmol)	8.6 ± 1.1	5.5 ± 0.7[a]
Conj. Bilirubin (mmol)	2.6 ± 0.5	2.0 ±0.0
Unconj. Bilirubin (mmol)	6.0±1.0	3.5±0.7 [a]
Sodium	135.2±1.9	135.0±1.4
Potassium	6.3±0.1	6.3±0.1
Chloride	102.0±1.6	101.5±0.7
Inorganic Phosphorus	3.3±0.1	2.5±0.1[a]

Superscripted items are significantly different from control at P<0.05.

3. Results and Discussion

Acute toxicity studies in mice showed that mortality was recorded in groups D and E. While an animal died in group D, group E recorded 33.3% mortality but groups A to C did not record any mortality. The behavioral change noted in these animals following extract administration is dullness though the animals later become active after some hours of extract administration (Table 1). The acute toxicity study in mice showed that at 1600 and 3200 mg/kg doses of the plant caused mortality in these animals. It therefore means that this extract should not be administered at a dose of 1600mg/kg and above, otherwise mortality will result.

In the sub-acute toxicity study, all the haematological parameters (except platelets) of the treated group (800 mg/kg) were within the reference range for rats and were not significantly different from the control group. The extract caused significant decrease in the level of the platelets when compared to the control (Table 3). From this study, it is inferred that the aqueous extract of *A. karroo* has no toxic effect on the haematological parameters i.e. red blood cell count, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, white blood cell counts and its differentials. Some plants are however known to cause destruction of red blood cells leading to anaemia. Examples of such plant include: *Solanum tuberosum, S. lycopersicum, Mercurialis perennis, M. annua* [12, 13]. The significant decrease in the level of platelets in this study is noted. Platelets also known as thrombocytes help to mediate blood clotting, which is a meshwork of fibrin fibres. The fibres also adhere to damaged blood vessels; therefore, the blood clot becomes adherent to any vascular opening and thus prevents further blood clot [14, 15, 16]. The extract could thus precipitate thrombocytopaenia which is the presence of low level platelets in the circulatory system. If someone suffers from thrombocytopaenia, there is tendency to bleed [17, 18]. This observation of decreased platelet level in the circulatory system by the extract also means that it has anticoagulant property. In some thromboembolic conditions, it is desirable to delay the coagulation process [18, 19]. It is known that low dose aspirin started immediately after myocardial infarction has been found to reduce the mortality and prevent reinfarction [20]. This plant may play similar role if further investigation is carried out.

Table 4. Effects of the graded doses of *A. karroo* on the body weights of rats.X±S.D.

Parameters	Control (n=5)	800mg/kg
Weight before extract administration (g)	126.6±12.0	136.7±22.7 (n=5)
Weight after 14 days		
% Difference in weight	188.2±10.7	164.6±62.3 (n=2)
	48.7	20.4

The extract caused significant decrease in the levels of total protein, albumin, globulin, ALT, AST, total bilirubin, unconjugated bilirubin and inorganic phosphates. On the other hand the extract did not cause any significant changes in the levels of ALP, GGT, conjugated bilirubin and the electrolytes (sodium, potassium, chlorides) (Table 3). Albumin is the protein with the highest concentration in plasma. It transports many small molecules in the blood (for example, bilirubin, calcium, progesterone, and drugs). It also prevents the fluid in the blood from leaking out into the tissues [21]. Since albumin is produced in the liver, decreased serum albumin may arise from liver and kidney diseases. Malnutrition or a low protein diet may also lead to low serum albumin. When compared with the control, the percentage difference in weight gain for the treated group (20.4%) is less than that of the control (48.7%) (Table 4). Since the study showed that rate of weight gain for the experimental group was far less than that of the control, it might mean that the extract has effect on feed conversion rate or the animals were off-feed. As a matter of fact, polyphenols particularly proanthocyanidins are known to interfere with intake and digestibility of feeds when fed to animals [2]. This plant is very rich in proanthocyanidins and studies have shown that animals feeding on *A. karroo* browse produce high faecal nitrogen and are often in negative nitrogen retention [2]. The effect of the extract on liver enzymes is that of significant decrease in their levels particularly ALT and AST. The bilirubin levels were also significantly reduced in this study. These observations of significant decrease in the levels of the liver enzymes may indicate that the extract of *A. karroo* has hepatoprotective effects. This is also corroborated by significant decrease in the serum levels of total and conjugated bilirubin. The effect of the extract on the electrolytes showed that the plant did not cause any impairment on the cardiovascular system.

Sixty percent (60%) mortality rate was recorded in the experimental group when sub-acute toxicity was carried out. Starry or rough hair coat and respiratory distress were prominent signs noticed also in this group. Histopathologically, a lung from one of the animals also displayed multiple abscesses throughout one of the lobes while the other lobe showed partial abscess distributed on it. This may have accounted for the respiratory distress noted. It thus showed that caution should be exercised in the use of this plant for medicinal purpose.

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