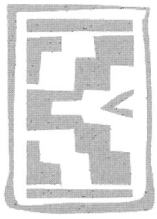

Biochemical and haematological changes in rats administered an aqueous extract of *Prunus africana* stem-bark at various dosage levels

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ABSTRACT

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An aqueous extract of *Prunus africana* (Hook. f.) Kalkm. (syn. *Pygeum africanum*) (Hook. f.) (Rosaceae) was administered daily at dosage rates of 10, 100 and 1 000 mg/kg body mass to randomized groups of Sprague Dawley rats. The extract caused a moderate rise in plasma alanine aminotransferase and creatine kinase mainly at rates of 1 000 mg/kg body mass, but it did not cause any significant variations in haematological parameters or in plasma levels of total proteins, albumin, aspartate aminotransferase, alkaline phosphatase and blood urea nitrogen at the dosage levels used. There were no overt clinical signs in any of the rats. It was concluded that the extract may contain components that are mildly toxic to the liver and heart of rats after repeated daily oral administrations of 1 000 mg/kg body mass.

Keywords: Biochemical data, haematological data, *Prunus africana* extract

INTRODUCTION

Prunus africana (Hook. f.) [(syn. *Pygeum africanum* (Hook. f.)], a plant in the family Rosaceae, has useful medicinal properties. The application of a chloroform extract made from it in the therapy of benign prostatic hyperplasia has received much attention in pharmacological, phytochemistry and clinical trial studies in an attempt to understand its mode of action and to isolate its active ingredients (Bombardelli & Morrazoni 1997). A water extract of its stem-bark is traditionally used in treatment of bovine babesiosis in Kenya (ITDG/IIRR 1996). Powdered stem-bark mixed in water is used traditionally as a remedy for stomachache and as a purgative in humans and animals (Kokwaro 1976). Toxicological investigations of water extracts from *Prunus africana* are few (Gathumbi 1995), despite their phytomedical applications.

The present report is based on the haematological and biochemical responses in rats that were administered various oral doses of an aqueous extract of *P. africana* stem-bark.

MATERIALS AND METHODS

Prunus africana stem-bark was obtained from the Nakuru district in Kenya, and was air-dried and milled into a fine powder. One kilogram of the powder was boiled in 5 ℓ of water to a final volume of 3 ℓ and filtered through cotton gauze and then through a double layer of Whatman number 1 filter paper. The filtrate was centrifuged at 5000 *g* for 10 min. The extract was then freeze-dried, yielding a cocoa-brown fine granular extract, 10 % (w/w). A 10 % stock solution of the extract was reconstituted in physiological saline for animal treatment.

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Animals

Forty uniformly inbred Sprague Dawley male rats aged 6–8 weeks were randomized into four groups of ten animals. The animals were individually identified and housed five to a cage. The cages were constructed of polycarbonate and contained cypress wood shavings as bedding. The rats were allowed free access to feed and water. A commercial pelleted diet for rats and mice (Unga Ltd, Kenya) was used as feed. The animals were allowed 2 weeks of conditioning before commencement of the experiment during which baseline data were obtained.

Treatment

The extract was orally administered to the rats in three of the groups at dosage rates of 10, 100 or 1 000 mg/kg of the freeze dried material each per animal, respectively, daily for 8 weeks. One of the groups served as the control; each rat was dosed daily with 2 mℓ physiological saline for the same period.

Parameters

Observations for clinical signs and deaths were undertaken three times daily. The body masses of the rats were recorded weekly, at which time EDTA blood samples for haematological analysis were obtained from the orbital sinus (Stone 1954). Haematological analysis was based on standard protocols (Jain 1986). Red blood cell counts (RBC), haemoglobin concentrations (HB) and white blood cell counts (WBC) were obtained in a Coulter Counter (Coulter Electronics). Packed cell volumes (PCV) was measured using the microhaematocrit method and total plasma proteins were measured in a hand refractometer. Differential leukocyte counts were determined by microscopical examination of Giemsa stained blood smears.

Heparinized blood samples were obtained weekly in the first 4 weeks and then fortnightly in the next 4 weeks, from the orbital sinus while the rats were under light diethyl ether anaesthesia. The plasma from each sample was separated and stored at –20 ° until the time of analysis. The biochemical parameters were assayed using Boehringer Mannheim kits. Plasma levels of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were determined using a standard procedure (Reitman & Frankel 1957). Alkaline phosphatase (AP) activity in plasma was assayed according to the method of Bessey (1946), while plasma lactate dehydrogenase (LDH) activity was measured according to the recommendations of the German society for clinical chemistry (1970). The activity of creatine kinase (CK) in plasma was determined according to a standard method (Szasz, Gruber & Bernt 1976). Blood urea nitrogen (BUN) was colorimetrically determined using the urease cleavage Berthelot's reaction (Fawcett & Scott 1960). Total plasma proteins (TP) and albumin (ALB) levels in plasma were determined using the Biuret (Coles 1986) and bromcresol green (Doumas, Wartson & Biggs 1971) methods, respectively.

All animals were sacrificed through exsanguinations under ether anaesthesia, for complete autopsy, at the end of the experiment. Representative samples, from the liver, kidneys, heart, skeletal muscles, lungs, stomach, intestines, adrenal glands, testicles, seminal vesicles, prostate gland, spleen, lymph nodes, bone marrow, brain and spinal cord, and skin at several sites, were preserved in 10 % neutral buffered formalin, for histopathological examination. Tissues were processed according to standard histological procedures and 5 µm sections were stained with haematoxylin and eosin (Luna 1968).

STATISTICAL ANALYSIS

Statistical testing was based on the analysis of variance by the use of least square means and separation of the means, using a Harvey (1990) computer programme.

RESULTS

Clinical signs

No deaths were recorded at any treatment level. There was a progressive increase in the body masses of the rats in all four groups (Fig. 1).

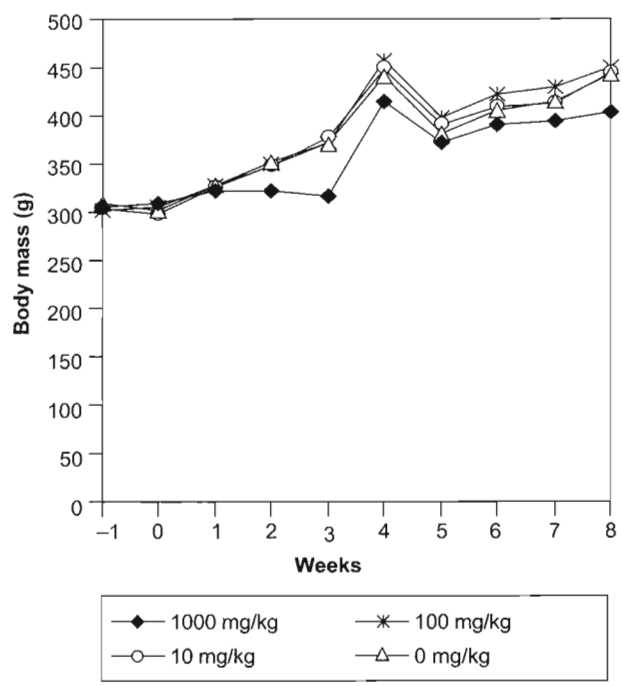

FIG. 1 Body mass changes in the rats in the four groups dosed orally with an aqueous extract of *Prunus africana* bark at various dosage levels

TABLE 1 Least squares mean values of biochemical parameters per group of rats dosed orally with an aqueous extract of *Prunus africana* bark at various dosage rates compared to those of the control group after 8 weeks

Dose	TP (g/dℓ)	ALB (g/dℓ)	ASAT (u/ℓ)	ALAT (u/ℓ)	AP (u/ℓ)	CK (u/ℓ)	LDH (u/ℓ)	BUN (mg/dℓ)
0	7,08 ± 0,11	4,02 ± 0,08	43,85 ± 0,87	64,55 ± 1,41[a]	389,64 ± 14,97	143,53 ± 9,77[a]	111,49 ± 5.59[a]	15,46 ± 0,33
10	7,05 ± 0,12	3,90 ± 0,08	43,61 ± 0,89	66,91 ± 1,44[c]	377,74 ± 15,34	153,23 ± 10,01[b]	137,72 ± 5,73[bc]	15,39 ± 0,33
100	7,46 ± 0,12	4,07 ± 0,08	45,14 ± 0,89	69,14 ± 1,44[bc]	415,14 ± 15,25	152,08 ± 9,95[b]	123,76 ± 5,70[b]	15,65 ± 0,33
1 000	7,19 ± 0,12	3,89 ± 0,09	45,56 ± 0,94	72,23 ± 1,53[b]	370,28 ± 16,19	196,91 ± 10,56[b]	124,41 ± 6,06[c]	16,02 ± 0,35

[a, b, c] Different superscripts within a column indicate significant differences between treatments

TABLE 2 Least squares mean values of haematological parameters in the groups of rats to which an aqueous extract of *Prunus africana* was orally administered at various doses, compared to those of the control group. These figures were obtained at the termination of the experiment after the analysis of the effect of each dosage rate on the parameters

Dose (mg/kg)	TP (g/dℓ)	PCV (%)	HB (g/dℓ)	RBC (x 10^6/uℓ)	WBC (x 10^3/uℓ)	N (%)	L (%)	M (%)	E (%)
0	7,23 ± 0,06	48,40 ± 0,32	16,56 ± 0,13	7,44 ± 0,09	17,36 ± 0,54	11,62 ± 0,81	87,98 ± 0,81	0,02 ± 0,02	0,27 ± 0,09
10	7,19 ± 0,07	47,93 ± 0,33[a]	16,63 ± 0,13	7,28 ± 0,10	16,77 ± 0,56	13,42 ± 0,84[a]	86,16 ± 0,84[a]	0,06 ± 0,02	0,27 ± 0,09
100	7,18 ± 0,06	49,20 ± 0,30[b]	16,82 ± 0,12	7,42 ± 0,09	18,08 ± 0,51	14,51 ± 0,75[b]	84,76 ± 0,76[b]	0,02 ± 0,02	0,36 ± 0,08
1 000	7,16 ± 0,07	48,60 ±,36	16,61 ± 0,14	7,41 ± 0,11	17,94 ± 0,61	14,59 ± 0,90	85,10 ± 0,90	0,01 ± 0,03	0,33 ± 0,10

[a, b, c] Different superscripts within a column indicate significant differences between treatments

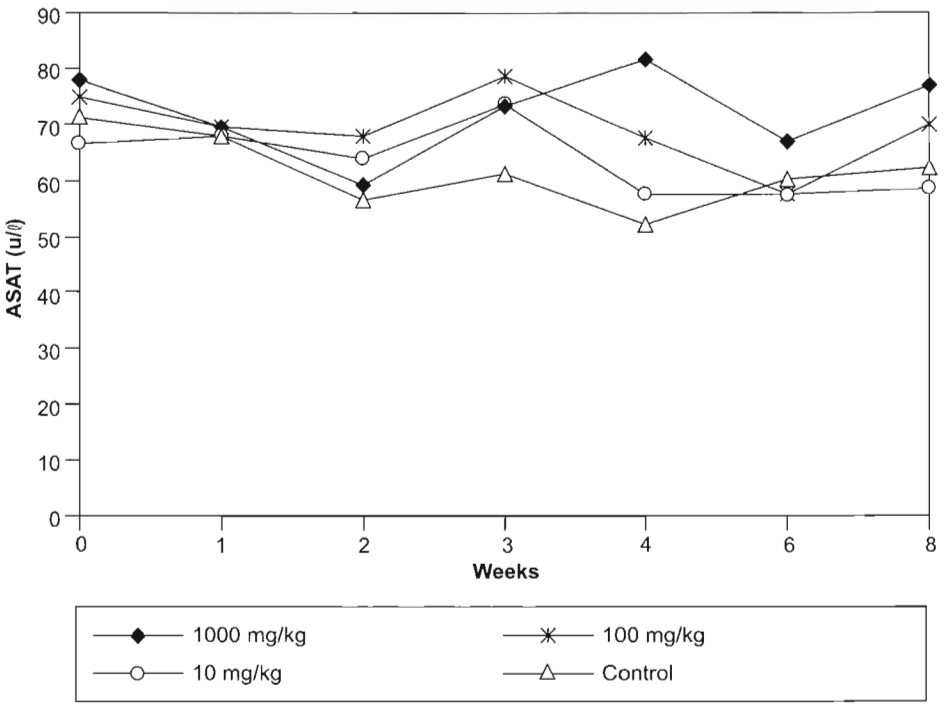

FIG. 2

Mean values of ALAT in progressive blood samples in the four groups of rats that were orally dosed with an aqueous extract of *Prunus africana* at various dosage levels

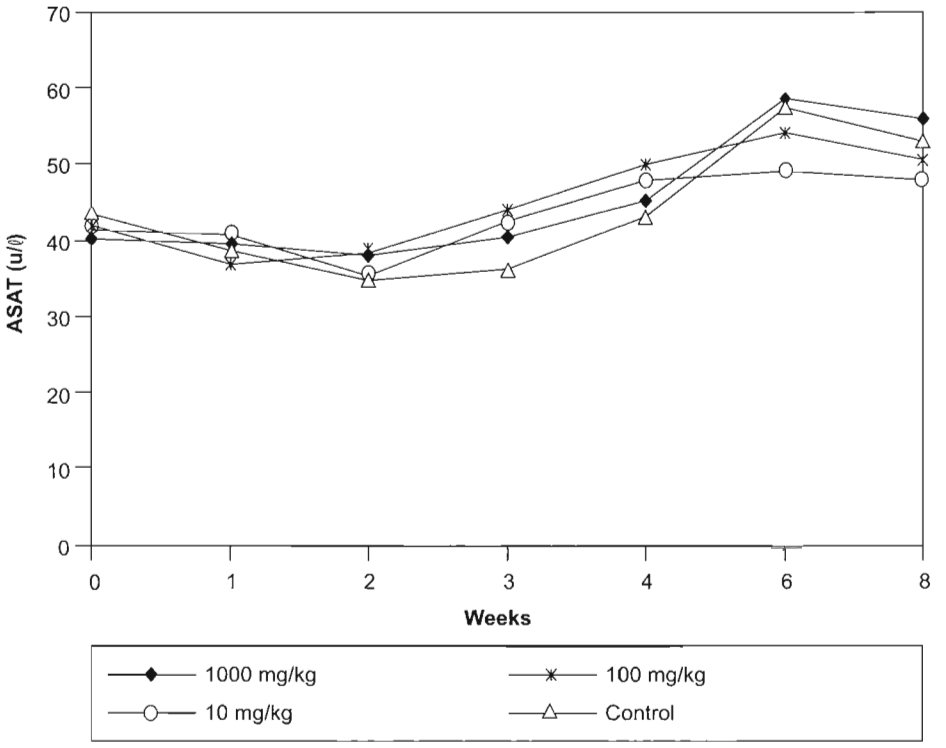

FIG. 3

Profiles of mean values of plasma ASAT in groups of rats that were orally dosed with an aqueous extract of *Prunus africana* bark at different dosage levels, compared to those of the controls

Biochemical changes

A summary of the biochemical data is presented in Table 1, while a graphic presentation of the average levels of some plasma enzymes at different sampling intervals is shown in Fig. 2–4.

ALAT levels increased moderately according to dosage rate during the course of the 8 weeks, the mean for each group being 66,91 ± 1,44 u/ℓ, 69,14 ± 1,44 u/ℓ and 72,23 ± 1,53 u/ℓ at dosage rates 10, 100 and 1 000 mg/kg body mass respectively at the end of the

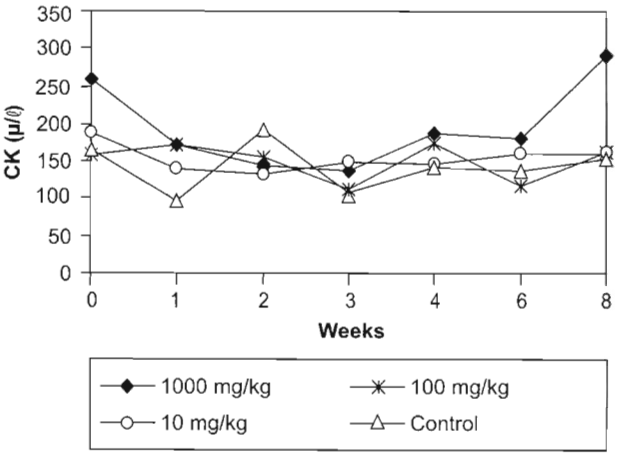

FIG. 4 Mean values of plasma CK at the respective sampling intervals in the four groups of rats that were dosed orally with an aqueous extract of *Prunus africana* bark at various dosage rates

experiment, against 64,55 ± 1,41 u/ℓ in the controls (Table 1).

From the profile of averages in progressive sampling intervals, it was observed that, although the levels fluctuated between blood samplings in each group, the extract caused a persistent and a moderately higher plasma ALAT activity, from the fourth week, at dosage 1 000 mg/kg body mass than at the other dosage levels (Fig. 2). On the other hand, although the profile of plasma ASAT levels showed a rising trend in each group, the extract did not cause a clear dose-related elevation in its plasma activity; the slight rise at dosage 1 000 mg/kg versus other dosage levels from week 6–8 notwithstanding (Fig. 3).

CK levels were moderately elevated at the dosage rate of 1 000 mg/kg body mass attaining a mean value of 196,91 ± 10,56 u/ℓ against 153,23 ± 10,01 u/ℓ, 152,08 ± 9,95 u/ℓ and 143,53 ± 9,77 u/ℓ at the rates 10 mg/kg, 100 mg/kg and controls respectively (Table 1). This increase in plasma CK activity at dosage 1 000 mg/kg, was eminent from fourth to eighth week (Fig. 4). TP, ALB, AP, and BUN were not altered by treatment at any dosage level tested. Plasma LDH levels did not reveal any apparent dose-associated responses.

Haematological changes

The results on the haematological changes are summarized in Table 2. There were no major variations in the haematological parameters at the different dosage levels in comparison with those of the controls and standard reference values (Jain 1986). TP fluctuated at a mean of 7g/dℓ. RBC and WBC had mean values of about 7 x 10^6/µℓ and 16–18x10^3/µℓ respectively in all groups. PCV ranged from about 47–49 % in the rats in all four groups, while the HB concentration varied from 16–17 g/dℓ. The differential leukocyte counts showed normal trends. Neutrophils (N) averaged 13,42 ± 0,84 to 14,5 ± 0,90 % in the rats at dosage levels of 10–1 000 mg/kg; that for control group being 11,62 ± 0,81. Lymphocytes (L) contributed the bulk of the leukocytes, being on average 84,76 ± 0,76 to 87,98 ± 0,81 % of the total leukocytes in the rats in all four groups including the control. Monocytes and eosinophils comprised less than 1 % of the total WBC, but basophils were not identified in the smears examined.

Pathology

The morphological features in each organ examined were predominantly unchanged. However, minute foci of mild hepatocellular vacuolation and single cell megalocytosis occurred in the liver at dosage 1 000 mg/kg, but not at 10 mg/kg, 100 mg/kg body mass or in the controls. These hepatocellular changes were nearly masked by the overwhelmingly normal appearance of the organ. There were no conspicuous lesions in other organs.

DISCUSSION

The administration of the aqueous extract of *Prunus africana* to the rats did not cause dramatic clinical signs or overt changes in haematological parameters when administered orally at dosage rates of 10, 100 and 1 000 mg/kg body mass, respectively.

The slight dosage rate-associated increase in plasma ALAT values that were obtained suggests that the extract may contain components that cause mild liver damage at rates as high as 1 000 mg/kg body mass. The mild effects of the extract on the liver at doses up to 1 000 mg/kg body mass, is further corroborated by the presence of an unaltered activity of ASAT, concurrent with increasing levels of plasma ALAT. ALAT, being a cytoplasmic enzyme, increases in the plasma after mild injury to hepatocytes, while ASAT is released into the plasma after severe necrotic injuries (Clampitt & Hart 1978). The occurrence of normal levels of plasma TP, ALB, ASAT and AP at dosage rates of 10–1 000 mg/kg body mass, further confirms that the extract causes minimal, if any, toxicity to the liver at dosage levels up to 1 000 mg/kg body mass. Plasma TP and ALB fall while ASAT and AP rise in animals suffering from liver damage (Kaneko 1988). The normal BUN values obtained exclude nephrotoxicity by this extract at the dosage levels given; while increasing plasma CK values, suggest that it may contain cardiotoxic components.

Absence of changes in haematological parameters at the dosage rates used indicates that the aqueous extract of *Prunus africana* does not cause significant effects in the haematopoietic system, when administered orally at the dosage levels used.

Based on the present observations, it can be concluded that, when administered orally, an aqueous extract of *Prunus africana* is mildly toxic to rats after repeated daily oral administration of 1 000 mg/kg body mass. The target organs of toxicity of this extract in rats, after oral administration, are the heart and the liver. Since there were no remarkable pathological changes at any dosage level, it can be suggested that the moderate rise in ALAT and CK at dosage 1 000 mg/kg body mass could have been caused by subcellular changes in the organs.

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