

Full Length Research Paper

Haematological studies on the ethanolic stem bark extract of *Pterocarpus erinaceus* poir (fabaceae)

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Accepted 21 February, 2008

Pterocarpus erinaceus (fabaceae) is used in the Nigerian folk medicine as well as in other African savanna countries to treat diarrhea, dysentery, urethral discharges, fever, and as an ingredient in abortifacient prescriptions. It is also used in arresting bleeding and as a dressing on ring worm of the scalp. The acute toxicity profile as well as possible haemostatic activity of the ethanolic stem bark extract of the plant after sub acute oral administration was studied in albino Wistar rats. The intraperitoneal LD₅₀ of the extract was found to be 447.21 mg/kg, while the oral LD₅₀ was > 5000 mg/kg. Administration of the extract for 30 days orally revealed a significant ($P < 0.05$) decrease in bleeding time in the group of rats treated with 200 mg extract/kg body weight compared to control. The clotting time and other haematological parameters like haemoglobin (Hb) concentration, platelet counts, packed cell volume (PCV), white blood cell count (WBC) were not significantly different from control. None of the rats used in the sub-acute toxicity studies died during the study period. The ethanolic stem bark extract of *P. erinaceus* may thus be practically non-toxic through the (oral) route and may contain some biologically active principle(s) which may be responsible for the haemostasis.

Key words: *Pterocarpus erinaceus*, Haemostasis, bleeding.

INTRODUCTION

The rich floral biodiversity of Africa has provided herbal health practitioners with an impressive pool of 'natural pharmacy' from which plants are selected as remedies or as ingredients in the preparation of herbal medicines for the treatment, management and/or control of an array of human disorders. The World Health Organization (WHO) consultative group defines a medicinal plant as one which in one or more of its organs contains substances that can be used for therapeutic purposes, or which are precursors of useful drugs (Sofowora, 1982). The problem with medicinal claims for some plants has been the insufficient scientific data on the plants (Briejer et al., 2001). There is therefore the need to establish scientific bases for their ethno medical uses and toxicological profiles.

P. erinaceus Poir (Fabaceae) is a small to medium size deciduous legume tree that is 12 – 15 m tall and 1.2 m

in diameter. The bark of the trunk is dark grey and rough with scales that curl up at the ends. The leaves are compound and imparipinnate. It is distributed throughout the West and Central African savanna and dry forest. It is popularly known as 'African rosewood'. In northern Nigeria, it is called "Madobiya" or "Shajini", while in the south; it is called "Apepe" or "Osun dudu". A decoction of the stem bark is used as astringent for severe diarrhea and dysentery and as an ingredient in abortifacient prescriptions. It is also used as a dressing on ring worm of the scalp and chronic ulcers (Dalziel, 1948). In some parts of Kano in Northern Nigeria, its decoction is used orally to arrest bleeding (Khamis Sangarib, verbal communication).

Previous studies by Aliyu et al. (2005) showed that the ethanolic stem bark extract of the plant possesses significant and dose-dependent analgesic and anti-inflammatory activities in laboratory rats and mice. The aim of this study was to investigate the safety profile and haemostatic property of *P. erinaceus* ethanolic stem bark extract using albino wistar rats.

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Table 1. Effect of *Pterocarpus erinaceus* on the bleeding time of rats.

Treatment (p.o.)	Mean bleeding time (s)
Normal saline (control)	17.75 ± 2.25
Extract 50 mg/kg	17.25 ± 1.70
Extract 100 mg/kg	16.75 ± 1.90
Extract 200 mg/kg	11.5 ± 1.20*

*Significantly different from control at $P < 0.05$; $n = 5$.

MATERIALS AND METHODS

Collection and preparation of plant material

The plant material was collected at Burunburun forest in Tudunwada local government area, Kano, Nigeria. The plant was identified and authenticated by Mallam Musa of the Botany Department of Ahmadu Bello University, Zaria, Nigeria where a voucher specimen (No. 900063) was deposited for future reference at the Herbarium.

The stem bark of the plant was cleaned, air dried and size-reduced to coarse powder with pestle and mortar. One hundred grams (160 g) of the powdered plant material was cold-macerated with 1 litre of 70% ethanol for 24 h with occasional shaking. It was then suction-filtered through Whatman #1 filter paper. The resulting filtrate was concentrated to dryness and the yield was 4.6% w/w.

Laboratory animals

Young adult Wistar rats of both sexes weighing 200 – 250 g were used. They were kept in clean cages and maintained under laboratory conditions of temperature, humidity, and light and were allowed free access to food (Pfizer feed) and water *ad libitum*.

Acute toxicity studies (LD₅₀)

LD₅₀ determination was conducted using modified method of Lorke (1983). The evaluation was done in two phases. In phase one, two sets of three groups of three rats each, were treated with 10, 100 and 1000 mg extract/kg body weight orally and intraperitoneally (ip) respectively. The rats were observed for clinical signs and symptoms of toxicity within 24 h and death within 72 h. Based on the results of phase one, another two sets of three (4) fresh rats per group were each treated with 1500, 3000 and 5000 mg extract/kg body weight orally and 300, 400, 500 and 600 mg extract /kg body weight (ip) respectively in the second phase. Clinical signs and symptoms of toxic effects and mortality were then observed for seven days.

The LD₅₀ was then calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose i.e. the geometric mean of the consecutive doses for which 0 and 100% survival rates were recorded in the second phase.

Sub-acute toxicity studies

Male and female rats were randomly divided into four groups of five rats each. Three groups were given 50, 100 and 200 mg extract /kg body weight respectively orally, through a cannula daily for 30 days. The last group received normal saline and served as the control. The rats were given feed and water *ad libitum*.

At the end of the study (day 31), each rat was euthanized under chloroform anaesthesia. Bleeding and clotting times were then determined using the method of Dacie and Lewis (1984). Blood

Table 2. Effect of *Pterocarpus erinaceus* on the clotting time of rats.

Treatment (p.o.)	Mean clotting time (s)
Normal saline (control)	45.5 ± 4.09
Extract 50 mg/kg	45.5 ± 2.39
Extract 100 mg/kg	45.75 ± 7.00
Extract 200 mg/kg	42.75 ± 3.17

samples were collected from the rats (hearts) into anticoagulant (EDTA)-containing bottles for estimation of the following haematological parameters: packed cell volume (PCV), haemoglobin concentration (Hb), platelet count, white blood cell count (WBC) and its differential counts using the method of Tietz (1985).

Bleeding time determination

The base of each rat tail was cleansed and pricked with a sterile lancet. A stop clock was started immediately. Blood was blotted every 15 s using Whatman filter paper until bleeding ceased. The time taken for the blood to stop flowing was recorded as the bleeding time.

Clotting time determination

A cut was made on the distal part of each rat tail using a sterile scissors. The blood was placed on a grease-free glass slide. A stop clock was started immediately. A needle was passed through the blood on the glass slide every 15 s until a thread-like structure was seen. The time taken to form the thread-like structure was taken as the clotting time.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Statistical analysis was carried out using student's t-test and differences between means were considered to be significant when $P < 0.05$.

RESULTS

All the rats in the groups treated with 500, 600 and 1000 mg extract/kg body weight and above intraperitoneally died. Behavioural changes observed in these rats were decreased mobility and respiratory distress. There was no convulsion prior to death. The intraperitoneal LD₅₀ of the extract in rats was calculated to be 447.21 mg/kg.

No mortality was recorded in the rats treated with the extract orally at all the doses used. The oral LD₅₀ is therefore greater than 5000 mg/kg.

The bleeding times obtained for rats treated with 50 and 100 mg extract /kg body weight were not significantly different from control, while significant ($P < 0.05$) decrease was observed in rats that received the highest dose of 200 mg extract/kg body weight (Table 1).

The clotting time obtained for the rats at all the doses used were not significantly different from the control (Table 2).

Table 3. Effect of administration (30 days) of *P. erinaceus* stem-bark extract on some haematological parameters in rats.

Treatment (p.o.)	Hb count (g/dl)	PCV (%)	WBC ($\times 10^7/L$)	Neutrophils (%)	Lymphocytes (%)	Platelets ($\times 10^9/L$)
Normal saline(control)	13.1 \pm 0.23	45.3 \pm 0.45	12.3 \pm 1.3	26.0 \pm 3.5	67.2 \pm 6.19	733.6 \pm 61.0
Extract 50 mg/kg	13.9 \pm 0.28	47.5 \pm 0.65	11.9 \pm 2.4	21.0 \pm 2.2	81.0 \pm 2.62	711.4 \pm 37.0
Extract 100 mg/kg	14.2 \pm 0.16	48.8 \pm 1.5	12.6 \pm 1.9	31.0 \pm 3.2	66.6 \pm 3.47	762.8 \pm 48.1
Extract 200 mg/kg	13.8 \pm 0.45	46.3 \pm 1.9	13.6 \pm 2.6	25.0 \pm 1.8	72.4 \pm 2.94	677.0 \pm 90.0

All the other haematological parameters (Hb concentration, PCV, platelet, WBC, neutrophil and lymphocyte counts) evaluated were also not significantly affected by the extract at all doses used compared to control group (Table 3).

DISCUSSION

A scale proposed by Lorke (1983) roughly classifies substances according to their LD₅₀ as follows: Very toxic (LD₅₀ < 1.0 mg/kg), toxic (LD₅₀ up to 10.0 mg/kg), less toxic (LD₅₀ up to 100.0 mg/kg) and only slightly toxic (up to 1000.0 mg/kg). Substances with LD₅₀ values greater than 5,000 mg/kg are practically non-toxic. Based on this proposal the intraperitoneal LD₅₀ of 447.21 mg/kg shows that the *P. erinaceus* stem-bark extract is slightly toxic. The high oral LD₅₀ (> 5000 mg/kg) obtained suggested that the extract is practically non-toxic through this route and is therefore safe in the rats and in its traditional use orally for treatment of the diseases it is indicated for. Since none of the rats used in the 30 days study died, it suggests that the plant can also be safely used in the treatment of diseases requiring long term drug administration.

Coagulation studies are of great importance considering the role of blood in life (Aballi and de-Lamerans, 1962). Arrest of bleeding from small blood vessels in the skin and elsewhere usually involves vasoconstriction and the formation of haemostatic plugs. Platelets are the blood cells involved in coagulation (Williams and Levine, 1982). Blood coagulation requires that platelets be in sufficient size, number and function, in the absence of which a satisfactory plug may not occur and haemorrhage may continue following a breach in the vascular endothelium.

Bleeding occurring in the presence of a normal platelet count and normal platelet function implies that haemorrhage is caused by pathological changes in the blood vessels. These may take the form of inflammation, infiltration and atrophy of supporting tissue (Winklemann and Ditto, 1964).

The inability of the extract to have effect on all the haematological parameters except the bleeding time implies the possible non-involvement of blood cells in the haemostatic effect of the extract. The significant reduction in bleeding time obtained however suggests that the

extract may possibly be acting in part on the integrity of the blood vessels to produce this effect.

Inhibition of formation of the smooth muscle-relaxing prostaglandin (prostacyclin [PGI₂]-like) by the vessel wall is produced as a normal vascular response to injury. Vessel injury is normally associated with transient vasoconstriction which is followed by the vessel relaxation. According to Bunting et al. (1976), vessel relaxation after constriction is contributed to by smooth muscle-relaxing prostaglandins that are released from vessel wall in response to injury and that the inhibition of these prostaglandins (PG_S) leads to a sustained vasoconstriction. This vasoconstriction has a haemostatic effect when the vessel wall is damaged in either normal or thrombocytopenic animals. Inhibition of PGE₂ synthesis and/or production was postulated to be responsible for the anti-inflammatory effect of the ethanolic extract of *Pterocarpus erinaceus* in rats by Aliyu et al. (2005). Generally, cellular stimulation results in the activation of phospholipase A₂ enzyme which can cleave arachidonic acid from its location. Once released, arachidonic acid can be oxygenated by cyclooxygenase enzyme to form prostaglandin G₂ (PGG₂). The peroxidase component of this enzyme reduces PGG₂ to PGH₂, the precursor of the production of several tissue specific PG_S eg PGI₂ by vascular endothelial cell, PGD₂ by mast cell and PGE₂ by the gastrointestinal tract, lung, etc.

Phytochemical analysis of the extract by Aliyu et al. (2004) revealed the presence of flavonoids, tannins, steroids, carbohydrates, proteins and amino acids. Flavonoids and tannins have been shown to protect body cells from damage and maintain capillary integrity by functioning as anti-oxidants (Frei et al., 1989). Flavonoids like quercetin and rutin are used as effective constituents of several pharmaceuticals in the treatment of capillary fragility and phlebosclerosis (Wild and Fasel, 1969). Orally administered flavonoids have also been observed to inhibit vascular permeability and prevent pulmonary haemorrhage. Acacatin at 25 - 100 mg/kg administered orally to mice was observed to reduce capillary fragility and vascular permeability (Gerdin and Srenso, 1983). Plants with high tannin concentration like *Rhus semialata*, *Punica granatum* and *Cedrel sinensis* have also been used to arrest functional bleeding by the Chinese (Subhuti, 2003). Inhibition of local synthesis and production of the vasodilating prostaglandin I₂ (prostacyclin) produced by the extract and the flavonoids and tannins

contained in it may thus be responsible for the haemostatic property of the ethanolic stem bark extract of *P. erinaceus*.

These results lend pharmacological support to the folkloric use and safety of the plant's stem bark decoction in the treatment of bleeding disorders.

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