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Archives of Applied Science Research, 2010, 2 (2):149-152
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ISSN 0975-508X
CODEN (USA) AASRC9

Blood cell indexes in wistar rats fed with the natural dye extracted from *Pterocarpus Osun*

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Abstract

Extracts of *Pterocarpus osun* have been used as a histological dye but its hazard to health is yet to be fully determined and its use has been with care. The study determined the haematological changes in Wistar rats fed with *Pterocarpus osun*. Concentrations ranging from 2.94g/kg, 5.88g/kg, 8.82g/kg, 11.76g/kg, 14.7g/kg, 17.64g/kg and 20.58g/kg were given orally to 35 rats in 5 groups daily for 28 days. Another group of 5 rats served as control. Blood samples were taken for full blood count and packed cell volume. The packed cell volume increased from $35 \pm 1.5\%$ to $44 \pm 1.5\%$, while the total leukocyte count and differentials remained relatively within normal limits. The film appearance of the red blood cells was also normal. We conclude that chronic intakes of extracts of *Pterocarpus osun* did not show any evidence of toxicity in haematological indices, rather, it increased the PCV and with normal blood film appearance in Wistar rats.

Key words: *Pterocarpus osun*, natural dye, full blood count.

INTRODUCTION

Pterocarpus osun belongs to the family papilionaceae and it occurs throughout the tropics. The Nigerian species are trees of about 30 meters tall, with a girth size of about 2.4 meters with spreading crown and with bright yellow and usually alternate leaflets. It has distinct pickles on twigs and young branches. The fruits are dark brown and velvety when young, with short soft and often sticky pickles mostly surrounding the centre [1,2]. The wood of *P. osun* contains 16% of a red colouring matter called santalin. It yields a blood red solution with alcohol, yellow with ether and violet with ammonia and caustic alkalis, but insoluble in water [3]. The leaves and stern-bark of *P. osun* are medicinally useful for superficial skin diseases such as Eczema [4]. The bark has also been used as a soup ingredient where it is said to increase production of red blood

cells [5]. It is also an active ingredient in a popular Nigerian antiseptic soap called Dudu Osun [4,5]. Some of the active constituents of *P. osun* are tannins [6] and saponin [7] which prevent the growth of fungi, and flavonoids which have antioxidant protective effects and retard the growth of tumours [8,9]. The medicinal value of drug plants is due to the presence in the plant tissue of some chemical substances that produce a definite physiological action on the human body. Some of these chemicals are powerful poisons [10]. The potential of toxicity depends on the dose and the conditions under which a person is exposed to a substance [11]. A natural histological stain for collagen fibres and red blood cells has been prepared from the alcohol-soluble fraction of *P. osun* [12]. Preliminary phytochemical analysis revealed that *P. osun* contains tannins, phenols, saponins and flavonoids [2]. Natural dyes are derived from organic substances or extracted from plants, insects and other creatures [13]. The most important histopathological natural dyes include haematoxylin, carmine and orcein. The recognition of inherent hazards in some synthetic dyes has resulted in the withdrawal of some dyes and the use of natural dyes has gained interest and popularity in the scientific world [14], particularly in poor nations where synthetic dyes are not within the reach of the ordinary scientists. The red pigment extracted from *Pterocarpus osun*, a forest tree [1], which is readily available and affordable has been investigated as a potential natural dye for histology [12]. The effects of *P. osun* on some organs of the body have been investigated histopathologically [15], but its toxicity on blood cells is yet to be determined. The aim of this study was to determine the haematological changes associated with accidental intake of the dye.

MATERIALS AND METHODS

The stem of *Pterocarpus osun* was collected fresh from Plant Science Department of University of Ado –Ekiti, Nigeria following ethical considerations. The stem was cut into tiny bits of about 0.5cm thick, dried at 60°C for 5 days in an open air oven (Selecta oven) milled until they became almost fine powder. 5kg of the powder was extracted with 1000 ml 70% alcohol under Soxlet at 78°C for 24 hours until completion. The extract was concentrated in vacuo at 50°C and finally dried in a decicator to get rid of the residual water. 200g of fine powder was obtained. Forty Wistar rats were obtained from the animal house at Obafemi Awolowo University, Nigeria. The animals, each weighing approximately 170g were grouped into eight. Each group had five rats. The first group was the control and they were fed with normal diet without the extracts. Rats in the other groups had the *P. osun* extract mixed with their diet in the following weights: 0.5g, 1.0g, 1.5g, 2.0g, 2.5g, 3.0g and 3.5g (equivalent to 2.94g/kg, 5.88g/kg, 8.82g/kg, 11.76g/kg, 14.7g/kg, 17.64g/kg, 20.58g/kg) daily for 28 days. The rats were sacrificed through cervical dislocation and 2 ml blood samples taken from their hearts into EDTA bottles while they were still active. PCV and full blood counts were then performed according to standard methods [16].

RESULTS

The PCV in the experimental rats increased substantially from 35±1.5% to 44±1.5% within 28 days reaching the peak at 2.94g/kg, which was maintained up to 20.58g/kg. There was a slight decrease in the total white blood cell count in the experimental animals when compared with the control but it was not significant. There was also no significant difference in the differential counts in the experimental and control groups. The appearance of the red blood cells in the stained films was normal.

Table 1: PCV, WBC and differential count of control and experimental rats fed with extract of *P. osun*

| g/ kg of rat | PCV (%) | WBC (Cx10 ³ /mm ³) | N | L | E | B | M | Film appearance of RBC |
|-----------------|---------|---|------|------|-------|------|------|------------------------|
| Control (0g/kg) | 35±1.5 | 11.00±0.50 | 14±3 | 86±2 | 01±01 | - | - | Normal |
| 2.94g/kg | 44±2.0 | 10.45±0.70 | 13±3 | 87±2 | 02±01 | - | 01±1 | Normal |
| 5.88g/kg | 44±1.5 | 10.00±0.50 | 12±4 | 84±3 | 02±01 | - | 01±1 | Normal |
| 8.82g/kg | 44±1.0 | 9.20±0.50 | 12±3 | 84±3 | 03±01 | - | 01±1 | Normal |
| 11.76g/kg | 44±1.5 | 9.20±0.60 | 13±3 | 87±2 | 02±01 | - | 01±1 | Normal |
| 14.7g/kg | 44±1.5 | 10.00±0.60 | 13±2 | 87±2 | 01±01 | - | 01±1 | Normal |
| 17.64g/kg | 45±1.0 | 9.50±0.70 | 12±4 | 88±2 | 02±01 | - | 01±1 | Normal |
| 20.58g/kg | 45±1.0 | 9.40±0.50 | 12±3 | 89±1 | 02±01 | 01±1 | 01±1 | Normal |

PCV-Packed cell volume, WBC-White blood cells, C-Control, M- Monocyte, B-Basophil, L-Lymphocyte, N-Neutrophils, E-Eosinophil,

DISCUSSION

P. osun has been used traditionally for the treatment of diseases [4] and recently for anaemia where it increased the PCV of Wistar rats [5]. Therefore, its medicinal usefulness is not in doubt but the recognition of hazards in some synthetic dyes has led to the withdrawal of some hazardous dyes [14] and has made it important to test for the toxicity of potential dyes before they can be used freely in the laboratory. The red pigment extracted from *Pterocarpus osun*, a forest tree [17], is readily available and affordable in Nigeria and has been used as a natural dye for histology [12] but the toxicity of the extract *in vivo* has not been studied. The leaves and stern-bark of *P. osun* are medicinally useful for superficial skin diseases such as Eczema [4] and it is a major constituent of a Nigerian antiseptic soap called Dudu osun. The use of Dudu osun as an antiseptic soap has been declared safe and approved by the National Agency for Foods, Drugs Administration and Control. The full blood count of Wistar rats fed with the extract was relatively normal when compared with the control animals but with an increase in PCV which confirms a previous work [5]. *Pterocarpus* species have been shown to contain high levels of calcium, magnesium, nitrogen, sodium and potassium [2]. These elements are said to be very important for nutrition and are required for strong bones, good teeth, production of red blood cells and maintenance of osmotic balance [2]. These minerals probably account for the increase in PCV in Wistar rats. It is however not clear if the intake of *P. osun* will cause certain biochemical alterations as these have not been studied.

CONCLUSION

Oral intakes of *P. osun* increases the packed cell volume and leaves the value of circulating leukocytes unchanged.

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