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Haematological Effects of Ethanolic Fruit Extract of *Tetrapleura tetraptera* in Male Dutch White Rabbits

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Abstract: *Tetrapleura tetraptera* fruit is widely used in Western Nigeria amongst men as a birth control medicine. It has been reported that feeding of extracts to animals produced some toxic effects and pathological lesions in some organs. This study was designed to investigate the haematological effects of 10 days oral administration of the ethanolic extract of *Tetrapleura tetraptera* (TTE) fruits in mature male rabbits. Twenty healthy acclimatized male rabbits (1.4-1.6 kg b.wt. were randomly assigned to 4 groups. Animals in groups 2, 3 and 4 were administered 50, 100 and 150 mg kg^{-1} b.wt. of TTE. Animals in group 1 served as the control and received only water and no extract. All animals were fasted for 18 h after withdrawal of treatment and sacrificed after anaesthesia. Venous blood sample from groups were analyzed for haematological parameters. The TTE caused significant ($p<0.05$) reduction in RBC and WBC.

Key words: *Tetrapleura tetraptera*, haematology, rabbits, phytochemical, toxicity

INTRODUCTION

Medicinal plants include plants or plants part which in one or more of its organs, contains substance that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (WHO, 1977). A number of medicinal plants have been used in traditional medicine for many years. Some do seem to work although there may not be sufficient scientific data to confirm their efficacy (Sofowora, 1993). Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value, which are yet to be discovered. Large numbers of plants are constantly been screened for their possible pharmacological value.

Tetrapleura tetraptera, commonly known as Aridan (fruit) in South Western Nigeria is a medicinal plant of the Mimosaceae family. It is generally found in the lowland forest of tropical Africa. The fruit consist of a fleshy pulp with small, brownish-black seeds. The fruit possess a fragrant, characteristic pungent aromatic odour (Aladesanmi, 2007). It is therefore, used as a popular seasoning spice in Southern and Eastern Nigeria (Okwu, 2003; Essien *et al*., 1994). Its fruit is used for the management of convulsions, leprosy, inflammation, rheumatism (Ojewole and Adesina, 1983), flatulence, jaundice and fevers (Bouquet, 1971). The anticonvulsant activity of the volatile oil from fresh fruits of *T. tetraptera* in mice has been reported (Nwawu and Akali, 1986). Its leaves are essential for the treatment of epilepsy (Aka and Nwabie, 1993) and present strong molluscicidal activity

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The fruit is also used traditionally in the management and control of adult-onset type 2 Diabetes mellitus. The aqueous fruit extract has also been shown to possess hypoglycaemic properties. Based on the studies using the dry fruit of this plant, it is suggested that the plant should be used in formulating drugs (Abii and Elegalam, 2007). The toxicological effect of this plant extract on haematological parameters in animals has not been studied. This study aimed at providing data on the effects of oral administration of this plant on haematological parameters in male Dutch-White rabbits.

MATERIALS AND METHODS

Plant Materials

One kilogram of *Tetrapleura tetraptera* fruits were purchased from the herbal market in Mushin, Lagos State, Nigeria and identified and authenticated in the Department of Pharmacognosy, College of Medicine, University of Lagos, Nigeria in December, 2007.

Preparation of Plant Extract

The fruit was shade dried and pounded in a mortar before being subjected to Soxhlet extraction using 80% ethanol as the solvent. Thereafter, the solvent was distilled off and the extract was successively rinsed with distilled water to eliminate any ethanol still present. The extract was further dried using a lyophilizer. The dried extract was stored in air tight amber bottles. The dried extract was weighed and percent yield was calculated using the expression:

$$\text{Yield}(\%) = \frac{\text{Weight of dried extract}}{\text{Weight of sample used}} \times 100\,\%$$

Phytochemical Screening

Phytochemical screening for alkaloids, saponins, flavonoids, tannins, anthraquinones and cardiac glycosides were carried out according to the methods of Sofowora (1993), Harborne (1984) and Evans (1989).

Experimental Animals

Twenty male Dutch-White rabbits weighing between 1.4 and 1.6 kg were obtained from and acclimatized in the animal house of the College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria. This study was conducted in January, 2008.

Treatment of Animals

The rabbits were allowed to acclimatize for 2 weeks prior to administration of extracts. They were randomly divided into four groups of 5 rabbits each such that differences in average body weights were minimal. Each group was kept in a metal cage at uniform temperature with 12 h dark/light periodicity and fed with standard rabbit pellets (Neimeth Livestock feeds Ltd, Ikeja, Lagos) and water *ad libitum*. Group 1 received orally distilled water only, while Groups 2, 3 and 4 were orally administered graded doses (50,100 and 150 mg kg^{-1} b.wt.) of ethanolic extract of *Tetrapleura tetraptera* fruits daily for 10 days. Treatment was stopped on the 10th day and animals were fasted overnight.

Collection of Blood

On the 11th day, all animals were sacrificed after anaesthesia with chloroform in a desiccator. The rabbits were quickly dissected and venous blood was collected via left ventricular cardiac puncture into heparinized sample bottles.

Haematological Analyses

The blood samples were analyzed for White Blood Cells (WBC), Red Blood Cells (RBC), haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) using the method described by Dacie and Lewis (1994).

Statistical Analysis

The SPSS 11.0 software was employed for data entry and validation. Statistical analysis was carried out between the groups and control using the student's t-test. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening of the plant extract revealed the presence of alkaloids, saponins, tannins, sugar, flavonoids and cardiac glycosides. Phlobatannins were however not detected (Table 1). The yield of the extract was 2.8%.

The extract of the fruits of *T. tetraptera* at 50, 100 and 150 mg kg^{-1} b.wt. caused a significant decrease in the RBC. In contrast, there was a significant increase in the MCV at all doses. However, there was no significant change in the levels of Hb, PCV, MCH and MCHC at all the doses investigated (Table 2). There were significant decreases in the WBC at doses of 50 and 100 mg kg^{-1} b.wt. However, there was no significant change in the WBC at a dose of 150 mg kg^{-1} b.wt. (Table 2).

Haematological analyses of plant extract in animals is one of the important methods of assessing the toxicity of plant extract in animals (Ashafa *et al*., 2009). The decrease in RBC by this extract at all doses is an indicator of the red blood cell-lysing effect of the extract. The extract also caused a reduction in the WBC at concentrations of 50 and 100 mg kg^{-1} b.wt. The extract could be said to lower immunity. Cytotoxic components present in this extract could be the cause of the cell-lysis observed. Results of phytochemical screening of the plant extract showed that alkaloids and saponins were present in large amount. Previous studies have reported that alkaloids are haemolytically active (Cheeke, 1989; Nwogu *et al*.,

Table 1: Phytochemical profile of the ethanolic extract of *Tetrapleura tetraptera* fruits

Phytochemical component	*Tetrapleura tetraptera* extract
Alkaloids	++
Saponins	++
Phlobatannins	ND
Tannins	+
Cardiac glycosides	+
Sugar	+
Flavonoids	+

++: Highly present, +: Present, ND: Not detected

Table 2: Haematological effect of *T. tetraptera* ethanolic extract in male Dutch-White rabbits

Groups	RBC (mm^{-3})	HB (g %)	PCV (%)	MCV ($x10^{-5}m^3$)	MCH ($x\ 10^{-5}$)	MCHC (g %)	WBC (mm^{-3})
1	5.80±0.06	8.00±0.58	45.00±1.16	7.47±0.32	1.40±0.12	17.87±1.73	5.23±0.15
2	4.50±0.06*	8.00±0.58	47.00±1.16	10.20±0.23*	1.80±0.17	17.10±1.65	3.47±0.15*
3	4.73±0.09*	9.00±0.58	43.00±0.58	9.07±0.03*	1.90±0.12	20.90±1.04	4.40±0.21*
4	4.30±0.12*	7.00±0.00	42.00±1.16	9.63±0.17*	1.63±0.03	16.70±0.46	5.00±0.12

n: 5 for each group. *p value <0.05 are considered significantly different from control. Results are mean of 5 determinations +standard error mean. RBC: Red blood cell, HB: Haemoglobin, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, WBC: White blood cell

2008). Saponins have been demonstrated to be haemolytic (Sodipo *et al*., 2000; Okwu, 2004; Okwu, 2005) in several studies and it has been reported in several others. Saponins and alkaloids present in the extract could therefore be the agents causing the red and white blood cell destruction. Packed Cell Volume (PCV) is a measure of the volume of blood consisting of solid cells. The PCV was unaffected by the extract indicating that the volume of red and white blood cells in the blood remain constant. The extract at all doses did not cause a change in the haemoglobin concentration. The oxygen-carrying capacity of the blood of the animals is therefore not affected by the different doses of the extract. The erythrocyte indices MCH and MCHC are used to mathematically define the concentration of haemoglobin within the cell. Extract administration at all doses did not produce any significant change in these parameters. This further supports the results indicating that haemoglobin concentration remains unchanged. The MCV is regarded as the average volume of a single red blood cell. However, there was a significant increase in the MCV at all doses. The other phytochemicals identified to be present in the extract could also play their part in affecting the blood of the animals. Flavonoids which are polyphenols have been shown to possess antioxidant properties and help in membrane stabilization (James and Nnacheta, 2008). The flavonoids present in this extract could be antagonistic to the action of the saponins which lyse red blood cells. This could possibly explain why the PCV was unaffected by the extract at different doses. Tannins also present in the extract bind to proteins and carbohydrates which are components of the erythrocyte membrane and therefore may prevent breakdown of the erythrocyte membrane.

The results of this study indicate that ethanolic extract of *T. tetraptera* at concentrations of 50 mg kg^{-1} b.wt. possess haemolytic properties and caused a reduction in RBC and WBC. It is suggested that this drug though has varied uses in the environment should be used with caution. Further work still needs to be done to study the appropriate dosage at which it can be safely administered.

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