

## EFFECT OF ETHANOLIC EXTRACT OF *DENNETTIA TRIPETALA* FRUIT ON HAEMATOLOGICAL PARAMETERS IN ALBINO WISTAR RATS

DANIEL E. IKPI and CLEMENT O. NKU

*Department of Physiology, College of Medical Sciences, University of Calabar, Calabar, Nigeria*  
E-mail: [ikpidanielewa@yahoo.com](mailto:ikpidanielewa@yahoo.com)

**Summary:** The effect of oral administration of ethanolic extract of *Dennettia tripetala* fruits on haematological parameters in albino Wistar rats was investigated. Lethality studies revealed that the extract had an LD<sub>50</sub> value of 251.19mg/kg mice intraperitoneal. Fifteen (15) male albino wistar rats weighing between 150 – 200g were used for the study and randomly assigned into three study groups of five animals each. The group 1 control received via oral route a placebo (4ml of normal saline), while test groups 2 and 3 received 85mg/kg body weight and 170mg/kg body weight of *D. tripetala* extract in 2.0ml and 4.0ml of the vehicle (normal saline) via oral route respectively. The administration of ethanolic extract of *D. tripetala* for 14 days produced a significant ( $P<0.05$ ) decrease in RBC and WBC counts in group 2 versus group 1 (control) but the decrease in RBC and WBC counts in group 3 were not significant compared to group 1. There was no significant difference in PCV and haemoglobin levels in groups 2 and 3 compared to control. The differential WBC results showed a significant increase ( $P<0.001$ ) in neutrophil count in group 2 versus group 1. While neutrophil count in group 3 was significantly decreased ( $P<0.001$ ) compared to group 1. There was a significant decrease ( $P<0.01$ ) in eosinophil count in groups 2 and 3 when compared to the control group. From the results, there was a significant decrease ( $P<0.001$ ) in lymphocyte count in group 2 while a significant increase ( $P<0.01$ ) in lymphocyte count was observed in group 3 when compared to the control group. There were no significant differences in basophils and monocytes counts in groups 2 and 3 compared to the control group. The study shows that *D. tripetala* extract, given at moderate to high doses may have hematotoxic effect, but the effect was worse with moderate doses.

**Key words:** *Dennettia tripetala*, haematological indices, red blood cell, white blood cell

### Introduction

*Dennettia tripetala* G. Baker (Annonaceae) also known as pepper fruit tree is a well-known Nigerian spicy medicinal plant. It is found in the tropical rainforest region of Nigeria and sometimes in Savana areas (Okwu *et al*, 2005). It is locally called “Nkarika” by the Efiks of Calabar. The young leaves and fruits have instinctive spicy taste (Achinewhu *et al*, 1995). The mature fruits constitute the main edible portions. Some communities in parts of Southern Nigeria also utilized the leaves and roots, in addition to the fruits for medicinal purpose (Iwu, 1989). *Dennettia triptala* is used as masticators, which when chewed produces unique peppery effect (Keay, 1989). The peppery spicy taste of mature *D. tripetala* fruits usually serves as a mild stimulant to the consumer. The fruits are sometimes taken with kolanut, garden egg and palm wine in parts of Nigeria, especially in Southern part of Nigeria where it serves also for cultural entertainment of guests, particularly during coronation, new yam festivals, weddings and marriage festivals

(Enwere, 1998; Keay, 1989). *Dennettia tripetala* fruit has also been reported to be used as spice in flavouring food, and as seasoning which are added to prepared food such as meat, sausages, soups and vegetable (Lebouef and Caver, 1972). The peppery fruits of *Dennettia tripetala* are applied to the food meant for pregnant women and are important in the diets of postpartum women, during which time it is claimed that spices and herbs aid uterine contraction (Okwu and Morah, 2004; Achinewhu *et al*, 1995). Okwu *et al* (2005) also reported that *D. tripetala* fruits contain important nutritive substances such as vitamins, minerals and fibre.

Despite the extensive use of the fruit, much work has not been done to study some of the toxicological implications on other related systems. Motivated by this, the aim of the present study was to assess the effect(s) the ethanolic extract of *D. tripetala* fruits would have on some haematological parameters of the wistar rats.

## Materials and Methods

### Preparation of plant extract

Mature ripe fruits of *Dennettia tripetala* were obtained from the University of Calabar Botanical Garden and identified by Mr. Frank Apejoye, the Chief Herbarium Officer of Botany Department, University of Calabar, Calabar. The fruits were rinsed with water to remove debris and sand and then sun dried. The dry sample was milled with an electric blender and finally ground into powder. 110g of the powdered plant sample was suspended in 660mls of ethanol (98.67%, BDH) (80% vol/vol). The suspension was agitated with an electric blender for about 10 minutes then allowed in a refrigerator (4°C). Twenty four hours later, the suspension was filtered with a chess cloth. The ethanolic filtrate was concentrated using a rotary evaporator at 40°C. This concentrate was allowed in a water bath at 37°C for complete dryness, yielding 15.5g (14.09% yields) of crude extract. This was reconstituted to an appropriate concentration in distilled water prior to administration.

### Acute toxicity

Seventy male albino wistar mice weighing 18 – 23g were randomly assigned to seven (7) cages of 10 animals per cage. Each group respectively received the following doses: 100, 200, 400, 800, 1600, 3200mg/kg body weight of the extract intraperitoneally (i.p). The maximum volume injected was 0.2ml and the control group received 0.2ml of normal saline i.p. The number of deaths in each group within 24 hours was recorded. The LD<sub>50</sub> was estimated from the graph of percentage (%) mortality (converted to probit) against log<sub>10</sub> of the dose of the extract (Eno *et al.*, 2001).

### Experimental design

Fifteen male albino wistar rats weighing between 150 – 200g were randomly assigned into three groups of 5 rats per group. They were maintained at room temperature of 30°C in the animal house of Physiology Department, Faculty of Basic Medical Sciences, University of Calabar, Calabar. The animals were fed with standard diet (product of Pfizer Nigeria, Ltd). There was free access to water. Group 1 served as control, groups 2 and 3 (extract treated groups) received orally the ethanolic extract of *D. tripetala* at doses of 85mg/kg bwt and 170mg/kg bwt respectively. After 14 days of extract administration, the animals from each group were sacrificed using chloroform anesthesia and blood obtained by cardiac puncture for analysis.

### Haematological estimations

The blood samples collected into heparinized tubes were immediately used for determination of haematological parameters. Total red blood cell and white blood cell counts were estimated according to the visual method of Dacie and Lewis (1975). The percentage packed cell volume was determined according to the hematocrit method of Alexander and Griffiths (1993a) while the blood haemoglobin concentration in all samples were estimated according to the cyanomethaemoglobin method of Alexander and Griffiths (1993b).

### Differential White Blood Cell counts

These were estimated using the method of Osim *et al.* (2004). A dry micropipette was used to suck in blood from the blood sample bottle, a small drop of blood was applied to one end of a slide and quickly placed on the bench holding it in position, the end of the second slide was then placed in the drop and held there until the blood had spread across it. It was then drawn slowly over the whole length of the first slide being held at an angle of 45°. After the blood had spread, it was dried before staining with Leishman's stain. The film which was washed off in a gentle stream of water was dried with filter paper and examined under low and high power microscope and the different kinds of cells counted.

## Results

### Acute toxicity

Lethality studies showed that the crude ethanolic extract of *D. tripetala* fruits had an LD<sub>50</sub> value of about 251.19mg/kg. mice, intraperitoneally (Fig. 1). From the results of the toxicity studies, convenient doses were chosen to preclude the lethal range.

### Haematological indices

Table 1 shows haematological indices of rats following oral administration of ethanolic extracts of *D. tripetala* fruits for 14 days. There were decreases in RBC counts of rats in group 2 ( $440.0 \times 10^4 \pm 0.13 \times 10^4$  cells per mm<sup>3</sup>) which received 85mg/kg bwt of extract and group 3 ( $487.2 \times 10^4 \pm 0.15 \times 10^4$  cells per mm<sup>3</sup>) which received 170mg/kg bwt of extract. The decrease in RBC count of group 2 was significantly different ( $P < 0.05$ ) when compared to Group 1 ( $510.0 \times 10^4 \pm 0.18 \times 10^4$  cells per mm<sup>3</sup> of blood) which served as control group, while the RBC count in group 3 was significantly higher ( $P < 0.05$ ) than that of Group 2.

Similarly, there was a decrease in WBC counts in groups 2 and 3 animals. However, the decrease was only significant ( $P < 0.05$ ) in Group 2 ( $3.05 \times 10^3 \pm 0.48 \times 10^3 \pm 0.87 \times 10^3$  cells per  $\text{mm}^3$  of blood) when compared to group 1 ( $5.61 \times 10^3 \pm 0.87 \times 10^3$  cells per  $\text{mm}^3$  of blood). There was no significant difference in packed cell volume of rats in all the groups. The values were  $34.99 \pm 1.40$  (%) for group 1,  $33.40 \pm 1.63$  (%) for group 2 and  $35.40 \pm 0.93$  (%) for group 3. Similarly, the blood haemoglobin concentration of group 2 ( $12.26 \pm 1.02$  g/dl) was lower when compared to group 1 ( $14.11 \pm 0.81$  g/dl) while that of group 3 ( $14.84 \pm 0.52$  g/dl) was higher compared to group 1. However, the differences were not statistically significant ( $P > 0.05$ ).

Table 2 shows the differential WBC counts of rats gavaged with test doses of the ethanolic extract of *D. tripetala*. From the

table, it is obvious that there was significant increase ( $P < 0.001$ ) in % Neutrophil count in group 2 ( $30.60 \pm 0.68$ ) compared to group 1 ( $22.80 \pm 1.07$ ). However, the result for group 3 ( $17.20 \pm 0.58$ ) was significantly lower ( $P < 0.001$ ) compared to group 1. There was a significant decrease ( $P < 0.001$ ) in % lymphocyte count in group 2 while a significant increase ( $P < 0.001$ ) in % lymphocyte was observed in group 3 ( $73.40 \pm 0.40$ ) when compared to that of group 1 ( $64.80 \pm 1.16$ ).

As shown in the table, there were no significant differences ( $P > 0.01$ ) in % Basophils and % Monocytes in groups 2 and 3 compared to group 1. There was a significant decrease ( $P < 0.01$ ) in % Eosinophil count in group 2 ( $3.20 \pm 0.20$ ) and group 3 ( $3.20 \pm 0.20$ ) when compared to group 1 ( $4.60 \pm 0.24$ ).

Table 1: Haematological indices of rats administered with ethanolic extract of *D. tripetala* fruits

Group	Treatment	RBC Count per $\text{mm}^3$ blood ( $N \times 10^4$ cells)	WBC count per $\text{mm}^3$ of blood ( $N \times 10^3$ cells)	PCV (%)	Hb Conc. (g/dl)
1 (control)	Normal saline	510 $\pm$ 0.18	5.61 $\pm$ 0.87	34.99 $\pm$ 1.40	14.11 $\pm$ 0.81
2	Extract (85mg/kg bw)	440.0 $\pm$ 0.13*	3.05 $\pm$ 0.48*	33.40 $\pm$ 1.63	12.26 $\pm$ 1.02
3	Extract (170mg/kg bw)	487.0 $\pm$ 0.15 <sup>a</sup>	4.59 $\pm$ 0.91	35.40 $\pm$ 0.93	14.84 $\pm$ 0.52

Mean  $\pm$  SEM, n = 5 \*  $P < 0.05$ , significant as compared with group 1 a  $P < 0.05$  significant as compared with group 2

Table 2: Differential white blood cell count of Albino Wistar rats treated with ethanolic extract of *D. tripetala* fruit

Group	Treatment	Neutrophils (%)	Lymphocytes (%)	Basophils (%)	Monocyte (%)	Eosinophil (%)
1 (control)	Normal saline	22.80 $\pm$ 1.07	64.80 $\pm$ 1.16	0.40 $\pm$ 0.24	7.40 $\pm$ 0.51	4.60 $\pm$ 0.24
2	Extract (85mg/kg bwt)	30.60 $\pm$ 0.68***	59.60 $\pm$ 1.03***	0.40 $\pm$ 0.24	6.20 $\pm$ 0.37	3.20 $\pm$ 0.24**
3	Extract (170mg/kg bwt)	17.20 $\pm$ 0.58 <sup>C</sup>	73.40 $\pm$ 0.40***	3.20 $\pm$ 0.20	5.80 $\pm$ 0.58	3.20 $\pm$ 0.20**

\*\*\* $P < 0.001$ , \*\*  $P < 0.01$  significant as compared with group 1 <sup>C</sup> $P < 0.01$  significant as compared with group 2.

## Discussion

The effects of *Dennettia tripetala* fruit extract on the formed elements (erythrocytes, leucocytes, thrombocytes) of blood have not been documented. The present study was therefore aimed at evaluating the effects of ethanolic extract of *D. tripetala* fruits on

haematological parameters viz – Rbc and Wbc counts, PCV, Hb concentration and differential WBC counts. The acute toxicity testing showed LD<sub>50</sub> value of ethanolic crude extract of *Dennettia tripetala* fruit to be moderately high.

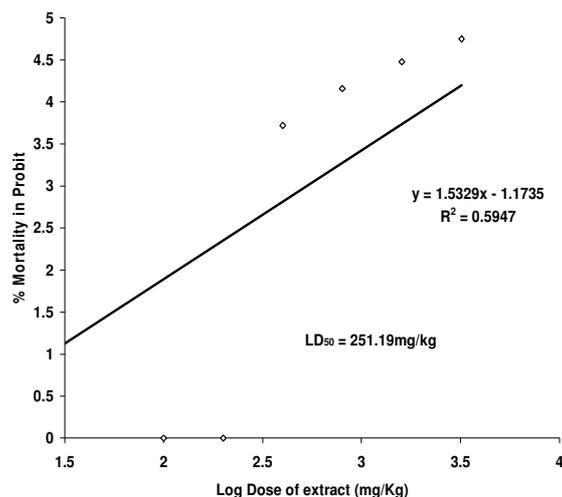


Fig. 1 Log dose probit curve showing percentage mortality for derivation of LD50 for *D. tripetala*, n = 10.

This suggests that the extract had a moderately high safety margin.

In this study, the RBC counts were significantly decreased in the group 2 rats and non-significantly decreased in the group 3 rats when compared to that of group 1. However, the group 2 rats showed a significant decrease in WBC count when compared to the control while WBC count in group 3 was not significantly different from that of group 1. However, PCV and blood Hb counts were not significantly different from that of group 1, although there was a slight non significant decrease in Hb and PCV counts in group 2 rats with respect to group 1.

The observed decrease in RBC counts in *D. tripetala* treated groups may have been due to the suppressive effect of some components of the extract on bone marrow. These components such as alkaloids, saponins, flavonoids, tannins and phenols (Muller, 1980) may have suppressed the growth and differentiation factors in the bone marrow.

Another probable reason for the observed decrease in RBC counts may be due to hemolysis mediated via the phytochemical components of the extract, or may be the extract caused failure of erythropoietin production. This could lead to anemia. The slight non-significant increase in Hb concentration in group 3 which received a high dose of the extract may therefore result due to the increase in hemolysis of RBC.

It is well known that PCV otherwise called haematocrit represents the percentage of RBC in blood. There is a direct relationship between erythrocytes, PCV and haemoglobin

concentration (Schalm *et al*, 1975) hence, an alteration in one parameter, alternately alters another. There was no significant change in PCV observed in this study, and this is not in agreement with the decrease in RBC count observed. The decrease in total WBC counts following oral administration of ethanolic extract of *D. tripetala* is not in line with the normal physiological response following perception of a foreign attack by body defense mechanism. The decrease observed may have resulted from suppression of leucocytes by the extract and also from the suppression of their production in the bone marrow.

However, there was a significant increase ( $P < 0.001$ ) in % Neutrophil count in group 2 ( $30.60 \pm 0.68$ ) compared to group 1 ( $22.80 \pm 1.07$ ), while there was a significant decrease ( $P < 0.001$ ) in % neutrophil count in group 3 ( $17.20 \pm 0.58$ ) compared to group 2. The observed increase in Neutrophil proliferation in group 2 which received a low dose of the extract may be related to the chemical composition of the extract, which later had a suppressing effect in group 3 rats that received a high dose of the extract.

There was a significant decrease ( $P < 0.001$ ) in % lymphocytes in group 2 while a significant increase ( $P < 0.001$ ) in % lymphocytes was observed in group 3 when compared to group 1. The observed increase in lymphocyte proliferation in group 3 may also be related to the chemical composition of the extracts. Lymphocyte proliferation is a common parameter which has been measured in several studies investigating the immunomodulatory effects of metabolites (Gossage, 2000).

However, there was a significant decrease ( $P < 0.01$ ) in % Eosinophil in groups 2 ( $3.20 \pm 0.2$ ) and 3 ( $3.20 \pm 0.2$ ) when compared to group 1 ( $4.60 \pm 0.24$ ). It may be that some component of the extract suppressed eosinophil proliferation.

## Conclusion

*Dennettia tripetala* fruit is rich in minerals and vitamins (ascorbic acid, thiamine, riboflavin and niacin as documented by Okwu and Morah (2005). The study has shown the effect of ethanolic extract of *D. tripetala* on haematological parameters of wistar rats. It shows that *D. tripetala* extract, given at moderate to high doses may adversely affect haematological parameters on account of its significant lowering effect on RBC and WBC counts. The effect was worse with moderate doses. Further studies to confirm this as well as evaluate its mechanism of action are suggested.

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