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EFFECT OF THEOBROMINE EXPOSURE ON HAEMATOLOGICAL PARAMETERS IN RATS

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Summary: This work was carried out to investigate the effect of theobromine exposure on haematological indices in rats and thus evaluate whether its use in chemotherapy may be associated with possible side effects such as anaemia. Theobromine in two doses was administered by oral gavage to albino Wistar rats of both sexes ($n=8$ for each group) for a four day period. Haematological parameters – indices of blood and erythropoietic status namely packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC) and mean cell haemoglobin concentration (MCHC) were assessed in whole blood obtained from the animals. The same parameters were assessed in the control group ($n=8$) administered with only the vehicle. Theobromine administration in moderate (600 mg/Kg body weight) to high (700 mg/Kg body weight) doses produced a significant ($P<0.05 - 0.001$) elevation in PCV, RBC and WBC when compared with the control. Blood haemoglobin concentration increased in treated animals relative to controls but the increase was not significant. No adverse alterations in haematological parameters were observed. The results indicate that there is normal erythropoietic function and the absence of anaemia following exposure to theobromine.

Key Words: theobromine, haematological indices, erythropoietic function, anaemia.

Introduction

Theobromine, a 3,7-dimethylxanthine is a purine-derived alkaloid. In recent times there has been an increased application of theobromine in chemotherapy. For instance, theobromine at the therapeutic dose of 500 mg is employed in the treatment of cardiac oedema and angina pectoris. It is used as a diuretic to eliminate excess water and toxic waste from vascular tissues, and is also employed in the treatment of asthma and chronic obstructive airway diseases (Trease and Evans, 1978, Hallworth, 1992). Its analogues like pentoxifylline, lisophylline, caffeine and suramin-theobromine complex find application in cancer therapy (Chang *et al.*, 1993, BBC Cancer News 1997).

The review by Eteng *et al.* (1997) has documented the reproductive toxicity of theobromine. The alkaloid induces placental vasoconstriction via the sympathetic nerve supply resulting in nutrient deprivation and retarded development of the embryo. It also crosses the placental barrier with ease into the environment of the developing embryo, and as an analogue of the purine bases, interact with the deoxyribonucleic acid (DNA) to induce embryo malformation. Its cardiotoxicity has also been reported (Eteng *et al.*, 1998). Following the increasing reports of the toxicity of this alkaloid, anxiety and fear have been expressed over its continuous use in chemotherapy

and its consumption in coffee, cocoa beverages and snacks such as chocolate and cocoa bread (Eteng, 1999). There is paucity of data on studies assessing the effect of theobromine exposure on haematological parameters. The objective of this study therefore is to investigate the effect of theobromine exposure on haematological indices namely packed cell volume, haemoglobin, mean cell haemoglobin concentration, red cell count, white cell count, and subsequently to evaluate whether its use in chemotherapy may have possible side effects such as anaemia, which is common with the use of most chemotherapeutic agents. We hypothesize that theobromine induces adverse changes in haematological parameters and subsequently anaemia.

Materials and Methods

Animals

Twenty-four albino rats (14 males and 10 females) of Wistar strain weaned at 30-42 days old were purchased from the disease-free stock of the Department of Biochemistry animal house, University of Calabar for the study. They were reared on a popular commercial stock diet (Pfizer Livestock Feeds, Abuja, Nigeria) until they were 60-85 days old and attained 100-150 g body weight when they were used for experimentation. Permission and approval for the animal studies were obtained from the College of Medical Science

Animal Ethics Committee, University of Calabar. The rats were weighed and randomly assigned on the basis of weight and litter origin to 3 groups of 8 animals each. The animals were housed individually in specially designed Perspex cages with plastic bottom grid and steel top. They were kept under adequate ventilation at room temperature and relative humidity of $28 \pm 2^\circ\text{C}$ and 46% respectively. Food and water were provided *ad libitum*.

Experimental Design

Animals in group 1 were gavaged with 0.5 ml of sodium acetate solution (placebo) and served as the control. Group 2 and 3 were gavaged with 600 mg/Kg and 700 mg/kg of theobromine in sodium acetate respectively. Dose administration continued daily for four days between 08:00 and 09:00 h each day. Twenty-four hours after the last administration, the animals were anaesthetized with chloroform vapour and dissected. Whole blood was obtained by cardiac puncture from each rat and collected into anticoagulant-treated (EDTA 0.77M) sterile screw-cap tubes in order to have plasma and suspended blood cells intact. This was used for haematological studies.

Blood haemoglobin (Hb) was determined by cyanomethaemoglobin method of Crosby *et al* (1954), as modified by Pla and Fritz (1971).

Packed cell volume (PCV) and mean cell haemoglobin concentration (MCHC) were both determined by the method of Dacie and Lewis (1975). White (WBC) and red (RBC) blood cell counts were estimated by light microscopy (Dacie and Lewis, 1975).

Statistics The student's t-test for unpaired groups was used for statistical analysis. Values are expressed as mean $\pm$ SD. Statistical significance was accepted at $p < 0.05$.

Results

The effect of orally administered theobromine on haematological parameters of experimental animals is summarized in Table 1. The percentage PCV for the test groups were $45.7 \pm 3.3\%$ (Mean $\pm$ SD) and $54.8 \pm 4.2\%$ for the low and high dose theobromine treatment respectively, whereas the PCV for the control group was $32.7 \pm 4.8\%$. This indicates a statistical significant ($P < 0.05$) increase in PCV in both low and high theobromine treatment groups relative to control. The blood haemoglobin concentrations (g/dl) were 8.40 ± 3.0 , 9.47 ± 3.0 and 11.21 ± 2.0 for control, low and high dose theobromine treatment groups respectively. The increase in haemoglobin concentration in the theobromine groups was not significant. The mean cell haemoglobin concentration (g/dl) decreased slightly from 28.6 ± 9.0 in controls to 21.4 ± 6.0 for the low dose, and 21.9 ± 5.0 for the high dose theobromine treatment. The mean values for red blood cell count ($\times 10^6$ cell/ mm^3) were 474.5 ± 4.1 , 641.8 ± 9.8 and 760.1 ± 5.6 for control, low and high dose theobromine treatments respectively. The white blood cell counts ($\times 50$ cells/ mm^3) for these respective groups were 61.4 ± 5.6 , 101.3 ± 8.5 and 145.0 ± 10.0 . This indicates a dose-dependent, statistically significant ($P < 0.001$ for RBC and $P < 0.05$ for WBC) increase relative to control.

Table 1: Effect of Theobromine on Haematological Parameters in Rats.

Treatment groups	Packed cell volume (PCV%)	Haemoglobin (g/100 ml)	Mean Cell Haemoglobin Concentration (g /100 ml)	Red Blood cell (RBC) Count (N/ mm^3) $\times 10^4$	White Blood Cell (WBC) Count (N/ mm^3) $\times 50$
Group I (Control) 0.5 ml sodium acetate	32.7 ± 4.8	8.4 ± 3.0	28.6 ± 9.0	$474. \pm 4.10$	61.42 ± 5.56
Group II 600 mg/Kg body weight theobromine	^b 45.7 ± 4.2	9.47 ± 3.0	21.4 ± 6.0	^a 641 ± 9.8	^b 101.28 ± 8.53
Group III 700 mg/Kg body weight theobromine	^b 54.8 ± 4.2	11.21 ± 2.0	21.9 ± 5.0	^a 760 ± 5.58	^b 145 ± 10.0

Discussion

Theobromine administration produced significant elevation in PCV, WBC and RBC counts, and Hb in treated animals relative to controls. The haematological parameters PCV, Hb, MCHC, RBC and WBC together provide information on the general state of the blood and reticular endothelial system. The results agree in part with the reports of other studies which assessed these parameters in anaemic and normal rats. For instance, Ifere (1986) has reported PCV values between 38.8 and 40% for normal rats, and values below 30% for anaemic rats. Similarly, McKee *et al* (1968) and Underwood (1977) have reported blood haemoglobin of 13.0 g/100 ml for normal rats. However, the control values of PCV and Hb for the present study appear to be low compared to the reference or reported control values, a factor which may be due to the differences in methodology and assay techniques. However, the increase in PCV, Hb and RBC observed in this study suggest that theobromine consumption in drugs or food is not associated with adverse haematological changes or anaemia in experimental animals.

The mechanism by which theobromine may act to stimulate erythropoiesis and WBC production is at present unknown. It is however known that theobromine inhibits cyclic adenosine monophosphate (c'AMP) phosphodiesterase leading to accumulation of c'AMP. Accumulating c'AMP has several actions the main one being the stimulation of phosphorylation of proteins. Structural proteins such as histones when phosphorylated, binds DNA less effectively and thus in some way 'free' DNA to enable replication and transcription to take place (Eteng *et al*, 1998). In this way theobromine may stimulate erythropoiesis. Exposure to theobromine significantly elevates PCV, WBC, RBC and haemoglobin and therefore enhances haemopoiesis generally. Further studies to confirm this as well as evaluate its mechanism of action and possible use in the management of anaemia are suggested.

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