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Haematological Changes Following Vitamin E Supplementation During caffeinated And Non-Caffeinated Paracetamol administration In Rats

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ABSTRACT

Haematological changes associated with caffeinaed and non-caffeinaed paracetamol administration with or without Vitamins A and E supplementation was investigated in albino Wistar rats using panadol extra and paracemol as caffeinated and non-caffeinated paracetamol respectively; and water soluble acetic acid derivatives of Vitamin E. administration of paracetamol alone caused a significant increase ($P < 0.05$) in Hb compared to the control while and panadol extra + vitamin E caused a significant decrease ($P < 0.05$) compared to paracetamol group. The PCV significantly decreased (($P < .0.05$) in the paracetamol + vitamin E group, while the RBC count significantly decreased in the panadol extra + vitamin E group compared to the paracetamol group. Caffeination, vitamin E produced significant decrease ($P < 0.05$) in WBC count when co-administered with paracetamol compared to the group on paracetamol alone. The result suggests that administration of paracetamol without caffeination or supplementated with vitamins E may result in anaemia and leucocytosis, and supplementtion with vitamins E may play a role in remitting these adverse changes. However, administration of vitamin E tended to potentiate the adverse haematological changes during caffeinated paracetamol therapy.

Keywords: Acetaminophen; Caffeine; Vitamin E; RBC; Hb; PCV; WBC.

Acetaminophen is a popular domestic analgesic and antipyretic for adult and children. It is effective in mild to moderate pain and particularly useful in patients who are to avoid aspirin because of gastric intolerance, bleeding tendency, allergy or those who are less than 12 years old (Laurence et al 1997). In therapeutic doses acetaminophen is usually well tolerated and free from adverse effects and interaction with other drugs. Its easy availability in pharmacies as well as ease of acquisition has led to the increase in reported cases of toxicity caused by paracetamol (Thomas 1993).

The most serious adverse effect of acute overdose of acetaminophen is a dose dependent potentially fatal hepatic necrosis (Thomas 1993; Mesembe et al 2004a). the less common features of paracetamol poisoning include thrombocytopenia (Thorton and Losowsky, 1990; Mesembe et al 2004b), subendocardial necrosis associated with haemorrhage (Mann et al 1989; Price et al 1991) and, a reduction in clotting factors (Gazzard et al 1974; Mesembe et al 2004b) and a prolongation of prothromb in time (Mesembe et al 2004).

Co-administration of paracetamol with caffeine is gaining popularity; and, caffeine has been shown to enhance the analgesic effect of paracetamol (Laurence et al 1997), as well as reduce the histopathological changes in the liver and kidney consequent upon paracetamol intoxication (Rainska et al 1992; Mesembe et al 2004a).

Furthermore, we have recently shown that supplementaion with vitamin E effectively reduces the toxicity of paracetamol (Mesembe et al 2004a; Mesembe et al 2004b).

Changes may occur in haematological parameters as a consequence of other systemic diseases (Mackie et al 1999). This study was therefore designed to ascertain the modulatory effect of concurrent administration of vitamin E with caffeinated paracetamol (paracetamol) on haematological parameters in adult Wistar rats.

MATERIALS AND METHODS

Thirty albino Wistar rats weighing between 200 to 300g, obtained from the animal house of the Department of Anatomy, University of Calabar were used for the experimental investigations. The animals were acclimatized for two weeks, kept in plastic cages in a ratio of

six animals to one cage and were fed with commercial rats chow and tap water *ad libitum* for two weeks. They were divided into seven experimental groups, each consisting of six rats and treated as follows:

Group 1	Distilled water (control)
Group 2	Paracetamol
Group 3	Panadol extra
Group 4	Paracetamol + vitamin E
Group 5	Panadol extra + vitamin E

Paracetamol tablets (Emzor pharmaceutical industries, Lagos) was dispersed in distilled water and administered by oral gavage daily doses of 171.43mg/kg body weight. Panadol extra tablets (Smithkline Beecham pharmaceutical company, Lagos) was dispersed in distilled water and administered by oral gavage on daily doses of 171.43mg/kg body weight. Vitamin E was administered as vitamin E acetate (Ephynal, tablets from Roch, France) dispersed in distilled water and administered on a daily oral dose of 4.286mg/kg body weight. The treatment period lasted for two weeks after which the animals were sacrificed after over-night fast. Blood was collected into heparinized screw cap bottles for estimation haemoglobin, red blood cell count, white blood cell countandhaematocrit.

Haematocrit (PCV) was estimated using micohaematocrit method according to Alexander and Griffiths (1993a); Haemoglobin (Hb) was determined using cyanomethamoglobin method according to Alexander and Griffiths (1993b); whereas the red blood cell (RBC) count and white blood cells (WBC) count were estimated by visual means using the new improved Neubauer counting chamber according to Dacie and Lewis (1991).

Statistical analysis

Students' test and analysis of variance (ANOVA) were used to analyze the data. Values of $P < 0.05$ were regarded as significant.

RESULTS

The effects of treatment on the haematological parameters are shown in table 1. the difference in the packed cell volume was significantly ($P < 0.05$) lower in the rest of the test group when compared with the paracetamol and panadol extra groups.

There was a significant increase ($P < 0.05$) in the haemoglobin concentration of the group on paracetamol when compared to the control group. The group on panadol extra + vitamin E showed significant decrease ($P < 0.05$) when compared to the paracetamol group.

The white blood cell count increased significantly ($P < 0.05$) in the groups on paracetamol, panadol extra and panadol extra + vitamin E when compared to the group receiving the paracetamol + vitamin E and the control group.

The red blood cell counts was

Table 1: Haematological parameters in rats given oral doses of paracetamol or panadol extra with or without vitamin E

Group	Treatment	Hb (g/dl)	PCV (%)	RBC (N x 10^6 per mm^3)	WBC (N x 10^3 per mm^3)
1	Control	12.40± 1.05	58.60± 4.28	4.70± 0.11	2.29± 0.10
2	Paracetamol	13.54± 0.24[a]	51.80± 3.96[a]	4.06± 0.10[a]	3.32± 0.57[a]
3	Panadol extra	13.15± 0.51[ab]	57.20± 6.65[b]	4.60± 0.22[ab]	3.80± 0.07
4	Paracetamol + vitamin E	13.10 ± 1.2[ab]	57.60± 6.73[ab]	4.51± 0.25[ab]	2.10± 0.08[ab]
5	Panadol extra + vitamin E	12.16± 0.65[ab]	56.80± 4.32[ab]	4.42± 0.20[ab]	3.44± 0.03[ab]

Values presented as mean ± SEM; N 6
[a] P<0.05 Compared to the control
[b] P<0.05 Compared to the paracetamol group.

Dose of oaracetamol = 171.43mg.kg
Dose of paracetamol in panadol extra = 171.43mg/kg
Dose of caffeine in panadol extra = 10.286mg/kg
Dose of vitamine E = 1428.57 iu/kg.

significantly decreased ($P<0.05$) in the paracetamol group as well as in other treatment groups when compared to the control.

DISCUSSION

This investigation has revealed alterations in the haematological parameters. The implications of these findings are discussed. The determination of haematological indices provides physiological information on the general blood picture and the reticuloendothelial system (Baker and Silverton, 1985). The administration of caffeinated and non-caffeinated paracetamol caused a significant increase inhaemoglobin levels. This increased Hb concentration observed in this study may be due to the increase in haemolysis of RBC. Hb concentrations are reported to be moderately increased in haemolytic anaemia and in event of rapid intravascular haemolysis (Bolarin, 1997).

Paracetamol administration caused a reduction in packed cell volume (PCV) and red blood cell (RBC) count. The observed decreased in RBC counts may be due to increase in haemolysis mediated via the reactive metabolite of paracetamol (NAPQI) which is believed to exert paracetamol toxicity (Raucey et al 1989; McClain et al 1999). NAPQI also may have suppressed the process of growth and differentiation in the marrow.

Failure of erythropoietin production may have caused the decrease in RBC in count. The kidney is the main source of erythropoietin which is produced by interstitial peritubular cells (Davidson et al 1999). Several studies have reported that paracetamol toxicity caused renal tubular necrosis (McJunkin et al 1976; Jones and Vale, 1993; Thomas, 1993). It has been shown that patient with renal dysfunction are anaemic due to failure of erythropoietin production (Edelstein et al 1997).

The PCV otherwise known as haemtocrit is a function of RBC. It represents the perentage of RBC in blood (Kiraly, 1980). Therefore, the decrease in PCV observed in this study is in agreement with the observed decrease in RBC counts. However, the exact mechanism responsible for the decrease in RBC count and PCV is yet to be established.

Paracetamol administration resulted in an increase in WBC counts the caffeination of paracetamol caused further increase in WBC count when compared to the paracetamol group. This is in line with normal physiologic response following the perception of an insult by the paracetamol is hepatoxic (McJunkin et al 1976; Thomas, 1993; McClain et al 1999; Lawson et al 2000). It is likely therefore that damage to the liver cells may have caused the insult that contributed to the observed increase in WBC count. Finlayson et al (1999) has demonstrated that leucocytosis may occur in hepatic damage.

There was a decrease in the Hb level of rats receiving paracetamol and vitamin E as well as those treated with panadol extra and vitamin E. the observed reduced RBC count and PVC remitted by the administration of vitamin E with paracetamol. There was also a concomitant reduction in WBC counts in these groups.

This study reveals that the administration of paracetamol alone without vitamin E supplementation might constitute a kind of metabolic stress through generation of free radicals. The generation of reactive oxygen intermediates by paracetamol in hepatocytes has been suggested to contribute to its hepatotoxic effect (Farber et al 1988; Thomas, 1993; McClain et al 1999). The caffeination of paracetamol has also been shown to reduce the haematoloigcal imbalance caused by paracetamol alone. The mechanism by which vitamin E remitted the haematological parameters may be linked to a reduction in the reactivity of reactive oxygen species and hence protection of the tissue against damage (Mitchell, 1977; Mansuy et al 1986; Thomas, 1993; Frei, 1994).

The co-administration of caffeinated paracetamol with vitamin E however resulted in further reduction in PCV and RBC and increase in WBC count. Although the mechanism is not clear; the result is agreement with our recent observation that vitamin E was not effective in remitting the hepatotoxicity of caffeinated paracetamol (Mesembe et al 2004a).

The results obtained in this study are in

concert with out recent reports where we demonstrated that supplementation with vitamin E is effective in remitting the histopathological changes in the liver and kidney, as well as changes in the haemostatic parameters consequent upon paracetamol intoxication in adult Wistar rats (Mesembe et al 2004a; Mesembe et al 2004b).

Although, co-administration of vitamin E and caffeinated paracetamol appeared to produce adverse effects as evidence by decrease Hb concentration, PCV,RBC count and increased WBC count. Inclusion, we propose that haematological assessment has revealed that supplementation with vitamin E is effective in remitting the toxicity of paracetamol.

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