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The Effect of Multivitamin-Haematinic Complex on Chloramphenicol-Induced Anaemia in Rabbits

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Abstract: A study on the haemotoxic effect of orally administered chloramphenicol palmitate (CAP) and the possible countering effect of multivitamin-haematinic complex (MVH) on chloramphenicol-induced anaemia was conducted using rabbits as the animal model. Twenty male rabbits were used in this study. They were randomly divided into four groups of five rabbits each according to the drug administered. Rabbits in group A were administered with 0.9% physiological saline; group B rabbits were administered with chloramphenicol only while rabbits in group C were given combination of chloramphenicol and MVH. Rabbits in group D were administered with MVH only. Chloramphenicol palmitate was administered at dosage of 50 mg kg⁻¹, 6 h interval per day for a period of three weeks. Chemiron® was the source of multivitamin- haematinics used in this study and 5 mL of the syrup was administered thrice daily for the same period of time. All the administration of drugs was done orally. Blood samples were collected from the rabbits in all the groups on the 7th, 14th and 21st of drug administration. Peripheral blood parameters such as the Red Blood Cell count (RBC), Packed Cell Volume (PCV), haemoglobin concentration (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and White Blood Cell count (WBC) were evaluated. The haemotoxic effect of CAP was evident from the 7th day to the 21st day of drug administration with the lowered haematological values of rabbits in group B when compared with those of the rabbits in control group A. Statistical comparison shows that the differences of the means of group A and B were significant for RBC ($p < 0.01$), PCV ($p < 0.05$) and Hb ($p < 0.05$) on day 7; for RBC ($p < 0.001$), PCV ($p < 0.001$) and Hb ($p < 0.01$) on day 14; for RBC ($p < 0.001$), PCV ($p < 0.001$) and Hb ($p < 0.05$) day 21. The administration of MVH to the rabbits in group D produced higher mean haematological values for the group compared with the mean values of the control group A and these changes were only significant for RBC ($p < 0.05$), PCV ($p < 0.05$) and MCH ($p < 0.05$) on day 21. The rabbits of group C administered with MVH and CAP exhibited significantly lower levels of PCV ($p < 0.05$) on day 7, RBC ($p < 0.01$) PCV ($p < 0.01$) and Hb ($p < 0.05$) on day 14 relative to the values obtained in the control group A. This study further confirmed the anaemic side effect of chloramphenicol and it also established the limitations of haematopoietic micronutrients in reducing or ameliorating this anaemic effect especially during prolonged administration of chloramphenicol.

Key words: Chloramphenicol, anaemia, multivitamin-haematinics, rabbits

INTRODUCTION

Chloramphenicol was first isolated in 1947 from the cultures of *Streptomyces venezuelae*. It was synthetically produced in 1948, thus becoming the first completely synthetic antibiotic of importance to be produced commercially (Bartz, 1948). It is used for the treatment of life-threatening infections like typhoid fever and meningitis (Holt *et al.*, 2001; Turton *et al.*, 1999). It is used extensively in developing countries because it is affordable and effective despite its known haemotoxicity

(Festing *et al.*, 2001; Robert and Adenis, 2001) and linkage to fatal aplastic anaemia (Turton *et al.*, 2002). In most of these countries the general approach to cushioning anticipated anaemic effect of chloramphenicol in man or animal, is by concurrent administration of multivitamins and haematinics.

This practice is aimed at enhancing or speeding up the physiological process of haemopoiesis in the body, which basically requires supplies of iron and copper and a number of vitamins, including folic acid, vitamin B₁₂, pyridoxine, ascorbic acid and riboflavin (Hillman, 1996;

Nynke, 2003). It is expected that increased delivery of newly formed red blood cells into the circulatory system, as products from increased rate of haemopoiesis caused by multivitamin-haematinics will serve to reverse the state of anaemia precipitated by chloramphenicol. In most developing countries the administration of such multivitamin-haematinics is often taken as sufficient where as it is usually advised to undertake monitoring of blood profile during therapy involving drugs known for their haemotoxicity (Kapusnik-Uner *et al.*, 1996; Sharp *et al.*, 1990).

The purpose of this study therefore is to investigate the level of impact that MVH have on chloramphenicol-induced anaemia, using rabbits as laboratory models.

MATERIALS AND METHODS

This study was conducted in University of Ibadan, Ibadan, Nigeria. Twenty apparently healthy adult domestic male rabbits (*Oryctolagus cuniculus*) were used in this research. They were housed in wooden cage system and fed *ad libitum* with commercially prepared rabbit pellets (Guinea Feeds Nigeria Limited). The animals were given free access to water all the time. The rabbits were randomly divided into four groups according to the type of drug administered: In group A (the control group), the rabbits (n = 5) were administered with 0.9% physiological saline. Group B consisted of rabbits (n = 5) administered with chloramphenicol alone while the rabbits in group C (n = 5) were administered with chloramphenicol and MVH (Chemiron®, Chemiron International Limited, Nigeria) and rabbits in group D (n = 5) were administered with MVH only. Each 5 mL of Chemiron® contains ferrous gluconate (140 mg), vitamins B₁ (1.4 mg), B₂ (2 mg) B₆ (5 mg) and B₁₂ (10% v/v).

Drug administration: Chloramphenicol was administered as chloramphenicol palmitate B.P (Afrab-Chem. Ltd., Nigeria) at a recommended dosage of 50 mg kg⁻¹, given at 6 h interval per day for a period of three weeks. Chemiron® was administered 5 mL thrice daily for the same period of time. Both drugs were administered orally.

Blood sample collection and analysis: Blood was collected from the rabbits in all the groups through the retro-orbital venous plexus into lithium-heparinized tubes on the 7th, 14th and 21st days of drug administration. The rabbits were first anaesthetized with ether to make for easier blood collection.

The RBC and WBC were counted with the use of the haemocytometer (Olayemi *et al.*, 2003). The packed PCV was determined using the haematocrit method. Hb concentration was measured by the cyanomethaemoglobin method (Pejrilova *et al.*, 2004). The values of the MCV and MCHC were calculated from the values obtained for RBC, WBC counts and PCV.

Statistical analysis: The data obtained were summarized as means±standard error of means and t-test was used to determine the level of statistical significance of the difference of the means (Steel and Torrie, 1996).

RESULTS

Haematology of the rabbits on day 7: Except for the MCV, the mean haematological values of rabbits in groups B and C were lower than the mean values of the rabbits administered with MVH only (i.e., group D) and the mean values obtained from rabbits in the control group (A) (Table 1).

Comparative statistical analysis showed that the difference of the mean values of RBC count was significant for groups A and B (p<0.01). The mean PCV values of the rabbits also differed significantly between the various groups as follows; A and B (p<0.05); groups B and C (p<0.05) and between groups B and D (p<0.001). The mean Hb concentration values of the rabbits also differed significantly between that of groups A and B (p<0.05) and group B and D (p<0.05) (Table 1).

Haematology of the rabbits on day 14: By the 14th day, the mean values obtained were further lower for groups B and C while the same parameters were observed to have increased in groups A and D (Fig. 1-3), except for the MCV values (Table 2). Comparison study among the four

Table 1: The mean value of haematological parameters obtained from rabbits groups administered with chloramphenicol, chloramphenicol plus MVH and physiological saline on day 7

Parameters	Control	Experiment		
	Group A (Saline) (n = 5)	Group B (CAP) (n = 5)	Group C (CAP + MVC) (n = 5)	Group D (MVC) (n = 5)
RBC (×10 ⁶ mL ⁻¹)	5.79±0.53 ^{a***}	4.80±0.21 ^{a**}	5.45±0.75	6.05±1.31
WBC (×10 ³ mL ⁻¹)	6.42±0.54	6.41±0.43	5.98±0.66	5.99±1.14
PCV (%)	36.20±4.88 ^{a*}	25.40±0.80 ^{a*b*,c***}	35.00±3.65 ^{b*}	39.32±2.21 ^{c***}
Hb (g dL ⁻¹)	10.96±0.81 ^{a*}	9.60±0.86 ^{a*b*}	10.98±1.30	11.01±0.88 ^{a*}
MCV (fC)	62.52±2.11	63.39±8.11	64.22±8.40	60.10±4.24
MCHC (%)	31.78±1.77	30.20±1.08	31.37±3.81	33.73±1.55
MCH (pg)	19.93±1.02	19.60±1.11	19.62±1.24	20.20±1.32

Difference of means in horizontal rows with the same superscripts are statistically significant at: *: p<0.05; **: p<0.01 and ***: p<0.001

Table 2: The mean value of haematological parameters obtained from rabbits groups administered with chloramphenicol, chloramphenicol plus MVH and physiological saline on day 14

Parameters	Control	Experiment		
	Group A (Saline) (n = 5)	Group B (CAP) (n = 5)	Group C (CAP + MVC) (n = 5)	Group D (MVC) (n = 5)
RBC ($\times 10^6 \text{ mL}^{-1}$)	6.10 $\pm$ 0.50 ^{a***, c**}	3.43 $\pm$ 0.33 ^{a***, b***}	4.15 $\pm$ 0.74 ^{c***, d**}	6.25 $\pm$ 0.82 ^{b***, d**}
WBC ($\times 10^3 \text{ mL}^{-1}$)	6.46 $\pm$ 0.67	6.01 $\pm$ 0.78	5.92 $\pm$ 1.54	6.63 $\pm$ 1.15
PCV (%)	39.60 $\pm$ 3.88 ^{a***, b**}	25.00 $\pm$ 0.89 ^{a***, c*, d***}	30.40 $\pm$ 4.41 ^{b***, c***}	39.82 $\pm$ 1.15 ^{d***, e***}
Hb (g dL ⁻¹)	11.88 $\pm$ 0.46 ^{a***, b*}	7.83 $\pm$ 0.58 ^{a***, c*, d***}	9.88 $\pm$ 1.03 ^{b***, c*}	12.23 $\pm$ 1.01 ^{d**}
MCV (fL)	61.92 $\pm$ 7.60	60.81 $\pm$ 5.59	64.55 $\pm$ 6.97	60.15 $\pm$ 4.34
MCHC (%)	32.08 $\pm$ 1.86	28.72 $\pm$ 1.17 ^{a*, b*}	32.67 $\pm$ 1.39 ^{a*}	33.78 $\pm$ 2.51 ^{b*}
MCH (pg)	20.48 $\pm$ 1.55	19.12 $\pm$ 0.87	21.22 $\pm$ 0.56	22.52 $\pm$ 1.02

Difference of means in horizontal rows with the same superscripts are statistically significant at: *: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.001$

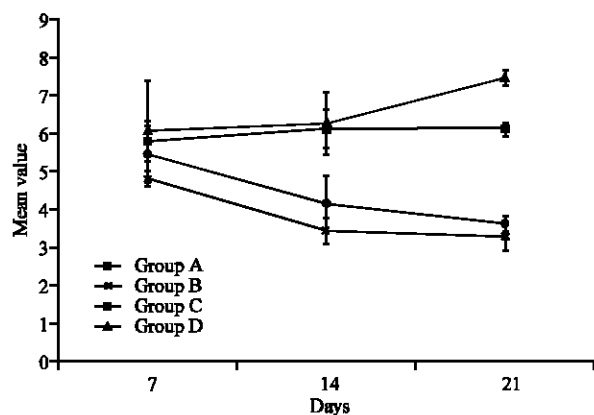


Fig. 1: Graphical comparison of the mean values of RBC count of rabbits administered with 0.9% Physiological saline (group A), chloramphenicol alone (group B), chloramphenicol + MVH (group C) or MVH alone (group D) from day 7 to day 21

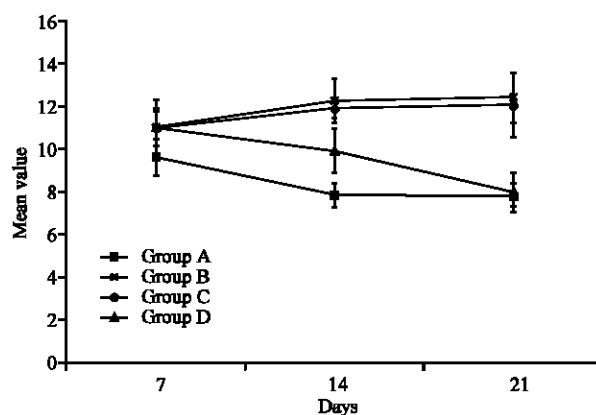


Fig. 3: Graphical comparison of the mean values of Hb concentration of rabbits administered with 0.9% Physiological saline (group A), chloramphenicol alone (group B), chloramphenicol + MVH (group C) or MVH alone (group D) from day 7 to day 21

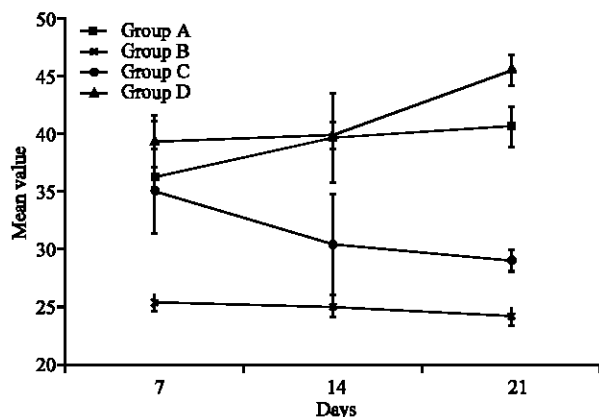


Fig. 2: Graphical comparison of the mean values of PCV of rabbits administered with 0.9% physiological saline (group A), chloramphenicol alone (group B), chloramphenicol + MVH (group C) or MVH alone (group D) from day 7 to day 21

groups showed that the difference of the means of RBC counts was statistically significant between the values of

the rabbits of groups A and B ($p < 0.001$), groups A and C ($p < 0.01$), groups B and D ($p < 0.001$) and groups C and D ($p < 0.01$) (Table 2).

The WBC count was also lower in groups B and C compared to the values obtained in group A and D but the difference was not statistically significant ($p > 0.05$) between any of the groups. The mean values of Hb concentration were recorded in this decreasing order as follows: Group D (12.23 ± 1.01) > group A (11.88 ± 0.46) > group C (9.88 ± 1.03) > group B (7.83 ± 0.58). The difference of the means of Hb concentration values was statistically significant between that of rabbits of group A and B ($p < 0.01$), group A and C ($p < 0.05$), group B and C ($p < 0.05$) and group B and D ($p < 0.01$) (Table 2).

The difference of the means values of MCV and MCH was not significant between any of the groups. However, the difference of the mean values of MCHC was statistically significant between that of group B and C ($p < 0.05$), group B and D ($p < 0.05$). The difference of the mean values of MCH was not significant between any of the groups (Table 2).

Table 3: The mean value of haematological parameters obtained from rabbits groups administered with chloramphenicol, chloramphenicol plus MVH and physiological saline on day 21

Parameters	Control	Experiment		
	Group A (Saline) (n = 5)	Group B (CAP) (n = 5)	Group C (CAP + MVC) (n = 5)	Group D (MVC) (n = 5)
RBC ($\times 10^6$ mL ⁻¹)	6.12 $\pm$ 0.15 ^{a***, b*}	3.28 $\pm$ 0.36 ^{a***, c***}	3.62 $\pm$ 0.19 ^{d***}	7.45 $\pm$ 0.18 ^{b*, d***, c***}
WBC ($\times 10^3$ mL ⁻¹)	6.47 $\pm$ 0.49	5.54 $\pm$ 0.98	5.53 $\pm$ 0.54 ^{a*}	7.52 $\pm$ 0.34 ^{a*}
PCV (%)	40.60 $\pm$ 1.72 ^{a***b*}	24.20 $\pm$ 0.75 ^{a***, c***}	29.00 $\pm$ 0.89 ^{d***}	45.42 $\pm$ 1.34 ^{b*, c***, d***}
Hb (g dL ⁻¹)	12.02 $\pm$ 1.51 ^{a*}	7.81 $\pm$ 0.53 ^{a*, b*, c***}	7.96 $\pm$ 0.92 ^{b*, d*}	12.38 $\pm$ 1.13 ^{c***, d*}
MCV (fL)	60.82 $\pm$ 2.39	61.61 $\pm$ 3.12	62.68 $\pm$ 2.59	60.05 $\pm$ 1.33
MCHC (%)	33.18 $\pm$ 4.29	28.22 $\pm$ 2.67 ^{a*}	30.02 $\pm$ 1.66 ^{b*}	34.56 $\pm$ 1.76 ^{a*, b*}
MCH (pg)	21.02 $\pm$ 1.15 ^{a*}	19.09 $\pm$ 1.12 ^{b***}	20.29 $\pm$ 1.33 ^{c*}	25.22 $\pm$ 1.10 ^{a*, b*, c***}

Difference of means in horizontal rows with the same superscripts are statistically significant at: *: p<0.05; **: p<0.01 and ***: p<0.001

Haematology of the rabbits on day 21: The haematological parameters obtained on 21st day of chloramphenicol administration shows that the mean values obtained for groups B and C were progressively lower than that of the rabbits in group A and D except for the MCV values. The mean values of RBC count were obtained in increasing order as follows; group B (3.28 $\pm$ 0.36) > group C (3.62 $\pm$ 0.19) > group A (6.12 $\pm$ 0.15) > group D (7.45 $\pm$ 0.18). The value obtained for the rabbits in group D was significantly higher than that of group A (p<0.05), group C (p<0.001) and group B (p<0.001). The mean RBC count of group B was also significantly (p<0.001) lower than that of the control group A. However, the difference of the mean values of group B and C was not significant (p>0.05) (Table 3).

The mean white blood counts were depressed in groups B and C compared with that of groups A and D but the difference of the means was only significant (p<0.05) for the rabbits of group C and D. While the PCV values of groups B and C were still depressed by the 21st day, the PCV values of group A and D increased (Fig. 2). The PCV value of rabbits in group B was significantly lower than that of group A (p<0.001) and D (p<0.001). While the mean PCV value of rabbits in group C was statistically significant lower than that of group D (p<0.001), the PCV value of group D was significantly (p<0.05) higher than that of group A, however the difference of the means of groups B and C was not significant (p>0.05) (Table 3). The mean haemoglobin concentration for group B was significantly lower than that of group A (p<0.05) and group D (p<0.01). In the same way the mean Hb concentration for group C was significantly lower than that of group D (p<0.05) however the difference of means was not significant between that of groups B and C (p>0.05) or groups A and D (p>0.05). There was only a marginal difference between the mean values of MCV of any two groups. The mean MCHC value of rabbits in both groups B and C were significantly lower than that of group D (p<0.05) while MCH of group B and C were significantly lower than that of group D (p<0.01, p<0.05) respectively and the mean value of MCH of group B was lower (p>0.05) than of

group A (p<0.05). There was also a significant (p<0.05) difference between the mean values of groups A and D (Table 3).

DISCUSSION

The results obtained from this research corroborate earlier studies (Holt *et al.*, 1998) that orally administered chloramphenicol lowers haematological parameters in both man and in animal models. In this research, the assessment of the peripheral blood parameters show significant depression of the RBC count, PCV, Hb concentration in rabbits administered with chloramphenicol. Though chloramphenicol is one of the cheapest and effective antibiotics for broad spectrum of bacterial infections; several workers have reported the haemotoxic side effect of the drug, which therefore limits its use or attract total ban in some situations (Kapusnik-Uner *et al.*, 1996). The significant reduction of the erythrocyte count and indices in this study was earliest observed at day 7 and was consistently observed at 14th and 21st days of chloramphenicol administration. The shortest onset of haemotoxic effect of chloramphenicol was reported by Holt *et al.* (1997) who demonstrated evidence of bone marrow depression 15 min after administration of chloramphenicol in mouse.

This result of this study also corroborate earlier reports that administration of MVH actually increases the circulating red blood cells and haemoglobin levels (Milman *et al.*, 2000; Das *et al.*, 2003). In this research, higher values of leukocyte, erythrocyte count and other indices were obtained from rabbits administered with MVH alone when compared with that of the rabbits in the control group. The difference of the means was significant for RBC count, PCV and MCH values on the 21st day of its administration.

However, the mean haematological values of rabbits administered MVH along with chloramphenicol was consistently lower than that of the control values. Though some of the values of rabbits in group B were significantly higher than that of group C for days 7 and 14. Figure 1-3 however show a steady decline of haematological values for both groups B and C throughout the course of the experiment. This is an

indication that administration of MVH did not mitigate against chloramphenicol-induced anaemia in this study.

Haemotoxic effect of chloramphenicol has been reported to be caused by bone marrow depression (Turton *et al.*, 2002; Holt *et al.*, 1997) and this depression may be masked by enhanced peripheral blood proliferation at the early stage (Holt *et al.*, 1998). The bone marrow depression is mostly reversible or sometimes irreversible but it is caused by inhibition of differentiation of immature committed erythroid progenitors (Holt *et al.*, 1998) and erythroid tissue cellular depletion (Turton *et al.*, 2002). This implies that, notwithstanding the erythropoietic potency of MVH, the responsiveness of erythroid tissue to the micronutrients or erythropoietin diminishes over time due to insufficient population of functional committed erythroid progenitors (Kapusnik-Uner *et al.*, 1996). More so, the mechanisms of haematopoietic action of micronutrients are only quite relevant in anaemia due to nutritional deficiency and when this is the case, the restoration to the normal haematocrit level is usually not immediate. Even in this study, the administration of MVH to rabbits in group D caused a significant increase in their haematological values compared to the control group values only at 21st day of its administration.

The conclusion from this study is that chloramphenicol will cause anaemia even though there is simultaneous administration of haematopoietic agents. Administration of multivitamin-haematinics for mitigation of possible occurrence of anaemia during chloramphenicol therapy as commonly practiced in some developing countries is a mere futility. It is therefore suggested that strict monitoring of the haematocrit level in the body be done when chloramphenicol or any other drugs known to cause bone marrow depression must be administered.

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