An *in vivo* assessment of the use of Haematopan B12® in the treatment of experimentally induced anaemia in domestic rabbits.

***Adedapo, A. A., Saba, A. B. and Dina, O. A.**

Department of Veterinary Physiology and Pharmacology, University of Ibadan, Ibadan, Nigeria.

** Corresponding author*

Abstract

A total of 15 domestic rabbits divided into 3 groups of 5 animals per group were used in this study. Group A animals (control) were not bled but those in groups B and C were bled to induce anaemia. Group B were left to recover by normal haemopoiesis while those in group C were treated with Haematopan B12®, a commercially prepared haematinic. The post-bleeding treatment haematologic values were obtained at given intervals (7 days) and compared with the pre-bleeding values earlier obtained. The result of this study showed that the group B animals recovered gradually by physiologic haemopoiesis while animals in group C showed accelerated recovery since these animals had a better and faster response in terms of white blood cell (WBC), red blood cell (RBC) count and haemoglobin (Hb) concentration. Thus Haematopan B12® is an effective haematinic.

Keywords: *Haematopan B12®, anaemia, rabbits.*

Introduction

Anaemia is defined as a reduction below normal of the concentration of haemoglobin in the blood (Smith, 1974; Macfarlane *et al*, 2000). It can also be defined as the reduction in the number of erythrocytes, haemoglobin or both in the circulating blood. It results from excessive red blood cell (RBC) destruction, RBC loss, or decreased RBC production (Coles, 1986; Jain, 1986; Straus, 1998).

Anaemia is of utmost importance in Nigeria because many of the causative agents abound in the environment due partly to the suitability of the climatic factors for their proliferation and partly to poor level of human and animal management practices (Taiwo and Anosa, 1995). For this reason, haematinics are employed in combating this menace. Haematinics are

pharmaceutical or chemical compounds functioning to enhance blood formation, and thus, maintain the normal haemoglobin level in the living systems. Elements such as iron, cobalt, zinc, copper, vitamins etc. usually form components of haematinics (Brander *et al*, 1982).

Therapeutic use of iron is indicated only in treatment and, in certain situations, prevention of iron deficiency. It should be recognized that indiscriminate administration of iron preparation is accompanied by the danger of iron toxicity or iron storage disease in healthy as well as unhealthy animals (Wilhelm, 1998). Vitamin B₁₂ is a cobalt-containing vitamin required by cells throughout the body for conversion of ribose nucleotides into deoxyribose nucleotides, a major step in formation of deoxyribonucleic acid

(DNA) (Adams, 1995). Thus, it is an essential nutrient for nuclear maturation and cell division, and deficiency of this vitamin results in general depression of cellular development and tissue growth (Adams, 1995). Since the erythropoietic centres of bone marrow are among the most rapidly growing and proliferating tissues, inadequate amounts of vitamin B₁₂ are especially manifested by decrease in red cell production (Adams, 1995). Erythrocytic precursors fail to mature properly under conditions of vitamin B₁₂ deficiency, and cell proliferation is inhibited (Adams, 1995). From the fore-going, knowledge about haematinic drugs should be integrated into a rational approach to management of anaemia dependent upon identification and treatment of aetiological factors.

This study was carried out to evaluate the haematinic potency of this drug because:

- a) This type of exercise should be the first stage in the process of introducing new drugs to the market and should therefore be a mandatory and legal exercise.
- b) This particular drug was manufactured in France, a different climatic environment, , , , thus, it is necessary to evaluate its suitability to a tropical environment like Nigeria.

Materials and Methods

Experimental Animals

A total of 15 domestic rabbits (*Oryctolagus cuniculi*) of both sexes, ranging in age from weaners to adults were used for this study. The animals were housed in the Experimental Animal house of the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. The animals were acclimatized for two weeks and health status was properly monitored to ensure that there were no haemoprotozoan infections such as coccidiosis before being used for this study. The animals were fed twice in a day with

commercially prepared rabbit's grower mash made by Guinea Feeds Nigeria Ltd. Good drinking water was provided *ad libitum* for the animals.

Haematopan B12^R

Haematopan B12[®] used in this study was manufactured by Rhone Merieux (France). Each 100 mls of this preparation contains:

Sodium cacodylate (crystals)	2.0g
Ammonium ferric citrate	2.0g
Methionine	1.0g
Histidine hydrochloride	0.5g
Tryptophan	0.2g
Cobaltoms acetate (crystals)	0.5g
Cyanocobalamin (Vitamin B12)	0.001g
Excipient q.s.	100 ml.

Pre-bleeding Sampling

About 1ml of blood was collected from each rabbit by venipuncture using the ear vein for the determination of pre-bleeding parameters.

Induction of Anaemia

It is an established fact that a rabbit has between 57.7-70 ml of blood per kilogram body weight (Kaplan and Timmons, 1979). Also, a loss of about one-third of the total blood volume of an animal is known to precipitate anaemia (Taiwo and Anosa, 1995). Therefore, based on their body weight, one-third of the total blood volume of the rabbit was collected through bleeding to induce clinical anaemia. The bleeding was carried out through veni-puncture via the ear vein. The punctured site was rubbed with some vaseline to prevent clotting and enhance continuous bleeding. The blood was then collected into a graduated bottle until the desired volume was obtained. To ensure that clinical anaemia has been induced, the rabbits were bled again 24 hours post-bleeding and the 2 mls of blood collected from each rabbit was used for

laboratory analysis for confirmation of clinical anaemia.

Treatment of Anaemia

The commercially prepared haematinic (Haematopan B12®) was administered intramuscularly at a dose rate of 1mg/kg body weight of the animal. Treatment lasted for 4 days.

Post-Treatment Blood Sample Analysis

The first sets of blood samples were collected for laboratory analysis 3 days following the completion of treatment. Thereafter, post treatment blood samples were collected at 7 days interval for follow-up laboratory analysis. Packed cell volume (PCV) estimation was done by conventional method of filling the capillary tubes with blood. One end of the tube was sealed

and the tubes centrifuged in a microhaematocrit centrifuge for 25 minutes. The PCV in percent was then read directly from a graphic reader. This is as described by Schalm *et al.*, (1975). The haemoglobin concentration was determined as described by Jain (1986) using the cyanomethaemoglobin method. After haemolysis of the red cells, the haemoglobin is converted to cyanomethaemoglobin by the cyanide in the diluting solution (Drabkin diluent). The red blood cell (RBC) and white blood cell (WBC) counts were determined by the use of Nebaur haemocytometer (Schalm *et al.*, 1975; Jain, 1986).

Statistical Analysis

Results were expressed as the mean parameters $\pm$ standard deviation of the mean. Differences

Table I: – Normal haematological values of experimental rabbits.

Measurement	T R E A T M E N T S		
	A	B	C
PCV	21.6 $\pm$ 4.10	21.4 $\pm$ 3.85	33.6 $\pm$ 2.07
WBC	2480 $\pm$ 1391.31	2603 $\pm$ 1365.94	5740 $\pm$ 1336.69
RBC	3.76 $\pm$ 2.17	3.176 $\pm$ 0.98	4102 $\pm$ 0.58
HB	9.78 $\pm$ 2.42	11.7 $\pm$ 3.42	9.34 $\pm$ 4.53

Table II: - Haematologic values of experimental rabbits on the 3rd day.

Measurement	T R E A T M E N T S		
	A	B	C
PCV	19.8 $\pm$ 3.90	15.6 $\pm$ 4.56	28.4 $\pm$ 2.16
WBC	2458 $\pm$ 1403.70	2011.6 $\pm$ 1316.84	5050 $\pm$ 2046.93
RBC	3.41 $\pm$ 2.14	3.234 $\pm$ 1.96	4.43 $\pm$ 1.11
HB	8.88 $\pm$ 1.78	6.1 $\pm$ 2.02	7.84 $\pm$ 2.78

Table III: -Haematological values of experimental rabbits on the 10th day.

Measurements	T R E A T M E N T S		
	A	B	C
PCV	21±2.83	20.6±6.15	28±2.74
WBC	2726±1232.02	2540±1038.03	5200±2590.37
RBC	4.328±1.24	4.01±1.62	4.38±0.71
HB	9.22±1.82	7.28±2.12	7.7±0.91

Table IV: -Haematological values of experimental rabbits on the 17th Day.

Measurements	T R E A T M E N T S		
	A	B	C
PCV	22.6±3.65	22.4±4.34	29.8±3.56
WBC	3224±1199.95	4870±3740.42	4400±2167.08
RBC	4.95±1.27	4.096±0.59	4.98±0.52
HB	10.004±1.15	6.54±0.96	7.76±1.30

between mean values were evaluated using the student's T- test. Differences were significant at $P<0.05$ (Bradford and Hill, 1991).

Results

The haematological parameters used to estimate the haematinic potency of this haematinic such as packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and Haemoglobin (Hb) concentration were affected in one way or the other in the course of this study in the different animal groups.

The PCV values increased in all the experimental animals until the 31st day after treatment. The RBC also followed similar pattern in that the RBC count that was decreased in level after initial bleeding gradually experienced an increase until the 31st day. Haemoglobin concentration also experienced an increase in the similar fashion (Tables 1-6). WBC count on the other hand

decreased with days but by the 31st day, it had increased beyond the pre-bleeding level. All the changes noted above were statistically significant at $P<0.05$.

When groups B and C animals were compared, it was noted that the animals in group C had higher values in terms of PCV, RBC and Hb concentration. All the changes noted were statistically significant at $P<0.05$. Since group A animals were not bled, it only served as control (Tables 1-6).

Discussion

The proprietary haematinic i.e. Haematopan B¹² was observed to cause some changes in the blood parameters. This was shown by the increase in the levels of the packed cell volume (PCV), red blood cell (RBC) count and haemoglobin (Hb) concentration which was initially decreased after

Table V: -Haematological values of experimental rabbits on the 24th Day.

Measurement	T R E A T M E N T S		
	A	B	C
PCV	22.8±3.42	22.4±4.56	26.4±1.82
WBC	3142±1433.64	4601.2±1761.18	4600±809.32
RBC	4.702±1.31	4.132±0.62	4.388±0.36
HB	9.32±1.22	6.76±0.43	7.28±0.94

Table VI: -Haematological values of experimental rabbits on the 31st Day.

Measurement	T R E A T M E N T S		
	A	B	C
PCV	22±2.74	22±3.74	28.8±1.79
WBC	2540±1145.86	4956±2388.74	7050±1126.94
RBC	3.88±0.94	4.04±0.55	50226±0.60
HB	9.42±1.81	6.54±0.94	7.82±1.10

bleeding, but gradually increased with days post treatment. It must be stressed however that by the 31st day of treatment, parameters like the PCV and haemoglobin concentration have not risen to the pre-bleeding values (Tables 1 and 6) in group C animals. The reverse was the case for RBC and WBC counts.

The difference in the pre-bleeding and post-bleeding PCV levels of group C rabbits was statistically significant ($P<0.05$). When the PCV values of group C animals were compared with those of group B animals, the difference was statistically significant ($P<0.05$). The implication of this is that the proprietary haematinic may have influence on the total percentage of blood volume. It should be recalled that most species of domestic animals have PCV values of 38% to

45 % with a mean of 40. Haemoconcentration due to dehydration, asphyxia, or excitement causing release of erythrocytes concentrated in the spleen can result in abnormally high PCV values (Swenson and Reece, 1993).

For white blood cell (WBC) count the difference in pre- and post-bleeding levels was statistically significant ($P<0.05$) for animals in group C. It was the same experience for group B animals that were not treated at all. It thus implied that the haematinic does have effect on the white blood cell count especially because when groups B and C animals were compared, the difference was statistically significant ($P<0.05$) with group C animals having higher value. There was a significant difference ($P<0.05$) when groups A and C animals were compared. This increase

was because the animals in group C were bled and the white blood cells, principally neutrophils and macrophages (derived from monocytes) found at sites of inflammation constitute the prime defensive features of the inflammatory response (Robins, 1974; Macfarlane, 2000). It thus implied that the increase in white blood cell (WBC) count is in response to inflammatory reaction initiated as a result of the bleeding and also may have something to do with the haematinic.

In the case of the red blood cell (RBC) count, the difference in the pre and post-bleeding levels was not statistically significant ($P>0.05$) for group C animals. As a matter of fact, as at 31st day, the red blood cell count was yet to reach the pre-bleeding level. When this parameter for groups C and B animals were compared however, the difference was statistically significant. It thus implied that Haematopan B12* has effect on the level of red blood cell count. This effect is particularly worth noting because the major function of the red blood cell (RBC) is to transfer oxygen from the lungs to the tissues. Also, the red blood cells are responsible for appropriate 70 % of all the buffering power of the whole blood (Forman and Thomas, 1986; Huebers and Finch, 1987; Young, 1987; Jelkman, 1992; Nikinma, 1992; Schaff and Foder, 1993; Staub, 1994).

For haemoglobin concentration, there was a difference in the level of this parameter between the pre- and post-bleeding blood sample in group C. As a matter of fact, the difference is statistically significant ($P<0.05$). Also when groups B and C animals were compared in terms of haemoglobin concentration, the difference was statistically significant ($P<0.05$) implying that Haematopan B12* may have some effect on haemoglobin concentration. Haemoglobin is a complex molecule formed of 4 haeme units

attached to 4 globins. Iron is added in the last step by ferrochelatase enzyme. Interference with the normal production of haeme or globin leads to anemia (Straus, 1998). It is shown that Haematopan B12* contains ammonium ferric citrate and this is necessary in haemoglobin formation.

Conclusion

It can be safely concluded that Haematopan B12* possesses a haematinic property because it improved the quality of the blood of the experimental animals in terms of erythrocytes counts and haemoglobin concentration.

References

- Adams, H. R. 1995. Antianaemic drugs. In: *Veterinary Pharmacology and Therapeutics*. Ed. Adams, H. R. Iowa State Univ. Press, Ames. 7th ed. Pp 531-543.
- Bradford, H. A. and Hill, I. D. 1991. *Principles of Medical Statistics*. 12th Ed. Edward Arnold, London.
- Brander, G. C.; Pugh, D. M. and Bywater, R. J. 1982. *Veterinary Applied Pharmacology and Therapeutics*, Balliere Tindall, London, pp. 141-144.
- Coles, E. H. 1986. *Veterinary Clinical Pathology*. 4th Ed., W.B. Saunders Co., Philadelphia, pp. 10-12.
- Forman, H. J. and Thomas, M. J. 1986. Oxidant production and bactericidal activity in phagocytes. *Annu. Rev. Physiol.* 48, 669.

-
- Huebers, H. A. and Finch, C. A. 1987. The physiology of transferring and transferring receptors. *Physiol. Rev.* 67, 520.
- Jain, N. C. 1986. *Veterinary Clinical Pathology*. 4th Ed., Lea and Fabiger, Philadelphia
- Jelkman, W. 1992. Erythropoietin: structure, control of production, and function. *Physiol. Rev.* 72, 449..
- Kaplan, H. M. and Timmons, E. H. 1979. *The rabbit, a model for the principles of mammalian physiology and surgery*. Academy Press, London, pp. 6-7.
- Macfarlane, P. S., Reid, R. and Callander, R. 2000. *Pathology Illustrated*. 5th ed. Churchill Livingstone, London. Pp 387-391.
- Nikinma, M. 1992. Membrane transport and control of haemoglobin-oxygen affinity in nucleated erythrocytes. *Physiol. Rev.* 72, 301.
- Robins, S. L. 1974. *Pathologic basic of Disease*. 1st Ed. W.B. Saunders Co., Philadelphia, pp. 704.
- Scharff, O. and Foder, B. 1993. Regulation of cytosolic calcium in blood cells. *Physiol. Rev.* 73, 547..
- Schalm, O. W.; Jain, N. C. and Carroll, E. J. 1975. *Veterinary Haematology*. 3rd Ed. Lea and Fabiger, Philadelphia.
- Staub, N. C. 1994. Pulmonary intravascular macrophages. *Annu. Rev. Physiol.* 56, 47.
- Smith, H. A.; Jones, T. C. and Hunt, R. D. 1974. *Veterinary Pathology*. 4th Ed., Lea and Fabiger, Philadelphia.
- Straus, J. H. 1998. Anaemia. In: *The Merck Veterinary Manual*. Aiello, S. E. ed. 8th ed. Pp 8-10.
- Swenson, M. J. and Reece, O. W. 1993. *Duke's Physiology of Domestic Animals*. 11th Ed., Comstock Publishing Associates, Ithaca, U.S.A, pp. 30.
- Taiwo, V. O. and Anosa, V. O. 1995. Fibrinogen, leukocyte and haematocrit value in cattle with various disease conditions. *Trop. Vet.* 13 (1 & 2), pp. 51-58.
- Wilhelm, R. 1998. Iron toxicity in newborn pig. In: *The Merck Veterinary Manual*. Aiello, S. E. ed. 8th ed. Pp 2071.
- Young, J. D. 1987. Killing of target cells by lymphocytes: a mechanistic view. *Physiol. Rev.* 69, 250.

(Received 06 November 2003, Accepted 24 November 2004)