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Full Length Research Paper

Haematinic potencies of the aqueous crude extracts of *Ficus mucoso* and *Senna occidentalis* in rabbits.

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ABSTRACT: A total of 20 domestic rabbits divided into 4 groups of 5 animals per group were used in this study to assess the haematinic potencies of the aqueous crude extracts of *Ficus mucoso* and *Senna occidentalis* and this was then compared with that of a proprietary haematinic, Haematopan B12®. Group A animals (control) were not bled but those in groups B, C and D were bled to induce anaemia. Group B animals were treated with Haematopan B12®, a commercially prepared haematinic, and groups C and D were treated with the aqueous crude extracts of *Senna occidentalis* (Linn) Link and *Ficus mucoso* Welw. ex Ficalho respectively. The post-haemorrhage treatment haematologic values were obtained at given intervals (7 days) and compared with the pre-haemorrhagic values earlier obtained. The study showed that all the experimental animals showed accelerated recovery since these animals had excellent response in terms of white blood cell (WBC) and its differentials, red blood cell (RBC) count, packed cell volume (PCV) and haemoglobin (Hb) concentration. Thus the extracts of *Ficus mucoso* and *Senna occidentalis* have comparative haematinic potencies as Haematopan B12®.

Keywords: Haematopan B12®, anaemia, rabbits, Senna occidentalis, Ficus mucoso

INTRODUCTION

The body of knowledge about plants, herbs and spices, and their respective and collective roles in promoting health is modest (Ballatine *et al.,* 1999; Craig, 1999). Herbs have been used as food and medicinal purposes for centuries. The use of medicinal herbs has however increased over the past few years and research interest has focused on various herbs that possess hypolipidemic, antiplatelet, antitumour or immune stimulating properties that may be useful adjuncts in helping reduced the risk of cardiovascular disease and cancer (Burkill, 1997).

Nwude and Ibrahim (1980) have compiled information on plants used in traditional veterinary medicine in Nigeria. More of this type of work should be done to identify plants used in various localities to treat animal diseases. These plants should be investigated for efficacy and toxicity. Those found effective with minimal toxicity should be processed for use in veterinary practice (Nwude, 1997). For instance, *Cassia occidentalis* was investigated and confirmed to have purgative, diuretic effects in dogs and galactogogue effects in goats. The methanol extract of *Morinda lucida* and aqueous extract of *Alstonia boonie* bark each have been reported to have activity against *Trypanosoma brucei* in mice (Asuzu and Chineme, 1990; Asuzu and Anaga, 1991). It has also been reported that *Azadirachta indica* leaf extract had trypanocidal potentials against *Trypanosoma brucei* (Nok et al., 1993).

Ficus mucoso Welw. ex Ficalho is a member of the family Moraceae. It is a large tree of about 40m high. It is a rain forest tree often found on river banks. The bole is cylindrical with short buttresses while the bark is usually smooth, green and exudes copious latex when slashed (Keay *et al.,* 1964; Burkill, 1997). Parts of this plant have been used for the treatment of various diseases. A bark and leaf decoction is drunk for diarrhoea or alternatively in the dried bark reduced to a meal; this preparation is also taken for dysmenorrhoea (Burkill, 1997). A melite of bark gratings is taken for bronchial infections (Bouquet,

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1969). The bark is smooth and on slashing, it exudes a copious white, sticky latex which quickly darkens to ochre, then brown (Burkill, 1995). The latex is given to children with convulsions, and is instilled into the ear for otitis (Bouquet, 1969). The bark is known to have analgesic properties hence used as embrocation. The bark and root macerates are separately drunk to fortify the blood in illness (Burkill, 1997).

Senna occidentalis (Linn.) Link synonymous as *Cassia occidentalis* Linn. is a member of the family Leguminosae. It is a glabrous tender shrub, annual or short-lived perennial commonly called the coffee senna or Nigerian senna. It is a weed of waste places found in many locations in Nigeria (Burkill, 1997). It is commonly planted around villages because of its importance in traditional medicine. As a legume, *Senna occidentalis* is known to improve soil fertility. While most of the parts especially the leaves are used in the treatment of various diseases, the effects are said to be analgesic, diuretic, laxative and diaphoretic. The root is considered to have restorative and tonic properties. However, the fresh seed is toxic containing tannins, a toxalbumin, an alkaloid, chrysophanic acid, chrysarobine, emodine and anthroquinonic derivatives (Oliver-Bever, 1986; Burkill, 1995; Burkill, 1997).

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 component of haematinics (Adams, 1995). The haematinic potency of Haematopan B12^{R} has been explored in Nigerian rabbits and its efficacy has thus been reported (Adedapo *et al.*, 2005).

The study is aimed at exploring the haematinic potencies of these plant extracts and compared their efficacies with that of Haematopan B12^{R}, a proprietary haematinic.

MATERIALS AND METHODS

Experimental Animals

A total of 20 domestic rabbits (*Oryctolagus cuniculi*) ranging in weight from 0.65 to 1.02kg were used for this study. The animals were of both sexes i.e. 12 males and 8 females. The animals were housed in the Experimental Animal house of the Faculty of Veterinary Medicine, University of Ibadan. 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.

Preparation of the plant extracts

The roots of *Ficus mucoso* and the bark of *Senna occidentalis* were collected freshly from the roadsides and pathways and washed with clean water to remove dirt. The plants were identified and authenticated at the herbarium of the Department of Botany and Microbiology of the University of Ibadan, Nigeria and the voucher specimens were deposited there and their numbers were UIH-22257 and UIH-22258 respectively.

The roots of *Ficus mucoso* and the bark of *Senna occidentalis* were each weighed (75g), and blended into liquefaction in 150ml of distilled water. These mixtures were then centrifuged at 1500 rpm. The supernatants were filtered through sterile filter papers into conical flasks as the study extracts. Thus the concentration of the extract is 500 mg/ml.

Haematopan B12^{R}.

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

Sodium cacodylatte (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 of the ear vein for the determination of pre-bleeding parameters.

Induction of Anaemia

It is said 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

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blood volume of an animal is known to precipitate anaemia (Smith *et al.,* 1974; 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 haemorrhagic anaemia has been induced, the rabbits were bled again 24 hours later and the 2mls of blood collected from each rabbit was used for laboratory analysis for confirmation of anaemia.

Animal grouping and treatment

The rabbits were divided into 4 groups: A, B, C and D. Each group consisted of 3 male and 2 female rabbits. Group A consisted of 5 animals that were healthy and did not receive any medication. Group B consisted of 5 animals that were bled and treated with Haematopan B12^{R}. Group C consisted of 5 rabbits, which were bled and administered with the aqueous crude extract of *S. occidentalis*, while the 5 rabbits in Group D were bled and administered with the aqueous crude extract of *F. mucoso*. The extracts and the commercially prepared haematinic (Haematopan B12®) were administered intramuscularly at a dose rate of 0.5-2.0mls per rabbit depending on the weight of these animals. 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 for about 4 weeks. 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 10 minutes. The PCV in percent was then read directly from a graphic reader (Schalm *et al.,* 1975). The haemoglobin concentration was determined using the cyanomethaemoglobin method (Jain, 1986). 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 Nebaeur haemocytometer (Schalm *et al.,* 1975; Jain, 1986). The white blood cell differentials were also determined as described by Jain (1986).

Statistical Analysis

Results were expressed as the mean parameters ± standard deviation of the mean. Differences between mean values were evaluated using the student's t test, analysis of variance (ANOVA) and Duncan multiple range 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), Haemoglobin (Hb) concentration; white blood cell (WBC) and white blood cell differentials were affected in one way or the other in the course of this study in the groups B, C and D animals.

The PCV values increased in all the experimental animals until the 24th day after treatment. Haemoglobin concentration also experienced an increase in the similar fashion. 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 24th day. It should be noted however that the RBC value for group C animals by the 17th day post-treatment experience a decrease (Figures I-V). For WBC count the result is similar to that of RBC because animals in group C also recorded a decrease in the value of WBC on the 17th day post-treatment, although by the 24th day post-treatment, there was an increase in the value for group C animals beyond the pre-bleeding level. The results for the WBC differentials also follow similar pattern (Figures IV-VIII). When these results were compared with the values obtained when the animals were bled prior to treatment and even 3 days post-treatment, the difference was statistically significant ($P<0.05$).

When groups B, C and D animals were compared, it was noted that though there were variations in the levels of their PCV, RBC and Hb concentration but by the 24th day, the difference in their levels were statistically insignificant. The same goes for the WBC and its differential. Since group A animals were not bled, it only served as control.

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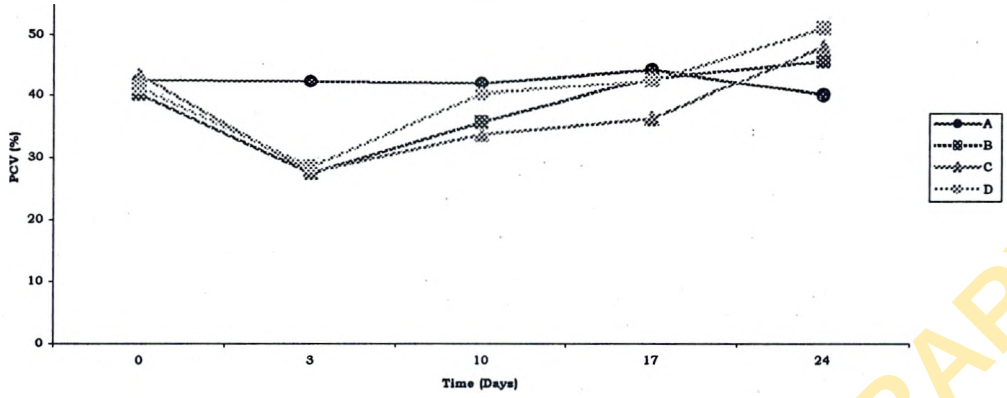

Figure 1
Changes in packed cell volume (PCV)

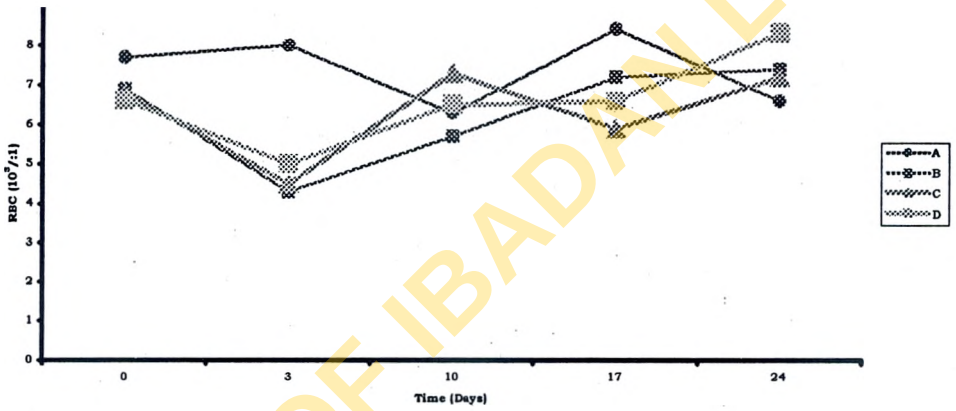

Figure 2
Changes in red blood cell count (RBC)

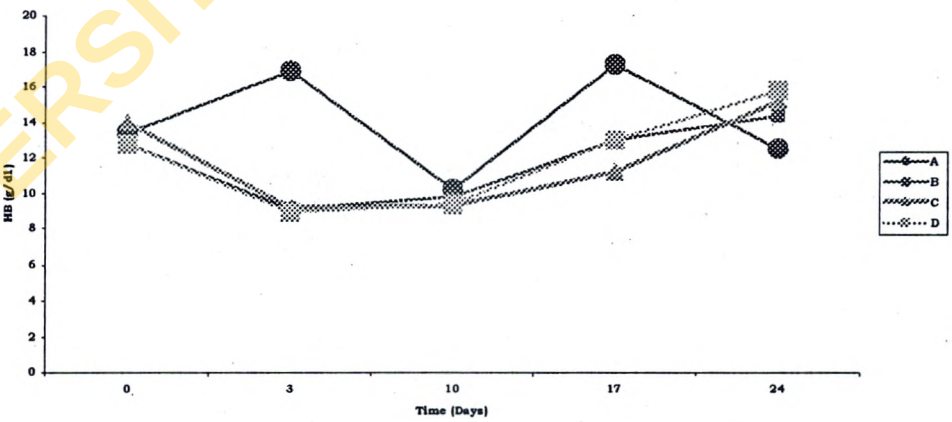

Figure 3
Changes in haemoglobin concentration (HB).
Note: A= Control; B= Haematopan; C= *Ficus mucoso*; D= *Senna occidentalis*

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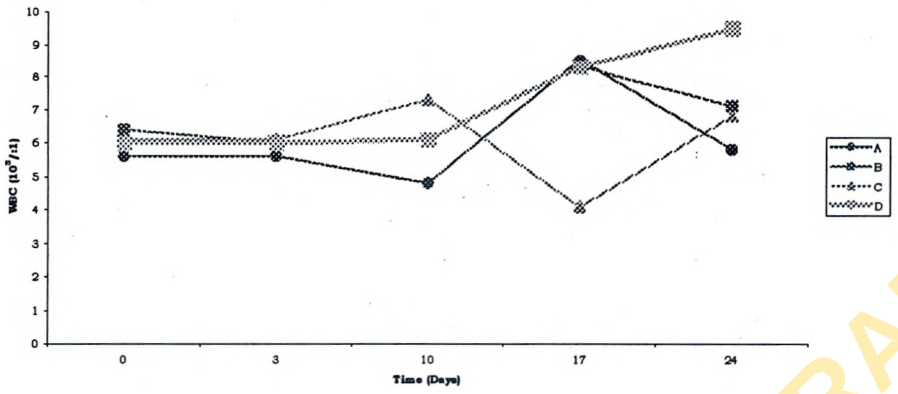

Figure 4-
Changes in white blood cell count (WBC).

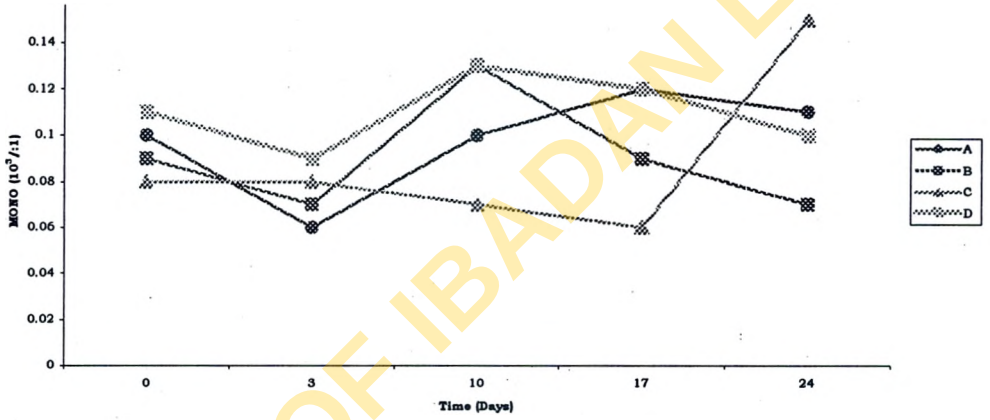

Figure 5-
Changes in monocytes level (MONO).

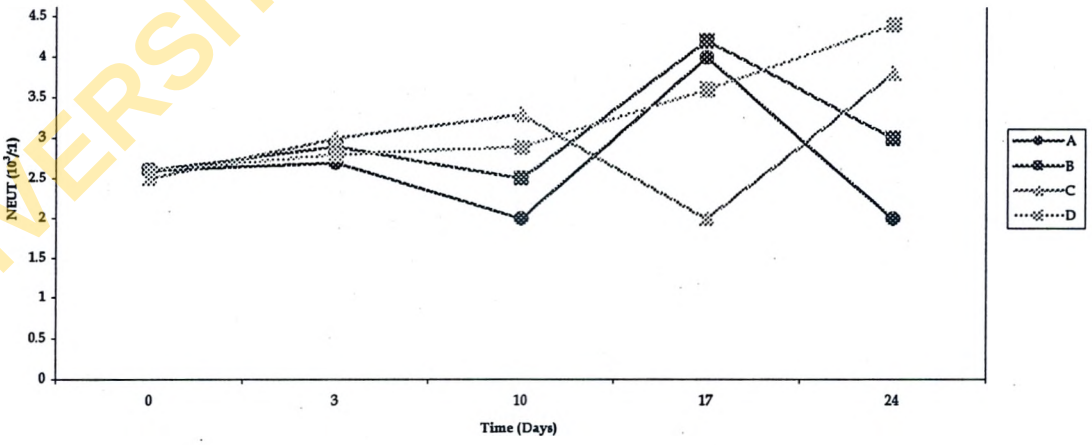

Figure 6
Changes in neutrophil level (NEUT).
Note: A= Control; B= Haematopan; C= *Ficus mucoso*; D= *Senna occidentalis*

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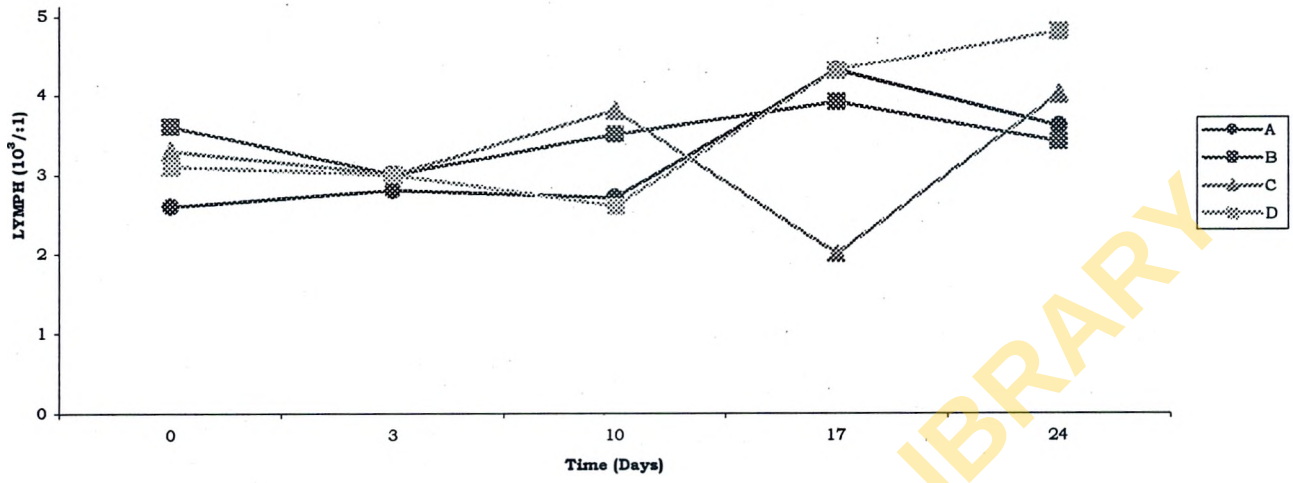

Figure 7-
Changes in lymphocyte level (LYMPH)

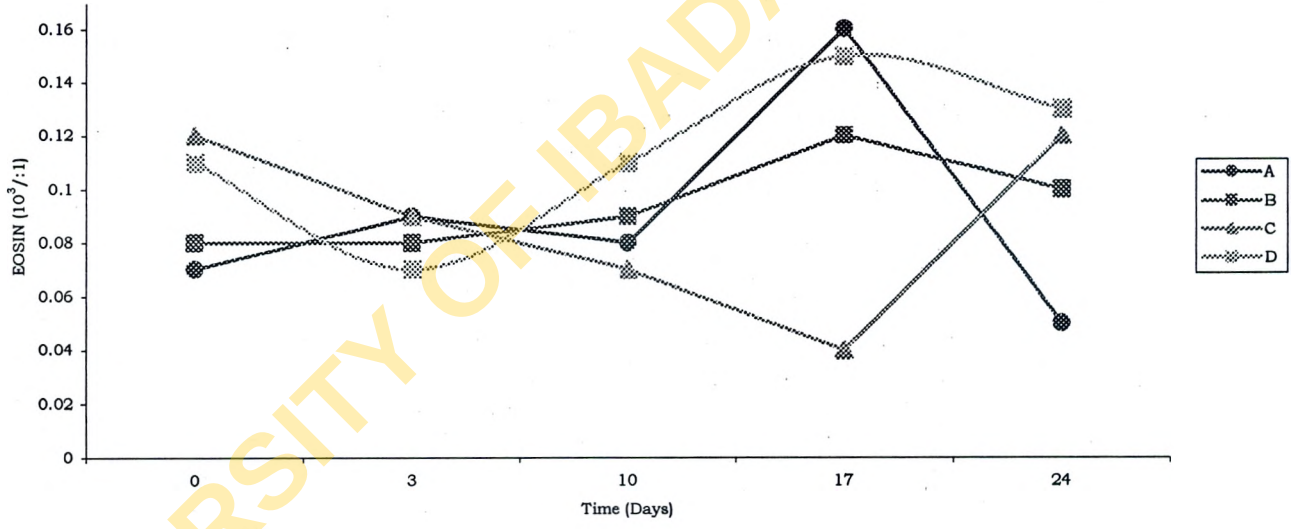

Figure 8-
Changes in eosinophils level (EOSIN).
Note: A= Control; B= Haematopan; C= *Ficus mucoso*; D= *Senna occidentalis*

DISCUSSION

The proprietary haematinic i.e. Haematopan B^{12} as well as the extracts of *Ficus mucoso* and *Senna occidentalis* were 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 bleeding, but gradually increased with days post treatment. It must be stressed that by the 24^{th} day of treatment, these parameters i.e. PCV, RBC and haemoglobin concentration have risen beyond the pre-bleeding values (Figures I - III) in all the experimental animals. The result was the same for WBC counts and its differential (Figures III - VIII).

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The difference in the pre-bleeding and post-bleeding PCV levels of all the experimental rabbits by the 24th day after treatment was statistically significant ($P<0.05$). When the PCV values of all the experimental rabbits were compared with those of group A animals, the difference too is statistically significant ($P<0.05$). The implication of this is that the proprietary haematinic and the extracts of *Ficus mucoso* and *Senna occidentalis* may have some 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 (Coles, 1986; Swenson and Reece, 1993; Guyton and Hall, 2000).

In the case of the red blood cell (RBC) count, the difference in the pre- and post-bleeding levels was statistically significant ($P<0.05$) for all the experimental animals. As a matter of fact, by the 24th day, the red blood cell count was higher than the pre-bleeding level. It thus implied that Haematopan B12® and the extracts have 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; Scharff and Foder, 1993; Staub, 1994; Guyton and Hall, 2000).

For haemoglobin concentration, there was a difference in the level of this parameter between the pre- and post-bleeding blood samples of all the experimental animals and this difference is statistically significant ($P<0.05$). Also when groups A and B, C, D animals were compared in terms of haemoglobin concentration, the difference was statistically significant ($P<0.05$) implying that Haematopan B12® and the extracts 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; Tripathi, 2003). It should be noted however that acute iron poisoning can occur in infants, children and newborn piglets (Wilhelm, 1998; Tripathi, 2003). It is shown that Haematopan B12® contains ammonium ferric citrate, cobaltoms acetate and vitamin B 12 that are necessary in haemoglobin formation (Rang *et al.*, 2003; Tripathi, 2003).

For white blood cell (WBC) count and its differentials, the difference in pre- and post-bleeding levels was statistically significant ($P<0.05$) for animals in groups B, C and D. It thus implied that the haematinic and the extracts do have some effects on the white blood cell count and its differentials especially because when groups A, B, C and D animals were compared, the difference was statistically significant ($P<0.05$) with group D animals having higher value. This increase was because the animals in groups B, C and D 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 *et al.*, 2001). It thus implied that the increase in white blood cell (WBC) count may be in response to inflammatory reaction initiated as a result of the bleeding and also may have something to do with the haematinic properties of these plants.

It will be noticed that in this study, there was no anaemic control because some studies have shown that even 31 days after bleeding, the haematological parameters of rabbits did not reach pre-bleeding values (Dina *et al.*, 1999; Dina *et al.*, 2000; Adedapo *et al.*, 2002; Adedapo *et al.*, 2005). It therefore showed that the extracts and the proprietary haematinic caused a restoration of all these parameters faster than it would have been in their absence. It can be safely concluded that Haematopan B12® and the extracts of *Ficus mucoso* and *Senna occidentalis* possess a haematinic property because it improved the quality of the blood of the experimental animals in terms of erythrocytes counts and haemoglobin concentration. This is the reason the haematinics are administered to anaemic patient (Brander *et al.*, 1991; Adams, 1995; Tripathi, 2003). The study also showed that the proprietary haematinic did not show superior haematinic potency relative to these preparations. As a matter of fact; the extracts of these plants recorded higher values on the 24th day post-treatment than the proprietary haematinic. Further study to determine the chemical constituents responsible for these observations will be carried out soon.

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