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Full Length Research Paper

Response of *Trypanosoma brucei brucei*–induced anaemia to a commercial herbal preparation

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Jubi Formula® is a herbal preparation made from three medicinal herbs (*Parquetina nigrescens*, *Sorghum bicolor* and *Harungana madagascariensis)*. It has been reported to have been successfully used in the treatment of anaemia in humans. A study was therefore carried out to determine the effect of the preparation on packed cell volume (PCV) and haemoglobin (Hb) concentrations in anaemic rabbits. The PCV and Hb concentrations of healthy rabbits infected with *Trypanosoma brucei brucei* were monitored for 49 days. *T. b. brucei* produced a significant reduction in PCV and Hb concentrations in all infected rabbits when compared with the controls ($p<0.05$). These hematological parameters were restored to normal levels in the anaemic rabbits by the herbal preparation. The anaemic rabbits not treated with the herbal preparation presented with a progressive decline in their PCV and Hb concentrations and majority of them died before the end of the study. Healthy rabbits that received daily doses of the herbal preparation showed gradual elevation in PCV and Hb concentrations which were maintained within normal range. Jubi Formula® can restore the PCV and Hb concentrations in anaemic conditions and is a potential substitute for blood transfusion. However, further studies are needed to investigate the potentials of the herbal preparation in reversing anaemia.

Key words: Anaemia, PCV, rabbits, haemoglobin, herbal preparation.

INTRODUCTION

Over the years, medicinal plants have been recognised to be of great importance to the health of individuals and communities. In many developing countries, herbal medicines are assuming greater importance in primary health care and their international trade has increased. However, the markets in these countries are not adequately regulated and many herbal products in circulation are unregistered by national regulatory bodies (WHO, 1996).

Jubi Formula®, manufactured by Health Forever Products Ltd, Lagos, Nigeria, is a commercial herbal preparation that recently made its way into the herbal medicine market in Nigeria. It is available as a powdered preparation formulated into capsules and suspension and contains iron, protein, fat, carbohydrates, tannins, saponins, and coumarins. The product was made from three medicinal herbs (*Parquetina nigrescens*, *Sorghum bicolor* and *Harungana madagascariensis)*. *Parquetina nigrescens* (family: Periplocaceae) is a shrub found in equatorial West Africa (Irvine, 1961; Mabberley, 1987) while *Sorghum bicolor* or Sorghum (family: Gramineae) is an important staple food crop in Africa, South Asia, and Central America, and is also grown in some developed nations for animal feed (ICRISAT, 1993). *Harungana madascariensis* (family: Clusiaceae) is a native of Madagascar, Mauritius and tropical Africa (Hutchinson, and Dalziel, 1954).

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The product is believed to elevate packed cell volume (PCV) and is recommended by the manufacturer for the support of treatment of moderate to severe anaemia as in sickle cell anaemia, cancer and HIV/AIDS. It is also said to be suitable as a nutritional supplement in stress, exhaustion and convalescent situations as well as helpful in diabetes, hypertension, arthritis and infertility. The sap of *H. madagascariensis* has been used in the treatment of skin diseases, leprosy spots and wounds while the leaves of *P. nigrescens* has been used for wounds in Africa (Irvine, 1961; Mabberley, 1987), and has sympathomimetic effects (Datté et al., 1999). However, no scientific data has been published to show the combined effects of the components of Jubi Formula on PCV and Hb concentrations in mammals.

The main objective of this study was to investigate the claim by the manufacturer that the product increases packed cell volume (PCV) in anaemia. The specific objective is to determine the effect of the herbal preparation on PCV and haemoglobin concentrations in rabbits with parasitemia-induced anaemia.

MATERIALS AND METHODS

Thirty-six locally bred rabbits – average weight, 1.18±1.73 kg; weight range, 0.94 – 1.46 kg purchased in Benin City were kept in standard rabbit cages (which were regularly cleaned and disinfected using standard procedures). The cages were located in the animal house in the Department of Pharmacology & Toxicology, University of Benin, Benin City, Nigeria. The rabbits were allowed unrestricted access to normal rabbit chow (Bendel Feed and Flower Mills Ltd., Ewu, Edo State, Nigeria) and water, and allowed to acclimatize to their new environment for four weeks. During the acclimatization period, the animals were treated with terramycin (200 mg/kg) (May and Baker Ltd., England) in the first week (to exclude bacteria infections) and blood samples were collected on microscope slides from each animal through the auricular vein every week, and examined under a microscope for presence of parasites (World Organisation for Animal Health, 2000) and bacteria. Six animals with blood infections were excluded from the experiment.

The thirty healthy rabbits were divided into five equal groups (groups A – E). Rabbits in groups A and B were used as controls; weekly blood monitoring indicated the absence of parasites and bacteria in their blood samples. The animals in groups C, D and E were infected with *Trypanosoma brucei brucei Federe Plateau* (*T. b. brucei*) and the presence of the parasite in blood was monitored daily through the auricular vein until parasitemia was established, usually within 5 – 10 days post-infection. Each animal in groups B, C and D received 3.4 ml/kg of aqueous suspension of Jubi Formula® powder (1 mg/60ml) orally every day from day 1, the day they were infected with *T. b. brucei* and the day parasitemia was established, respectively. Parasitemia in the rabbits in groups C, D and E were induced by mouse inoculation technique (World Organisation for Animal Health, 2000) using a single dose of 0.25 ml of *T. b. brucei*-infected blood of albino rat. The blood samples were diluted (50:50) with normal saline before injecting intraperitoneally into the rabbits. The infected albino rats were obtained from the National Institute for Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria.

Uncoagulated blood samples of the rabbits were obtained every week from the animals. The samples were collected in 5 ml EDTA tubes and, the PCV and haemoglobin concentrations were determined within 24 h of collection using the microhaematocrit and cyanomethaemoglobin methods (Omotainse and Anosa, 1992; Schalm et al., 1975), respectively. The proportional changes in PCV and Hb concentrations of the rabbits were determined as follows:

$$\% \text{ change in PCV} = \frac{P_t - P_o}{P_o} * 100 \quad \ldots\ldots (1)$$

$$\% \text{ change in Hb} = \frac{H_t - H_o}{H_o} * 100 \quad \ldots\ldots (2)$$

where P_t and P_0 are the PCV at time, t and day 1, respectively and H_t and H_o are the Hb concentrations at time, t and day 1, respectively. The differences in these hematological parameters were analysed statistically using one-way analysis of variance (ANOVA) with Instat® software (GraphPad Inc., USA). Statistical estimates were made at confidence interval of 95% and probability values of ≤0.05 were considered significant.

The ethical approval for this study was obtained from the Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria.

RESULTS

The changes in packed cell volume (PCV) and haemoglobin (Hb) concentrations of the rabbits during the period of the study are presented in Tables 1 and 2, respectively. The control rabbits had PCV and Hb concentrations within the normal range throughout the duration of the experiment (PCV: mean, 37.0±2.8%; median, 37%; 95% confidence limit, 36.1 - 39.9%; Hb: mean, 11.45±1.18 g/dl; median, 11.73 g/dl; 95% confidence limit, 11.115 – 13.575 g/dl). The rabbits in group B had slightly higher (but not significantly different, $p > 0.05$) PCV and Hb concentrations from the third week of treatment with the herbal preparation than those in group A. Groups A and B animals had PCV and Hb concentrations within the normal range. As much as 17.2±2.0% elevation in PCV and 15.0±13.0% elevation in Hb concentrations were achieved in the group B rabbits treated with Jubi Formula® within six weeks. The PCV and Hb concentrations were maintained within normal range by the Jubi Formula®. The PCV and Hb concentrations of animals in groups C, D and E were significantly lower than those of the animals in groups A and B ($p<0.05$). During the course of the experiment (49 days), the PCV of the parasite-infected rabbits not treated with Jubi Formula® (Group E) progressively fell to as low as 25% and five of the rabbits died before the end of the experiment; one dying on the 15th day and the others dying between the 30th and 48th days. The parasite infected rabbits treated with Jubi Formula® from day 1 gradually developed lower PCV and Hb concentrations, with PCV values of 27% recorded in one rabbit and 28% recorded in three other rabbits. However, the PCV and Hb concentrations of these animals increased progressively from the fifth week of treatment to normal

Table 1. Packed cell volume (l/l) of healthy (groups A and B) and *T. b. brucei* infected rabbits (groups C – E) either treated (groups B – D) or untreated (groups A and E) with Jubi Formula®.

Day	Packed cell volume (l/l) of rabbits (mean±sd)				
	Group A[a]	Group B[b]	Group C[c]	Group D[d]	Group E[e]
-14	0.360±0.05	0.363±0.03	0.370±0.04	0.383±0.03	0.375±0.03
-7	0.365±0.04	0.365±0.04	0.358±0.02	0.368±0.02	0.368±0.02
1	0.375±0.03	0.348±0.02	0.350±0.02	0.370±0.01	0.378±0.03
7	0.365±0.02	0.378±0.02	0.360±0.02	0.358±0.03	0.350±0.02
14	0.363±0.02	0.365±0.01	0.338±0.01	0.345±0.03	0.318±0.02
21	0.368±0.03	0.375±0.02	0.308±0.02	0.313±0.01	0.295±0.02
28	0.375±0.03	0.390±0.01	0.300±0.03	0.295±0.02	0.268±0.02
35	0.385±0.02	0.403±0.04	0.305±0.02	0.288±0.03	0.260±0.01
42	0.365±0.02	0.408±0.05	0.325±0.01	0.315±0.03	0.255±0.01
49	0.383±0.04	0.403±0.02	0.323±0.03	0.323±0.02	0.253±0.01

[a,c]p<0.05; [a,d]p<0.05; [a,e]p<0.001; [b,c]p<0.05; [b,d]p<0.01; [b,e]p<0.001Group A, healthy rabbits, controls, no Jubi Formula® administered; Group B, healthy rabbits, Jubi Formula® administered from day 1 – 49; Group C, rabbits infected with *T. b. brucei* and Jubi Formula® administered from days 1 - 49; Group D, rabbits infected with *T. b. brucei* and Jubi Formula® administered from day 10 - 49; Group E, rabbits infected with *T. b. brucei*, no Jubi Formula® administered.

Table 2. Haemoglobin concentrations (g/dl) of healthy (groups A and B) and *T. b. brucei* infected rabbits (groups C – E) either treated (groups B – D) or untreated (groups A and E) with Jubi Formula®.

Day	Packed cell volume (l/l) of rabbits (mean±sd)				
	Group A[a]	Group B[b]	Group C[c]	Group D[d]	Group E[e]
-14	11.50±0.91	11.38±1.11	11.50±1.08	11.7±1.19	12.13±1.18
-7	12.08±0.98	11.75±0.87	11.37±1.14	11.88±0.63	12.50±0.82
1	12.50±0.71	11.80±0.36	12.00±0.41	12.25±0.65	12.95±0.84
7	12.05±0.90	12.55±0.42	11.63±0.85	11.63±1.49	11.70±0.81
14	12.03±0.92	12.08±0.51	11.25±0.65	11.75±0.87	10.63±0.85
21	12.13±1.11	12.43±0.85	10.25±0.65	10.75±0.29	10.25±1.44
28	11.88±0.25	12.78±0.26	10.2±0.81	10.00±0.71	9.13±0.48
35	12.28±1.44	13.13±1.49	10.38±0.63	9.88±0.75	9.00±0.71
42	11.78±0.63	13.58±1.65	11.13±0.75	11.00±0.41	8.63±0.48
49	12.61±1.14	13.23±0.89	10.25±0.50	10.88±0.85	8.38±0.25

[a,c]p<0.05; [a,d]p<0.05; [a,e]p<0.01; [b,c]p<0.01; [b,d]p<0.05; [b,e]p<0.001Group A, healthy rabbits, controls, no Jubi Formula® administered; Group B, healthy rabbits, Jubi Formula® administered from day 1 – 49; Group C, rabbits infected with *T. b. brucei* and Jubi Formula® administered from days 1 - 49;Group D, rabbits infected with *T. b. brucei* and Jubi Formula® administered from day 10 - 49; Group E, rabbits infected with *T. b. brucei*, no Jubi Formula® administered.

levels. Similarly, the parasite-infected rabbits that were treated with Jubi Formula® from the 10th day progressively developed lower PCV and Hb concentrations, with a PCV value of 26% recorded in one of the rabbits. There were significantly lower PCV and Hb concentrations in the animals in Group D than those in Group C ($p<0.05$). Like the rabbits in Group C, the rabbits in Group D had PCV and Hb concentrations which increased gradually from the fifth week of treatment to normal levels. The PCV and Hb concentrations of

animals in Group E fell significantly below the normal levels. Proportional changes in PCV and Hb concentrations of the rabbits during the course of the experiment (as calculated in equations 1 and 2) are shown in Figures 1 and 2. The maximum reductions in PCV and Hb concentrations in the untreated rabbits infected with *T. b. brucei* in this study were 32.8±5.8% and 35.2±3.0%, respectively.

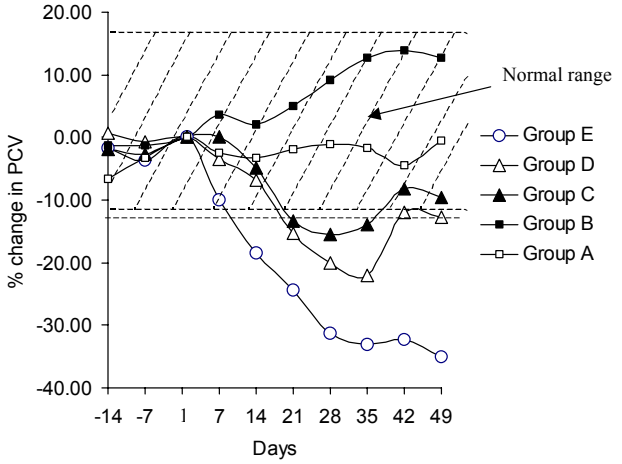

Figure 1. Proportional change in PCV levels of healthy and *T. b. brucei*-infected rabbits either treated or untreated with Jubi Formula®.

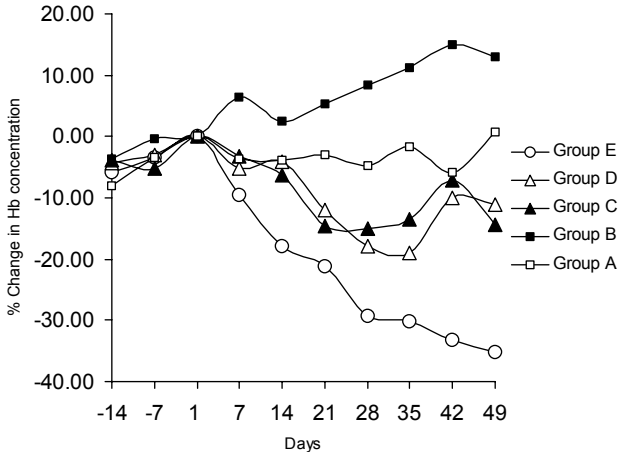

Figure 2. Proportional change in Haemoglobin concentrations of healthy and *T. b. brucei*-infected rabbits either treated or untreated with Jubi Formula®.

Microscopic examination of the rabbit blood revealed macrocytic normochromic anemia within three weeks post-infection with the *T. b. brucei*. Microcytes and hypochromia were evident from the third week of infection in some infected animals. The macrocytic anaemia later changed to microcytic hypochromic anaemia in the infected rabbits. The infected rabbits also showed evidence of polychromia from the third week post-infection but this was not sustained in the rabbits treated with Jubi Formula. Moderate nucleated red cells, which were also present in the control groups (group A and B) throughout the study period, declined in the parasitemia rabbits (groups C – E) from the third week post-infection. There were no noticeable abnormalities in the red blood cells of the rabbits in groups A and B.

DISCUSSION

Trypanosoma brucei brucei is a tissue parasite which induces anaemia in infected rabbits, as in other susceptible animals such as cattle, dogs, rats, mice (Jenkins et al., 1980; McCrorie et al., 1980). As reported previously (Jenkins et al., 1980; Mwangi et al., 1995), *T. b. brucei* successfully produced anaemia in all infected rabbits resulting in the significant reduction in PCV and haemoglobin (Hb) concentrations. The maximum reduction in the PCV and Hb concentrations were, however, not as low as the 50% that has been reported to be possible. Occurrence of parasitemia within 5 to 10 days post-inoculation with *T. b. brucei* is in line with the parasite's known incubation period of 5 to 10 days (Maré, 2000). The PCV and Hb concentrations of healthy rabbits treated with Jubi Formula® did not exceed normal levels and no abnormalities in the red blood cells of the animals were observed suggesting that the herbal preparation can be used in healthy rabbits without hematological disorder. Nucleated red blood cells found in the blood of the treated rabbits are a normal feature of rabbits (Emeribe and Anosa, 1991). These observations suggest that Jubi Formula® could have a therapeutic role in cases of anemia.

Consistent with previous reports (Emeribe and Anosa, 1991) is the development of microcytic hypochromic anaemia observed in the rabbits within five weeks of inoculation of the rabbits with the parasite. Polychromasia observed in some of the rabbits infected with *T. b.* brucei is also consistent with previous report of anisocytosis, poikilocytosis, polychromasia and punctate basophilia which may all occur together, in part or not at all in infected animals (Maré, 2000). Iron deficiency anaemia observed in the infected rabbits has been reported previously (Mwangi et al., 1995) and Jubi Formula® appeared to have eliminated the presence of polychromia in the rabbits with *T. b. brucei*-induced anaemia as polychromia was not observed in those animals treated with the preparation.

T. b. brucei is known to cause fever, reduced food and water intake within 4 to 6 days post-inoculation, anaemia, fibrinogenemia, hypertriglyceridemia, hyperproteinemia, transient alteration in the number of neutrophils and lymphocytes (January et al., 1991; Nakamura, 1998),

kidney damage (Amole et al., 1990), weight loss and severe illness leading to death if untreated (Luckins, 2000). The parasites are also known to invade brain, eyes and skin causing nervous signs, discharge from the eyes and oedematous swellings under the skin. In this study, Jubi Formula® was able to produce useful effects in reducing the mortality of the parasite infected rabbits, and prolonging the life span of the animals. The mechanism by which the herbal preparation produced its effect on the elevation of PCV and Hb concentrations in the experimental rabbits is yet to be determined. The herbal preparation may be producing its effects by strengthening the body immune system, invigorating the defense mechanism, and reducing parasite load in infected animals.

We conclude that Jubi Formula® can increase the PCV and Hb concentrations in anemiac laboratory rabbits. The progressive recovery of *T. b. brucei* infected rabbits treated with Jubi Formula® is suggestive of progressive reduction of parasitemia in the infected animals and restoration of PCV and Hb concentrations. The herbal preparation may be useful in the treatment of anaemia as claimed and may be a potential substitute for blood transfusion. The product needs to be further investigated for any toxicity prior to clinical trials to ascertain the full potentials of the product in anaemia and other disease states in human.

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