

# HAEMATOLOGICAL EVALUATION OF NORMAL RABBITS TREATED WITH AQUEOUS STEM BARK EXTRACTS OF *IRVINGIA GABONENSIS* FOR TWENTY-FOUR WEEKS

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## ABSTRACT

The use of *Irvingia gabonensis* in the treatment of several ailments is documented. Our previous studies have established the long-term anti-obesity, hypoglycaemic and hypolipidaemic effects of aqueous stem bark extracts in normal rabbits. This study was designed to evaluate the long term (24 weeks) effect of daily oral administration of aqueous bark extracts of *I. gabonensis* on haematological parameters, assessed at pre-determined intervals, of normal rabbits as part of the biochemical evaluation of the plant. Plant extract administration caused significant ( $p < 0.05$ ) increases in red blood cell (RBC) count, packed cell volume (PCV) and haemoglobin (Hb) concentration, as well as platelet count. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of *I. gabonensis* treated rabbits were mostly similar to control throughout the period of monitoring. White blood cell (WBC), and differential counts were largely unaffected by medicinal plant administration. This study revealed that long-term treatment of rabbits with *I. gabonensis* bark extracts improved haematopoietic system of rabbits and did not negatively affect their immune system.

## INTRODUCTION

Apart from the use of *Irvingia gabonensis* (African/Wild mango) as food in Nigeria, it is also used as a medicinal plant for the treatment of several ailments.<sup>1,2</sup> Several studies have been undertaken on the

nutritional and medicinal value of *I. gabonensis*.<sup>3,4,5,6,7,8,9</sup> The bark of *I. gabonensis* mixed with palm oil has been used in the treatment of diarrhea,<sup>10</sup> it is also administered for colic and dysentery<sup>6</sup> as well as for hernias, yellow fever and as an antipoison.<sup>11</sup> Antibiotic properties for healing scabby skin have also been reported for the bark of *I. gabonensis*<sup>10</sup> and it has been demonstrated that the bark contains a narcotic-type analgesic agent that validates its folkloric use as a pain killer.<sup>6</sup> *I. gabonensis* seeds also possesses anti-diabetic properties<sup>4,5,8</sup> and the aqueous bark extract has been shown to have sustained (24 weeks) hypoglycaemic

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**KEY WORDS:** *Irvingia gabonensis*; Haematological evaluation; Medicinal plants; Toxicity; Rabbits.

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This study was designed to evaluate the long term (24 weeks) effect of daily oral administration of aqueous bark extracts of *I. gabonensis* on haematological parameters of normal rabbits as part of a toxicological evaluation of the plant.

## **MATERIALS AND METHODS**

### **Plant materials**

The bark of *I. gabonensis* was obtained from open forest at Akungba-Akoko, Ondo State, Nigeria and identified by Dr A. E. Ayodele of the Department of Microbiology and Botany, University of Ibadan, Ibadan, Nigeria. Herbarium specimen (voucher number UIH 22286) was deposited at the University of Ibadan Herbarium. The aqueous plant extract was prepared by the method described by Onoagbe et al<sup>7</sup>.

### **Experimental animals**

Twelve (12) rabbits of the New Zealand strain, weighing between 800-1200g, purchased from the Animal Unit of Federal University of Technology, Akure, Ondo State, were used for this research. The rabbits were examined by a veterinary doctor and allowed to acclimatize for three weeks before the commencement of experiments. The animals were placed on commercial feed (Ewu growers from the Bendel Feed and Flour Mill Ewu, Nigeria) and allowed to drink water freely. Treatment of the animals was in accordance with the Principles of Laboratory Animal Care (NIH Publication 85-93, revised 1985).

The rabbits were divided into two groups:

**GROUP I:** Control (Normal rabbits)

**GROUP II:** Normal rabbits treated with *Irvingia gabonensis* aqueous bark extracts

The plant extract was orally administered to the rabbits at 200 mg/kg body weight daily for 24 weeks. This dose was chosen from a pilot study of varying doses and it reflects the lowest dose that presents significant therapeutic effect.

### **Blood collection**

Blood was collected from the ventral vein of the rabbits' ear at pre-determined intervals (weeks 1, 2, 3, 4, 6, 8, 10, 12, 15, 18, 21 and 24) for 24 weeks. Approximately 1 ml of whole blood was collected in EDTA bottles and assessed for haematological parameters within 24 hours.

### **Determination of haematological parameters:**

Determination of packed cell volume (PCV) was carried out using the Haematocrit method as described by Schalm et al.<sup>12</sup>; Dacie and Lewis<sup>13</sup>. Haemoglobin concentration was determined using the cyanomethaemoglobin method.<sup>14</sup> The total white blood cells (WBC), white blood cell differentials, red blood cell (RBC) and the platelet counts were estimated using the improved Neubauer counting chamber.<sup>14,13</sup> The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated by the formula described by Bauer et al<sup>15</sup>.

### **Statistical analysis**

Results are presented as means of 4 – 6 determination  $\pm$  SEM. The differences between means of control and test group were analyzed by the independent samples t-test. The SPSS 11.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis. A value of  $P < 0.05$  was considered as statistically significant.

## RESULTS

The results obtained in this study are presented in figures 1 to 13

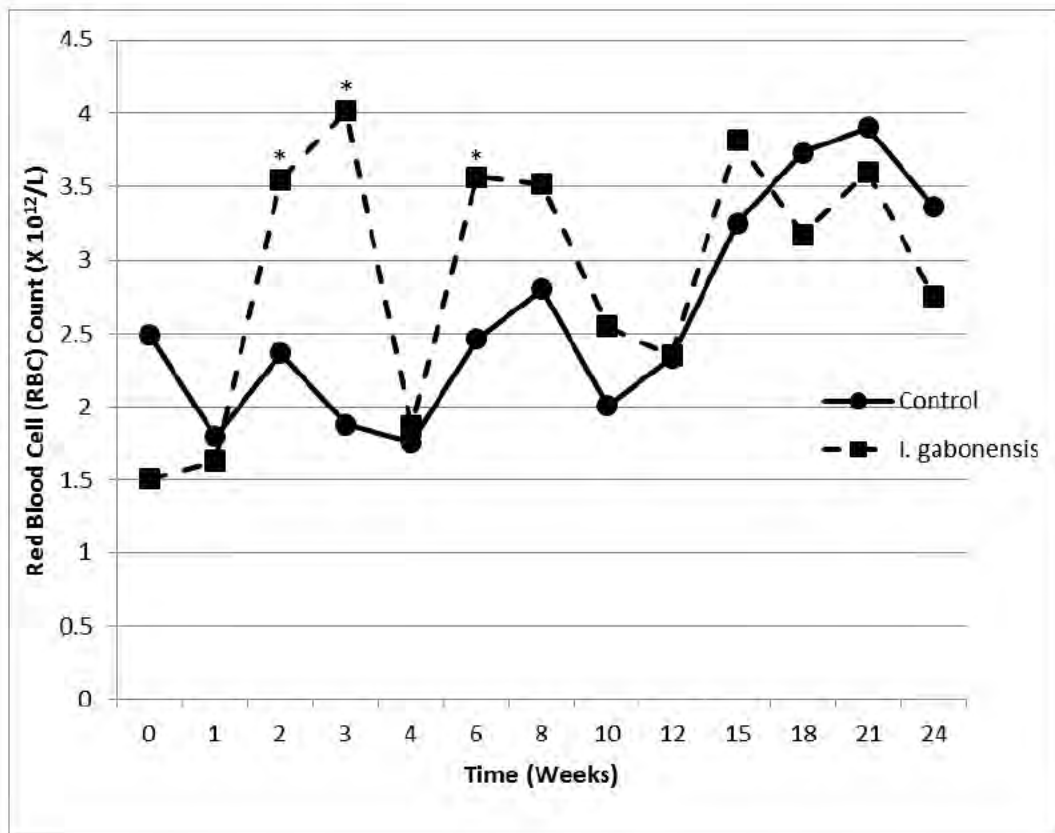


Figure 1: Effects of *I. gabonensis* on red blood cell (RBC) count of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

The values obtained for RBC (Figure 1) in the *I. gabonensis* treated group were either significantly ( $p < 0.05$ ) higher (weeks 2, 3 and 6) or statistically similar to control. Most of the values that were statistically similar to control, were numerically higher.

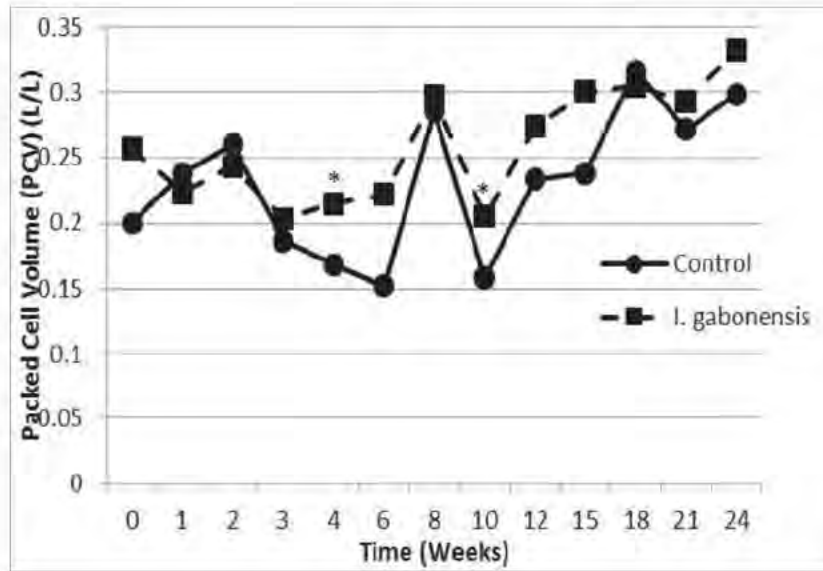


Figure 2: Effects of *I. gabonensis* on packed cell volume (PCV) of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

PCV levels were either statistically ( $p < 0.05$ ) higher, or similar to control (Figure 2).

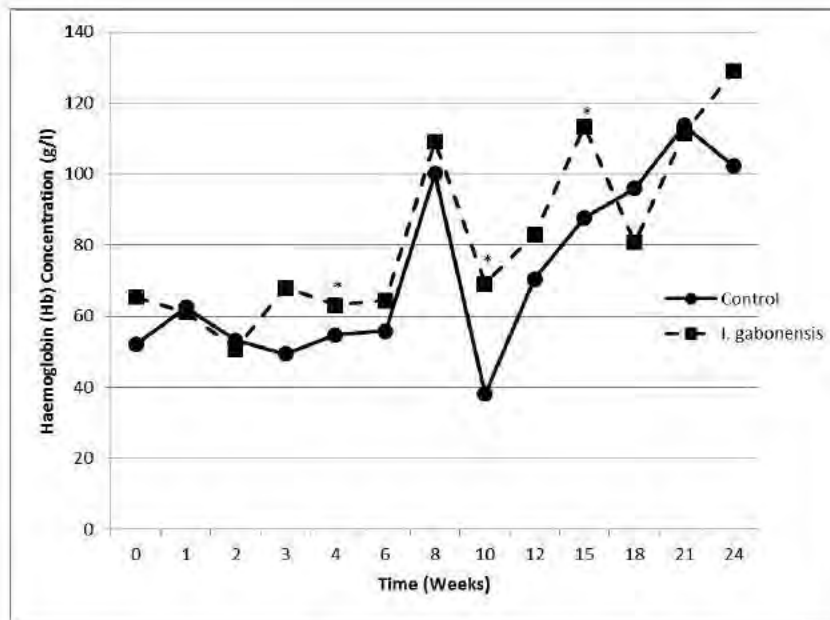


Figure 3: Effects of *I. gabonensis* on haemoglobin (Hb) concentration of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

Hb levels were either statistically ( $p < 0.05$ ) higher, or similar to control (Figure 3).

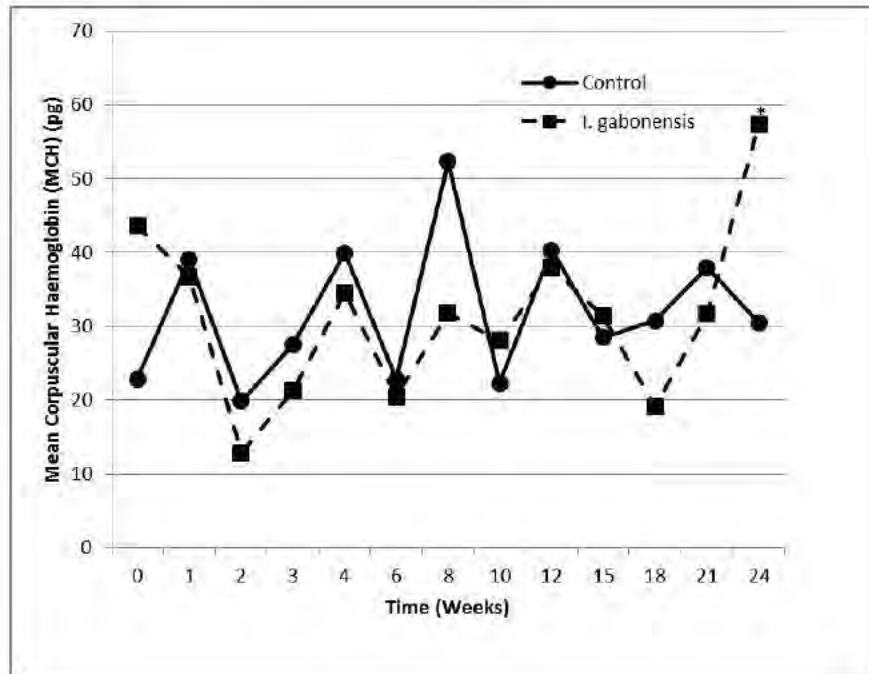


Figure 4: Effects of *I. gabonensis* on mean corpuscular haemoglobin (MCH) of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

As shown in Figure 4, the MCH values for *I. gabonensis* treated rabbits were statistically similar to control until week 24 when they were significantly ( $p < 0.05$ ) higher than control.

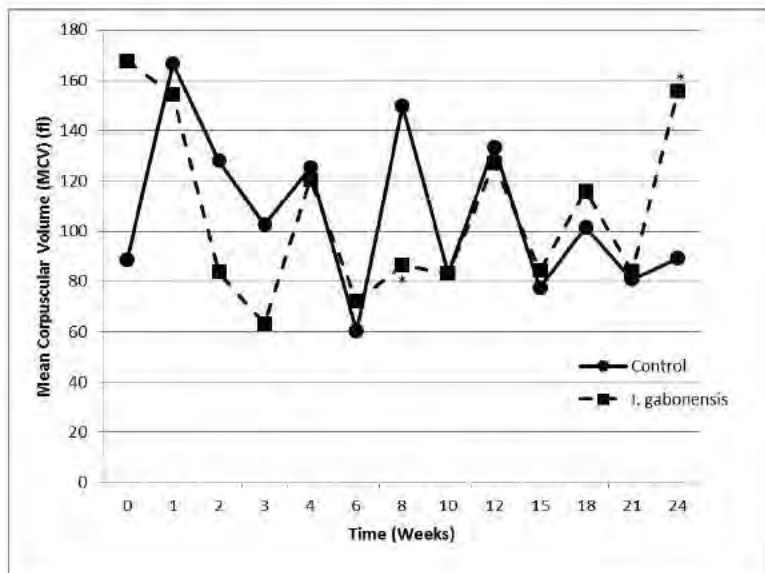


Figure 5: Effects of *I. gabonensis* on mean corpuscular volume (MCV) of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

The MCV of week 8 significantly ( $p < 0.05$ ) decreased while week 24 significantly ( $p < 0.05$ ) increased (Figure 5). The other values for the *I. gabonensis* treated rabbits were mostly comparable to control.

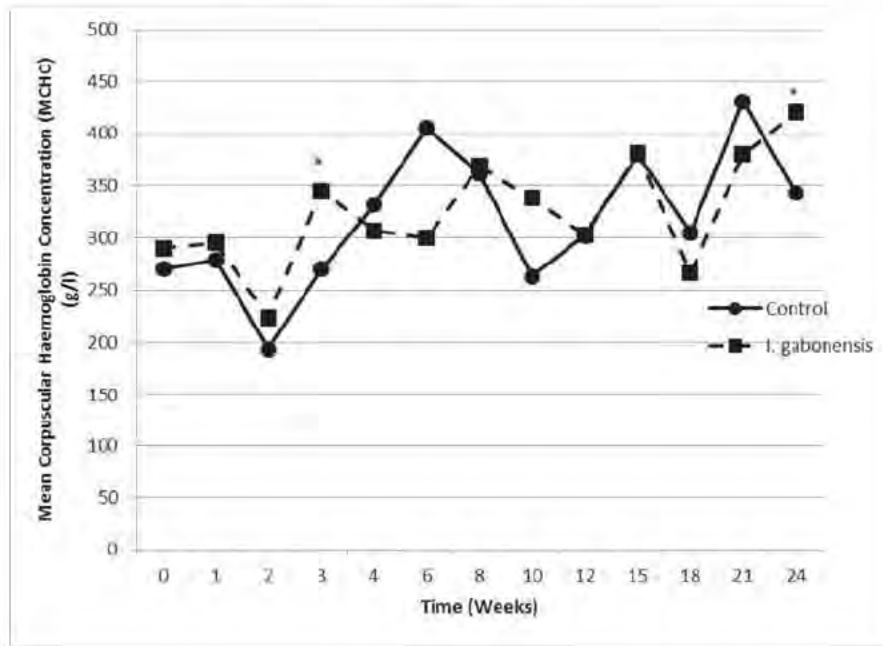


Figure 6: Effect of *I. gabonensis* on mean corpuscular haemoglobin concentration (MCHC) of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

From Figure 6, it can be seen that with the exception of weeks 3 and 24 which significantly increased, the MCHC values of the *I. gabonensis* treated rabbits were similar to control.

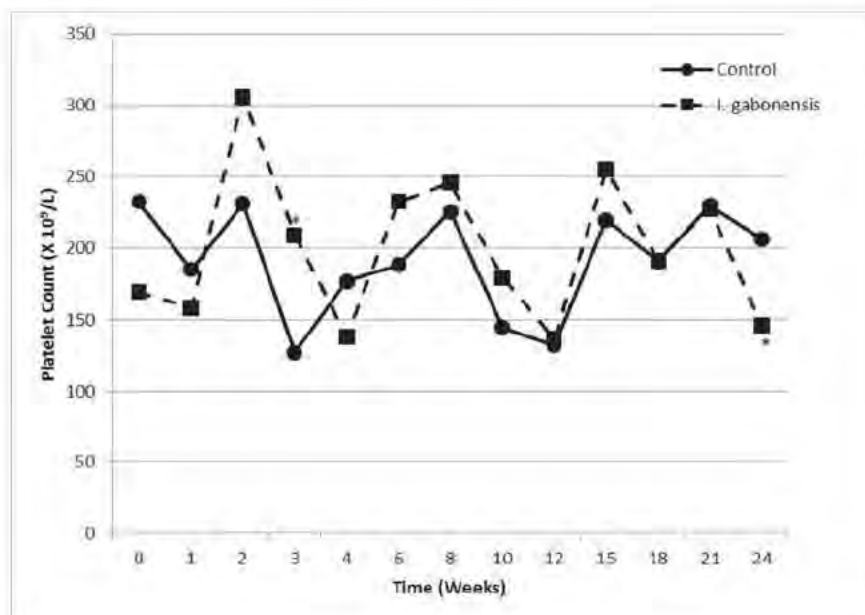


Figure 7: Effects of *I. gabonensis* on platelet count of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

The platelet counts of the *I. gabonensis* treated rabbits were slightly higher or comparable to control (Figure 7), however it decreased in week 24.

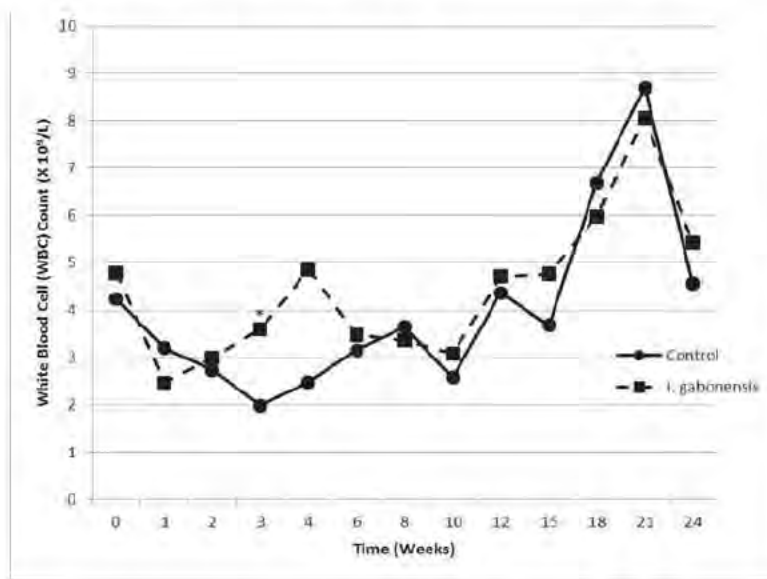


Figure 8: Effects of *I. gabonensis* on white blood cell (WBC) count of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

Apart from weeks 3 and 4, most of the values for WBC of the *I. gabonensis* treated rabbits were comparable to control (Figure 8).

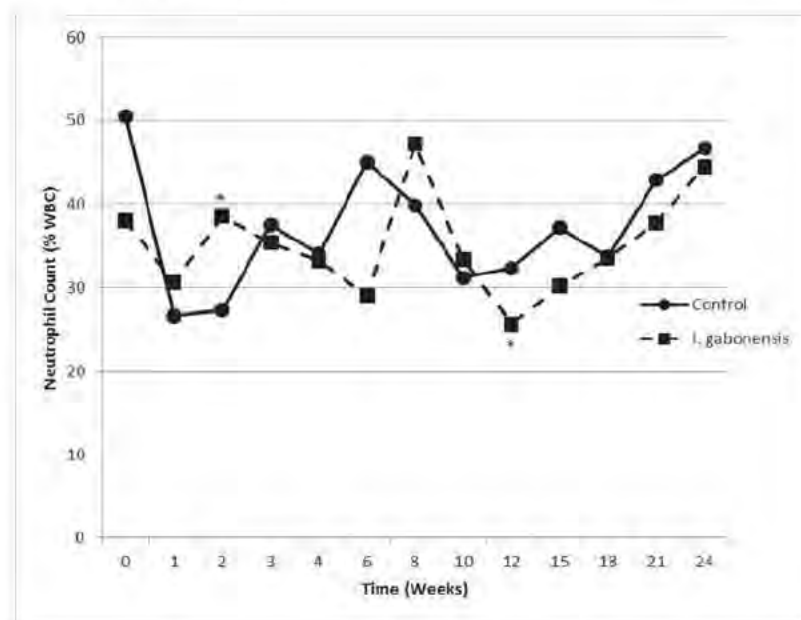


Figure 9: Effect of *I. gabonensis* on neutrophil count of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

Week 2 recorded an increase while week 12 neutrophil count significantly decreased (Figure 9). The other values were mostly similar to control.

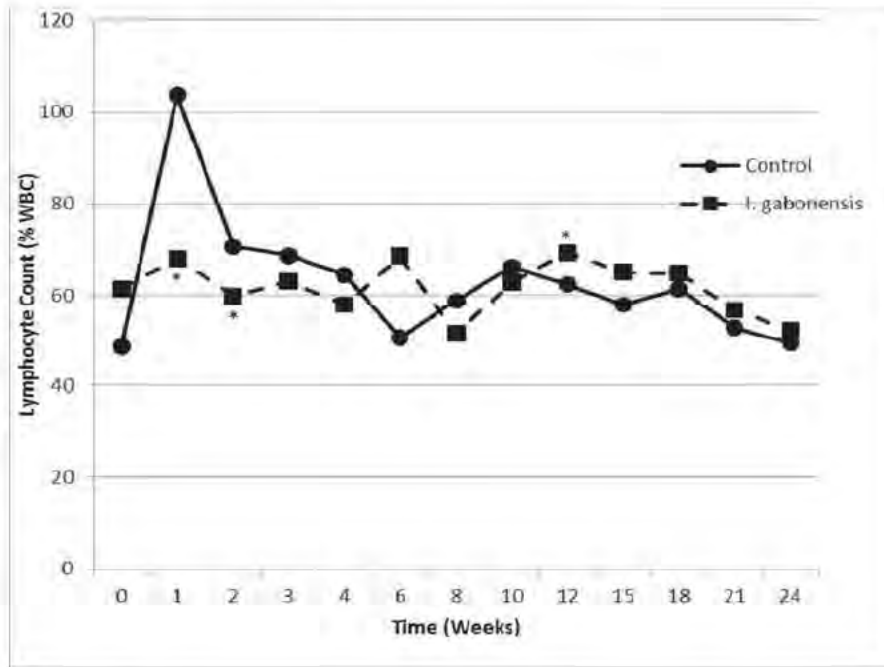


Figure 10: Effects of *I. gabonensis* on lymphocyte count of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

Figure 10 shows significant ( $p < 0.05$ ) decreases in week 1 and 2, and a significant increase in week 12, in the lymphocyte count of *I. gabonensis* treated rabbits. Other values are similar to control.

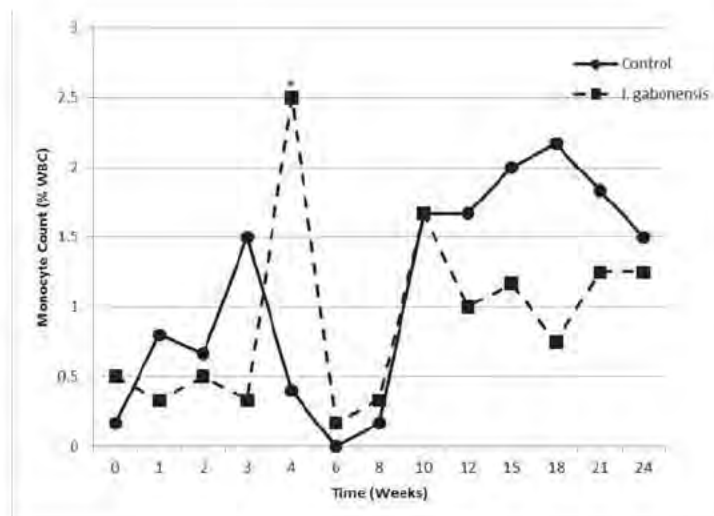




Figure 11: Effects of *I. gabonensis* on monocyte count of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

As shown in Figure 11, at week 3 a decrease in monocyte count was recorded. In week 4 a significant ( $p < 0.05$ ) increase were recorded. From week 12 monocyte counts of the medicinal plant treated rabbits were numerically lower than control.

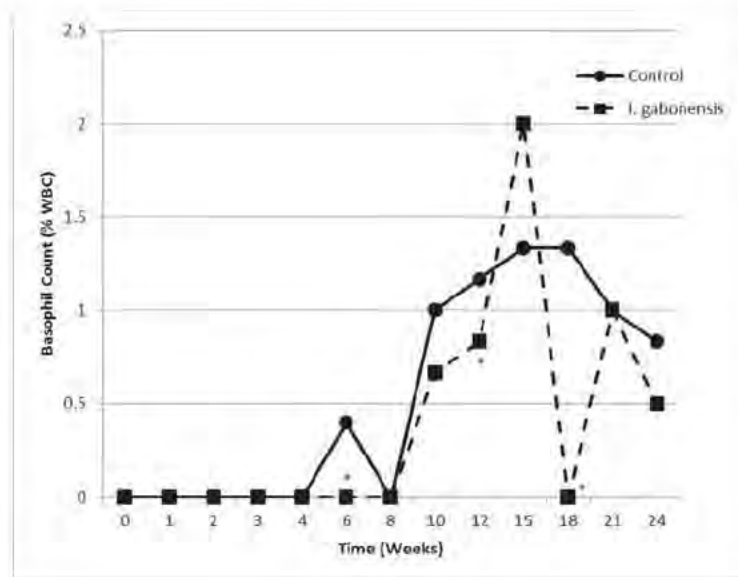


Figure 12: Effect of *I. gabonensis* on basophil count of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$ .

As seen in Figure 12, the values obtained for basophil count in the *I. gabonensis* treated groups from week 6 were mostly lower than control.

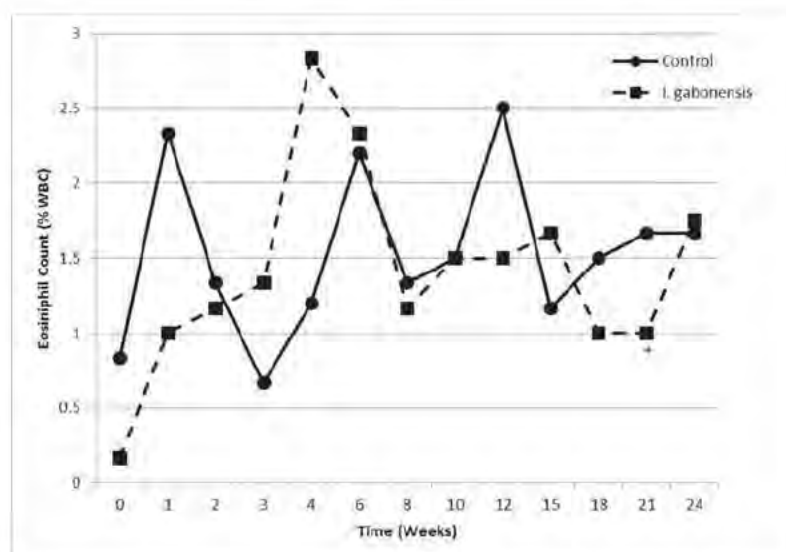


Figure 13: Effect of *I. gabonensis* on eosinophil count of normal rabbits. Values carrying notations are statistically different from control at  $p < 0.05$

With the exception of week 21 when a decreased eosinophil count was recorded (Figure 13), the eosinophil count of the *I. gabonensis* treated group were statistically similar to control.

## DISCUSSION

The complete blood count is an analysis of the blood that provides much information. It consists of a red blood cell count, haemoglobin and haematocrit measurement, platelet and a white blood cell count, including differential count. Evaluation of haematological parameters can be used to assess the extent of damaging effect of foreign compounds, including plant extracts, on the blood constituents of an animal.<sup>16</sup> The general increases recorded in RBC, PCV and Hb concentrations of the *I. gabonensis* treated rabbits indicate that the medicinal plant either improved haematopoiesis or reduced RBC destruction. MCH, MCHC and MCV relates to the size and Hb content of individual red blood cells, the values recorded for these parameters in this study were mostly similar to control, suggesting that neither the incorporation of haemoglobin into the red blood cells nor the morphology and osmotic fragility of the red blood cells was altered.<sup>17</sup> The implication of this is that the increase seen in RBC count is a reflection of an actual increase in RBC production (erythropoiesis). Platelet count was either higher or similar to control, indicating that these medicinal plants did not negatively affect platelet production or facilitate their destruction. These results are similar to that of Nwinuka et al<sup>18</sup> who reported increases in the levels of PCV (haematocrit), erythrocyte and platelet counts in normal rats given *Mangifera indica*, a hypoglycaemic plant, for 14 days. The improvement seen in the haematopoietic

system of *I. gabonensis* treated rabbits could be a reflection of the presence of polyphenolic anti-oxidants in the plant<sup>19</sup>, which have the potential of protecting RBCs, which are particularly susceptible to oxidants, from oxidative damage, thus prolonging their health-span. High neutrophil levels may indicate an active infection; a low count may indicate a compromised immune system or depressed bone marrow.<sup>20</sup> Elevated lymphocyte levels may indicate an active viral infection and a depressed level may indicate an exhausted immune system or if the neutrophils are elevated an active infection.<sup>20</sup> Elevated monocyte levels are seen in tissue breakdown or chronic infections, carcinomas, leukemia (monocytic) or lymphomas. Low levels are indicative of a state of health.<sup>20</sup> High basophil and eosinophil levels are found in allergic reactions, low levels are normal.<sup>20</sup> The increases observed within the first 4 weeks of medicinal plant administration in WBC and neutrophil count, as well as decrease in lymphocyte count, suggests an initial immune stimulation by the medicinal plant. This event was however transient as the values were subsequently restored to control levels. This claim is supported by the increase observed in week 4 for monocyte count and its subsequent reduction from week 6 to 24.

The basophil and eosinophil counts for the *I. gabonensis* treated rabbits were mostly lower or similar to control. These low basophil and eosinophil counts indicate that the rabbits did not exhibit any allergic

response to the administered extract. Generally, the results obtained for WBC and differential count in this study indicate that the administered medicinal plant caused a slight initial stimulation of the immune system which was not sustained and did not elicit an allergic response.

Long-term haematological evaluation of daily oral administration of aqueous extracts of *I. gabonensis* bark revealed an improvement of haematopoietic system of rabbits and it did not compromise the immune system of rabbits.

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