Effect of aqueous extract of *Bulbine natalensis* Baker stem on haematological and serum lipid profile of male Wistar rats

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*Bulbine natalensis* stem extract (25, 50 and 100 mg/kg body weight for 14 days) did not significantly alter the red blood cell count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and red cell distribution width in male Wistar rats. In contrast, the white blood cell count increased by the end of the experimental period. While the levels of neutrophils, lymphocytes and eosinophils decreased after the administration of single dose of the extract (day 1), those of the platelets and monocytes increased. The extract also reduced the levels of basophils and large unstained cells after the seven daily doses. All the doses increased the serum concentrations of cholesterol and triacylglycerols. Whereas the serum concentration of low-density lipoprotein was unaffected throughout the experimental period, the decrease in high-density lipoprotein cholesterol was accompanied by increase in the atherogenic index. The results showed that aqueous extract of *B. natalensis* stem exhibited localized systemic toxicity mainly on the white blood cell count and related indices. The alterations in the serum lipid profile may predispose the animals to atherosclerosis especially when consumed repeatedly for two weeks.

**Keywords:** Bulbine natalensis, Haematological parameters, Localized systemic toxicity, Serum lipids

*Bulbine natalensis* Baker (Asphodelaceae) locally known as *ibhucu* (Zulu) *ingcelwane* (Xhosa) and *rooiwortel* (Afrikaans), is widely distributed in the eastern and northern parts of South Africa. The leaf sap is used in the management of wounds, burns, rashes, itches, ringworms and cracked lips. The infusion of the root is taken to quell vomiting, diarrhoea, convulsion, venereal diseases, diabetes and rheumatism. The plant stem consists of tannins, anthraquinones, cardiac glycosides, saponins and alkaloids. Recently, the acclaimed folkloric use of the stem as an aphrodisiac and sexual invigorator in male rats was scientifically validated.

Medicinal plants with aphrodisiac potentials such as *Asparagus racemosus* Willd and *Fadogia agrestis* (Schweinf. Ex Hiern) stem have been shown to alter serum lipids in hypocholesterolic and normal rats. Alterations in the serum lipids of animals administered with extract of medicinal plants appear to be a significant factor in the development of premature atherosclerosis. Elevated serum lipids are risk factors in cardiovascular problems. Similarly, ingestion of some plant materials (either in the raw form or their extracts) have been reported to cause anaemia, which may result from sequestration of red blood cell in the spleen, impaired red blood cell production or primary bone marrow dysfunction. Specifically, *F. agrestis* have been reported to exhibit localized systemic toxicity on the white blood cells and indices relating to it while *Allium ascalonium* have been shown to decrease most of the parameters relating to red blood cells and also increased most of those relating to white blood cells. Considering the use of *B. natalensis* for the treatment of various diseases, there is the need to investigate the possible toxic effect of the plant on some biochemical parameters in animals. In this study, we present information on the effect of *Bulbine natalensis* stem extract on the haematological parameters and serum lipid profile of male rats.

**Materials and Methods**

*Plant material and authentication*— Plants collected from Sikusthwana village, near Alice in the Eastern Cape, of South Africa were authenticated by Prof DS Grierson of the Department of Botany, University of Fort Hare, South Africa. A voucher specimen of the plant (Yakmed. 2008/1) was deposited at the Giffen Herbarium of the University.

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Animals—Healthy, three months old male albino rats (*Rattus norvegicus*) weighing 206.00 ± 4.67 g were obtained from the Animal House of the Agricultural and Rural Development Research Institute, University of Fort Hare. The animals were housed in clean metabolic cages placed in well-ventilated house conditions (23 ± 1°C; 12:12 hr photo period 45-50% RH). They were also allowed free access to Balanced Trusty Chunks (Pioneer Foods (Pty) Ltd, Huguenot, South Africa) and tap water. The cleaning of the cages was done daily.

Assay kits — The assay kits for cholesterol, triacylglycerols, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were obtained from Roche Diagnostic GmbH, Mannhein, Germany. All other reagents used were of analytical grade and were supplied by Merck Chemicals (Pty) Ltd., Bellville, South Africa.

Preparation of extract — The dried plant material was pulverized with an electric blender. Twenty gram of the powder was extracted in 1000 ml of distilled water for 48 hr at room temperature with constant shaking on a Stuart Scientific Orbital Shaker (SO1, United Kingdom). The extract was filtered with Whatman No. 1 filter paper and the resulting filtrate was freeze-dried (Vir Tis benchtop K, Vir Tis Company, Gardiner, NY) to give a yield of 4.71 ± 0.04 g. This was reconstituted separately in distilled water to give the required doses used in this study.

Animal grouping and extract administration—Male albino rats (60) were completely randomized into four groups of 15 each and were orally administered as follows: Group A (control) was administered with 0.5 ml of distilled water while groups B, C and D were given equal volume of the extract corresponding to 25, 50 and 100 mg/kg body weight, respectively. The administration was done with the aid of metal oropharyngeal cannula. Five rats from each of the groups were sacrificed 24 hr after 1, 7 and 14 of their respective daily doses. The study was carried out following the approval from the ethical committee on animal use and care of University of Fort Hare, South Africa.

Preparation of serum — The procedure described by Yakubu et al. was adopted for the preparation of serum. Briefly, under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to prevent blood contamination by interstitial fluid) were sharply cut with sterile scapel blade and an aliquot (2 ml) of the blood was collected into BD vacutainer® (BD Plymouth, UK) sample bottles for the haematological analysis. Another 5 ml of the blood was allowed to clot for 10 min at room temperature and thereafter centrifuged at 1282 g for 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were later aspirated with Pasteur pipettes into sample bottles and used within 12 hr of preparation for the lipid assay.

Determination of biochemical parameters — Adopting the method of Tietz et al., the levels of cholesterol, triacylglycerol, HDL-C and LDL-C were determined in the serum using assay kits from Roche Diagnostics on Roche modular (model P800) Mannheim, Germany. The Horiba ABX 80 Diagnostics (ABX pentra Montpellier, France) was used for the determination of red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), large unstained cell (LUC), red cell distribution width (RCDW), white blood cell (WBC), neutrophils, monocytes, lymphocytes, eosinophils, basophils and platelets.

Statistical analysis — Data obtained were subjected to one way analysis of variance (ANOVA) and means were separated by the Duncan Multiple Range Test. Percentage data were transformed to arcsine before analysis. Significant levels were tested at P < 0.05.

Results The aqueous extract of *B. natalensis* stem in either of the doses (25, 50 and 100 mg/kg body weight) did not significantly alter the red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RCDW) throughout the experimental period (Table 1). In contrast, the extract increased the white blood cell count (WBC) throughout the period of administration. By the end of the experimental period, the WBC had increased by 41.9, 26.1 and 38.5% in the 25, 50 and 100 mg/kg body weight groups respectively. While the levels of neutrophils, lymphocytes and eosinophils
decreased after the administration of single dose of the extract (day 1), those of the platelets and monocytes increased. The extract also reduced the levels of basophils and LUC after the seven daily doses (Table 1).

All the doses significantly increased the serum concentrations of cholesterol and triacylglycerols (Table 2). By the end of the experimental period, the 25, 50 and 100 mg/kg body weight of the extract increased the concentration of cholesterol by 51.9, 38.9 and 55.6% respectively whereas the triacylglycerol increased by 115.8, 82.5 and 45.6% respectively. Although, the extract did not alter the serum concentration of low-density lipoprotein cholesterol throughout the period of administration, the decrease in high-density lipoprotein by the end of the experimental period was accompanied by increase in the atherogenic index (Table 2).

### Table 1 — Effect of *Bulbine natalensis* stem extract on haematological parameters of male rats

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>DAY 1 (Control)</th>
<th>DAY 7 (Control)</th>
<th>DAY 14 (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (× 10^{12}/l)</td>
<td>7.34±0.48a</td>
<td>7.13±0.49a</td>
<td>7.44±0.72a</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.30±0.76a</td>
<td>14.36±0.65a</td>
<td>14.72±0.74a</td>
</tr>
<tr>
<td>PCV (l/l)</td>
<td>0.46±0.02a</td>
<td>0.47±0.02a</td>
<td>0.47±0.04a</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>62.74±1.02a</td>
<td>62.66±1.09a</td>
<td>63.06±1.27a</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.46±0.34a</td>
<td>19.86±0.24a</td>
<td>19.56±0.47a</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>30.76±0.28a</td>
<td>31.06±0.34a</td>
<td>30.10±0.43a</td>
</tr>
<tr>
<td>RCDW (%)</td>
<td>13.12±0.85a</td>
<td>13.08±0.103a</td>
<td>12.58±0.101a</td>
</tr>
<tr>
<td>LUC (%)</td>
<td>11.78±1.34a</td>
<td>10.00±0.66a</td>
<td>10.88±1.22a</td>
</tr>
<tr>
<td>WBC (× 10^{9}/l)</td>
<td>8.82±1.81a</td>
<td>11.24±1.09b</td>
<td>12.24±1.62a</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>8.10±1.40a</td>
<td>7.56±0.97a</td>
<td>7.78±0.87a</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>23.30±1.51a</td>
<td>23.36±2.54a</td>
<td>28.22±3.64a</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>55.18±2.04a</td>
<td>57.46±2.90a</td>
<td>56.62±2.16a</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.28±0.07a</td>
<td>1.34±0.08a</td>
<td>1.33±0.08a</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.34±0.09a</td>
<td>0.28±0.01a</td>
<td>0.29±0.04a</td>
</tr>
<tr>
<td>Platelet (× 10^{7}/l)</td>
<td>723.80±6.20a</td>
<td>725.40±7.39a</td>
<td>737.40±8.85a</td>
</tr>
</tbody>
</table>

^a-d^ Test values carrying superscripts different from the control across each parameter are significantly different (P < 0.05).

WBC=white blood cell, RBC=red blood cell, Hb=Haemoglobin, PCV=packed cell volume, MCV-mean corpuscular volume, MCH-mean corpuscular haemoglobin, MCHC-mean corpuscular haemoglobin concentration, LUC-large unstained cell, RCDW- red cell distribution width

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Discussions

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. It can also be used to explain blood relating functions of chemical compounds/plant extracts. Such analysis is important to risk evaluation as the changes in the haematological system have higher predictive value for human toxicity, when the data are translated from animal studies.

The non-significant effect of the extract on the RBC may be an indication that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) was not unaltered. This may imply that the extract was not capable of stimulating the release of erythropoietin in the kidney, which is the humoral regulator of RBC production. Since MCHC, MCH and MCV relate to individual red blood cells while Hb, RBC, PCV, LUC and RCDW are linked to the total population of red blood cells, the non-effect of the extract on these indices may imply that neither the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cells was altered. Therefore, it is unlikely that the extract will affect the oxygen-carrying capacity of each of the RBC and the total population. In addition, since the anaemia diagnostic indices of MCV, MCH and MCHC were not altered by the extract, it may be logical to infer that the extract had no effect on the average size of RBC (microcytes) as well as the weight of haemoglobin per RBC.

Interestingly, WBC and all indices relating to it were significantly altered during the experimental period. The increase in WBC may be due to enhancement in the rate of entrance of the haematological parameter into the blood from the bone marrow and a diminished rate of removal from circulation. Granulocyte-macrophage colony stimulating factor, macrophage colony stimulating factor, interleukins IL-2, IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBC. Therefore, such increase in the blood parameter may be due to overproduction of these haematopoietic regulatory elements by the stromal cells and macrophages in the bone marrow.

White blood cell differentials are indicators of the ability of an organism to eliminate infection. An increase in the number of circulating leukocytes is rarely due to an increase in all the types of leukocytes. Neutrophils attack and destroy bacteria in the blood. The reduced neutrophils will adversely affect the phagocytosis activity in the animals. Lymphocytes are the main effector cells of the immune system. The reduction in the lymphocytes in the present study may affect the effector cells of the immune system. Similarly, decreased levels of eosinophils and basophils observed in the present study may suggest adverse effect on the immune system. Since monocytes have been shown to increase in cases of infection, the increase in monocytes observed with the extract in this study may be ascribed to challenges on the immune system.

### Table 2 — Effect of *B. natalensis* stem extract on serum lipid profile of male rats

<table>
<thead>
<tr>
<th>Lipid parameters (mmol/l)</th>
<th>Control</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>Control</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>Control</th>
<th>25</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>1.04 ± 0.22</td>
<td>2.26 ± 0.09</td>
<td>2.16 ± 0.06</td>
<td>2.28 ± 0.08</td>
<td>1.04 ± 0.05</td>
<td>1.66 ± 0.15</td>
<td>2.06 ± 0.05</td>
<td>2.12 ± 0.04</td>
<td>1.08 ± 0.02</td>
<td>1.64 ± 0.05</td>
<td>1.50 ± 0.08</td>
<td>1.68 ± 0.08</td>
</tr>
<tr>
<td>TAG</td>
<td>1.14 ± 0.08</td>
<td>1.84 ± 0.03</td>
<td>2.20 ± 0.03</td>
<td>1.46 ± 0.09</td>
<td>1.12 ± 0.11</td>
<td>1.48 ± 0.29</td>
<td>1.74 ± 0.05</td>
<td>1.92 ± 0.16</td>
<td>1.14 ± 0.01</td>
<td>2.46 ± 0.49</td>
<td>2.08 ± 0.08</td>
<td>1.66 ± 0.04</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.76 ± 0.11</td>
<td>1.76 ± 0.05</td>
<td>1.76 ± 0.06</td>
<td>1.86 ± 0.09</td>
<td>1.72 ± 0.08</td>
<td>1.68 ± 0.17</td>
<td>1.62 ± 0.11</td>
<td>1.66 ± 0.05</td>
<td>1.73 ± 0.07</td>
<td>0.90 ± 0.00</td>
<td>1.04 ± 0.03</td>
<td>1.02 ± 0.06</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.83 ± 0.05</td>
<td>0.86 ± 0.04</td>
<td>0.86 ± 0.05</td>
<td>0.86 ± 0.05</td>
<td>0.78 ± 0.04</td>
<td>0.82 ± 0.04</td>
<td>0.80 ± 0.00</td>
<td>0.78 ± 0.04</td>
<td>0.79 ± 0.01</td>
<td>0.82 ± 0.04</td>
<td>0.78 ± 0.04</td>
<td>0.80 ± 0.00</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>0.47 ± 0.05</td>
<td>0.49 ± 0.04</td>
<td>0.47 ± 0.03</td>
<td>0.46 ± 0.03</td>
<td>0.45 ± 0.04</td>
<td>0.50 ± 0.04</td>
<td>0.49 ± 0.03</td>
<td>0.47 ± 0.04</td>
<td>0.46 ± 0.04</td>
<td>0.91 ± 0.03</td>
<td>0.75 ± 0.02</td>
<td>0.78 ± 0.02</td>
</tr>
</tbody>
</table>

### Note

TAG-triacylglycerol, HDL-C-high density lipoprotein cholesterol, LDL-C-low density lipoprotein cholesterol

\[a-d\] Test values carrying superscripts different from the control across each parameter are significantly different \((P < 0.05)\).
Platelets are the blood cells involved in coagulation\textsuperscript{19}. Coagulation of blood requires that the platelets be in sufficient size, number and function. The increase in the platelet levels may be explained by stimulatory effect on thrombopoietin\textsuperscript{20}.

Bone marrow is responsible for the production of red blood cells, white blood cells and platelets\textsuperscript{6,23}. The alterations produced by the extract of \textit{B. natalensis} stem on the WBC, platelets as well as the non-effect on the red blood cells suggest selective, stimulatory effect on the bone marrow. This may be an indication of localized systemic toxicity of the extract which may affect the normal functioning of the WBC and its related indices. This agrees with the findings of Adebayo \textit{et al}\textsuperscript{14} on ethanolic extract of \textit{Bougainvillea spectabilis} leaves and Yakubu \textit{et al.}\textsuperscript{8} on aqueous extract of \textit{Fadogia argrestis} stem.

Alterations in the concentration of major lipids like cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides could give useful information on the lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated coronary heart diseases\textsuperscript{5}. Elevated levels of all lipids except the HDL-C are associated with increased risk of atherosclerosis.

The increase in serum cholesterol observed with the extract may be due to increase in the concentration of acetyl CoA arising probably from enhanced \(\beta\)-oxidation stem of fatty acid, since acetyl CoA is a key substrate in the biosynthesis of cholesterol\textsuperscript{22}. High blood cholesterol concentration is one of the important risk factors for cardiovascular disease\textsuperscript{23}. Therefore, such increase in the lipid contents may not be beneficial to the animals as it may enhance atherosclerosis and hypertension\textsuperscript{24}.

Triacylglycerol are the main storage form of fatty acid. The increase in triacylglycerol by the extract of \textit{B. natalensis} stem may be due to accelerated lipolysis. This may deplete the store of fatty acids. It has been reported that cardiovascular diseased patients had markedly elevated levels of triacylglycerol but reduced HDL-C. This however, resulted from the metabolism of TAG rich lipoproteins on the HDL-C, particularly the subfraction HDL\textsubscript{2}\textsuperscript{25}. Therefore, the inverse relationship which existed between the TAG and HDL-C in the present study by the end of the experimental period may suggest cardiovascular risk, thus, the repeated administration of the extract may not be beneficial to the animals.

Low-density lipoprotein cholesterol (LDL-C) is a primary carrier of plasma cholesterol. The non-effect of the extract on this lipid parameter may imply that the transportation of plasma cholesterol was unimpaired.

HDL-C is considered to have anti-atherogenic properties. It has also been shown that increase in HDL-C correlates inversely with coronary heart disease\textsuperscript{26}. The reduced levels of HDL-C by the end of the experimental period may portend cardiovascular risk. This was, however supported by the increased atherogenic index, a useful indicator of cardiovascular diseases\textsuperscript{27}. Therefore, the repeated consumption of the extract at all the doses investigated for 14 days may predispose the animals to atherosclerosis and its associated coronary artery diseases. In the present study, the HDL-C and atherogenic index which were not altered within the first week shows that the extract may not predispose the rats to atherosclerosis at this period.

The results of the present study has shown that aqueous extract of \textit{Bulbine natalensis} stem exhibited localized systemic toxicity mainly on the WBC and indices relating to it. The alterations in the serum lipid profile may predispose the animals to atherosclerosis especially when consumed repeatedly on daily basis for two weeks.

\textbf{Acknowledgement}

This research was supported with grants from Govan Mbeke Research and Development Centre, University of Fort Hare, and the National Research Foundation, South Africa. The authors are also grateful to the University of Ilorin, Nigeria for supporting the Postdoctoral Fellowship Programme of Dr M T Yakubu.

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