Investigation of haematological effects of *Terminalia macroptera* stem bark via platelet-to-lymphocyte ratio among other blood parameters in *Wistar* rats

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**ABSTRACT:** This study investigated the effects of aqueous and ethanol extracts of *Terminalia macroptera* stem bark (TMSB) in *Wistar* rats via white blood cell count and its differentials; erythrocytic parameters (EP) [red blood cell count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration]; platelet count and platelet-to-lymphocyte ratio (PLR). Daily oral administration of extracts at low (50 and 200 mg/kg), mid (400 and 600 mg/kg) and high doses (1000 and 1200 mg/kg) were delivered to four female rats in six groups respectively for 28 days while control group received distilled water. Thereafter, animals were sacrificed and harvested blood analysed using automated systems. The results showed that high doses (1000 and 1200 mg/kg) of both extracts respectively caused granulocytic leukocytosis. However, only ethanol extract (EE) induced lymphocytopenia (1000 mg/kg, p=0.005; 1200 mg/kg, p=0.013) while granulocytosis observed with low doses had no corresponding increases in white blood cell counts. Aqueous extract (AE) had no effect on EP whereas significant increases were observed in groups administered 50 – 400 mg/kg of EE. PLR was 44.34 (± 6.58) in 1200 mg/kg aqueous extract dosed rats, significantly lower (p=0.01) than control group [73.03 (±5.14)], but higher in rats dosed with EE at 1000 mg/kg [PLR=175.55 (± 3.84); p<0.001] and 1200 mg/kg [PLR=117.28 (±3.90); p=0.005] respectively. These findings revealed that TMSB(AE) possesses immunoimpressive potential at high doses without significant erythropoietic effects while high doses of TMSB(EE) exhibits possible induction of systemic inflammation inaddition to erythropoietic boost at low to mid dose ranges.

**KEYWORDS:** *Terminalia macroptera*; platelet-to-lymphocyte ratio; lymphocytopenia; monocytes; granulocytic leukocytosis.
INTRODUCTION

Terminalia macroptera Guill. & Perr. (Combretaceae) is a perennial plant that is used to treat a number of ailments in northern Nigeria and other West African countries (Pham et al., 2011a). Previous investigations have revealed the chemical constituents of its stem bark to contain triterpenes, polyphenols and polysaccharides (Conrad et al., 1998; Conrad et al., 2001; Zou et al., 2014; Akpovona et al., 2016a). The extracts also show potential in alteration of food conversion rate without stimulation of gross toxic responses (Akpovona & Onoagbe, 2015). Although, its parts are known for antibacterial properties (Silva et al., 1996; Silva et al., 1997; Silva et al., 2012; Traore et al., 2015a; Traore et al., 2015b) and antioxidant activities (Pham et al., 2011b), its use as a veritable therapeutic agent still remained to be sufficiently proven to be safe for human consumption. Beyond the acute toxicity study of the stem bark as reported in other findings (Akpovona et al., 2016b), there is the need to examine its systemic toxicity in order to obtain a holistic clearance on its consumption.

Blood is a primary target of xenobiotics when introduced into the body system and evaluation of its profile is important in determining their effect in animals (Anderson, 2001). Screening blood also gives insight into the functionality of the bone marrow (Badole & Kotwal, 2015). Studies have shown that the administration of phytochemical compounds can alter the haematological status of animals (Omonkhua et al., 2013; Geidam et al., 2004). Similarly, in diseased conditions, the administration of herbal extracts have shown positive alterations in blood profiles that indicated ameliorative effects (Sakuljaitrong et al., 2012).

Platelet-to-lymphocyte ratio (PLR) is a simple marker of subclinical inflammation that can be easily obtained from differential white blood cell count (WBC) (Ilgun et al., 2016). This study examines the response of haematological parameters (HP) to infused Terminalia macroptera stem bark (TMSB) extracts with special emphasis on PLR. Determination of the PLR values has been documented to indicate rise in inflammation related to nephrological and cardiovascular diseases (Tsiara et al., 2003). An attempt is made in this work to utilize this ratio to examine possible inflammation at the systemic level as may be caused by the ingestion of TMSB extract (Zahorec, 2001).

MATERIALS AND METHODS

Materials

Matured Terminalia macroptera stem bark (TMSB) were harvested from Ilorin, Kwara State, Nigeria. Absolute ethanol was purchased from JHD, China while distilled water was obtained from Pyrex-IG Scientific Company, Benin City, Nigeria. Pelletized rodent chow was obtained from UAC, Nigeria. The chow contained protein (13 % min.), fat (8 % max.), fibre (15 % max.), Ca (0.9 % min.), P (0.35 % min.) and energy (2,600 kcal/kg, min.).

Preparation of Plant Extract

Matured TMSB (voucher number: UBHT 0232) was used (Akpovona et al., 2016a). The aqueous and ethanolic plant extracts were obtained by cold extraction of 200 g sample weight of pulverized stem bark in 800 ml of extractant (water and ethanol) in a ratio of 1:4 for three days at 8 °C and 25 °C respectively.

Phytochemical Composition of Plant Extracts

Findings from preliminary investigations revealed that the TMSB extracts (aqueous and ethanol) contained alkaloids, cyanogenic glycosides, anthraquinones, terpenoids, flavonoids, tannins and saponins respectively with alkaloids, terpenoids and tannins having higher extractive values in ethanol. Both extracts recorded very high concentrations of tannins (Akpovona et al., 2016a).
Animal and Experimental Protocol

Fifty two (52) inbred female Wistar albino rats of 6 weeks old (weight 150-200 g) were purchased from a rodent breeder in the Department of Biochemistry, University of Benin, Benin City, Nigeria. These were housed in capacious wooden framed cages with iron-meshed borders where acclimatization was allowed for one week before experimentation. The rats were maintained at 21 to 27 °C, conditioned for 12-hours diurnal and nocturnal cycle with a relative humidity that fluctuated between 58–65% in the animal house of the Department of Biochemistry, University of Benin. The rats were fed with standard pelletized rodent chow and allowed free access to tap water. Randomization of the animals was done into 7 groups of 4 rats each. The crude extracts were administered orally by use of orogastric tube to 6 groups according to the doses: 50, 200, 400, 600, 1200 mg/kg respectively, while control rats (seventh group) received 1 ml of distilled water. Powdery extracts were reconstituted every day in distilled water before administration.

Blood Collection and Analysis

At the end of the experiment, the animals were euthanized under mild chloroform, dissected and whole blood samples obtained by heart puncture using a 5 ml hypodermic syringe and needle while the rats were still under the effect of the anaesthetic. Animals were thereafter humanely sacrificed immediately to reduce pain in accordance with the guidelines of European Economic Committee (EEC, 1986). Blood was then placed in EDTA-containing sample bottles and analysed by means of a standard blood analyser (model: PCE 210 N, ERMA INC., Tokyo, Japan) at the University of Benin Teaching Hospital, Benin City.

Statistical Analysis

The statistical evaluation of data was done utilizing SPSS version 23 software package for windows. Paired sample t-test was used to compare the means of the each group with control. All values were expressed as the mean ± SEM with significance set at p < 0.05.

RESULTS

Table 1 shows the effect of TMSB aqueous extract (TMSB(AE)) on white blood cells count (WBC), lymphocytes count (LPC), monocytes count (MNC), granulocytes count (GNC), red blood cells count (RBC), haemoglobin (HGB), packed cell volume (PCV), platelets count (PLT), mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) of the rats after 28 days of administration. The extract at high doses showed elevated WBC population (1000 mg/kg; p=0.005 and 1200 mg/kg; p = 0.046); LPC and MNC had no change in concentrations when compared with control group. However, GNC in all the groups were significantly increased (p < 0.05), except for the mid-doses (400 mg/kg; p = 1.000 and 600 mg/kg; p = 0.423). There were no significant differences in the values of RBC, HGB, PCV, MCV and MCHC of all AE tested rats when compared to control group. Although decreased PLT population was noticed in the high dosed groups, only 1200 mg/kg group showed significance (p=0.018). Interestingly, high dosed groups showed significant increases in MCH levels (1000 mg/kg; p=0.031 and 1200 mg/kg; p = 0.008).

Table 2 shows the effect of the TMSB ethanol extract (TMSB(EE)) on WBC, LPC, MNC, GNC, RBC, HGB, PCV, PLT, MCV, MCH and MCHC of Wistar rats after 28 days of administration. Groups dosed with EE showed numeric increases in WBC population that were only significantly higher than control at 600 mg/kg (p=0.019), 1000 mg/kg (p=0.022) and 1200 mg/kg (p=0.017). Higher doses (1000 mg/kg and 1200mg/kg) of the extract caused decreases in LPC concentration (p=0.005 and p=0.013) and
contrasting increases in MNC concentrations (p-values tending towards significance as the dose level increases: low doses, 50 mg/kg, p=0.252; 200 mg/kg, p=0.149; mid doses, 400 mg/kg, p=0.103; 600 mg/kg, p=0.079 and high doses, 1000 mg/kg, p=0.001; 1200 mg/kg, p=0.069) when compared to control group respectively. Though numeric increases in GNC concentrations were recorded in all the dosed groups in a non-monotonic fashion, only 200 mg/kg (p=0.040), 600 mg/kg (p=0.001), 1000 mg/kg (p<0.001) and 1200 mg/kg (p=0.001) were significantly higher than control group. Groups dosed with 50 and 400 mg/kg of TMSB(EE) respectively showed significant increases in RBC (p=0.018 and 0.017) when compared to control group respectively. PCV of groups administered 50 – 400 mg/kg were significantly increased above control group. Significant increases in PLT concentration were observed in groups dosed with 1000 mg/kg (p=0.002) and 1200 mg/kg (p=0.013), while 600 mg/kg caused a significant reduction (p=0.007). There were significant increases in the PCV, MCV and MCH concentration of groups administered 50 – 400 mg/kg of the extract while other doses only caused insignificant increases when compared to control. No significant differences were observed in the level of MCHC of the dosed groups when compared to control group.

Table 1. Haematological profile of rats administered aqueous extract of *Terminalia macroptera* stem bark for 28 days compared with control rats

<table>
<thead>
<tr>
<th>Extract Dose</th>
<th>Control (0 mg/kg)</th>
<th>50 mg/kg</th>
<th>200 mg/kg</th>
<th>400 mg/kg</th>
<th>600 mg/kg</th>
<th>1000 mg/kg</th>
<th>1200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x10^7/l)</td>
<td>6.93±0.04</td>
<td>6.66±0.27</td>
<td>6.60±0.20</td>
<td>6.62±0.19</td>
<td>7.23±0.18</td>
<td>7.88±0.13</td>
<td>7.31±0.13</td>
</tr>
<tr>
<td>LPC (x10^3/l)</td>
<td>6.55±0.39</td>
<td>5.83±0.25</td>
<td>5.98±0.20</td>
<td>6.13±0.20</td>
<td>6.83±0.20</td>
<td>6.80±0.30</td>
<td>5.93±0.18</td>
</tr>
<tr>
<td>MNC (x10^3/l)</td>
<td>0.13±0.01</td>
<td>0.18±0.03</td>
<td>0.13±0.03</td>
<td>0.13±0.02</td>
<td>0.11±0.01</td>
<td>0.26±0.08</td>
<td>0.22±0.03</td>
</tr>
<tr>
<td>GNC (x10^3/l)</td>
<td>0.23±0.05</td>
<td>0.55±0.03</td>
<td>0.48±0.03</td>
<td>0.27±0.07</td>
<td>0.23±0.03</td>
<td>0.70±0.12</td>
<td>0.70±0.10</td>
</tr>
<tr>
<td>RBC (x10^6/l)</td>
<td>6.95±0.54</td>
<td>6.77±0.17</td>
<td>6.65±0.4</td>
<td>6.05±0.47</td>
<td>6.96±0.36</td>
<td>6.21±0.43</td>
<td>5.94±0.50</td>
</tr>
<tr>
<td>HGB (g/dl)</td>
<td>13.15±0.96</td>
<td>14.0±0.7</td>
<td>13.7±0.82</td>
<td>12.37±1.49</td>
<td>14.33±0.19</td>
<td>13.38±0.72</td>
<td>13.0±1.33</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>40.78±2.41</td>
<td>44.45±1.82</td>
<td>42.88±3.44</td>
<td>41.93±3.64</td>
<td>46.1±0.86</td>
<td>42.7±1.88</td>
<td>38.05±3.73</td>
</tr>
<tr>
<td>PLT (x10^3/l)</td>
<td>474.00±22.70</td>
<td>486.5±21.33</td>
<td>478.75±50.52</td>
<td>472.33±42.72</td>
<td>456.33±37.13</td>
<td>416.00±21.42</td>
<td>263.65±41.44</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>59.00±2.19</td>
<td>64.87±2.15</td>
<td>64.30±2.41</td>
<td>69.07±1.50</td>
<td>67.13±4.40</td>
<td>69.15±3.44</td>
<td>63.83±1.08</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.96±0.35</td>
<td>20.67±0.68</td>
<td>20.63±0.58</td>
<td>20.39±0.63</td>
<td>20.84±1.74</td>
<td>21.65±0.51</td>
<td>21.98±0.75</td>
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<tr>
<td>MCHC (%)</td>
<td>32.20±0.72</td>
<td>31.47±0.54</td>
<td>32.16±1.07</td>
<td>29.32±0.20</td>
<td>31.32±0.62</td>
<td>31.32±0.91</td>
<td>34.56±1.44</td>
</tr>
</tbody>
</table>

Superscripts (*) attached to mean ± SEM values on the same row indicate significant differences with control values at *p<0.05*, †*p<0.01* and ‡*p<0.001* for n=4. Where WBC: White Blood Cell Count; LPC: Lymphocyte Count; MNC: Monocyte Count; GNC: Granulocyte Count; RBC: Red Blood Cell Count; HGB: Haemoglobin; PCV: Packed Cell Volume; PLT: Platelet Count; MCV: Mean Cell Volume; MCH: Mean Cell Haemoglobin and MCHC: Mean Cell Haemoglobin Concentration.
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<td>7.75±0.15</td>
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</tr>
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<td>LPC (x10^3/ul)</td>
<td>6.55±0.39</td>
<td>6.70±0.41</td>
<td>6.36±0.18</td>
<td>6.20±0.04</td>
<td>6.37±0.43</td>
<td>4.47±0.18</td>
<td>5.01±0.15</td>
</tr>
<tr>
<td>MNC (x10^3/ul)</td>
<td>0.13±0.01</td>
<td>0.18±0.03</td>
<td>0.19±0.03</td>
<td>0.20±0.03</td>
<td>0.23±0.03</td>
<td>0.83±0.05</td>
<td>0.21±0.03</td>
</tr>
<tr>
<td>GNC (x10^3/ul)</td>
<td>0.23±0.05</td>
<td>0.35±0.03</td>
<td>0.58±0.06</td>
<td>0.40±0.04</td>
<td>1.06±0.06</td>
<td>2.10±0.04</td>
<td>1.68±0.09</td>
</tr>
<tr>
<td>RBC (x10^6/ul)</td>
<td>6.95±0.54</td>
<td>7.46±0.11</td>
<td>6.57±0.04</td>
<td>7.14±0.10</td>
<td>6.05±0.33</td>
<td>5.75±0.01</td>
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<td>HGB (g/dl)</td>
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<td>51.73±0.59</td>
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<td>49.87±1.23</td>
<td>38.93±1.06</td>
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<tr>
<td>PLT (x10^3/ul)</td>
<td>474±22.70</td>
<td>551.00±3.79</td>
<td>466.33±33.72</td>
<td>547.67±26.46</td>
<td>360.50±10.67</td>
<td>782.00±23.09</td>
<td>586.25±7.66</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>59.00±2.19</td>
<td>69.40±0.93</td>
<td>71.88±1.37</td>
<td>69.83±1.13</td>
<td>64.72±1.47</td>
<td>65.48±1.80</td>
<td>60.81±3.57</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.96±0.35</td>
<td>21.78±0.58</td>
<td>22.46±0.57</td>
<td>22.33±0.50</td>
<td>22.26±0.89</td>
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Superscripts (*) attached to mean ± SEM values on the same row indicate significant differences with control at *p*<0.05, **p**<0.01 and ***p***<0.001 for *n*=4. Where WBC: White Blood Cell Count; LPC: Lymphocyte Count; MNC: Monocyte Count; GNC: Granulocyte Count; RBC: Red Blood Cell Count; HGB: Haemoglobin; PCV: Packed Cell Volume; PLT: Platelet Count; MCV: Mean Cell Volume; MCH: Mean Cell Haemoglobin and MCHC: Mean Cell Haemoglobin Concentration.
Figure 1: Platelet-to-lymphocyte ratio (PLR) of rats administered aqueous extract of Terminalia macroptera stem bark [TMSB(AE)] for 28 days. Results are mean SEM, n=4. Differences between test and control values were set at significance *p < 0.05, **p < 0.01, ***p < 0.001. A trend of gradual reduction in ratio was observed from the lowest to the highest dosed group.

Figure 2: Platelet-to-lymphocyte ratio (PLR) of rats administered ethanol extract of Terminalia macroptera stem bark [TMSB(EE)] for 28 days. Results are mean ± SEM, n=4. Differences between test and control values were set at significance *p < 0.05, **p < 0.01, ***p < 0.001.
DISCUSSION

Previous phytochemical analysis of TMSB has shown the presence of secondary metabolites that are known to exhibit therapeutic effects (Conrad et al., 1998; Conrad et al., 2001; Zou et al., 2014; Akpovona et al., 2016a). However, a holistic investigation of its possible toxic effects is needed for proper pharmaceutical use. Evaluation of blood parameters has been used to predict the effects of foreign compounds (such as plant extracts) on body systemic functions (Anderson, 2001). Blood as a tissue, is one of the most sensitive targets of xenobiotics and an important index of pathological status (Diallo et al., 2010). Its components can also provide information on the haematopoietic functions of the bone marrow as well as give a general picture of the immunological status of the animal (Badole & Kotwal, 2015). Consequently, this study examined the effect of TMSB on blood indices in female Wistar albino rats.

WBC count rises on sensing the presence of alien compounds (Anderson, 2001). The selective granulocytosis caused by low doses of the extract without a gross change in WBC indicates a differential subpopulation adjustment which signals subclinical stimulation of immunological indices that however lie within the boundaries of normal haemostasis. This definitely was offset by the high doses of the administered extract. The granulocytic leukocytosis observed at high doses of TMSB(AE) was below acute phase inflammatory response since there was neither a supportive increase in MNC nor marked alteration in LPC levels. The rise therefore, was a function of the increased number of GNC. Such characteristic increase in leukocytes has been reported for immunological responses associated with low-grade inflammation (Ptaczewska et al., 2014).

Observed insignificant differences in the erythocytic parameters at low doses when compared to control group suggest that TMSB(AE) did not alter the process of erythropoiesis. The depression in PLT (thrombocytopenia) as recorded in the highest administered dose of TMSB(AE) may be due to production defects in the bone marrow, most likely caused by the excess extract, an observation which may be linked to decline in food consumption (Izak & Bussel, 2014). Consistent with this reasoning, a previous study showed that high concentration of TMSB depresses food intake in Wistar rats (Akpovona & Onoagbe, 2015). This could cause intestinal malabsorption of folate and vitamin B12 hence instigating megaloblastic changes in the bone marrow (Cheesebrough, 2006). This is supported by the significant reduction in the population of RBC, LPC and PLT (Table 1). A conclusion on macrocytic anaemia based on the above remains doubtful since the increased MCH values in the high dosed rats were not complemented by the non-significant increases in MCV. However, a possible occurrence of this condition upon prolonged administration of high doses of TMSB(AE) appears undeniable. Megaloblastic changes can only be justified by the examination of megakaryocytes in the bone marrow aspirate, an aspect that is currently beyond the scope of the present study. These observations suggest that a case of an alarmed systemic inflammation was absent.

Recent studies have shown that there is a relationship between PLTs and LPCs, and that an increase in PLR could lead to increased vascular endpoints (Gary et al., 2013). In this study, PLR was investigated (Figure 1) to determine the probable inflammatory level accruing from the extract and its likelihood in causing thrombosis. The low level of PLR recorded at the highest administered dose as well as the maintenance of near normal ratio observed with the low dosed groups indicates that TMSB(AE) most likely would not stimulate thrombosis in tested rats, at least at the highest examined dose (Suades et al., 2012).

Unlike TMSB(AE), TMSB(EE), while causing an increase in GNC and MNC which were highly expressed in WBC of the high dosed groups, showed a contrasted decrease in the population of LPC (Table 2), an indication of inflammation (Ramaekers et al., 1975; Campbell, 1996). LPC has the highest
proportion in total WBC count of rats which is in agreement with its composition in the control rats (age 6-10 weeks) used in this study (Shayne, 2006). This specifically high proportion became reduced in tested rats administered high doses of TMSB(EE) in a manner observed with hypersecretion of corticoid hormones under stressed conditions (Dougherty & Frank, 1953; Cox & Ford, 1982; Black, 2002). TMSB(EE) may contain a factor that acts as a stimulator of cortisol or a direct stressor to the homeostasis of the blood which assumes a cortisomimetic function characterized by granulocytosis and lymphocytopenia (Ramaekers et al., 1975; Toft et al., 1994; Guyton & Hall, 2010). The extract at high doses possibly causes LPC to be evacuated and sequestered in tissues like lymph nodes and spleen in readiness for stress response (Dhabhar et al., 2012). The severity of the inflammation can be judged from the gradual increase in the MNC from low to high doses (where significance was attained). These findings readily supports the observations of a previous study which detected presence of inflammatory cells in the liver photomicrographs of rats administered TMSB(EE) (Akpovona et al., 2016b).

Similarly, high doses of EE simultaneously induced secondary thrombocytosis among the experimental animals. This culminated to a high PLR (figure 2). High PLR values are indicative of increased inflammation and are reported to be linked to cardiovascular risks (Tsiara et al., 2003). The elevated PLR values at high doses of the EE augument the suspicion already portrayed by the leucocyte differentials (MNC and GNC) that these range of doses possibly induce subchronic inflammatory responses. Interestingly, the results showed that the extract does not have any effect on RBC indices at high doses.

In contrast to the observed effect above, dose ranges between low and mid values imparted positively on RBC and its indices (PCV, MCV and MCH) in the test rats which suggest that the TMSB(EE) has erythropoietic properties. TMSB probably contains erythropoietin-stimulating principles which are implicated in the increased production of erythrocytes. This positive effect of the low doses of EE could be utilized therapeutically to upregulate red blood cell level under anaemic condition.

Conclusion

This study shows that high doses of TMSB(AE) produced immunostimulatory effects while similar doses of TMSB(EE) yielded strong induction of systemic inflammation in female Wistar albino but low to mid-doses of EE alone exhibited erythropoietic effect as seen in the increased erythrocytic parameters. However, further work would be needed to understand the mechanism behind the erythropoiesis so observed while caution should be taken against administration of high doses of the EE at 1000 mg/kg and above when used therapeutically.

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