Clinical observations and haematological changes following subchronic administration of methanolic leaf extract of *Crotalaria lachnosema* Stapf. (Fabaceae) in male Wistar rats

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**Abstract**

The aim of this study was to determine the clinical observations (Visually and via laboratory analysis) and haematological changes induced by subchronic administration of methanolic leaf extract of *Crotalaria lachnosema* (*C. lachnosema*) leaf (MLECL) on male Wistar rats. Four groups designated as Groups 1(control), 2, 3 and 4, of ten rats each, which were exposed to the extract in feed at dose levels of 0, 40, 200 and 1000 mg/kg for 42 days respectively. The clinical observations were recorded. At the end of the study period, the surviving rats were sacrificed following light ether anaesthesia and blood was collected from each rat via jugular venesection. The extract was found to cause impairment in both the body weight and relative organ weights of the exposed rats at termination of the experiment (P < 0.05) compared to the control group. The extract was found to affect the erythron only in group 2 with total red blood cell count (7.72 ± 0.25 × 10°/L), haemoglobin concentration (127.00 ± 2.66 g/L) and packed cell volume (48.44 ± 2.11 %) being significantly (P < 0.05 ) lower relative to those of the control (8.86 ± 0.32 × 10°/L, 150.80 ± 6.03 g/L and 48.44 ± 2.11 %, respectively). It was concluded from these studies that exposure to MLECL caused toxic effects in male Wistar rats (toxic effect like the decreased erythron; haematopoietic system is one of the most sensitive targets of toxic compounds, and thus considered an important index in pathophysiological status in man and animal). Human food or animal feed products contaminated with the plant *C. lachnosema*, even at very low concentrations, with the LD\(_{50}\) of 1300 mg/kg should be discouraged.

**Keywords**: *Crotalaria lachnosema*, Haematology, LD\(_{50}\), Toxicity, Weight changes

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**Introduction**

The genus *Crotalaria* belongs to the family Fabaceae (Leguminosae) and consists of about 700 species (Polhill, 1982; Lewis et al., 2005; Adema, 2006; Mabberley, 2008), distributed throughout the tropical and subtropical regions of the world with highest concentration in Africa. Toxicity of *Crotalaria* species, though highly documented in other parts of the world, is not recognized in Nigeria as potentially hazardous (Nuhu et al., 2009). *Crotalaria* poisoning can be either chronic or acute (Srinivasan and Liu, 2012). The foliage and seeds of many *Crotalaria* plants contain Pyrrolizidine alkaloids (PAs) (Maia et al., 2013) which poses potential hazard to grazing livestock. The *Crotalaria* plant occurs in Nigeria (Mattocks and Nwude, 1988), and it is found in damp sites along forest margins (Burkill, 1995). The plant is known as ‘fara bi rana’, ‘Akedimwo’, ‘Korupo’ and ‘Birijibe’ in Hausa, Igbo, Yoruba, and Fulani, respectively (Nuhu et al., 2009). *Crotalaria lachnosema* (*C. lachnosema*) Stapf. is a woody shrub about 1m to 2m high, it is tawny, covered with hairs all over and has orange streaked flowers as shown in plate I. In Nigeria, there are several *Crotalaria* species which are freely used in traditional medication and as animal feeds without any information or studies on their toxicological potentials, even though conditions which favours poisoning in livestock by this plants are prevalent (Adedapo et al., 2007). These conditions include periodic long annual dry seasons and drought, overgrazing, and nomadism, and the trekking of herds of cattle from Northern Nigeria to the South through unfamiliar territory.
voucher botanical number 1885 at the Herbarium section of the Department of Biological Science, Ahmadu Bello University, Zaria. The sample was air dried in the Laboratory to a constant weight and pulverized using a Laboratory milling machine (5 mm sieve mesh). The ground leaves were stored in a black polythene bags under room temperature.

**Plant extraction**
The extraction ((95% methanol in soxhlet apparatus for 12 hours of the plant material was conducted according to the method described by Mattocks & Nwude (1988).

**Phytochemical determination**
Identification of alkaloid and other phytoconstituents in the plant extract was done by qualitative analysis using appropriate reagents as described by Trease & Evans (1983) and Nash et al. (1992). The LD<sub>50</sub> value was 1300 mg/kg, while the doses used was the maximum tolerated dose (MTD) which was 1000 mg/kg and two lower doses; 1/5<sup>th</sup> of MTD and 1/25<sup>th</sup> of MTD, with the lowest dose being a predicted no effect level (Adaudi, 2005).

**Subchronic toxicity study**
The subchronic toxicity study was conducted for 6 weeks (42 days), as described by WHO (1978), Wester et al. (1986) and Barros et al. (2005). Four groups of male Wistar rats consisting of 10 rats per dose levels were used for the toxicity testing. The crude MLECL was added to the feed in a uniform mixture of selected doses from acute toxicity study as described by Adaudi (2005) and OECD (2010). The selected doses 40, 200 and 1000 (in mg) of extract per kilogram feed was fed to groups 2, 3 and 4 rats, respectively, while the standard feed formulation (which is the untreated control) was fed to group 1. The rats were weighed weekly throughout the study period, also the feed and water consumption were recorded for each group. The dose used was the maximum tolerated dose (MTD) and two lower doses, of 0.2 MTD and 0.04 MTD with the lowest dose being a predicted no effect level (Adaudi, 2005). The observed and recorded end points of the test included: feed intake, water consumption, weight changes, clinical signs, target organ toxicity and hematology.

**Evaluation of feed and water intake and body weight changes**
The feed intake and the body weight of rats were estimated using digital-weighing scale (QE – 400). A known amount of feed (X gram) was given to the rats, and the feed consumed (Y gram) was

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**Plate I: Crotalaria lachnosema** growing in farmland along Ahmadu Bello University streams in Zaria, Nigeria

Despite the reported potential hazards posed by the PAs containing plants worldwide, and in Nigeria, there is dearth of information on cases of the disease condition in Nigeria (Pieters & Vietinck, 1991; Omojokun, 2013). This may be due to lack of knowledge on the clinicopathological presentations of the disease (In domestic animals, syndromes such as sluggishness, weakness, loss of appetite, wasting, ascites, jaundice, photosensitization and behavioural abnormalities associated with PAs poisoning are related to hepatic insufficiency (Keeler et al., 1978).
calculated by subtracting the amount of the left over feed (Z gram) from the X gram as follows: Y gram = X − Z.

The water consumption was determined in a similar fashion using a calibrated (millilitres) rat drinker. Similarly, the body weight changes were determined. A container was placed on the weighing scale and set at zero reading. The rat was placed inside the container, allowed to stabilize, and the readings were taken (grams).

**Evaluation of hematological parameters**

At the end of the study period, the surviving rats were sacrificed following light ether anaesthesia and 1 ml of blood was collected from each rat via jugular venesection into heparinized sample bottles for determination of hematological parameters such as packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) and total leucocyte counts, using auto hematology analyzer (SHENZHEM MINDRAY; BC-3200) at the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

**Data analysis**

Data was expressed as mean ± standard deviation. The data was subjected to analysis of variance (ANOVA) followed by Tukey’s post hoc test for comparisons using Graphpad prism Version 4.0 for windows (Graphpad Software April 2003, San Diego, CA, USA). Values of p < 0.05 were considered significant. The data were presented in tables, figures and plates.

**Results**

**Test Plant Preparation, Extraction and Yield**

The weight of the ground leaves of *Crotalaria lachnosema* was 800 gram from a freshly collected sample of 2269 gram. The crude methanolic soxhlet extract obtained from this was 118.48 gram with a percentage yield of 14.81 %.

**Phytochemical qualitative determination**

The phytochemical qualitative analysis revealed that the crude methanolic leaf extract of *C. lachnosema* contained alkaloids (Mayer’s and Dragendorff’s tests), carbohydrates (Molisch’s test), cardiac glycosides (Kella-killiani test), flavonoids (Sodium hydroxide test), saponins (Frothing test), steroids and triterpenes (Lieberman-Burchards test), and tannins (Ferric chloride test).

**Clinical signs of toxicity**

Rats treated subchronically manifested signs of diarrhea and rough hair coat.

**Effect of crude MLECL on feed intake**

Figure I shows the result of feed consumption of rats exposed to 0, 40, 200, and 1000 mg extract of *C. lachnosema* per kg feed. There was significant (p<0.05) reductions in feed intake of group 2 (40 mg/kg) rats at week 5, when compared to the control group.

**Effect of crude MLECL on water intake**

Figure II shows the result of weekly oral water (milliliters) intake by male Wistar rats exposed to crude MLECL at 0, 40, 200, and 1000 mg extract per kg feed for 6 weeks. There were significant (p<0.05) reductions in the water intake recorded for all the treated groups at weeks 2 and 5.

**Effect of methanolic leaf extract of Crotalaria lachnosema (MLECL) on weekly body weight**

Figure IIIa shows the effect of exposure to 0, 40, 200, and 1000 mg/kg crude methanolic leaf extract of *Crotalaria lachnosema* (MLECL) on the mean weekly body weights of rats for 6 weeks. There were significant (p<0.05) reductions in the mean body weights of treated rats in groups 2 (40 mg/kg) during weeks 2, 3 and 5 of exposure. The rats treated at 200 mg/kg showed...
significant (p<0.05) reductions in the mean body weight at weeks 2 and 4. The rats treated at 1000 mg/kg (group 4) showed significant (p<0.05) reductions in the mean body weight at weeks 1 and 4.

**Effect of MLECL on the body weight for 6 weeks**

Figure IV shows the effect of rats exposed to: 0, 40, 200, and 1000 mg/kg crude MLECL on feed. The mean differences in body weights of rats for 6 weeks, caused significant (p<0.05) reductions in the body weights of treated rats with the highest reductions recorded for group 4 (1000 mg/kg).

**Effect of crude MLECL on relative organ weight ratio**

Figure V shows significant differences between control values and extracts of *C. lachnosema* exposed for 6 weeks periods. There were significant (p<0.05) reduction in the weights of liver, kidneys, and heart of the treated groups, with the highest reduction in the relative organ body weight ratio recorded for group 2 (40 mg/kg) rats for both liver and kidneys and group 4(1000 mg/kg) rats for the heart.

**Effect of crude MLECL on haematological parameters**

Table 1 shows non-significant (p>0.05) differences in White blood cell (WBC), mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelet (PLT) values in all the treated groups as compared to the control values at the end of 6 weeks toxicity study. However, there were significant (p<0.05) reductions in RBC, Hb, and PCV indices for group 2 (40mg/kg) rats as compared to the control values.

**Discussion**

Phytotoxicological studies have always been considered vital prior to plant usage for foods, cosmetic and medicine; bearing in mind that plants are often consumed indiscriminately without resort to the potential side effects which could vary from mild, moderate, and severe to life threatening (WHO, 2000). The present toxicity studies of absolute methanolic leaf extract of *Crotalaria lachnosema* Stap. (MLECL) were evaluated in male Wistar rats using clinical signs, weight changes and haematological parameters.
Similarly, rats exposed to 200 mg MLECL / kg feed also had a significant decrease in body weight in week 2, and an increase in week 3. There were no significant decrease in body weights for rats exposed to 1000 mg MLECL / kg feed in weeks 2 and 3. In weeks 4, 5 and 6, the body weights of the treated rats did not change significantly compared to that of the control. The observations that 40 mg and 200mg MLECL caused pronounced decrease in body weights when compared to the 1000 mg dosed group, could be due to a nucleotoxic effects of the low dose alkylating agent pyrrolizidine alkaloid content of the MLECL. In alkylating agents like plants containing PAs, at high dose levels the repair processes would have started or the subject would have died. The increase in body weight in week 3 observed could be explained by repair process on DNA nucleic damage by the third week.

Similarly, the differences in feed and water consumption in week 2 at the dose of 40 and 200 mg/kg respectively may probably be related in part to differences in hepatic metabolism (Conney et al., 1965). Food consumption per unit body weight decreases, as the adult animal gets older (WHO, 1978). Body weight changes serves as a sensitive indication of the general health status of test agents (Adaudi, 2005).

The subchronic oral exposure of rats to MLECL over the period of 6 weeks had shown significant decreases in the body weights of MLECL exposed groups of rats. This outcome suggests that the extract could be having weight losing effect as also observed with significant (p<0.05) decreases in relative weights of liver, kidneys and heart in the extract-exposed groups. Pyrrolizidine alkaloids are known to exhibit radiomimetic, nucleotoxic, cytotoxic and carcinogenic effects. The application of dose response data obtained for such agents on studies of their effects on individual cells and also on whole animals and man do not yet have an adequate explanation for their modes of action (BEIR, 1980). PAs are alkylating agent hence manifesting their toxic effects even at very low level. As at present, only theoretical approaches have been used primarily to develop some understanding of the effects of low doses of radiation and cytotoxic alkylating agents. In the present studies, the low level doses effects of C.lachnosema Stapf. extract on

Figure IV: Treatment of Wistar rats with MLECL causes significant reduction (p<0.05) with resultant highest reduction at 1000 mg/kg of extract

Figure V: Exposure of Wistar rats to MLECL caused significant decreases (p<0.05) in relative organ body weight ratio of liver, kidneys and heart, with highest reduction seen at dose level of 40 mg/kg of extract
Table 1: Effects of crude MLECL on Mean ± SD haematological parameters of male Wistar rats exposed to crude MLECL, n=5

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>40mg/kg</th>
<th>200mg/kg</th>
<th>1000mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10⁹/L)</td>
<td>13.84±1.90</td>
<td>11.52±3.61</td>
<td>10.70±3.37</td>
<td>10.80±3.35</td>
</tr>
<tr>
<td>RBC (×10¹²/L)</td>
<td>8.86±0.72 ( ^a )</td>
<td>7.72±0.57 ( ^b )</td>
<td>8.23±0.26</td>
<td>8.57±0.56</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>150.80±13.48 ( ^a )</td>
<td>127.00±7.23 ( ^b )</td>
<td>136.00±7.11</td>
<td>139.60±5.96</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>48.44±4.72 ( ^a )</td>
<td>40.44±2.48 ( ^b )</td>
<td>44.06±1.69</td>
<td>44.40±2.54</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>54.70±1.16</td>
<td>52.54±2.64</td>
<td>53.66±2.44</td>
<td>51.94±3.23</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>16.96±0.30</td>
<td>16.42±0.57</td>
<td>16.48±0.81</td>
<td>16.28±1.13</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>311.00±5.79</td>
<td>313.80±6.06</td>
<td>308.20±10.66</td>
<td>314.20±6.76</td>
</tr>
<tr>
<td>PLT (×10⁹/L)</td>
<td>575.80±43.58</td>
<td>623.60±173.80</td>
<td>614.80±173.80</td>
<td>614.80±94.89</td>
</tr>
</tbody>
</table>

Means with the similar superscripts (a, b) within a row are not significantly different (p > 0.05); Tukey’s post hoc test.

the target organs; liver and kidneys, fits into this anomalous effects of radiomimetic agents (BEIR, 1980). The functional expression of observation or data relevant to risk at very low doses, express upward positive effect at low doses and downward negative effect at high doses (that is sigmoidal) for cell-killing effect (BEIR, 1980). This could also be attributed to perhaps organs damage by the toxins resulting in inflammatory processes and fibrosis, leading to eventual shrinkage and weight loss. However literature has shown that; typically, pyrrolizidine alkaloids intoxication is accompanied by hepatic fibrosis, biliary proliferation, and to some circumstances nodular regeneration of parenchyma (McGavin and Zachary, 2007). In this study, rats fed with the extract; a pyrrolizidine alkaloid in Crotalaria species causes hepatotoxicity, and increased reduction in body weight. Conversely, literatures have shown that, rats fed with monocrotaline, a pyrrolizidine alkaloid in Crotalaria species causes hepatotoxicity, and increased reduction in body weight (Mingatto et al., 2008). Thus, the presence of pyrrolizidine alkaloids in MLECL alone and/or in combination with the clinical signs like initial restlessness, diarrhea, and later weakness following feed exposure of the extract could justify for the weight losing effect of MLECL.

The hematopoietic system is one of the most sensitive targets of toxic compounds and it is thus considered an important index of pathophysiological status in man and animals (Feldman et al., 2000; Adeneye et al., 2006). Also, hematological parameters provide vital information regarding the status of bone marrow activity and intravascular effects such as hemolysis and anaemia (Voigt, 2000). In conclusion, subchronic exposure induced deleterious effects on body weights and haematological parameters. The observation that low level of MLECL is very toxic at low level is responsible for rating PAs in C. lachnosema as a very hazardous plant toxin known to man and animals. The probable daily intake of pyrrolizidine alkaloids contaminated food and feed should be investigated. Herbal medicinal products containing toxic pyrrolizidine alkaloids (even at lower levels) should be regarded as very hazardous.

References


