Gongronema latifolium Crude Leaf Extract Reverses Alterations in Haematological Indices and Weight-loss in Diabetic Rats

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ABSTRACT

The effect of Gongronema latifolium crude leaf extract (GL) on weight-loss, growth-depression and haematotoxicity was assessed in male diabetic rats. Normal and diabetic rats were gavaged with 200, 300 and 400 mg kg^{-1} b.wt. of GL day^{-1} for two weeks. The levels of haemoglobin (Hb), haematocrit or Packed Cell Volume (PCV), Red Blood Cells (RBC), white blood cells (WBC) and Platelet Count (PC) in Diabetic Rats (DR) were significantly higher, (p<0.05 for PCV%; p<0.001 for Hb, RBC, WBC and PC), compared, respectively to the levels obtained for the Non-Diabetic Rats (NDR). However, GL at dose levels of 200 and 400 mg kg^{-1} b.wt., respectively, caused significant decrease in the level of WBC in diabetic treated rats when compared to control. At 300 and 400 mg kg^{-1} b.wt., PCV% and WBC levels in NDR were significantly different (p<0.05 for both levels at 300 mg kg^{-1} b.wt.; p<0.05 and p<0.001 for PCV% and WBC at 400 mg kg^{-1} b.wt.) compared to their controls. The results also showed a significant decrease (p<0.001) in weight and growth-rate of diabetic test groups when compared to non-diabetic test groups following increased treatment with doses of GL. These observations indicated that diabetic condition produces alterations in haematological indices, weight-loss and growth-depression which may be reversed by treatment with GL at 400 mg kg^{-1} b.wt. in rat model. The significant (p<0.001) increase in WBC counts in alloxan-induced diabetic rats may likely be due to alloxan poisoning, which is in line with the normal physiological response following the perception of an insult to the body defense mechanisms. The results of this study suggest that GL may be used to reverse, prevent or reduce weight-loss, growth-depression and haematotoxicity in diabetic subjects.

Key words: Alloxan-induced diabetes, Gongronema latifolium, haematological indices, weight-loss, growth-depression

INTRODUCTION

Gongronema latifolium Benth et Hook (Asclepiadaceae) is an herbaceous shrub, with flowers usually yellow and the stem yields characteristic milky exudates. It is commonly grown in Nigeria and is locally called Utasi by the Efiks, Ibibios and Quas; utazi by the Igbos and Arokeke by the Yorubas. The Efiks and Quas in Cross River State of Nigeria use Gongronema latifolium leaf extract in the treatment of malaria, laxative, diabetes and hypertension. The studies of herbal
medicinal studies and the use of plants: leaves, stem, roots, seed and even the latex for human benefits is a age long event (Edet et al., 1985; Okafor et al., 1994; Hoareau and Dasilva, 1999; Ananthan et al., 2003; Viana et al., 2004; Aritajat et al., 2004; Sy et al., 2004; Akpanabiatu et al., 2005a, b). Though Gongronema latifolium is in very common usage, the volume of information found in literature on lipid peroxidative activity (Nwanjo et al., 2006); its chemical composition and antibacterial activity (Elleyini, 2007) is very limited. Alloxan is widely used to induce experimental diabetes in animals. The cytotoxic action of alloxan is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide and thereafter cause a massive release of cytosolic calcium concentration, which causes rapid necrotic destruction of pancreatic β cells (Szkudelski, 2001) hence diabetes and its attendant haematotoxicity to the vascular system. Before the advent of insulin therapy in 1922, starvation diets and traditional plant treatments were the cornerstone of anti-diabetic therapies (Day and Bailey, 1998). The renewed and increasing interest in traditional antidiabetic plants like Gongronema latifolium may be due to several contributing factors including changes in the epidemiology of diabetes and attitudes to its control (Day, 1998). As illustrated by Gray and Flatt (1998), there is a re-gathering of scientific validation for the use of certain anti-diabetic plants which has encouraged botanical exploration in the quest for new anti-diabetic drugs. In addition, there is the wider appeal of natural dietary adjuncts as functional foods through which patients can gain added benefits to the management of their disease (Swanson-Flatt et al., 1991). Administration of Gongronema latifolium to animals is reported to reduce reaction oxygen species concentration, modify liver and renal oxidative stress, as well as cause antihyperglycaemic effects (Ugochukwu and Cobourne, 2003; Ugochukwu and Babady, 2003; Ugochukwu et al., 2003). Hence this study is aimed at assessing the effect of water soluble fraction of ethanol extract of the leaf on haematotoxicity and weight-loss associated with alloxan diabetogenesis.

MATERIALS AND METHODS
Preparation of plant materials: Fresh leaves of Gongronema latifolium were obtained from Akpabuyo local Government of Cross River State, Nigeria in the month of June, 2005. The leaf was identified and authenticated by the Botanist in the Department of Botany, University of Calabar, Nigeria. The leaves were picked, sun-dried initially and then oven-dried in a Plus 11 oven and crushed using laboratory KENWOOD blender (Kenwood Electric, KENWOOD LTD, England). The ground leaf powder was stored in a glass bottle with a plastic screw cap and kept in a refrigerator (4°C).

Animals and animal care: Fifty-six mature male rats of the Wistar strain were obtained from the animal stock in the Department of Biochemistry, University of Calabar, Nigeria. The animals were 90 days old and weighed between 180 and 300 g each and were kept in a well-ventilated animal house. The fifty-six male rats were divided into 2 major (diabetic and non-diabetic) groups of 28 rats each and 8 (4 diabetic and 4 non-diabetic) sub-groups of 7 rats each, consisting of one group of control and three test groups. The animals in group 1 were designated control and received distilled water only (1.5 mL b.wt. of rat) as placebo for two weeks, while the test groups 2, 3 and were gavaged 1, 1.5 and 2 mL, corresponding to 200, 300 and 400 mg kg⁻¹ b.wt. of the extract. Both feed and water were given ad libitum. Diabetes was induced by a single dose (ip) of 150 mg kg⁻¹ b.wt. of alloxan to the rats after a 24 h fast (Esmerino et al., 1998). Diabetes
was confirmed after 7 days, in rats which showed Fasting Blood Glucose (FBG) levels of >300 mg dL⁻¹. The extract was administered once daily or 14 days. The animal house temperature fluctuated around 25-30°C. Normal rat chow was used in feeding the animals during the period of experiment. The Biochemistry department research/seminar committee approved the animal experimental protocols prior to implementation.

**Chemicals:** All reagents and chemicals used in this work were of analytical grade. Randox reagents and instrument kits (UK) were used for the determination of serum aspartate and alanine amino transferase (AST and ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LD) respectively. Gamma glutamyl transferase activity was determined using Biolabo SA (France) reagent kit. Ninety six percent ethanol (BDH, England) diluted to 80% was used in preparing the crude extract of Gongronema latifolium leaves.

**Preparation of plant extracts:** Bulk ethanol extraction of dried, ground and powdered leaf of Gongronema latifolium was under taken by regular shaking in bottles for 12 h. The extract was thereafter concentrated using a rotary evaporator (Labo Rotavap 3000 Resona) and a vacuum pump (Edwards model) and received into a round bottomed flask from where it was allowed to evaporate under pressure at 50°C. The ethanol vapour was channeled through an in-built condenser under pressure which enabled ethanol to be condensed. A trap between the vacuum pump and the rotary evaporator was provided to prevent the entry of the vapour into the vacuum pump in case of reverse suction. Evaporation was kept at 60°C in order to prevent denaturation of the bioactive ingredients inherent in the crude extract. The extract was dried in an oven at 60°C to remove the ethanol and kept in a beaker, stored in a refrigerator until required for use. The concentration of the extract was determined by drying a known volume and measuring the dry weight. sacrificed using chloroform fumes for anaesthesia.

**Collection and analysis of blood:** Blood samples were collected by cardiac puncture EDTA tubes. Whole blood samples were stored in the refrigerator at 4°C until required while haematological analyses were carried out within 24 h of sample collection. The whole blood specimens were used for the determination of the levels of Hb, haematocrit, red blood, white blood and platelet counts. Haemoglobin and haematocrit levels were determined by the methods described by Alexander and Griffiths (1993). All absorbance readings for haemoglobin determination was taken using Optima SP-300, spectrophotometer. The total red and white blood cells were counted by the microscopic visual identification methods described by Dacie and Lewis (1975). Platelet count was carried on the whole blood by diluting the blood with Rees and Ecker solution as was done for the RBC count and visual observation of the platelet under the microscope.

**Determination of weight increase and growth rate:** Total body weight of diabetic and non-diabetic and diabetic male Wistar albino rat was measured using a digital chemical balance, before and after the experimental period and recorded as initial (IBW) and Final Body Weight (FBW), respectively. The mean body weight for each group of rats was measured from the total weights. Weight changes were expressed as % weight increase and % growth rate respectively, where % weight increase was obtained as follows:

\[
\frac{FBW-IBW}{IBW} \times 100
\]
% growth rate was obtained as follows:

\[
\frac{FBW-IBW}{N} \times 100
\]

where, N represented the experimental period.

**Statistical analysis:** Statistical analysis of data was determined with the use of standard student’s t-test method on \(p<0.05\) was regarded as significant. The group data were regarded as Mean±standard deviation (SD) of seven (7) determinations.

**RESULTS**

The results of this study show that the levels of Hb, PCV%, RBC, WBC and PC in diabetic rats (Table 2) were significantly higher \((p<0.001\) for Hb, RBC, WBC and PC; \(p<0.005\) for PCV%) when compared to the levels obtained for non-diabetic rats (Table 1). The levels of Hb, PCV%, RBC and PC in respective treated rats were not significantly different \((p>0.05)\) when compared to their respective controls. However, at dose levels of 200 and 400 mg kg\(^{-1}\) b.wt., respectively, the value of WBC in diabetic rats (Table 2) decreased significantly \((p<0.005\) for 200 mg kg\(^{-1}\) and \(p<0.05\) for 300 mg kg\(^{-1}\) b.wt.) when compared to the diabetic control. Similarly, at dose levels of 200 and 400 mg kg\(^{-1}\) b.wt., respectively, the level of WBC in diabetic rats (Table 2) decreased significantly \((p<0.05)\) when compared to the non-diabetic control. The level of PC in diabetic rats was significantly lower \((p<0.05)\) when compared to the non-diabetic control. Increased treatment of diabetic rats with doses of the leaf showed non-dose dependent decreases in Hb, PCV%, RBC and WBC values respectively while PC showed a non-dose dependent increase in value. At a test dose of 300 and 400 mg kg\(^{-1}\) b.wt. of the leaf extract, PCV% and WBC values in non-diabetic rats

![Image](https://example.com/image1)

Table 1: Some haematological indices of non-diabetic rats

<table>
<thead>
<tr>
<th>Indices</th>
<th>Group 1 (Control)</th>
<th>Group 2 200 mg kg(^{-1}) b.wt.</th>
<th>Group 3 300 mg kg(^{-1}) b.wt.</th>
<th>Group 4 400 mg kg(^{-1}) b.wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g dL(^{-1}))</td>
<td>7.97±0.09</td>
<td>8.64±0.62</td>
<td>9.47±0.51</td>
<td>9.60±0.59</td>
</tr>
<tr>
<td>Packed cell volume (PCV%)</td>
<td>49.00±3.37</td>
<td>49.57±1.49</td>
<td>43.86±1.90(^b)</td>
<td>43.14±3.34(^b)</td>
</tr>
<tr>
<td>Red Blood Cells (RBC) (x10(^6) mm(^{-3}))</td>
<td>5.62±0.14</td>
<td>6.15±0.20</td>
<td>5.86±0.19</td>
<td>5.56±0.38</td>
</tr>
<tr>
<td>White Blood Cells (WBC) (x10(^3) mm(^{-3}))</td>
<td>5.95±0.60</td>
<td>5.26±4.29</td>
<td>4.30±0.60(^a),160.73(^b)</td>
<td>4.12±3.39(^a),143.43(^b)</td>
</tr>
<tr>
<td>Platelet Count (PC) (x10(^9))</td>
<td>3.86±0.59</td>
<td>4.46±0.19</td>
<td>3.45±0.20</td>
<td>3.38±0.14</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±standard deviation of 7 determinations. \(^a\): Significant difference b/w non-diabetic test group and non-diabetic control, \(I = p<0.05\) (level of significance), \(II = p<0.001\) (level of significance)

Table 2: Some haematological indices of diabetic rats

<table>
<thead>
<tr>
<th>Indices</th>
<th>Group 1 (Control)</th>
<th>Group 2 200 mg kg(^{-1}) b.wt.</th>
<th>Group 3 300 mg kg(^{-1}) b.wt.</th>
<th>Group 4 400 mg kg(^{-1}) b.wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g dL(^{-1}))</td>
<td>3.11±0.79(^a)</td>
<td>9.74±0.57(^d)</td>
<td>9.09±0.68(^a)</td>
<td>9.61±0.41(^a)</td>
</tr>
<tr>
<td>Packed cell volume (PCV%)</td>
<td>47.43±2.37(^a)</td>
<td>49.14±5.30(^a)</td>
<td>49.86±7.34(^a)</td>
<td>46.86±2.19(^a)</td>
</tr>
<tr>
<td>Red Blood Cells (RBC) (x10(^6) mm(^{-3}))</td>
<td>5.97±0.23(^a)</td>
<td>5.88±0.23(^a)</td>
<td>6.30±0.45(^a)</td>
<td>5.91±0.25(^a)</td>
</tr>
<tr>
<td>White Blood Cells (WBC) (x10(^3) mm(^{-3}))</td>
<td>4.942±0.71.76(^a)</td>
<td>4.750.00±87.60(^a),(^c)</td>
<td>5.150.07±87.32(^a)</td>
<td>4.857.14±31.34(^a),(^c)</td>
</tr>
<tr>
<td>Platelet Count (PC) (x10(^9))</td>
<td>2.70±1.01(^a)</td>
<td>3.48±0.49(^a)</td>
<td>4.46±0.34(^a)</td>
<td>3.64±0.22(^a)</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±standard deviation of 7 determinations. \(^a\): Significant difference b/w diabetic test group and non-diabetic control group, \(^c\): Significant difference b/w diabetic test group and diabetic control group, \(I = p<0.05\) (level of significance), \(II = p<0.001\) (level of significance)
showed significant decrease (p<0.05 for PCV%; p<0.05 p<0.001 for WBC) when compared to non-diabetic control. Increased treatment of non-diabetic rats with doses of the leaf showed dose-dependent decreases in PCV%, RBC, WBC and PC values, respectively while Hb alone showed a dose-dependent increase in value. The results also showed significant increases in weight loss and growth depression in diabetic rats (Table 2) when compared to the non-diabetic rats (Table 1). There was a significant decrease (p<0.001) in weight changes in diabetic control rats (Table 2) when compared to non-diabetic control rats.

DISCUSSION

The total amount of drug entering the extracellular fluid depends on the balance between that entering into and that leaving the compartment. The plasma concentration depends on the volume of fluid. Thus drugs produce clinical and biochemical effects, e.g., carbamazepine may cause hyponatraemia; primidone may partially metabolize to phenobarbital. Some drugs may bind to plasma albumins. Digoxin- a cardiac anti-arrhythmic drug is used in congestive cardiac failure. Plasma digoxin concentrations even within the therapeutic range are very difficult to interpret in the presence of conditions that may alter receptor sensitivity such as hypokalaemia, hypocalcaemia or hypomagnesaemia, hypoxia or acidosis and hypothyroidism which are associated with blood toxicity. Phenytoin is used in the treatment of partial and generalized conditions of tonic-clonic seizures by inhibiting voltage-gated sodium channels. However phenytoin in plasma inhibits saturation kinetics. Thus a small dose of phenytoin may result in a large increase in plasma concentration and could be associated with haematotoxicity. Similarly, plasma concentrations of anticoagulants in children and pregnant women if not monitored, are more likely to develop haematotoxicity. Some xenobiotics in blood are converted to reactive intermediates which react covalently with macromolecules in blood provoking different types of toxicity and poisoning. It was observed that the level of platelet counts changed significantly. Some drugs have been reported not to have adversely influenced some of the haematological and biochemical indices in experimental animals (Maurice and Pearce, 1987; Udosen and Ekpo, 1992; Akpanabia, 2001).

Garlic fed rats were reported to have resulted in a steady fall in Hb and PCV% level indicating that some plant phytochemical principles are capable of increasing or decreasing some haematological indices. The insignificant differences in Hb concentrations observed in this study was indicative of the fact that gavaging the experimental rats with doses of Gongronema latifolium crude leaf extract in diabetic and non-diabetic rats did not affect the erythropoietic activity or haem synthesis negatively. It could also mean that the effect of the extract was not potent enough to alter the level of erythropoietin, the hormone required for increased stem cell differentiation into erythrocyte precursors. The non-significant increase in the level of RBC count reported in this study may follow the same suggested mechanism of increased erythropoiesis. This could also suggest that there was a mild synthesis of RBC in the bone due possibly to the influence of the leaf extract components on bone marrow activity. Earlier studies reported decreases in haematological indices in experimental animals exposed to different chemical agents or extract-based active principles. Since the same/ closely related pattern of haematological indices was observed for Hb, PCV% and RBC in both diabetic and non-diabetic rats, this may rule out megaloblastic anaemia and still implicate increase in erythropoietic mechanism. Platelets play a key role in blood clotting and the
insignificant increase in platelet counts in this study strongly indicates that the active principles present in the leaf extract may not have been potent enough to stimulate platelet synthesis. The clotting capacity of animals in this study may have been unaffected by the platelet count when compared to the control. Akpanabiatu (2001) reported increase in platelet count in normal rats treated with *Nuclea latifolia* extract- an antihypertensive herb. The significant increase in Hb concentration observed in diabetic rats when compared to non-diabetic rats might result due to increased haemolysis of RBC. Hb concentration was reported to be moderately increased in haemolytic anaemia and in any other clinical condition associated with rapid intravascular haemolysis and haemoglobinuria (Bolarin, 1997). Within the diabetic group of rats, there was a dose-dependent decrease in Hb level following increased intragastric treatment with the extract. This observation may be an attempt by the leaf extract to stop haemolysis of RBC caused by alloxan.

The significant increase in WBC count in diabetic rats when compared to the control may be due to alloxan poisoning. This is in line with normal physiological response following the perception of an assault by the body defense mechanisms. The observed significant decrease in serum WBC count in diabetic rats at a test dose of 200 mg kg\(^{-1}\) b.wt. when compared to the control gave credence to the efficacy of the leaf extract at that dose to control and contain some haematological abuse in the body defense system. Alloxan diabetogenesis may cause perturbation on growth or differentiation inducers involved in erythropoiesis. Similarly, alloxan metabolites on further metabolism in rats body may produce reactive species which may interact with body tissues to exhibit their toxic or hazardous effects in the blood. This could bring down body weight and enhance growth depression in experimental rats. According to Synder and Hadli (1996), haematotoxic effect associated with toxic substances, such as benzene involves both bone marrow depression and leukaemogenesis, caused by damage to multiple classes of haematopoietic cells and a variety of haematopoietic functions. The molecular events in cell growth cause or influence changes in total body weight and growth rate which bring about complication and involve an increasing array of molecules and intracellular pathways. According to Cotran et al. (1999), aberrations in such pathways may the uncontrolled growth such as cancer and abnormal responses to a variety of diseases. According to Murray et al. (2006), growth stimulation and inhibition proceed through a variety of intracellular signaling systems. From the foregoing it is apparent that the significant decrease in weight-loss and growth depression, positive improvements and alleviation of some haematotoxicity due to *Gongronema latifolium* crude leaf extract in diabetic rats exposed to alloxan diabetogenesis are hereby reported in this study. Although, further study to identify the specific mechanism(s) responsible for some cases whereby the recorded results in this study slightly differ from those of the previous studies is in progress, the differences in the quality and quantity of the chemical constituents of the plant extract may be suggested to be implicated.

**CONCLUSION**

The result of this study indicate that the soluble fraction of ethanol extract of *Gongronema latifolium* leaf could be used to alleviate weight loss, growth depression and haematotoxicity in alloxan-induced diabetes. Further work is on-going in our laboratory to evaluate the histological effects with some biochemical correlations in diabetic rats treated with *Gongronema latifolium* crude leaf extract.
REFERENCES


