AMELIORATIVE EFFECTS OF METHANOL TUBER EXTRACT OF CHLOROPHYTUM ALISMIFOLIUM BAKER ON HYPERGLYCAEMIA-INDUCED HAEMATOLOGICAL AND HEPATO-RENAL ALTERATIONS IN RATS

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ABSTRACT

Diabetes mellitus is a metabolic disorder associated with debilitating complications which affect the blood and other organs of the body including the liver and kidney. The tubers of the plant Chlorophytum alismifolium are widely used in herbal medicine in the management of diabetes mellitus. This study was aimed at evaluating the ameliorative effects of methanol tuber extract of C. alismifolium on hyperglycaemia-induced haematological and hepato-renal alterations in rats. Experimental hyperglycaemia was induced following the administration of a single dose of streptozotocin (50 mg/kg) in rats. The study was carried out on normal and hyperglycaemic rats for 28 days using six groups of 6 rats each: group I (normal control, received normal saline 1 ml/kg), group II (hyperglycaemic control), groups III, IV and V (test groups administered C. alismifolium extract at doses of 150, 300 and 600 mg/kg p.o. respectively) and group VI (Standard, administered 10 mg/kg glimepiride). Blood samples were collected from the jugular vein for haematological, hepatic and renal indices examination. C. alismifolium extract at all the doses tested increased the levels of the red blood cells, pack cell volume and platelets while the white blood cells counts were reduced. The extract significantly \( p<0.05 \) reduced the lymphocyte counts at the doses tested compared to the hyperglycaemic group. There was no significant difference \( p>0.05 \) observed in the levels of liver enzymes. The extract at all the doses tested significantly \( p<0.001 \) reduced the sodium levels. The urea levels were significantly \( p<0.001 \) reduced at 300 mg/kg. In conclusion, the results obtained showed that the methanol tuber extract of Chlorophytum alismifolium ameliorated some haematological and hepato-renal alterations associated with hyperglycaemia in rats.

Keywords: Chlorophytum alismifolium, Diabetes mellitus, Hyperglycaemia, Renal function, Liver function

INTRODUCTION

Diabetes mellitus is ranked seventh among the leading causes of death globally and it is considered third when its complications are taken into account (Trivedi et al., 2004). Diabetes can result in a host of complications that can affect nearly all organs in the body including the liver and kidney (Barathmanikanth et al., 2010). There are reports of a wide range of diseases associated with diabetes mellitus such as diabetic nephropathy and liver diseases (Leeds et al., 2009; Lee et al., 2014). Similarly, oral hypoglycaemic agents are...
associated with serious side effects which affect the blood, liver and kidney (Hellmuth et al., 2000). These shortcomings have led to the increased patronage of herbal medicine.

The use of natural products from herbs forms an important component of the health care delivery system in African countries (Cragg and Newman, 2013). Herbal products are extensively used in the management of diabetes mellitus and recently there has been a resurgence of interest in medicinal plants with hypoglycemic potential (Mamun-or-Rashid et al., 2014). One of such plants widely used in Northern Nigeria in the management of diabetes mellitus is Chlorophytum alismifolium (Abubakar et al., 2016; Abubakar et al., 2017).

Chlorophytum alismifolium Baker (Liliaceae) is a short stem herb with tuberous root stocks and white flowers found around stony sites in forest streams (Burkill, 1995) commonly known as Alimsa-ground lily or Morton. The vernacular names include “Rogon Makwarwa” (Hausa) and “Cigorodi” (Fulfulde). The tubers are used in folk medicine for the management of diabetes mellitus by the Fulani’s of North-eastern Nigeria and also in the management of pains and inflammation (Abubakar et al., 2016). Previous reports had shown that the methanol tuber extract of Chlorophytum alismifolium possessed antihyperglycaemic activity in streptozotocin (STZ)-induced hyperglycaemic rats (Abubakar et al., 2017). To the best of our knowledge, there is no report on the effects of the extract on haematological, hepatic and renal parameters in hyperglycaemic rats. Therefore, the aim of this study is to ascertain the ameliorative effects of Chlorophytum alismifolium extract (CAE) on hyperglycaemia-induced haematological and hepato-renal alterations in rats.

MATERIALS AND METHODS

Drugs and chemicals
Streptozotocin (MP Biomedicals M 3219k, France), Glimipiride (Sanofi Aventis, D-65926 Frankfurt, Germany), 10% Dextrose (Dana Pharmaceuticals, Nigeria), Normal saline (Dana Pharmaceuticals, Nigeria)

Experimental animals
Male Wistar rats weighing 150-200 g were used for the studies. The rats were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were fed with standard animal feed and water ad libitum, and maintained under standard laboratory conditions. All experimental protocols were approved by the Ahmadu Bello University Animal Ethics Committee (Protocol number: DAC/IW-OT/212-15). The study was carried out according to the regulations governing the care and use of experimental animals as contained in “Principles of laboratory animal care” published by the National Institute of Health (NIH Publication No. 85-23, revised, 1996).

Collection and identification of plant material
The whole plant of Chlorophytum alismifolium was collected from Tudun Fulani River in July, 2014 in Toro Local Government Area of Bauchi State, North-Eastern Nigeria. The botanical identification and authentication was done by Musa Muhammed of the Herbarium Unit of the Department Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen number of 6785 was issued for future reference.

Preparation of plant extract
The plant material was washed, size-reduced and then dried under shade after which it was pulverized into coarse powder. About 1 kg of the powdered tubers was then
extracted with 90% methanol for 72 h using a soxhlet extractor. The extract was then concentrated to dryness over a water bath maintained at 45 ºC. The percentage yield of the extract was calculated and later stored in a desiccator. The extract was reconstituted in distilled water at appropriate concentrations prior to the experiment.

**Preliminary phytochemical studies**
Preliminary phytochemical screening of the methanol tuber extract of *Chlorophytum alismifolium* was carried out using standard screening tests (Evans, 2002; Sofowora, 2008).

**Acute toxicity studies**
The method described by Lorke (1983) was employed in the determination of the oral median lethal dose (LD₅₀). In phase one, three groups of three rats were administered widely differing doses of CAE (10, 100 and 1000 mg/kg) respectively and were observed for signs of toxicity and mortality for 24 h. In the second phase, 3 rats were administered 1600, 2900 and 5000 mg/kg of the extract (based on the outcome of the first phase) and then observed for signs of toxicity and mortality for 24 hrs. The LD₅₀ was calculated as the geometric mean of the lowest lethal dose and highest non-lethal dose.

**Experimental induction of hyperglycaemia**
Experimental hyperglycaemia was induced using the method described by Virendra *et al.* (2011). Streptozotocin (STZ) was dissolved in ice cold citrate buffer (pH 4.5) immediately before use. The solution was injected intraperitoneally at the dose of 50 mg/kg into rats fasted for 12 hours. The rats were given 10% glucose solution for 24 hours to prevent mortality due to initial hypoglycaemia caused by STZ. The animals were given food and water and then observed over a period of 72 hours for development of hyperglycaemia. The determination of blood glucose concentration was done using test strips and glucometer (Accu-check Active, Roche, Germany), which follows the glucose oxidase principles (Beach and Turner, 1958). The animals with glucose level above 200 mg/dL were considered hyperglycaemic and selected for further study (Liu *et al.*, 2008).

**Experimental design**
The studies were conducted on six groups of six rats each (n=6). The first group of rats were normal control; while the STZ-induced hyperglycaemic rats were assigned accordingly into the remaining five groups. Group I rats (normal control) were administered normal saline (1 ml/kg), group II rats served as hyperglycaemic control and were also administered normal saline (1 ml/kg). Groups III, IV and V were hyperglycaemic rats treated with CAE at doses of 150, 300 and 600 mg/kg respectively, while group VI hyperglycaemic rats served as positive control and were treated with glimepiride (10 mg/kg). The normal saline, graded doses of CAE and glimepiride were administered orally and daily for 28 days.

**Haematological evaluation**
At the end of the 28th day, the rats were sacrificed and blood samples were collected from the jugular vein into sample bottles containing EDTA as anticoagulant and evaluated for haematological parameters viz: Red blood cells count (RBC), white blood cell count (WBC), pack cell volume (PCV), lymphocytes (LYMP)and Platelets (PLT) using an automated haemotogy machine (Cell-Dyn, Abbott, USA).
Evaluation of liver enzymes, renal indices and electrolytes
Another portion of blood was collected into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 min. The serum thus obtained were investigated to determine the effect of the extract on liver enzymes: aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TB) using a photoelectric colorimeter (AC-115 Optima, Japan). The effect of the extract on renal biomarkers such as urea and creatinine, and electrolytes (sodium, potassium, chloride and bicarbonate) were also determined using Hitachi 902 analyzer.

Statistical analysis
Data obtained were statistically analyzed using Statistical Package for Social Science (SPSS) Version 20. Differences between means were analyzed by One Way Analysis of Variance (ANOVA) followed by Bonferroni post hoc test. Values of \( p < 0.05 \) were considered statistically significant. The results were expressed as Mean ± S.E.M. and were presented in tables.

RESULTS

Extractive value and phytochemical constituents
The percentage yield of methanol extract of \textit{C. alismifolium} was 5.16 \%\textsubscript{w/w}. The preliminary phytochemical test revealed the presence of carbohydrates, saponins, flavonoids, glycosides, cardiac glycosides, alkaloids and triterpenes (Table 1).

Table 1: Phytochemical components of \textit{Chlorophytum alismifolium} extract

<table>
<thead>
<tr>
<th>Test</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>–</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>–</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>–</td>
</tr>
</tbody>
</table>

Key: Absent – , Present +

LD\textsubscript{50} determination
The oral LD\textsubscript{50} of the crude methanol extract of \textit{C. alismifolium} was estimated to be greater than 5000 mg/kg in rats and no adverse symptoms or death was recorded.

Effects of 28-day daily administration of methanol tuber extract of \textit{Chlorophytum alismifolium} on haematological parameters of streptozotocin-induced hyperglycaemic rats
Administration of streptozotocin produced elevation in the levels of WBC and lymphocyte counts. The lymphocyte count was significantly \( p<0.05 \) increased when compared to the normal control. Administration of \textit{C. alismifolium} extract at all the doses tested increased the levels of RBC, PCV and platelets though not statistically significant \( p>0.05 \), while the WBC levels were reduced. However, a significant \( p<0.05 \) decrease in lymphocyte counts was observed at doses of 150, 300 and 600 mg/kg when compared to the hyperglycaemic group. Similarly, the standard drug, glimepiride (10 mg/kg) significantly decreased the lymphocyte counts when compared to the hyperglycaemic group (Table 2).
Table 2: Effects of 28-day daily administration of methanol tuber extract of Chlorophytum alismifolium on haematological parameters of streptozotocin-induced hyperglycaemic rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBC (×10^9/L)</th>
<th>WBC (×10^9/L)</th>
<th>PCV (%)</th>
<th>LYMP (%)</th>
<th>PLT (×10^9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS (1 ml/kg)</td>
<td>6.40±0.45</td>
<td>7.55±3.60</td>
<td>37.75±2.49</td>
<td>5.53±2.64</td>
<td>424.50±49.12</td>
</tr>
<tr>
<td>H/Control</td>
<td>6.45±0.74</td>
<td>12.23±3.10</td>
<td>24.73±9.81</td>
<td>19.78±5.39</td>
<td>167.25±9.26</td>
</tr>
<tr>
<td>H+CAE (150)</td>
<td>5.63±0.72</td>
<td>9.00±1.06</td>
<td>31.55±4.15</td>
<td>6.40±0.88*</td>
<td>483.50±59.02</td>
</tr>
<tr>
<td>H+CAE (300)</td>
<td>4.06±2.03</td>
<td>2.28±0.50</td>
<td>26.13±11.05</td>
<td>1.40±0.21**</td>
<td>305.50±53.22</td>
</tr>
<tr>
<td>H+CAE (600)</td>
<td>8.25±0.44</td>
<td>9.10±1.57</td>
<td>44.98±2.90</td>
<td>6.50±1.19*</td>
<td>455.75±117.23</td>
</tr>
<tr>
<td>GPD (10)</td>
<td>4.92±0.76</td>
<td>7.85±2.22</td>
<td>28.15±4.54</td>
<td>5.68±1.14*</td>
<td>310.00±80.47</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± S.E.M., #= p<0.05 compared to normal control, *= p<0.05, **= p<0.01 compared to hyperglycaemic control- one way ANOVA followed by Bonferroni test, n = 6, CAE = Chlorophytum alismifolium extract, RBC = Red blood Cell, WBC = White blood Cell, PCV = Pack cell volume, LYMP = Lymphocytes, PLT = Platelets, GPD = Glimepiride, H = Hyperglycaemic

**Effects of 28-day daily administration of the methanol tuber extract of Chlorophytum alismifolium on liver function of streptozotocin-induced hyperglycaemic rats**

Administration of streptozotocin produced an increase in AST, ALT, ALP and TB levels though not statistically significant (p>0.05) when compared to the normal control. There was no statistical significant difference (p>0.05) in levels of the liver enzymes of all the animals administered graded doses of the extract but there was elevation of alkaline phosphatase in the glimepiride treated group which was significant (p<0.01) compared to hyperglycaemic control (Table 3).

**Effects of 28-day daily administration of the methanol tuber extract of Chlorophytum alismifolium on renal indices of streptozotocin-induced hyperglycaemic rats**

Streptozotocin-induced hyperglycaemia did not produce significant (p>0.05) changes in the renal indices when compared to the normal control. The extract at all the doses tested significantly (p<0.001) reduced the sodium levels. The urea levels at 300 mg/kg was significantly (p<0.001) reduced compared to the hyperglycaemic control. However, the levels of potassium, chloride, bicarbonate and creatinine were not significantly different (p>0.05) when compared to the hyperglycaemic control (Table 4).
Table 3: Effects of 28-day daily administration of the methanol tuber extract of *Chlorophytum alismifolium* on liver function of streptozotocin-induced hyperglycaemic rats

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>AST (I.U/L)</th>
<th>ALT (I.U/L)</th>
<th>ALP (I.U/L)</th>
<th>TB (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS (1 ml/kg)</td>
<td>148.00±6.48</td>
<td>66.50±8.97</td>
<td>525.50±25.44</td>
<td>16.00±0.00</td>
</tr>
<tr>
<td>H/control</td>
<td>174.75±38.88</td>
<td>70.50±2.72</td>
<td>546.50±108.90</td>
<td>24.50±8.50</td>
</tr>
<tr>
<td>H + CAE (150)</td>
<td>155.25±12.74</td>
<td>53.75±5.68</td>
<td>392.75±37.69</td>
<td>20.25±4.25</td>
</tr>
<tr>
<td>H + CAE (300)</td>
<td>144.75±34.82</td>
<td>78.25±7.89</td>
<td>466.75±63.65</td>
<td>16.00±0.00</td>
</tr>
<tr>
<td>H + CAE (600)</td>
<td>215.00±34.33</td>
<td>106.25±7.87</td>
<td>938.00±137.63</td>
<td>21.67±5.67</td>
</tr>
<tr>
<td>GPD (10)</td>
<td>149.00±15.42</td>
<td>76.75±7.8</td>
<td>1317.00±256.37**</td>
<td>27.33±5.67</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± S.E.M **= p< 0.01 compared to hyperglycaemic control - one way ANOVA followed by Bonferroni post hoc test. AST= Aspartate amino transferase, ALT= Alanine amino transferase, ALP= Alkaline Phosphatase, TB=Total bilirubin, CAE=*Chlorophytum alismifolium* extract, n=6, GPD = Glimepiride, NS =Normal saline, I.U=International unit, H = Hyperglycaemic

DISCUSSION

The phytochemical constituents present in the methanol tuber extract of *C. alismifolium* in this study were largely corroborative of the findings of Abubakar *et al.*, (2016). The biological actions produced by plant extract are usually attributed to the presence of their secondary metabolites (Kensa and Yasmin, 2011). The genus Chlorophytum for example has been reported to possess pharmacologically important saponins which have attracted the interest of the scientific community (Nutan, 2005). Similarly, phytoconstituents like flavonoids and saponins have been linked to the antihyperglycaemic activity of *C. nimonii, C. borivilianum* and *C. alismifolium* (Kunal and Prashat, 2013; Neli *et al.*, 2014; Abubakar *et al.*, 2017).

Determination of the median lethal dose value of plants used in herbal medicine using acute toxicity study is of paramount importance because it provides information regarding the margin of safety of the plant. The acute toxicity study revealed that *C. alismifolium* extract at a dose of 5000 mg/kg caused neither visible signs of toxicity nor mortality, suggesting that it is practically non-toxic when administered orally (Matsumura, 1985).

The assessment of haematological parameters could be used to reveal the harmful effects of foreign compounds including plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology and normal functioning of the organs (Magalhaes *et al.*, 2008). The alterations of these parameters are well known to cause anaemic conditions in man (Balasubramanian *et al.*, 2009). The occurrence of anaemia in diabetes mellitus for example, has been linked to the increase in non-enzymatic glycosylation of RBC membrane proteins (Oyedemi *et al.*, 2011). Furthermore, kidney damage at several levels is a complication of diabetes and also associated with impairment of erythropoietin activity and reduction in haematocrit values (Mehdi and Toto, 2009). In this study, the beneficial effects of *C. alismifolium* extract on the anaemic status of the hyperglycaemic rats were investigated. The levels of PCV in the hyperglycaemic control were reduced compared to the normal rats. Following *C. alismifolium* extract administration, the
levels of PCV and RBC were appreciably improved especially at 600 mg/kg. According to Bigoniya et al. (2013), important phytochemicals like flavonoids have anti-anemic and hematopoietic activities. This gives an indication that some of the bioactive compounds present in C. alismifolium extract can boost blood formation and thus prevent anaemia. High lymphocyte level (lymphocytosis) in some cases indicates certain types of diseases such as cancer, autoimmune disorder (like diabetes mellitus) and infections (Kraine and Tisch, 1999). The destruction of beta cells is characterized by two factors; autoantibodies and lymphocyte infiltration of the pancreas (Notkins, 2002). This infiltration leads to the production of insulitis and decreased insulin production (Bending et al., 2012). In this study, the lymphocyte counts were elevated following hyperglycaemia, however, the administration of C. alismifolium extract significantly lowered the counts. This could be beneficial in ameliorating some of the aforementioned complications associated with lymphocytosis.

Individuals with type 2 diabetes have a higher incidence of liver function test abnormalities than those who do not have (Harris, 2005). In the current study, elevations in markers of liver injury (AST, ALT, ALP and bilirubin) reflect the hepatocytes injury in experimental diabetes. Administration of C. alismifolium extract did not produce significant changes in the levels of the liver enzymes. However, ALP levels were significantly elevated in the glimepiride treated group. This is expected because sulphonylureas like glimepiride cause elevation of liver enzymes in diabetics (Omar et al., 2009).

Blood tests for urea and creatinine are the simplest way to monitor kidney function (Dabla, 2010). Creatinine (a product of body metabolism which is normally excreted by the kidneys) is a marker for assessing the function of the kidney by comparing its amount in the blood with the amount appearing in the urine (Singh et al., 2001). The extract at all doses tested did not produce significant changes in the creatinine levels when compared to the diabetic control which indicates that it does not have much adverse effect on the kidneys. Blood urea nitrogen is a normal metabolic waste product excreted by the kidneys. In diabetic kidney disease, urea is not excreted normally, and so it accumulates in the body thus causing an increase in its blood levels (Molitotis, 2007). The rats in the hyperglycaemic group had the highest level of urea while C. alismifolium extract at 300 mg/kg significantly reduced the urea levels. This reduction in urea level is beneficial in ameliorating renal function in diabetics because an elevation in the serum urea level increases the chances of developing diabetic nephropathy (Bamanikar et al., 2016).

Diabetes mellitus (DM) is included among the diseases with increased frequency of electrolyte abnormalities (Elisaf et al., 1996). DM is a well-known cause of dysnatremias via several underlying mechanisms (Liamis et al., 2008; Liamis et al., 2013). The significant reduction in sodium ion levels produced by C. alismifolium could benefit diabetics that have comorbidity with hypertension because higher sodium level is associated with higher blood pressure (Mackay and Mensah, 2004); similarly, low sodium diets maximize albumin decreasing effect which could ameliorate some complications of diabetes mellitus like nephropathy (Bakris and Smith, 1996).
TABLE 4: Effect of 28-day daily administration of the methanol tuber extract of *Chlorophytum alismifolium* on renal indices and electrolytes in streptozotocin-induced hyperglycaemic rats

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Urea</th>
<th>Creatinine</th>
<th>Sodium</th>
<th>Potassium</th>
<th>Chloride</th>
<th>Bicarbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.13±0.13</td>
<td>70.75±4.70</td>
<td>131.00±1.08</td>
<td>4.35±0.29</td>
<td>93.00±1.91</td>
<td>22.00±1.58</td>
</tr>
<tr>
<td>H/ control</td>
<td>6.63±0.85</td>
<td>67.75±5.53</td>
<td>131.25±2.14</td>
<td>4.68±0.27</td>
<td>95.00±2.38</td>
<td>23.25±1.49</td>
</tr>
<tr>
<td>H+CAE (150)</td>
<td>4.58±0.22</td>
<td>67.75±5.53</td>
<td>100.25±1.31***</td>
<td>4.52±0.26</td>
<td>100.25±1.31</td>
<td>24.75±1.25</td>
</tr>
<tr>
<td>H+CAE (300)</td>
<td>1.88±0.13***</td>
<td>68.00±3.00</td>
<td>101.50±0.50***</td>
<td>6.50±1.35</td>
<td>101.50±0.50</td>
<td>20.25±1.44</td>
</tr>
<tr>
<td>H+CAE (600)</td>
<td>3.75±0.85</td>
<td>85.50±8.72</td>
<td>99.50±3.10***</td>
<td>6.50±0.39</td>
<td>99.50±3.10</td>
<td>19.25±1.31</td>
</tr>
<tr>
<td>GPD (10)</td>
<td>3.91±0.24</td>
<td>67.75±7.39</td>
<td>125.25±1.80**</td>
<td>5.48±0.24</td>
<td>104.00±1.96</td>
<td>25.00±2.04</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± S.E.M; **= p<0.01, ***= p<0.001 compared to hyperglycaemic control - one way ANOVA followed by Bonferroni post hoc test. CAE= *Chlorophytum alismifolium* Extract, n=6, GPD = Glimepiride, H=Hypoglycaemic
CONCLUSION

The findings of this study suggest that the methanol tuber extract of *Chlorophytum alismifolium* ameliorated the haematological and hepato-renal disturbances produced by streptozotocin-induced hyperglycemia.

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