Effect of aqueous leaf extract of Senecio biafrae on liver and kidneys function indices of alloxan-induced diabetic rats

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ABSTRACT
The objective of this research work is to investigate some biochemical parameters in the liver and kidney of alloxan-induced diabetic rats treated with Senecio biafrae leaf aqueous extract. Alloxan-induced diabetic rats (150mg/kg) were administered orally with Senecio biafrae leaf aqueous extract (200mg/kg and 400mg/kg) for fifteen days after which some biochemical indices in the serum, liver and kidney were measured and compared with the control. The results shows that serum and tissues alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), serum urea, direct bilirubin and creatinine of untreated diabetic group were significantly higher (p<0.05) when compared with the normal control group and extract treated group, while significant reduction were observed in serum total protein, albumin and globulin of diabetic untreated group when compared to both extract treated and normal control groups. It can be concluded that administration of aqueous leaf extract of Senecio biafrae to diabetic rats did not have any adverse effect on the liver and kidney functions indices in rats, instead it ameliorate the adverse effects of diabetes complications.

Keyword: Senecio biafrae, liver function, kidney function, diabetic rats.

INTRODUCTION
Diabetes mellitus (DM) is a metabolic disorder which occurs when the pancreas produces insufficient amounts of insulin, or in when individual’s system fail to respond appropriately to insulin (due to defects in reactive oxygen species scavenging enzymes and high oxidative stress impairing pancreatic beta cells). DM is characterized with increases in glucose levels build up in the blood and urine, causing excessive urination, thirst, hunger and problems with carbohydrate, fat and protein metabolism [15], [1], [8]. Hyperglycemia leads to long-term tissue damages and complications, such as liver-kidney dysfunctions, often associated with serious diseases [27], [16].

The disease is ranked among the leading causes of death and is a major health problem in developed and developing countries [25], [1]. The number of diabetic patients is increasing globally because of diverse changes in diets in all cultures. It has been predicted that the number of diabetic patients will double from 171 million in 2000 to about 366 million by 2030 mainly because of dietary intake and other lifestyle factors [32].

The prevalence of this disease worldwide is at alarming rates, which has led to the uses of several therapeutic strategies (currently available) for the management of this chronic metabolic disorder, including the stimulation of endogenous insulin secretion, enhancement of insulin action at the target tissues, inhibition of dietary starch and lipid degradation, and treatment with oral hypoglycemic agents [8], [9]. These therapeutic strategies are associated with different degrees of side effects. This has led to searching for more efficient and less cost-effective alternatives. This trend has been further intensified by increasing doubts surrounding current dietary and other lifestyle behaviors together with growing interests in functional foods and nutraceuticals [8], [18]. Complementary and alternative medicine applications have attracted special attention in recent research because they offer new promising opportunities for the development of efficient, side effect-free and lower cost alternatives to existing synthetic hypoglycemic agents [8], [23]. A wide range of medicinal plants have been used by various cultures
to treat diabetes mellitus because of their hypoglycaemic properties [1].

Senecio biafrae (called “worowo” in Yoruba speaking region of Nigeria) has been believed to be endowed with medicinal properties. It is also a vegetable found mainly under cocoa and kolanut trees plantation [5]. Therefore, the aim of this study is to evaluate the effect of aqueous leaf extract of Senecio biafrae on liver and kidneys of alloxan-induced diabetic rats.

**MATERIALS AND METHOD**

**Preparation of Senecio biafrae leaf aqueous extract**

Sample of Senecio biafrae leaf was obtained from Oja-Oba Market in Ado-Ekiti, Ekiti State, Nigeria. This was then authenticated at the Department of Biological Sciences in the University of Ilorin where a voucher number was given. The leaf was washed thoroughly under running tap water, and cut into small pieces, dried in an oven at 500°C. The leaf was then milled using an automatic electrical blender (model MS-223, China) to powder.

100 g of Senecio biafrae leaf powder was added to 1000 ml of distilled water (at normal room temperature) inside a conical flask and plugged with cotton wool for 24 hours. The mixtures were filtered using cheese cloth and then through Whatman No.1 filter paper. The filtrate was then concentrated using combination of rotary evaporator and freeze drier [14]. This was used to prepare 2mg/ml and 4mg/ml doses to deliver 200mg/kg and 400mg/kg respectively for rats daily.

**Chemicals and drugs**

Alloxan, enzyme kits and all other chemicals used in this study were obtained from Sigma chemical company and Randox respectively.

**Experimental animals and grouping:**

Twenty five albino rats (Rattus novergicus) of both sexes weighing between 140-200g were obtained from Animal Holding Unit of the Department of Biochemistry, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria. The animals were exposed to 12 hours of natural daylight and darkness and given rat chow and water ad libitum. They were divided into five groups as follows:

- **Group A:** Non-diabetic rats (normal control)
- **Group B:** Diabetic control rats
- **Group C:** Diabetic rats treated with metformin
- **Group D:** Diabetic rats treated with Senecio biafrae leaf extract (200mg/kg)
- **Group E:** Diabetic rats treated Senecio biafrae leaf extract (400mg/kg)

**Induction of diabetes mellitus**

This was done by intraperitonea administration of a single dose of freshly prepared alloxan monohydrate (2, 4, 5, 6 tetraoxypyridine 5, 6-dioxouracil) of 150mg/kg bodyweight, dissolved in 0.9% sterile NaCl solution of pH 7 [12], [22], [11] to rats in group B to E to induce type II diabetes. Prior to this, their blood glucose levels have been determined. After 48 hrs rats that had blood glucose level above 200 mgdL-1 were considered diabetic and selected for the study.

**Serum and Tissue Homogenate preparation**

Rats were anaesthetized with diethyl-ether, blood were obtained by cardiac puncture (inside plane test tubes). The blood were then left on the laboratory desk to clot at room temperature for one hour and then put in a refrigerator for another one hour. Furthermore, the sera were collected after centrifugation at 3000rpm for 5 minutes for each sample. This was kept inside the refrigerator (at 0OC) and used within 12 hour of preparation. The animals were quickly dissected, liver and kidneys were removed and rinsed in ice-cold 0.25M sucrose solution, which were thereafter cut finely with sterile blade and homogenized in ice-cold 0.25M sucrose solution (1:5 w/v). The homogenates were kept frozen (inside the freezer) overnight to ensure maximum release of the enzymes [6], [1].

**Enzymes assay determination and measurement of some serum metabolites**

Alkaline phosphatase (ALP) (EC. 3.1.3.1) activity (in the serum, liver and kidney) was determined using Para- Nitrophenyl phosphate (PNPP) [33]. Alanine aminotransferase (ALT) (EC. 2.6.1.2) and aspartate aminotransferase (AST) (EC. 2.6.1.1) (in the serum, liver and kidney) were assayed as described by [24]. Serum albumin, bilirubin, globulin, protein, creatinine and urea were determined using [34], [26], [28], [13], [29], [30] respectively. Also, all measurements were done using Spectronic 21 digital Spectrophotometer. While serum sodium and potassium ions were determined by flame photometry using the Jenway Clinical PFP7 Flame Photometer.

**Statistical Analysis**

All the data were analyzed using Duncan Multiple Range Test, alongside with one-way analysis of variance (ANOVA), while differences were considered at P<0.05 [19].

**RESULTS**

Table 1, 2 and 3 show the activities of ALP, ALT and AST respectively in the tissues of interest (liver and kidney) and serum of the experimental animals. There were significant different (p<0.05) in the activities of ALP, ALT and AST in the serum, liver and kidney of diabetic animals treated with Senecio biafrae aqueous leaf extract (200mg/kg and 400mg/kg body weight) when compared with the control, untreated diabetic group and metformin treated groups, whereas, the activities of the three enzymes in the serum of the diabetic groups (diabetic untreated, metformin, 200mg/kg and 400mg/kg body weight) were significantly higher (p<0.05) than the normal control group.

Table 4 shows the concentration of urea, creatinine and direct bilirubin. In urea concentration there were no significant difference (p>0.05) in control, metformin, 200mg/kg and 400mg/kg body weight groups with differences in others (p<0.05). There were no significant different (p>0.05) in the concentration of creatinine at 200mg/kg and 400mg/kg while in direct bilirubin there also no significant difference (p>0.05) in the concentration metformin, 200mg/kg and 400mg/kg body weight groups, but significance difference in others (p<0.05).

Table 5 shows the concentration of total protein, albumin and globulin in the serum of the experimental animals. There were significant difference (p<0.05) in the concentration of total protein of diabetic untreated when compared to both metformin and extract treated groups. In albumin there were no significant difference (p>0.05) between the metformin, 200mg/kg and 400mg/kg body weight whereas there were significant different in others (p<0.05), while globulin shows no significant difference (p<0.05) in the control and 400mg/kg body weight, also, in metformin and 200mg/kg body weight.
Table 1: Effects of Senecio biafrae aqueous leaf extract on Alkaline Phosphatase (ALP) activity (IU/L) of alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Kidney</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>189±0.1</td>
<td>342±1.20</td>
<td>200±1.20</td>
</tr>
<tr>
<td>Untreated</td>
<td>374±0.4</td>
<td>600±1.42</td>
<td>488±1.20</td>
</tr>
<tr>
<td>Metformin (treated)</td>
<td>214±0.1</td>
<td>347±2.1</td>
<td>298±1.0</td>
</tr>
<tr>
<td>Senecio biafrae (200mg/kg)</td>
<td>220±0.12</td>
<td>406±2.13</td>
<td>299±1.20</td>
</tr>
<tr>
<td>Senecio biafrae (400mg/kg)</td>
<td>200±2.40</td>
<td>356±1.20</td>
<td>279±0.10</td>
</tr>
</tbody>
</table>

Column values with different superscripts are significantly (p<0.05) different
Each values is a mean of five determination ± SEM

Table 2: Effects of Senecio biafrae aqueous leaf extract on Alanine Amino Transferase (ALT) activity (IU/L) of alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.013±0.01</td>
<td>0.080±1.20</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.02±0.20</td>
<td>0.010±2.10</td>
</tr>
<tr>
<td>Metformin (treated)</td>
<td>0.014±1.40</td>
<td>0.089±2.60</td>
</tr>
<tr>
<td>Senecio biafrae (200mg/kg)</td>
<td>0.018±1.21</td>
<td>0.096±2.60</td>
</tr>
<tr>
<td>Senecio biafrae (400mg/kg)</td>
<td>0.015±0.18</td>
<td>0.088±1.01</td>
</tr>
</tbody>
</table>

Column values with different superscripts are significantly (p<0.05) different
Each values is a mean of five determination ± SEM

Table 3: Effects of Senecio biafrae leaf aqueous extract on Aspartate Amino Transferase (AST) activity (IU/L) of alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.012±0.02</td>
<td>0.075±0.10</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.085±0.01</td>
<td>0.095±0.01</td>
</tr>
<tr>
<td>Metformin (treated)</td>
<td>0.030±0.09</td>
<td>0.061±0.03</td>
</tr>
<tr>
<td>Senecio biafrae (200mg/kg)</td>
<td>0.038±0.22</td>
<td>0.063±0.02</td>
</tr>
<tr>
<td>Senecio biafrae (400mg/kg)</td>
<td>0.026±0.06</td>
<td>0.075±0.01</td>
</tr>
</tbody>
</table>

Column values with different superscripts are significantly (p<0.05) different, Each values is a mean of five determination ± SEM
Table 4: Effects of Senecio biafrae aqueous extract on the kidney function parameters of alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mmol/l)</th>
<th>Creatinine (mmol/l)</th>
<th>Direct bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.05±0.12a</td>
<td>69.29±2.10a</td>
<td>8.80±0.20a</td>
</tr>
<tr>
<td>Untreated</td>
<td>4.28±1.24c</td>
<td>98.10±1.20b</td>
<td>12.42±1.20b</td>
</tr>
<tr>
<td>Metformin (treated)</td>
<td>2.21±1.21b</td>
<td>72.31±1.30b</td>
<td>9.30±1.12a</td>
</tr>
<tr>
<td>Senecio biafrae (200mg/kg)</td>
<td>1.89±0.12b</td>
<td>78.50±1.30a</td>
<td>9.20±0.80a</td>
</tr>
<tr>
<td>Senecio biafrae (400mg/kg)</td>
<td>1.78±0.16b</td>
<td>76.20±1.40a</td>
<td>9.90±1.20a</td>
</tr>
</tbody>
</table>

Column values with different superscripts are significantly (p<0.05) different
Each values is a mean of five determination ± SEM

Table 5: Effects of Senecio biafrae aqueous leaf extract on the liver function parameters of alloxan - induced diabetic rats (g/dl)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein</th>
<th>Albumin</th>
<th>Globulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48.42±0.13a</td>
<td>24.85±0.15a</td>
<td>23.57±0.21a</td>
</tr>
<tr>
<td>Untreated</td>
<td>28.93±0.45e</td>
<td>13.81±0.34c</td>
<td>15.13±0.14c</td>
</tr>
<tr>
<td>Metformin (treated)</td>
<td>42.20±0.10b</td>
<td>21.75±0.21b</td>
<td>20.45±0.25b</td>
</tr>
<tr>
<td>Senecio biafrae (200mg/kg)</td>
<td>38.48±0.10c</td>
<td>26.26±0.20b</td>
<td>22.10±0.15b</td>
</tr>
<tr>
<td>Senecio biafrae (400mg/kg)</td>
<td>49.32±0.50d</td>
<td>24.44±0.21b</td>
<td>23.42±0.20a</td>
</tr>
</tbody>
</table>

Column values with different superscripts are significantly (p<0.05) different
Each values is a mean of five determination ± SEM

DISCUSSION

Enzyme activities in the tissues are usually used as a ‘marker’ to ascertain early toxic effects of administered foreign compounds to experimental animals [6], [2], [1]. ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes. These enzymes are highly concentrated in the liver and kidney and are found only in the serum in significant quantities when the cell membrane becomes leaky and completely ruptured [1], [10], [21]. [20] has documented that rise in serum level with corresponding decrease in tissue level of these intracellular enzymes is an index of damage to liver and kidney cells.

In this present study, increase in serum enzymes activities without concomitant alteration in the tissue levels of the enzymes may implied that the serum elevation in the diabetic groups might be from single intraperitoneal administration of alloxan at a dose of 150mg/kg body weight has been reported to cause oxidative damage to pancreatic beta cells in rats [31], which was gradually reversed in dose dependent manner by Senecio biafrae leaf extract administration.

In addition, significant increase observed in the serum urea, creatinine and bilirubin of diabetic untreated group might due to kidney damage caused by alloxan injection (due to cell injury as a result of free radical production). This might be responsible for frequent urination observed in diabetic groups. Senecio biafrae leaf extract improved renal function by reversing these effects. Also, decrease in the total protein, albumin and globulin of alloxan induced rat, might may due to decrease due to microproteinurea and albuminurea, which are important clinical markers of diabetic nephropathy [17], and/or may be due to increased protein catabolism [7] as a result of insulin deficiency from free radical generation due to alloxan induction, since it has been established that insulin stimulates the incorporation of amino acids into protein [7].

The excellent performance of Senecio biafrae leaf extract in reversing the negative effects of alloxan on diabetic rats may due to the present of some antioxidant vitamins and minerals in the plant as reported by [3], as well as present of phenol, flavonoids and alkaloids in Senecio biafrae leaf [4]. Furthermore, Senecio biafrae leaf extract may have exert its action through insulinomimetic.
REFERENCES:


