Antioxidant Status and Organ Function in Streptozotocin-Induced Diabetic Rats treated with Aqueous, Methanolic and Petroleum Ether Extracts of Ocimum basilicum leaf

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ABSTRACT
The effects of various extracts of Ocimum basilicum leaf on biochemical indices of organ damage and oxidative stress status of streptozotocin-induced diabetic rats were examined. Oral administration of 200mg/kg of aqueous, methanolic and petroleum ether extracts of the leaf for 35 days resulted in a significant (P<0.05) reduction in thiobarbituric acid reactive substances (TBARS) and an increase in catalase (CAT) and superoxide dismutase (SOD) activities in streptozotocin-induced diabetic rats from diabetic levels. The leaf extracts brought about a significant (P<0.05) increase in serum protein and albumin as well as decreases in urea and creatinine levels of STZ – induced diabetic rats compared with diabetic control levels. The Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels increased significantly (P<0.05) in diabetic control group. The extracts caused a significant reduction in levels of AST and ALT in treated diabetic groups and maintained the normal level observed in normal rats. In this study a significant decrease in PCV was observed in diabetic control group and increase in the PCV in rats given extracts. It was concluded that the extracts have in vivo antioxidant, hepatoprotective and nephroprotective effects in STZ – induced diabetic rats. These results support its traditional use in the management of diabetes and cardiovascular diseases.

INTRODUCTION
Free radicals are potentially important in a number of disease states that can have severe effects on the cardiovascular system, either through lipid peroxidation or vasoconstriction (Lachance et al., 2001). Hyperglycemia causes over production of free radical thereby creating oxidative stress (Arango et al., 2000). Oxidative stress is an imbalance between the levels of pro-oxidants and antioxidants in the biological systems, leading to cellular injury (Villa-Caballero et al., 2000). Excessive oxidative stress has been implicated in the pathology and complications of diabetes mellitus (Wolff, 1993). The high prevalence of diabetic complications poses a serious public health problem, especially in our setup, where health care resources are limited. Oral hypoglycaemic agents, especially the sulphonylureas and biguanides have been commonly employed in the management of type 2 diabetes. Many of the synthetic drugs like sulphonylureas and biguanides can produce side effects including hematological, cutaneous and gastrointestinal reactions and disturbances in liver and kidney (Arango et al., 2000). Ocimum basilicum is an erect and herbaceous branched plant, 1 meter high or less. It is a plant belonging to the Lamiaceae family. It is a small shrub with many branches, commonly found in many gardens around village huts in Nigeria and planted for its medicinal uses. The phytochemical investigations and elemental analysis of the aqueous leaf extract of O. basilicum indicated the presence of pharmacologically useful classes of compounds such as saponin, alkaloids, flavonoids, cardiac glycosides, terpenes, steroids, tannins and carbohydrates (Sanni et al., 2008). Tannins were reported to possess physiological astringent properties, which hasten wound healing and ameliorate inflamed mucus membrane (Gupta, 1994). It also has hemostatic properties (Awosika, 1991). Saponin present in plants is cardiotonic in nature (Trease and Evans, 1989).
There are claims in traditional circles that *O. basilicum* can be used in management of the complications of diabetes mellitus when continuously used for weeks. To establish the veracity of these claims, this study was designed to evaluate the hepato- and nephro-protective effects and long term antioxidants activities of aqueous, methanolic and petroleum ether extracts of *O. basilicum* streptozotocin-induced diabetic rat models.

**MATERIALS AND METHODS**

**Plant material**

Fresh leaves of *Ocimum basilicum* L. were harvested from Enugu, South-East, Nigeria. This species of basil were grown in a local farm at Edem-Ani in Nsukka Local Government Area of Enugu state. The numbers of plants harvested are about one hundred and nine.

The plant was identified at the herbarium unit of the Department of Biological Sciences A.B.U, Zaria. The fresh leaves were collected, washed with clean water and dried under the shade for six days. The dried leaves were milled using pestle and mortar to get a powder that was used for extraction.

**Animals**

Forty-five male Wistar strain albino rats weighing between 150 – 200g obtained from the Department of Pharmacology, Ahmadu Bello University, Zaria were used. The animals were kept and maintained in well ventilated cages under standard laboratory conditions.

They were maintained on grower’s mash (Vital feeds Nigeria Ltd) and provided with water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for two weeks before the experiment.

**Induction of diabetes**

Diabetes mellitus was induced by single intraperitoneal dose of 60 mg/kg of streptozotocin (Sigma chemicals, St Louis U.S.A) dissolved in 0.1 M fresh cold citrate buffer, pH 4.5; into 12 h-fasted rats (Burcelin et al., 1995). After 3 days the fasting blood sugar levels were monitored with a glucometer (Accu-chek Advantage, Roche Diagnostics GmbH, Germany) and the rats having fasting blood glucose levels more than 200 mg/dl (11.1mmol/L) were selected for experimentation.

**Acute Toxicity test**

The LD$_{50}$ for all the different extracts was determined using the method of Lorke (1983).

**Experimental design**

The rats were divided into 9 groups of 5 rats each. Group 1 rats were diabetic rats treated with 200mg/kg of the aqueous extract daily for 35 days.

The extract was given orally using an oral cannula. Group 2 animals were diabetic rats similarly treated with 200mg/kg of the methanolic extract. While group 3 consisted of diabetic rats similarly treated with 200mg/kg of the petroleum ether extract. The fourth group consisted of diabetic rats given no further treatment.

The last group of diabetic rats (group 5) was similarly treated daily with Glibenclamide at a dose of 2.5 mg/kg body weight. The groups 6, 7 and 8 animals were non-diabetic rats similarly treated with 200mg/kg of aqueous extract, methanolic extract and petroleum ether extract, respectively. Group 9 rats served as Normoglycemic controls given no further treatment.

On the 35th day post-treatment the animals were fasted overnight, anesthetized with chloroform and sacrificed by humane decapitation. The blood was collected in test tubes and serum prepared. Liver and kidney tissues were removed, weighed and placed in ice-cold containers.

Then the organ homogenates were prepared as follows: to 1.0g of the liver and kidney tissues, 10ml of 0.01 M phosphate buffer (pH 7.0) was added and homogenized using pestle and mortar. The supernatants collected, after centrifuging at 3000xg, were used for analysis.

**Methodologies**

**Determination of Weekly Packed Cell Volume (PCV)**

Tail blood was collected in heperinized capillary tubes and the haematocrit determined.

**Estimation of serum biochemical parameters (organ function)**

The determination of the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, total protein, albumin, urea and creatinine were carried out using Randox assay kits (Antrim, UK).

**Determination of oxidative stress markers in organs**

Thiobarbituric acid reactive substances (TBARS), as malondialdehyde (MDA), in tissues were estimated by the method of Fraga, et al; (1988). Calatase was assayed according to the method of Machly and Chance (1954). Superoxide Dismutase (SOD) activity assay was carried out according to the method described by Martin et al., (1987).

**Statistical analysis**

Data are presented as means + SD and analyzed by Analysis of Variance and Students’t – test.

**RESULTS**

A significant decrease in the level of PCV in diabetic control groups was observed (P<0.05) when compared with the normal control group.

Treatment with extracts or standard drug (Glibenclamide) prevented, to a significant (P<0.05) degree, the diabetes-induced fall in PCV. The non-diabetic animals did not record any decrease in PCV. All groups, except the diabetic control group, recorded final PCVs higher than the PCVs on day 0 (Figure 1).
Table 1: Effects of Extracts of *Ocimum basilicum* Leaf or Glibenclamide on Serum AST, ALT, Creatinine, Total protein and Albumin in Diabetic and Non-diabetic Rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>D+AQ</th>
<th>D+MET</th>
<th>D+PET</th>
<th>D+STD</th>
<th>N+AQ</th>
<th>N+MET</th>
<th>N+PET</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (u/l)</td>
<td>33.91±9.51a</td>
<td>42.70±12.2a</td>
<td>60.00±8.29a</td>
<td>65.99±3.13a</td>
<td>35.00±2.67a</td>
<td>31.91±6.75a</td>
<td>32.87±6.58a</td>
<td>32.70±7.72a</td>
</tr>
<tr>
<td>ALT (u/l)</td>
<td>29.87±5.23a</td>
<td>30.82±8.85a</td>
<td>30.00±4.87a</td>
<td>46.07±1.15a</td>
<td>29.96±2.90a</td>
<td>27.93±2.10a</td>
<td>28.07±1.21a</td>
<td>28.61±3.05a</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>29.23±7.30a</td>
<td>32.72±6.50a</td>
<td>32.67±5.58a</td>
<td>42.12±6.98a</td>
<td>31.73±6.88a</td>
<td>24.77±2.42a</td>
<td>21.80±3.58a</td>
<td>23.74±1.84a</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.46±0.21a</td>
<td>1.65±0.15a</td>
<td>1.62±0.15a</td>
<td>2.10±0.12a</td>
<td>1.50±0.23ab</td>
<td>1.36±0.06a</td>
<td>1.36±0.11b</td>
<td>1.35±0.10a</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.96±0.43a</td>
<td>7.10±0.46a</td>
<td>7.56±0.30a</td>
<td>3.49±0.37a</td>
<td>7.53±0.31a</td>
<td>7.61±0.23a</td>
<td>7.78±0.64a</td>
<td>7.74±0.53a</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>5.34±0.35a</td>
<td>5.23±0.17a</td>
<td>5.41±0.14a</td>
<td>2.62±0.12a</td>
<td>4.56±1.57a</td>
<td>5.64±0.14a</td>
<td>5.52±0.09a</td>
<td>5.46±0.30a</td>
</tr>
</tbody>
</table>

Values are means ±SD of five replicate determinations. Values with different superscript (a, b, c, d, e) on the same row are statistically different (P<0.05). D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET: Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract, N+PET: Normal rats + Petroleum Ether, NC: Normal Control rats.

Table 2: Effects of Extracts of *Ocimum basilicum* Leaf or Glibenclamide on Relative Organ Weights in Diabetic and Non-diabetic Rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>D+AQ</th>
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<th>N+AQ</th>
<th>N+MET</th>
<th>N+PET</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (%)</td>
<td>3.93±0.4a</td>
<td>3.50±1.5a</td>
<td>4.24±0.7a</td>
<td>6.05±1.20a</td>
<td>3.32±0.49a</td>
<td>3.38±0.08a</td>
<td>3.28±0.52a</td>
<td>3.32±0.53a</td>
</tr>
<tr>
<td>Kidney (% x 10^4)</td>
<td>6.90±0.34a</td>
<td>6.40±0.67a</td>
<td>7.20±0.50a</td>
<td>7.90±1.00a</td>
<td>6.50±0.59a</td>
<td>6.20±1.00a</td>
<td>5.80±1.30a</td>
<td>6.10±0.55a</td>
</tr>
</tbody>
</table>

Values are means ±SD of five replicate determinations. Values with different superscript (a, b, c, d, e) on the same row are statistically different (P<0.05). D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET: Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract, N+PET: Normal rats + Petroleum Ether, NC: Normal Control rats.

Table 3: Effects of Extracts of *Ocimum basilicum* Leaf or Glibenclamide on Some Markers of Oxidative Stress in Liver of Diabetic and Non-diabetic Rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>D+AQ</th>
<th>D+MET</th>
<th>D+PET</th>
<th>D+STD</th>
<th>N+AQ</th>
<th>N+MET</th>
<th>N+PET</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mg protein)</td>
<td>5.72±0.99a</td>
<td>6.44±2.03a</td>
<td>5.96±0.86a</td>
<td>8.90±1.62a</td>
<td>6.94±1.66a</td>
<td>5.29±0.67a</td>
<td>5.23±0.45a</td>
<td>5.14±0.76a</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>57.75±5.67a</td>
<td>50.38±3.02a</td>
<td>54.85±6.15a</td>
<td>46.63±6.06a</td>
<td>56.72±3.94a</td>
<td>63.95±1.07a</td>
<td>61.50±2.01a</td>
<td>64.13±1.54a</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>2.31±4.2a</td>
<td>2.39±0.70a</td>
<td>2.21±0.45a</td>
<td>1.34±0.16a</td>
<td>1.87±0.49a</td>
<td>4.17±0.62a</td>
<td>4.04±0.23a</td>
<td>3.66±0.25a</td>
</tr>
</tbody>
</table>

Values are means ±SD of five replicate determinations. Values with different superscript (a, b, c, d, e) on the same row are statistically different (P<0.05). D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET: Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract, N+PET: Normal rats + Petroleum Ether, NC: Normal Control rats.

Table 4: Effects of extracts of *Ocimum basilicum* Leaf or Glibenclamide on Some Markers of Oxidative Stress in Kidney of Diabetic and Non-diabetic Rats.

<table>
<thead>
<tr>
<th>GROUPS</th>
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<th>D+MET</th>
<th>D+PET</th>
<th>D+STD</th>
<th>N+AQ</th>
<th>N+MET</th>
<th>N+PET</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mg protein)</td>
<td>9.21±0.41a</td>
<td>9.43±0.28a</td>
<td>9.36±0.49a</td>
<td>10.61±2.23a</td>
<td>8.64±1.20a</td>
<td>8.64±1.26a</td>
<td>8.27±1.15a</td>
<td>8.54±1.02a</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>48.97±0.87a</td>
<td>43.45±0.86a</td>
<td>46.38±1.23a</td>
<td>38.34±2.06a</td>
<td>51.50±1.01a</td>
<td>54.60±4.73a</td>
<td>52.52±2.14a</td>
<td>57.10±2.71a</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>1.76±0.30a</td>
<td>1.78±0.38a</td>
<td>1.61±0.27a</td>
<td>1.11±0.10a</td>
<td>1.62±0.36a</td>
<td>2.77±0.63a</td>
<td>2.63±0.49a</td>
<td>1.88±0.33a</td>
</tr>
</tbody>
</table>

Values are means ±SD of five replicate determinations. Values with different superscript (a, b, c, d, e) on the same row are statistically different (P<0.05). D+AQ: Diabetic rats + Aqueous Extract, D+MET: Diabetic rats + Methanolic Extract, D+PET: Diabetic rats + Petroleum Ether, DC: Diabetic rats Control D+STD: Diabetic rats + Standard Drug, N+AQ: Normal rats + Aqueous Extract, N+MET: Normal rats + Methanolic Extract, N+PET: Normal rats + Petroleum Ether, NC: Normal Control rats.
Table 1 presents the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities as well as urea, creatinine, total protein and albumin levels in the blood. Significant (P<0.05) increases in serum AST, ALT, urea and creatinine, above normal levels, were recorded in diabetic control, while total protein and albumin fell below normal levels in the same group. Treatment of diabetic rats with extracts of Ocimum basilicum leaf brought about significant (P<0.05) increases in total protein and albumin and significant decreases in AST, ALT, urea and creatinine compared with diabetic levels.

The intraperitoneal LD<sub>50</sub> of aqueous, methanolic and petroleum ether extracts were 470mg/kg, 3,800mg/kg and 3,800mg/kg, respectively. Table 2 shows the relative liver and kidney weights of all the rats. A significant increase (P<0.05) in liver to body weight ratio was observed in diabetic control group when compared with the normal control. Treatment of diabetic rats with extracts or Glibenclamide significantly (P<0.05) prevented this disease-induced enlargement of the liver. The kidney size was not significantly affected by any of the treatments.

The level of Thiobarbituric acid reactive substance (TBARS), catalase and superoxide dismutase (SOD) in the liver and kidney of the rats are shown in Tables 3 and 4. There was a significant increase (P<0.05) in Malondialdehyde (MDA) in the liver and kidney of diabetic control group. Treatment with different extracts of Ocimum basilicum leaf and the standard drug (Glibenclamide) tended to reduce the MDA level in the liver and kidney of diabetic rats. There was an observed significant reduction (P<0.05) in the activity of catalase in the liver and kidney of the diabetic control group and treatment with different extracts of Ocimum basilicum leaf and the standard drug tended to increase the catalase activity in the liver and kidney of diabetic rats. Also there was a significant reduction(P<0.05) in the activity of SOD in the liver and kidney of the diabetic control group and treatment with different extracts of Ocimum basilicum leaf and the standard drug tended to increase the SOD activity in the liver and kidney of diabetic rats.

**DISCUSSION**

All the rats treated with streptozotocin recorded fasting blood glucose levels ranging from 10.5 – 20.0mM at the beginning of experiment, as reported (Ugwu, et al., 2011). The untreated diabetic rats maintained hyperglycemia throughout the period of experiment, indicating the induction was successful and there were no reversions. Lipid peroxide-mediated tissue damage has been observed in the development of both types 1 and 2 Diabetes (Stanely et al., 1998). It has been observed that insulin secretion is closely associated with lipoxynegase derived peroxides (Gupta, 1994). Low levels of lipoxygenase peroxides stimulates the secretion of insulin, but when the concentration of endogenous peroxides increase it may initiate uncontrolled lipid peroxidation leading to cellular infiltration and islet cell damage in type 1 diabetes (Wolff, 1993). Increased concentration of MDA level was observed in liver and kidney of diabetic control group when compared to diabetic groups treated with extracts or standard drug and with normal control group. Wilson et al., (2001) have reported that the concentration of lipid peroxides, increases in the kidney of diabetic rats. This present work shows that administration of Ocimum basilicum leaf extracts tends to bring the kidney and liver MDA back to normal.

Reduced activity of SOD and catalase (CAT) in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals (O<sub>2</sub>·) and hydrogen peroxide (Wilson et al., 2001). In this study administration of Ocimum basilicum leaf extracts resulted in the activities of SOD and CAT returning to normal in the groups of diabetic rats (Tables 3 and 4). The results of SOD and CAT activities clearly showed that Ocimum basilicum leaf extracts contain a free radical scavenging activity, which could exert a beneficial action against pathological alterations caused by the presence of O<sub>2</sub>·and OH·. This action, predominantly due to the extract, could involve mechanism related to scavenging activity. Ocimum species has been extensively reported for its essential oil content (Roberto et al., 2003); however, the antioxidant capacity of the plant extracts is mainly dependent on phenolic compounds (Ramarathnam et al., 1997; Pitchersky and Gang, 2000). Antiradical activity of phenolic compounds seen in Ocimum species depend on their molecular structure; that is, on the availability of phenolic hydrogens, which result in the formation of phenoxy radicals due to hydrogen donation (Ramarathnam et al., 1997).

It has been suggested that the anemia occurring in DM is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycemia (Twaij and Al-Badr, 1988). Oxidation of these glycosylated membrane proteins and hyperglycemia in DM cause an increase in the production of lipid peroxides, which in turn cause the hemolysis of RBCs. In this study a decrease in PCV was observed in diabetic control group. The increase in the PCV in rats given extracts of Ocimum basilicum leaf may be due to the decreased level of blood glucose and/or due to lowered lipid peroxide level in RBC membrane leading to a decreased susceptibility of RBC to hemolysis.

Renal disease is one of the most common and severe complications of diabetes (Tierney et al., 2002). The blood urea level increased significantly in diabetic control group. These results were consistent with those reported by Urmila and Goyal (2003). A relationship between treatment related alterations in urea concentration and histopathology of the kidney has been reported in rats (Laakso, 1999). Creatinine, a marker of renal function (Gross et al., 2005) is significantly increased in diabetic control group. Aqueous, methanolic and petroleum ether extracts of Ocimum basilicum leaf brought about a significant (P>0.05) increase in serum protein, albumin and a decrease in urea and creatinine levels of STZ-induced diabetic rats (Table 1).

Increases in serum urea and creatinine concentrations were rectified by administration of these extracts in STZ-induced diabetic rats. An increase in serum urea and creatinine levels in STZ-diabetic rats may indicate diminished ability of the kidneys to
filter these waste products from the blood and excrete them in the urine. Based on these findings, the extracts of this plant may have enhanced the ability of the kidneys to remove these waste products from the blood as indicated by the reduction in serum urea and creatinine levels and thus, confer a protective effect on the kidney of diabetic rats. Taken together, it is also possible to suggest that these extracts might directly improve the structural and functional integrities of cells of the blood, liver and kidney.

The present work also showed that injection of STZ – induces diabetes caused hepatocellular damage, which is another characteristic change in diabetes as evidenced by decrease in total protein and albumin in the serum as well as high serum levels of AST and ALT in untreated diabetic group (Table 1). The liver releases ALT and an increased plasma concentration is an indicator of liver damage.

The liver, kidney and heart release AST and ALT and elevation in their plasma concentrations indicate liver and heart damage (Ogbonnia et al., 2008). The extracts caused a significant reduction in level of AST and ALT in treated diabetic groups and maintained the normal level observed in normal rats showing that at the doses used the extracts have hepato-and nephro-protective effects. Therefore, it is possible to suggest that these extracts are safe and might confer protection against diabetes – induced hepatocellular damage as evidenced by normal serum levels of AST and ALT in treated diabetic groups.

In conclusion, the study showed that treatment with various extracts of Ocimum basilicum leaf increased the packed cell volume and protected organs against damage. The extracts did not show signs of toxic effects on the organs for 35 days the experiment lasted. It also exhibited free radical scavenging effect. These results support its traditional use in the management of diabetes and cardiovascular diseases.

**REFERENCES**


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