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The Effect of Ethanolic Extract of *Larpotea ovalifolia* Plants Growing in Calabar on Antioxidants Status of Streptozocin Induced Diabetic Rats

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Abstract: In this study, the effect of the administration of extract of *Laportea ovalifolia,* on the antioxidant status of diabetic rats was determined. Thirty (30) male albino rats with a mean weight of 166.96±1.93g were divided into three groups thus: Group I (normal control), Group II (diabetic control) and Group III, (*Laportea ovalifolia* treatment). Treatment with L*aportea ovalifolia* significantly reduced ($P<0.001$) the level of plasma glucose and significantly increased ($P<0.05$) levels of the antioxidant enzymes when compared with the untreated group. The results indicate that *Laportea ovalifolia,* in addition to being antihyperglycemic, is effective in reducing oxidative stress in diabetes.

Key words: Larpotea ovalifolia · Antioxidative potential · Ethanolic extract · Diabetes

INTRODUCTION

Diabetes is a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose caused by a relative or absolute deficiency in insulin [1]. In line with the above, [2] defines diabetes as a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both.

Diabetes is associated with a number of complications including ketoacidosis, recurrent infections, weight loss, cardiovascular disease, diabetic neuropathy, diabetic nephropathy and diabetic retinopathy. The oxidative stress which is associated with diabetes mellitus might play an important role in the initiation and progression of diabetic complications [3]. Free radicals trigger cataract, one of the degenerative manifestations of diabetes [3]. Patients with diabetes have decreased antioxidant defenses with lower levels of antioxidants such as vitamin C and E or reduced activities of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase [4]. One promising aspect of understanding the role of oxidative stress in diabetes-mediated disorders is the ability of antioxidant supplementation to attenuate diabetes adverse effects [5].

Several studies have reported that reactive oxygen species cause damage to biological macromolecules leading to lipid peroxidation, protein oxidation, enzyme inactivation, DNA base modifications and DNA strand breaks [6]. Because much of this damage occurs within cells, the antioxidant activity in tissue is considered to be more relevant than that in plasma. The liver is the main organ involved in the metabolism of biological toxins and medicinal agent and such metabolism is always associated with the disturbance of hepatocyte biochemistry and generation of reactive oxygen species [7]. Therapy using antioxidants as contained in medicinal plants can prevent, delay or ameliorate these complications.

The mechanisms of action of antidiabetic plants have been identified by several researchers to include one or a combination of the following: inhibition of carbohydrate hydrolyzing enzymes in the GIT [8], insulin-like activity, insulinase inhibition, increased glycogen storage, improved peripheral glucose utilization and antioxidant activity [9].

There is an increasing interest among diabetic patients and health professionals in using medicinal herbs. These natural products are available in abundance and can provide safe, cheap, stable, standardized and efficacious medicinal preparations [10]. The plant *Laportea ovalifolia* belongs to the nettle family, *Urticaceae*). The people of Ibibio tribe in Nigeria use the leaves and tender shoots of the plant as pot herb or vegetables in soups [11]. Some households use the plant

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as vegetables when preparing food for babies. Several studies have shown that nettles have been used to treat many infections. When administered 30 minutes before glucose loading aqueous extract of stinging nettle (*Urtica dioca*), a plant of the same family as *Laportea ovalifolia,* showed a strong glucose lowering effect [12]. This effect according to [12] may be caused in part by the reduction of intestinal glucose absorption. In the diabetic rats, 2 weeks of daily, intragastric treatment with *Laportea ovalifolia* not only produced a significant reduction in the fasting serum glucose concentrations but also lowered the serum concentrations of total cholesterol, triglycerides and low-density lipoprotein cholesterol, [13].

It is important therefore to carry out a study on the antihyperglycemic and antioxidative potential of this common plant. The result obtained from this study will go a long way to helping the poor and uneducated folks in our locality who tend to prefer herbal therapy to conventional medicine to manage diabetes at lower cost.

MATERIALS AND METHODS

Materials and Equipment: The materials and equipment used in the course of this study include: wooden cages, water bottles, feeding tube, syringes (1ml and 5ml), test tubes, beakers, glass pipettes, automatic micropipettes, glucometer, lancet, cannula, electric blender, centrifuge, water bath, refrigerator, deep freezer, water bath, timer, rotary evaporator (RE-52A, Shangai Ya Rong Biochemistry Instrument Company, England), visible spectrophotometer (SP-300 Optima, Japan).

Chemicals and Reagents: All chemicals used for this research were of analytical grade. Ethanol was from James Burrough Limited, England. Streptozotocin used for induction of diabetes was obtained from Sigma, St Louis, Mo, U.S.A. Glucometer strips were from Lifescan Inc., USA. Assay kits for the determination of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and malondialdehyde (MDA) were obtained from OXIS International, Inc., USA. An assay kit for glucose determination from Randox laboratories Ltd, United Kingdom was used.

Collection and Preparation of Plant Materials: The plant used for this study was identified and authenticated as *Laportea ovalifolia* with voucher number 359, in the herbarium of the Department of Botany, University of Calabar, Calabar. Fresh leaves of *Laportea ovalifolia* were collected from the botanical garden of the Department of Botany, University of Calabar. The leaves were rinsed with several changes of clean tap water and allowed to drain completely.

The plant materials of *Laportea ovalifolia* (700g) were chopped into bits with a knife then homogenized in 1400 liters of 80% (v/v) ethanol using an electric blender. The homogenized mixtures were kept for 48hours in the refrigerator at 4°C, after which the mixtures were filtered with cheese cloth then with Whatman No. 1filter paper. The filtrates were concentrated at 37-40°C using rotary evaporator. The concentrates were placed in water bath at 40°C for complete dryness. *Laportea ovalifolia* yielded 23.3g of extract.

Table 1: Weights of rats before and after treatment

Groups	Before Treatment (g)	After Treatment (g)	Weight Change (g)	Percentage Weight Change	N
NC	168.57±6.36^{a}	182.92±5.56^{a}	14.35±3.46^{a}	8.94±2.24^{a}	10
DC	168.96±3.98^{a}	155.82±6.08^{b}	-13.14±4.86^{b}	-7.76±2.80^{b}	10
LO	177.03±3.35^{a}	190.28±5.40^{a}	13.25±4.05^{a}	7.48±2.17^{a}	10

NC: Normal control; DC: Diabetic control; IN: Insulin treated group; LO: *Larpotea ovalifolia* treated group. Means on the same column with different superscripts are significantly different (P<0.05)

Table 2: Glucometer readings of fasting blood glucose before and after treatment

Groups	Before Treatment (mmol/L)	After Treatment (mmol/L)	N
NC	3.08±0.16	2.88±0.20^{NS}	10
DC	11.12±1.12	12.12±0.94^{NS}	10
LO	11.56±0.90	3.61±0.43*	10

NC: Normal control; DC: Diabetic control; IN: Insulin treated group; LO: *Larpotea ovalifolia* treated group; Results are presented as mean ± standard error; * = Significantly different when compared with blood glucose before treatment (P<0.001); NS= Not Significant

Table 3: Effect of treatment on the levels of antioxidant enzymes, malondialdehyde and fasting plasma glucose

Groups	CAT (U/ml)	SOD (U/ml)	GPx (mU/ml)	MDA (µmol/L)	Glucose (mmol/L)	N
NC	12.56±0.19^{a}	158.98±38.46^{a}	171.32±22.14^{a}	7.11±2.56^{c}	2.09±0.15^{c}	10
DC	11.14±0.65^{b}	29.78±15.23^{b}	12.83±4.87^{b}	25.08±4.17^{a}	9.38±1.21^{a}	10
LO	12.57±0.44^{a}	63.22±14.58^{b}	133.04±20.20^{a}	14.77±4.06^{b}	3.73±0.43^{b}	10

NC: Normal control; DC: Diabetic control; LO: *Larpotea ovalifolia* treated group; CAT: Catalase; SOD: Superoxide Dismutase; GPx: Glutathione Peroxide; MDA: Malondialdehyde; GL: Fasting Plasma Glucose; Means on the same column with different superscripts are significantly different (P<0.05)

Animals: Thirty male albino wistar rats of average weight 166.96±1.93g were used for this study. The animals were obtained from the animal house of the department of pharmacology, University of Calabar. The animals were allowed to acclimatize for 7 days in the animal house of the department of Biochemistry where they were housed during the study. The animals were housed in well ventilated cages and kept under environmental temperature of (25 ±5°C), relative humidity of (50±5%) and 12 hour light/ dark cycle.

Induction of Diabetes: The animals were subjected to 12 hour fast after which 65mg/kg body weight streptozotocin (STZ) reconstituted in normal saline was injected intraperitoneally to induce diabetes. Diabetes was not induced in animals that were used as normal control. Confirmation of diabetes was done after seven days of induction. Blood was collected from the tail by a prick with a lancet for fasting blood glucose estimation. Estimation of fasting blood glucose was done using one touch glucometer and animals with fasting blood glucose >126 mg/dl (7.0 mmol/l) were regarded as diabetic.

Experimental Design : After induction and confirmation of diabetes, rats were randomly divided into 3 groups containing 10 rats each. Each group received different treatments twice daily for 28 days as follows:

Group 1: Normal control (Received 0.2ml of distilled water).
Group II: Diabetic control (Received 0.2ml of distilled water).
Group III: *Laportea ovalifolia* treated group (Received 200mg/kg body weight of the extract).

Sample Collection and Processing: After 28 days of treatment, the animals were subjected to 12 hour fast. The rats were euthanized by anesthesia under chloroform vapor and dissected. Blood was collected by cardiac puncture, transferred into fluoride oxalate bottle and mixed thoroughly. After two hours this was centrifuged at 4000g for 10 minutes and the plasma was obtained and stored frozen until used. The liver was removed surgically, immediately blotted using filter paper to remove traces of blood.

The liver was first rinsed with ice-cold heparinized saline (0.9% NaCl containing 0.16mg/ml heparin) to remove blood cells and thereafter blotted with blotting paper. One gram of the tissue was weighed and thoroughly homogenized in 10ml of ice-cold phosphate buffered saline (20mM; pH 7.4). The homogenate was then centrifuged at 3,000g for 10 minutes and the supernatant decanted into clean tubes and stored frozen until used for the assay of catalase, SOD, GPx and MDA.

Statistics: The results were expressed as mean ± standard deviation (SD). Student t-test was used to compare group means before and after treatment. The mean values for all parameters between groups were compared using analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD). The difference was considered to be significant when p value was less than 0.05.

RESULTS

Table 1 shows the mean weight before treatment and after treatment for normal control (NC), diabetic control (DC), *Laportea ovalifolia* treated group (LO). The mean weights before treatment were: 168.57 ± 6.36g, 168.96 ± 3.98g, 177.03 ± 3.35g for NC, DC and LO respectively. While the mean weights after treatment were: 182 ± 5.56g, 155.82 ± 6.08g, 190.28 ± 5.40g for NC, DC and LO respectively. The differences in weights before treatment and after treatment were used to determine the percentage weight increase. Values obtained for weight change and percentage weight change for NC, DC, LO, GL and IN were: (14.35 ± 3.46g, 8.94 ± 2.24%); (-13.14 ± 4.86g, -7.76 ± 2.80%); (13.25 ± 4.05g, 7.48 ± 2.17%). The result shows that untreated diabetic rats recorded a reduction in weight whereas normal control and rats receiving *Laportea ovalifolia* treatment recorded increase in weight. The percentage weight gain of untreated diabetic rats was significantly lower (P<0.05) when compared with normal control and treated group. No significant difference in percentage weight gain (P<0.05) was observed between normal control and *Laportea ovalifolia* treated group after treatment.

Table 2 shows the result obtained for glucometer readings of the blood glucose of rats before treatment and after treatment The mean levels of blood glucose before treatment were: 3.08 ± 0.16mmol/L, 11.12±1.12mmol/L, 11.56 ± 0.90mmol/L for NC, DC and LO respectively. After treatment, the blood glucose levels recorded were: 2.88 ± 0.20mmol/L, 12.12±0.94mmol/L, 3.61 ± 0.43mmol/L for NC, DC and LO respectively. Treatment with, *Laportea ovalifolia* significantly reduced (P<0.001) and returned the high level of blood glucose found before treatment to normal. On the other hand, untreated diabetic rats recorded a high level of blood glucose even at the end of the experiment.

The result of the effect of treatment with *Laportea ovalifolia* on the levels of catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), malondialdehyde (MDA) and plasma glucose is shown on Table 3. The mean levels of catalase in the hepatocytes were:

12.56 ± 0.19 U/ml, 11.14 ± 0.65 U/ml, 12.57 ± 0.44 U/ml for NC, DC and LO respectively. The mean levels of SOD were: 158.98 ± 38.48 U/ml, 29.78 ± 15.22 U/ml, 63.22± 14.58 U/ml for NC, DC and LO respectively. The mean levels of GPx were: 171.32 ± 22.14 mU/ml, 42.83 ± 4.87 mU/ml, 133.04 ± 20.20 mU/ml for NC, DC and LO respectively. The mean levels of MDA in the hepatocytes were: 7.11 ± 2.56 μmol/L, 25.08 ± 4.17 μmol/L, 14.77 ± 4.06 μmol/L for NC, DC and LO respectively. Values obtained for the mean plasma glucose were: 2.09 ± 0.15 mmol/L, 9.38 ± 1.21 mmol/L, 3.73 ± 0.43 mmol/L for NC, DC and LO respectively. There was a significant difference ($P<0.001$) among the groups for all parameters. The diabetic control group had a significantly higher ($P<0.05$) plasma glucose than all other groups. Plasma glucose level of normal control was significantly lower ($p<0.05$) than those of LO treated group.

Diabetic control rats recorded a significantly lower ($P<0.05$) levels of antioxidant enzymes when compared with normal control and LO treated. No significant difference ($P>0.05$) was found in catalase activity between normal control and LO treated. There was a significantly higher SOD activity in normal control when compared with diabetic control and LO treated. The difference in the level of Gpx between normal control and LO treated was not significant ($P>0.05$). There was a significantly lower ($p<0.05$) MDA in normal control than diabetic control and treated group. Treatment with LO resulted in a significantly lower MDA concentration in the hepatocytes when compared with the untreated diabetic rats.

DISCUSSION

Weight reduction has been known to be one of the symptoms of diabetes mellitus. In the diabetic control rats, deficiency of insulin led to decreased amino acids uptake by tissues with a consequent reduction in the level of protein synthesis. Also insulin deficiency results in lipolysis in adipose tissues and protein breakdown in tissues [14]. The increase in weight observed in the group treated with *Laportea ovalifolia* and normal control group at the end of the experiment, is a reflection of efficient insulin action. Hyperglycaemia in diabetes mellitus is responsible for the development of oxidative stress via glucose auto-oxidation and protein glycation [15]. Bioactive molecules present in *Laportea ovalifolia* extract may probably possess insulin- like effect or stimulate the β cells of the pancreas to produce insulin which in turn lowers the blood glucose level. A possible mechanism of action of *Laportea ovalifolia* could be by increasing pancreatic secretion of insulin [13].

In the hepatocytes, *Laportea ovalifolia* has efficiently reduced enzyme protein oxidation which may occur as a result of accumulation of hydrogen peroxide and other free radicals. Superoxide dismutase converts superoxide radical to hydrogen peroxide and the hydrogen peroxide is destroyed by catalase or glutathione peroxidase. Treatment with *Laportea ovalifolia* has therefore prevented depression of the activity of these antioxidant enzymes which would have occurred as an adaptive response to oxidative stress. Diabetic control rats on the other hand recorded the lowest levels of these enzymes. The free radicals generated in this group, in addition to protein glycation as a result of hyperglycemia could inactivate the enzymes. In line with the findings of this study, [16] from their research had observed that superoxide dismutase and catalase are inactivated by sugars. Also, [3], observed that superoxide dismutase, catalase and glutathione peroxidase decrease in liver, kidney and heart of tissues of patients with diabetes mellitus while the level of reactive oxygen species such as superoxide anion radicals increase.

Mineral elements form part of some enzymes. For example, catalase contains iron, superoxide dismutase contains copper and zinc while glutathione peroxidase is a selenium containing enzyme. *Laportea ovalifolia* belongs to the plant family, *urticaceae* found to be rich in minerals [17]. The results obtained for diabetic rats treated with extract of *Laportea ovalifolia* may be an indication of the availability of these metals for the formation of the enzymes. It is interesting to discover from a study carried out by [18] that the excretion of these important metals is higher in diabetic patients than in normal controls. This observation could explain why there are significantly reduced levels of these metal containing antioxidant enzymes in the untreated diabetic rats.

The study shows that MDA, a lipid peroxidation product and a marker of oxidative stress was significantly lower in the group treated with *Laportea ovalifolia* when compared with diabetic controls. This indicates that the diabetic rats were exposed to an increased oxidative stress and lipid peroxidation. Similar result was also found in diabetic patients by [19]. Abnormally high levels of free radicals, lipid peroxidation and simultaneous decline in antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes [19]. Lower levels of MDA in normal controls when compared with *Laportea ovalifolia* treated group could be a reflection of increase in the formation of free radicals as a result of diabetes.

The result obtained from this study shows that *Laportea ovalifolia* increases the levels of antioxidant enzymes and inhibits lipid peroxidation. *Laportea ovalifolia* therefore has great potential to reduce complication that occurs in diabetes as a result of oxidative stress.

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