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Evaluation of the Effects of Aqueous Whole Plant Extract of *Lepidium sativum* L. (Brassicaceae) on some Biochemical and Hematological Parameters in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Lepidium sativum is widely accepted in folkloric medicine to be effective in the treatment of diabetic mellitus among the Hausa dwellers in Jos North Local Area of Plateau State, Nigeria yet, there was no scientific evidence to support this claim. This study was carried out to evaluate some biochemical and haematological parameters that could be used to determine the antidiabetic activity of the aqueous extract of Lepidium sativum whole plant in both normal and streptozocin-induced diabetic rats. After the safety of the plant was established using LD_{50}, Streptozotocin-induced diabetic and non-diabetic rats were administered with aqueous extract of the whole plant at 100 mg/kg and 200 mg/kg body weight through intragastric tube for four weeks. There was a statistically significant ($p<0.05$) reduction in the level of blood glucose of the rats as compared to the normal control after administration with respect to the biochemical and hematological parameters analysed. The results from this study suggest that the aqueous extract of the whole plant possesses some hypoglycemic properties and could have potential to be used as antidiabetic.

Keywords: *Lepidium sativum*, Brassicaceae, Streptozotocin, Glibenclimide, Diabetes, Blood and glucose

INTRODUCTION

Lepidium sativum L. (Brassicaceae) popularly known as Garden cress is an annual, fast growing, edible plant botanically related to watercress and mustard and sharing their peppery, tangy flavor and aroma [1]. Various parts of the plant namely; seeds, leaves and roots have been used in treating various human ailments such as inflammation, pyretism, pain, hemorrhage, diabetes, hypertension, etc. [2,3]. The leaves of *Lepidium sativum* known locally as "Lansir" by the Hausa in Northern Nigeria are commonly used in folk medicine for the treatment of diabetes. This study seeks to investigate the effect of *Lepidium sativum* (Garden cress) aqueous extract on some biochemical parameters in Streptozotocin-induced diabetic Rats. The aim therefore is to provide some scientific basis if any for the use of this plant as anti-diabetic agent.

MATERIALS AND METHODS

Plant collection, identification and preparation

Lepidium sativum whole plant was collected on the 21st January, 2016 from 'Farin gada' locality, near the permanent site of the University of Jos, Jos North Local Government Area of Plateau state, Nigeria. The plant was identified in the field using the descriptions and keys by the 'Flora of West Tropical Africa' [4] and the 'Woody plants of Ghana" [5]. The identity of the plant was authenticated at the Department of Horticulture and Landscape Technology, Federal College of Forestry, Jos, Nigeria and assigned Voucher specimen Number (FHJ 221). The plant was collected and air dried at room temperature under shade until a constant weight was obtained for a period of three weeks. The plant was then pounded to coarse powdery form using local pestle and mortar. The powdery form was sieved with a mesh of size-20 to obtain fine powder and stored in an air-tight container until when required for use.

Plant extraction

The powdered whole plant of *Lepidium sativum* (120 g) was extracted by maceration using water as the extraction

solvent for 72 h with intermittent shaking at 2 h intervals. The extract was filtered with Whatman No. 1 filter paper. A rotary evaporator (Rotavapour R 210, Buchi, Switzerland) was used to concentrate the extract at 4°C and the yield determined. The dried extract was then stored in a refrigerator until it was required for use.

Animals

Healthy White male albino rats (Wistar Strain) weighing between 180-210 g purchased from the animal house of the Department of Pharmacology, University of Jos, Jos Nigeria were used for the study. The experimental animals were fed with standard pellet feed and water *ad-libitum* and kept in standard cages under laboratory condition. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals and the study protocol was approved by the Institutional Animal Care and Use Committee and was assigned BCHJ302 as the ethical number.

Chemicals and reagents

General purpose solvents used were from Sigma-Aldrich (St. Louis, MO, USA).

Acute toxicity studies

The method described by Lorkes [6] and Onwusonye et al. [7] with slight modifications was used for Acute Toxicity Studies. The study was conducted in two phases. In the first phase, three groups of four mice each were administered with the whole plant extract of *Lepidium sativum* at the respective oral doses of 10, 100 and 1000 mg/kg body weight. The rats were observed for signs of toxicity such as any change in skin and fur, eyes and mucus membrane (nasal), breathing and changes like salivation, lacrimation, perspiration, piloerection, urinary incontinence ptosis, drowsiness, gait, tremors and convulsion and possible deaths for 24 h, 72 h, 2 weeks and then, 4 weeks. There was no death recorded in the first phase so, the experiment was preceded to the second phase. In the second phase, another three groups of four rats each were given respective doses of 1500, 2900 and 5000 mg/kg body weight of the extract and were also monitored as in phase one for toxicity signs and possible deaths. From the data obtained, LD_{50} was then calculated.

Experimental animals

Fifty adult male rats (50) were obtained from the animal housing unit of the University of Jos. Twenty five (25) of the rats were scheduled to be used for the experiment. The animals were fed for three (3) weeks until they attained a weight of 180 to 210 g. They were maintained in normal and standard laboratory conditions of temperature with a 12 h light-dark cycle and adequate ventilation. The rats were fed with standard pellet feed and water *ad libitum*. Diabetes was experimentally induced after fasting was prescribed for the animals overnight by administering intraperitoneally (i.p) streptozotocin (55 mg/kg). The animals were then left for 72 h after which the blood glucose levels were measured. Diabetes was confirmed from the fasting blood glucose using On Call Plus Glucometer. The rats with plasma glucose >200 mg/dl were classified as diabetic and were included in the study.

Induction of diabetes

The method used by Kumaresan et al. [8] was adopted with some modifications. Following induction of diabetes, diabetic animals were randomly distributed into five groups of five animals each. The fifth group served as the control. The animals were treated daily for 28 days as follows:

Group A: Normal Control Rats (NC): Non-diabetic rats fed with normal diet for four weeks.

Group B: Diabetic Control rats (DC): Rats fed with normal diet for four weeks.

Group C: Diabetic treated rats (DT_E): Rats fed on normal diet+*Lepidium sativum* extract (100 mg/kg b.w) for four weeks.

Group D: Diabetic treated rats (DT_E): Rats fed on normal diet+*Lepidium sativum* extract (200 mg/kg b.w) for four weeks.

Group E: Diabetic treated rats (DT_G): Rats fed on normal diet+glibenclimide (Hovid) for four weeks.

Animals in Groups C and D were given 100 mg/kg/day and 200 mg/kg/day of *Lepidium sativum* extract through intragastric tube.

The standard drug glibenclimide (Hovid) was administered to Group E at 2 mg/kg/day through intragastric tube.

Blood collection

On the 28th day, the experimental animals were fasted for 24 h and then sacrificed by decapitation. The blood was collected in clean dry centrifuge tubes and was allowed to clot for 40 min and spun at 5,000 rpm for 10 min. The serum was collected, transferred to bijou bottles and kept for analysis.

Assay of biochemical parameters

Protein concentrations of the various samples were determined by means of the Biuret method as described by Jarald et al. [9] with some modifications. Potassium iodide was added to the reagent to prevent precipitation of Cu^{2+} ions as cuprous oxide. Plasma was analyzed for Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) by standard enzymatic assay method. High density lipoprotein-cholesterol was determined in serum by the method of Laxmiverma et al. [10]. The Randox HDL-cholesterol precipitant Kit was used. The levels of cholesterol were determined using Randox kits, a method of enzymatic hydrolysis described by Emeka et al. [11]. Serum Bilirubin was determined by Colorimetric method based on that described by Rajaram [12]. The method of Sambo et al. [13] was used in the determination of serum creatinine. Determination of Serum Chloride (Cl^-) was by Mercuric Nitrate titrimetric Method of Iweala et al. [14].

Assay of hematological parameters

The haematological parameters and other blood chemistries were determined using blood collected with EDTA containers.

Statistical analysis

The results were expressed as mean ± Standard Deviation (SD) using one-way Analysis of variance (ANOVA) and student's t-test to evaluate the significant difference between the mean value of the measured parameters in the respective test and control groups. A significant change was considered acceptable at $p<0.05$.

RESULTS

Results of aqueous extracts of *Lepidium sativum* whole plant on blood glucose, protein and serum albumin are shown in Table 1 and on blood glucose, protein and serum albumin alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)are shown in Table 2 and Table 3.The Results of the effect of aqueous extract of *Lepidium sativum* whole plant on uric acid, urea and creatinine and total bilirubin and direct bilirubin in streptozotocin-induced diabetic rats are shown in Table 4 and Table 5. The Results of the effect of aqueous extract of *Lepidium sativum* whole plant on some serum electrolytes and Results of the effect of aqueous extract of *Lepidium sativum* on some haematological parameters of streptozotocin-induced diabetic rats are shown in Table 6 and Table 7.

Table 1: Results of aqueous extracts of *Lepidium sativum* whole plant on blood glucose, protein and serum albumin in streptozotocin- induced diabetic rats

Treatment Groups	Glucose (mmol/L)	Protein (g/L)	Albumin (g/L)
Normal Control (A)	4.04 ± 0.07	77.85 ± 0.50	38.81 ± 0.50
Diabetic Control (B)	18.80 ± 0.30[a]	59.46 ± 0.67[a]	28.88 ± 0.14 [a]
Diabetic Treated 100 mg/kg (C)	11.02 ± 0.33[ab]	67.63 ± 0.38 [ab]	33.49 ± 0.08 [ab]
Diabetic Treated 200 mg/kg (D)	9.64 ± 0.30 [ab]	72.08 ± 0.20[ab]	36.11 ± 0.18 [ab]
STD Drug Treated (E)	7.88 ± 0.34 [ab]	75.22 ± 0.58 [ab]	38.12 ± 0.97 [ab]

n=5

a=statistically significant difference ($p<0.05$) compared to normal control

b=statistically significant difference ($p<0.05$) compared to diabetic control

Table 2: Results of the effects of aqueous extract of *Lepidium sativum* whole plant on total cholesterol, triglyceride, HDL and LDL

Treatment Groups	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Normal Control (A)	3.75 ± 0.05	0.89 ± 0.03	1.25 ± 0.02	1.83 ± 0.016
Diabetic Control (B)	5.88 ± 0.04 [a]	2.64 ± 0.19 [a]	0.41 ± 0.04 [a]	3.10 ± 0.06 [a]
Diabetic Treated 100 mg/kg (C)	4.92 ± 0.02 [ab]	1.89 ± 0.04 [ab]	0.65 ± 0.02[a]	2.58 ± 0.03 [ab]
Diabetic Treated 200 mg/kg (D)	4.70 ± 0.04 [ab]	1.44 ± 0.02 [ab]	0.86 ± 0.01 [ab]	2.23 ± 0.02 [ab]
STD Drug Treated (E)	4.43 ± 0.11 [ab]	1.19 ± 0.01 [ab]	1.08 ± 0.04[b]	2.02 ± 0.07 [ab]

n=5
a=statistically significant difference ($p<0.05$) compared to normal control
b=statistically significant difference ($p<0.05$) compared to diabetic control

Table 3: Results of the effects of aqueous extract of *Lepidium sativum* whole plant on alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP)

Treatment Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal Control (A)	12.54 ± 0.10	17.16 ± 0.26	158.31 ± 1.61
Diabetic Control (B)	36.81 ± 0.25[a]	58.72 ± 0.32[a]	490.41 ± 0.83 [a]
Diabetic Treated 100mg/kg (C)	29.57 ± 0.81 [ab]	43.42 ± 0.46 [ab]	379.47 ± 2.59 [ab]
Diabetic Treated 200mg/kg (D)	25.01 ± 0.31 [ab]	38.63 ± 0.02 [ab]	350.16 ± 3.52 [ab]
STD Drug Treated (E)	16.40 ± 0.55 [ab]	19.65 ± 0.67 [ab]	262.61 ± 2.17 [ab]

n=5
a=statistically significant difference ($p<0.05$) compared to normal control
b=statistically significant difference ($p<0.05$) compared to diabetic control

Table 4: Results of the effect of aqueous extract of *Lepidium sativum* whole plant on uric acid, urea and creatinine in streptozotocin-induced diabetic rats

Treatment Groups	Uric Acid (umol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Normal Control (A)	198.90 ± 0.78	4.68 ± 0.03	79.82 ± 0.33
Diabetic Control (B)	574.19 ± 481.68 [a]	18.43 ± 0.62[a]	348.77 ± 12.99 [a]
Diabetic Treated 100mg/kg (C)	481.68 ± 4.26 [ab]	14.92 ± 0.23[ab]	342.29 ± 41.74[a]
Diabetic Treated 200mg/kg(D)	450.52 ± 0.84 [ab]	12.73 ± 0.44 [ab]	227.48 ± 7.32 [ab]
STD Drug Treated (E)	422.05 ± 0.76 [ab]	9.06 ± 0.30 [ab]	141.78 ± 0.95 [ab]

n=5
a=statistically significant difference ($p<0.05$) compared to normal control
b=statistically significant difference ($p<0.05$) compared to diabetic control

Table 5: Results of the effect of aqueous extract of *Lepidium sativum* whole plant on total bilirubin and direct bilirubin

Treatment Groups	Total-Bilirubin (umol/L)	Direct-Bilirubin (umol/L)
Normal Control (A)	8.03 ± 0.32	3.90 ± 0.03
Diabetic Control (B)	29.74 ± 0.35[a]	10.30 ± 0.06[a]
Diabetic Treated 100 mg/kg (C)	24.13 ± 0.46[ab]	13.05 ± 0.31[ab]
Diabetic Treated 200 mg/kg (D)	17.76 ± 0.32[ab]	8.71 ± 0.29 [ab]
STD Drug Treated (E)	15.13 ± 0.59[ab]	5.49 ± 0.40 [ab]

n=5
a=statistically significant difference ($p<0.05$) compared to normal control
b=statistically significant difference ($p<0.05$) compared to diabetic control

Table 6: Results of the effect of aqueous extract of *Lepidium sativum* whole plant on some serum electrolytes in streptozotocin-induced diabetic rats

Treatment Groups	Phosphate (mmol/L)	Calcium (mmol/L)	Sodium (Na+) (mmol/L)	Potassium (K+) (mmol/L)	Chloride (Cl-) (mmol/L)	Bicarbonate (HCO_3^-) (mmol/L)
Normal Control (A)	1.12 ± 0.01	2.45 ± 0.02	144.80 ± 0.84	3.73 ± 0.2	113.00 ± 1.58	24.60 ± 1.14
Diabetic control (B)	1.25 ± 0.08[a]	1.59 ± 0.06[a]	132.40 ± 1.14[a]	5.88 ± 0.04 [a]	102.60 ± 1.14 [a]	17.40 ± 1.14 [a]
Diabetic Treated 100 mg/kg (C)	1.25 ± 0.02[a]	1.85 ± 002 [a]	136.20 ± 1.30[a]	4.63 ± 0.016	106.40 ± 1.14[ab]	19.60 ± 1.14[ab]
Diabetic Treated 200 mg/kg (D)	1.43 ± 0.01[b]	1.98 ± 0.01[a]	138.60 ± 1.14[ab]	4.71 ± 0.021[ab]	108.40 ± 1.14[ab]	21.80 ± 0.84[ab]
STD Drug Treated (E)	1.33 ± 0.02[b]	2.09 ± 0.02[b]	140.40 ± 1.14[ab]	4.45 ± 0.98 [ab]	110.60 ± 1.14[ab]	22.40 ± 1.14[ab]

n=5

a=statistically significant difference ($p<0.05$) compared to normal control
b=statistically significant difference ($p<0.05$) compared to diabetic control

Table 7: Results of the effects of aqueous extract of *Lepidium sativum* whole plant on some haematological parameters of streptozotocin-induced diabetic rats

Treatment Groups	Normal Control (A)	Diabetic Control (B)	Diabetic Treated 100 mg/kg (C)	Diabetic Treated 200 mg/kg (D)	Std Drug Treated (E)
PCV (%)	47.80 ± 0.45	31.80 ± 1.30[a]	36.00 ± 0.71[ab]	37.20 ± 1.30[ab]	41.00 ± 1.00[ab]
Haemoglobin (g/dl)	16.00 ± 0.71	10.60 ± 1.14[a]	12.00 ± 0.71[ab]	12.51 ± 0.17[ab]	13.49 ± 0.29[ab]
RBC(mm^3)	9.79 ± 0.02	4.72 ± 0.13[a]	6.52 ± 0.13[ab]	7.02 ± 0.22[ab]	8.54 ± 0.21[b]
WBC (mm^3)	6540.00 ± 114.02	10120.00 ± 311.45[a]	8140.00 ± 114.02[ab]	7540.00 ± 114.02[ab]	6900.00 ± 158.11[b]
Platelet (mm^3)	166200.00 ± 836.66	258000.00 ± 1000.00[a]	248200.00 ± 836.66[a]	202120.00 ± 99243.00[ab]	238600.00 ± 1140.18[ab]
Neutrophil (%)	30.00 ± 0.71	18.60 ± 1.14[a]	22.00 ± 0.71[ab]	25.20 ± 1.30[ab]	28.40 ± 1.14[b]
Lymphocyte (%)	65.00 ± 0.71	78.40 ± 1.14[a]	75.60 ± 1.14[ab]	69.60 ± 1.14[ab]	67.00 ± 0.71[b]
Eosinophil (%)	2.20 ± 0.45	0.40 ± 0.55[a]	0.60 ± 0.55[a]	1.40 ± 0.55	1.40 ± 0.55
Basophil (%)	0.00 ± 0.00	0.40 ± 0.55	0.60 ± 0.55	0.60 ± 0.55	0.40 ± 0.55
Monocyte (%)	3.00 ± 0.00	2.00 ± 0.00	1.60 ± 0.55[a]	3.60 ± 0.55[b]	2.60 ± 0.55

n=5
a=statistically significant difference ($p<0.05$) compared to normal control
b=statistically significant difference ($p<0.05$) compared to diabetic control

DISCUSSION

The LD_{50} was calculated to be 2000 mg/kg body weight. The acute toxicity study using the loading dose of 2000 mg/kg body weight of the aqueous whole plant extract of *Lepidium sativum* did not result in any observable symptoms or in death. No toxic effects were observed throughout the study period. No rats showed signs of toxic effect such as changes on skin and fur, eyes and mucus membrane, behavior pattern, tremors, salivation, diarrhea, sleep and coma or death. The drug is said to be relatively safe when taken for a short period of time.

On the induction of diabetes, there was a rise in the serum glucose level and a significant fall in the protein and albumin level. Increased glycosylated hemoglobin is used as an indicator of elevated glucose level in the blood. When the aqueous whole plant extract of *Lepidium sativum* was given, there was a gradual fall in the serum level of glucose and an increase in the serum level of protein and albumin indicating the hypoglycemic effect of the extract [15]. When Streptozotocin was administered in the diabetic control rats, there was an increase in the total cholesterol, Triglyceride, HDL and LDL in the blood. The increase in blood LDL and triglycerides may be due to the action of hormone sensitive lipase, which promotes lipolysis and subsequently increases the level of plasma free fatty acids and triglycerides. When the extract was administered to the rats, there was a significant reduction in the serum level of these parameters ($p<0.05$) compared to the normal control rats in a dose dependent way. The significant reduction in serum cholesterol and triglyceride levels may be due to reduction or inactivation of the multienzyme complex of fatty acid synthesis HMG-CoA reductase. These free fatty acids are catabolized to acetyl Co A which is further channeled to cholesterol synthesis thus, increasing blood cholesterol level [16].

As diabetes was induced, there was a progressive increase in the serum level of these enzymes showing evidence of hyperglycemia. The aqueous whole extract of *Lepidium sativum* on the other hand significantly ($p<0.05$) reduced the different serum enzyme levels in a dose dependent form. Elevated activities of the serum transferase enzymes are common signs of liver diseases and are frequently observed among people with diabetes mellitus than the general population. Furthermore, diabetic complications such as restricted joint mobility, retinopathy and neuropathy were shown to be associated with liver enzyme activities, alcohol consumption and body mass index among others [17]. On induction of diabetes, there was a drastic increase in the serum level of these parameters showing evidence of hyperuricemia, hypercreatinaemia and ureamia in the diabetic control rats but following treatment with the extract, the serum level reduced significantly ($p<0.05$) compared to the normal control rats in a dose dependent pattern. An increased level of creatinine has been associated with a low level of Hemoglobin, often leading to anaemia. The reduced creatinine level in the diabetic rats was observed to be inversely proportional to the Hemoglobin level, thus correlating with the antianemic potential of the extract [18].

Furthermore, direct and total bilirubin levels increased significantly ($p<0.05$) in streptozotocin-induced diabetic rats, suggesting that Diabetes affects bilirubin excretion. On the administration of the extract, the serum level reduced significantly ($p<0.05$) compared to the normal control rats in a dose dependent pattern indicating that extracts of *Lepidium sativum* enhances the excretion of bilirubin and reduced oxidation. Bilirubin is an excretory product formed by the catabolism of heme which is excreted by the liver [19]. When diabetes was induced, there was a decrease in the composition of Phosphate, Calcium, Sodium, Potassium, Chloride and Bicarbonate minerals in the serum but with the administration of the extract, a very high concentration of the electrolytes ($p<0.05$) was observed, with the concentration of sodium being the highest and phosphate being the lowest in a dose dependent manner. Electrolytes such as sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and bicarbonate (HCO_3^-) are involved in the maintenance of osmotic pressure of the heart and other muscles, electron transfer reactions and in the catalysis and cofactors for enzymes. The significant increase in the serum Na^+ seems to suggest that *Lepidium sativum* aqueous whole plant enhances Na^+ ion balances, thus preventing hyperosmolar non-ketotic state. Furthermore, the increase in serum Cl^- and HCO_3^- observed in this study would suggest that the extract enhances rehydration and/or prevent metabolic acidosis. In this study it may be possible that the decrease in serum calcium in diabetic control rats may also be due to impaired calcium hemeostatis [20].

Induction of diabetes with streptozotocin led to a decrease in the RBCs, WBCs, Platelets, Neutrophils, Lymphocytes, Eosinophil, Basophils and Monocytes on the rats but a significant increase ($p<0.05$) was observed when the rats were treated with the aqueous whole plant extract of *Lepidium sativum* in a dose dependent fashion. The degree of anemia in diabetes patients can be associated with a number of factors, including glomerular filtration rate, urinary albumin excretion rate and glycated (HbA1c) levels. Anemia has been reported to be due to diminished erythropoietin production by failing kidneys and increased nonenzymatic glycosylation of RBC membrane proteins. In this study, alterations in haematological parameters' levels of the diabetic rats suggest occurrence of anemia. The observed increase in these parameters on the administration of the extract suggests its use in the management of the ailment. The reduced levels of WBC, Platelets, and Lymphocytes in diabetic rats indicate a suppression of the immune system. These cells identify and eliminate pathogens, either by attacking larger pathogens through contact or by phagocytosis. They form part of the innate immune system, which is also an important mediator in the activation of the adaptive immune system. The reduced immunity can contribute to the various complications associated with diabetes mellitus but the extract was able to increase the level to an appreciable level compared to the control, suggesting a boost in the immune system [21].

CONCLUSION

The whole plant aqueous extract of *Lepidium sativum* was demonstrated to have hypoglycemic, hypolipidemic, and hypocholerestorlaemic properties, lowers the elevated activities of serum enzymes in diabetic rats and enhances protection against renal dysfunction by reducing the creatinine levels. These properties provide some biochemical and hematological basis for its use in the management of Diabetes.

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