Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.12, No.2, 2021

Effect of Aqueous Fruit Extract of Cucumis Sativus on Some Biochemical Parameters in Streptozotocin Induced-Diabetic Rats

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Abstract
Diabetes mellitus is among the major global public health problems and its prevalence is currently increasing at an alarming rate. The research was carried out to evaluate the effect of aqueous fruit extract of *Cucumis sativus* on blood glucose, total protein, total bilirubin, cholesterol, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), urea, creatinine levels as well as aspartate aminotransferase (AST), alkaline Phosphatase (ALP) and electrolytes (Na^+, Cl^- and HCO_3^-) levels on normal and streptozotocin induced-diabetic rat. The aqueous fruit extract was administered orally at a dose of 400mg/kg body weight to both normal and streptozotocin induced-diabetic rat. Twenty adult male rats were divided into four groups of five rats each, two groups were made diabetic and the other two groups were non-diabetic. One of the diabetic groups were administered with the aqueous fruit extract and the second served as diabetic control. The streptozotocin was administered intraperitoneal at a dose of 55mg/kg per body weight. The administration of the aqueous fruit extract lasted for 28 days. Effect of aqueous fruit extract on blood glucose, total protein, total bilirubin, cholesterol, triglycerides, LDL, HDL, urea, creatinine, and electrolytes (Na^+, Cl^- and HCO_3^-) levels were analysed. The toxic effect of the aqueous fruit extract was determined using biochemical enzymes markers. The photochemical screening of the aqueous fruit extract showed the presences of Alkaloids, Balsam, Cyanogenic glycosides, Flavonoids, Saponins, Resins and Carbohydrate. Administration with the fruit extract showed significant ($P<0.05$) reduction on the serum blood glucose level and other biochemical parameters analyzed. The extract possesses no toxic effect as indicated by lowered AST and ALP levels and may be used for the management of diabetes mellitus.
Keywords: *Cucumis sativus,* Phytochemicals, Diabetic Mellitus, Liver enzymes, hypoglycemic activity.
DOI: 10.7176/JNSR12-2-04
Publication date: January 31st 2021

1. Introduction

Diabetes mellitus is a non-communicable disease which have been shown to improve with medicinal plants. It is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fats and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO, 2006). Diabetes is also referred to as a syndrome of disorder in metabolism usually due to the combination of hereditary and environmental causes resulting in abnormally high blood sugar levels (hyperglycemia) (Mohammed *et al*., 2017). Blood glucose levels are controlled by a complex interaction of multiple chemical and hormones in the body including the hormones insulin made in beta cells of the pancreas. Diabetes mellitus develops due to diminished production of insulin (in type I) or resistances to its effects (in type II and gestational), both leads to hyperglycemia, which largely causes the acute signs of diabetes and changes in energy metabolism (Alhassan *et al*., 2017). As a result of the deficiency of insulin or inadequate insulin function there is an inadequate transfer of glucose into the cells; the utilization of glucose for energy and cellular products and its conversion to glycogen or fat and storage as such are depressed, thereby leading to accumulation of glucose in the blood, causing hyperglycemia (Muhammad *et al*., 2016). Fat may be mobilized from adipose tissue and broken down to provide a source of energy, which is eventually withdrawn from the body by the liver and broken down to glycerol and fatty acids leading to oxidation by the hepatic cells to ketone bodies and metabolizes by cells to produced energy, carbon dioxide and water (Rui, 2014). Only a limited amount of ketone acids can be utilized by cells as such if ketogenesis proceeds rapidly, exceeding the rate at which they can be metabolized, the ketone acids accumulate in the blood causing ketosis or ketone acidosis (Luka and Mohammed, 2012). Tissue protein may also be broken down to amino acids which are used in gluconeogenesis contributing to the hyperglycemia. Both the uptake of amino acids by the cells and body protein synthesis are decreased. Insulin-dependent diabetes mellitus (IDDM) usually has a sudden onset in a severe, acute form. In non-insulin-dependent diabetes mellitus (NIDDM) the onset is most often insidious going undetected and untreated for a considerable period of time (Rui, 2014).

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnosis, improve or treat physical and mental illness (Mahomoodally, 2013). Traditional medicines that have been adapted

by other populations (outside its indigenous culture) are often termed alternative or complementary medicine. Herbal preparations and finished herbal products that contain parts of plants or other plants materials as active ingredients (Pan *et al*., 2014). *Cucumis sativus* is an annual climber growing up to 2 m. The fruit is roughly cylindrical, elongated with tapered ends, and may be as large as 60 cm long and 10 cm in diameter (Uzodike and Onuoha, 2009). The cucumber is a common ingredient of salads, being valued mainly for its crisp texture and juiciness. However, it is very watery, with little flavor and is not very nutritious.

2. Materials and Method

2.1 Sources of Plant Material and Preparation of Cucumber Juice

The plant Cucumber (Cucumis sativus) fruits was obtained from Farin-gada area in Jos North Local Government, Plateau State. The plant was identified and authenticated at the Federal College of Forestry Jos North, Plateau State. The fresh Cucumber (Cucumis sativus) friuts were washed with clean water, sliced with knife and homogenized using an electric blender without addition of water. Grinder for 3 minutes and sieved to extract the filtrate.

2.2 Source of Experimental Animals

Twenty (20) adult male albino rats weighing approximately 80-150g were obtained from Animal House Unit Department of Pharmacology, University of Jos. The rats acclimatized to the laboratory condition for two weeks before any experimental work was undertaken, they were fed with standard feed.

2.3 Preparation of Streptozotocin

One gramme of Streptozotocin was dissolved in 10mls of distilled water for standardization and was used at once for inducing the experimental rats.

2.4 Induction of Experimental Diabetes

Diabetes was induced in groups A and B rats by intraperitoneal injection (IP) of streptozotocin at doses of 55mg/kg body weight. Diabetes was confirmed in the animal after 48 hours by estimation of blood glucose level. Animals with blood glucose level above 200mg/dl were selected.

2.5 Administration of the Extract

The Cucumis sativus fruit extract was administrated through the oral route at a dose of 400mg/kg body weight daily for 28days.

2.6 Experimental Design

Twenty male rats were randomly divided into four groups of five rats each and fed with standard feed as follows:
Group A - Diabetic control rats with no administration of extract (negative control).
Group B - Diabetic rats given extract (400mg/kg) body weight daily for 28 days.
Group C - Normal rats with no administration of extract (positive control).
Group D - Normal rats given extract (400mg/kg) body weight daily for 28 days.

2.7 Sample Collection and Preparation

At the end of 28 days of extract administration, blood from the animals (both treated and control groups) were collected from the jugular vein into plan bottles. The blood in the plain bottle were allowed to clot at room temperature. The clotted blood sample were ringed and centrifuged for 10 minutes at 5,000 r.p.m. Pasteur pipette were used to separate the serum (supernatant) into clean bottles. The serum were used for the biochemical assay.

2.8 Statistical Analysis

Results were expressed as mean ± standard deviation and analyzed using ANOVA, with p value <0.05 considered significant followed by Tukey´s post hoc test. A component of Graph Pad Instat3 Software 2000 version 3.05 by Graph Pad Inc was used to analyze the data.

3.0 Results

Table 1 shows the results obtained when aqueous extracts was screened for phytochemicals such as Alkaloids, balsam, cardiac glycosides, flavonoids, saponins, terpenes and steroids, phenols, resins, carbohydrates and tannins. All were detected except for terpenes and steroids, phenols and Tannins.

Table 1: Result of phytochemical screening of freshly prepared *Cucumis sativus* extract.

Bioactive constituents	Chemical test	Aqueous extract
Alkaloids	Dragendrorff	+
Balsam	Alcoholic FeCl3	+
Cyanogenic glycosides	Salkowski	+
Flavonoids	Lead acetate	+
Saponins	General test	+
Terpenes and steroids	General test	–
Phenols	General test	–
Resins	General test	+
Carbohydrate	General test	+
Tannins	Ferric chloride	–

+ = present, - = absent.

Table 2 shows serum glucose, total protein levels in the diabetic and normal groups of rats. The diabetic rats showed a significant (P<0.05) increase in serum glucose when compared with normal control rats while significant (P<0.05) decrease in total protein was observed when compared to normal control rats. On administration of *Cucumis sativus* aqueous extract there was a significant decrease (P<0.05) in serum glucose when compared with the diabetic control rats. However, there was significant (P<0.05) increase in total protein level when compared with diabetic control rats.

Table 2: Effect of *Cucumis sativus* aqueous extract on serum glucose and total protein levels in streptozotocin induced diabetic rats.

Groups	Glucose (mmol/L)	Total Protein (g/L)
Diabetic Control	17.83±0.175	65.55±0.286
Diabetic + Extract	6.85±0.198[a]	76.88±0.029[a]
Normal Control	3.39±0.223[a]	78.86±0.085[a]
Normal + Extract	4.70±0.151[a]	75.38±0.089[a]

Values are presented as mean ± SD, n=6
a = statistically significant when compared with diabetic control (P<0.05)

Table 3 shows total cholesterol, triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL) levels in the diabetic and normal groups of rats. The diabetic rats showed a significant (P<0.05) increase in all the parameters when compared with normal control rats except for high density lipoprotein level which showed a significant (P<0.05) decrease when compared with normal control rats. On administration of *Cucumis sativus* aqueous extract there was a significant (P<0.05) decrease when compared with the diabetic control rats except for high density lipoprotein which showed significant (P<0.05) increase.

Table 3: Effect of *Cucumis sativus* aqueous extract on serum lipid profile levels in streptozotocin induced diabetic rats.

Groups	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	LDL (mmol/L)	HDL (mmol/L)
Diabetic Control	5.44±0.263	2.20±0.062	2.70±0.200	0.38±0.017
Diabetic + Extract	3.97±0.188[a]	1.22±0.035[a]	1.59±0.014[a]	1.27±0.053[a]
Normal Control	3.20±0.072[a]	0.99±0.005[a]	1.00±0.037[a]	1.75±0.073[a]
Normal + Extract	3.52±0.098[a]	1.20±0.053[a]	1.11±0.054[a]	1.55±0.042[a]

Values are presented as mean ± SD, n=6
a = statistically significant when compared with diabetic control (P<0.05)

Table 4 shows result of ALT, AST, ALP, Albumin, Total Bilirubin and Direct bilirubin levels in diabetic and normal groups of rats. The diabetic rats administered with the aqueous extract showed significant (P<0.05) decrease in all the parameters analyzed except for albumin which showed significant (P<0.05) increase when

compared with diabetic control rats.

Table 4: Effect of *Cucumis sativus* extract on liver marker enzymes on streptozotocin induced diabetic rats.

Groups	ALT(U/L)	AST (U/L)	ALP(U/L)	Albumin (g/L)	T. Bilurubin (µmol/L)	D. Bilurubin (µmol/L)
Diabetic Control	44.37±0.221^{b}	46.30±15.227^{b}	389.99±0.066^{b}	30.64±0.069^{a}	34.25±0.189^{b}	21.95±0.077^{b}
Diabetic + Extract	15.83±0.164^{bc}	17.48±0.198^{bc}	160.99±0.559^{bc}	36.71±0.179^{ad}	14.90±0.095^{bc}	4.51±0.269^{bc}
Normal Control	11.22±0.098	15.41±0.188	134.43±0.322	38.77±0.112	9.55±0.152	3.82±0.103
Normal + Extract	16.09±0.066^{bc}	18.24±0.148^{bc}	246.64±0.234^{bc}	37.74±0.179^{ad}	10.27±0.173^{bc}	4.16±0.127^{bc}

Values are presented as mean ± SD, n=6

a = statistically significant when compared with diabetic control (P<0.05)

Table 5 shows the result of Urea, creatinine, Na^+, K^+, Cl^- and HCO_3^- levels in diabetic and normal groups of rats. The diabetic rats administered with the extract showed significant (P<0.05) decrease in Urea, creatinine and K^+ levels when compared to diabetic control rats while Na^+, Cl^- and HCO_3^- levels where significant (P<0.05) increase when compared with diabetic control rats.

Table 5: Effect of *Cucumis sativus* on kidney parameters in streptozotocin induced diabetic rats.

Groups	Urea (mmol/L)	Creatinine (µmol/L)	Na^+ (mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)	HCO_3^- (mmol/L)
Diabetic Control	19.94±0.059	378.44±0.303	135.56±0.550	6.38±0.181	98.52±0.321	15.44±0.294
Diabetic + Extract	5.60±0.066^{a}	104.73±0.204^{a}	138.58±0.385^{a}	4.24±0.085^{a}	109.12±0.072^{a}	25.25±0.163^{a}
Normal Control	3.98±0.020^{a}	69.93±0.110^{a}	144.59±0.305^{a}	3.93±0.208^{a}	115.61±0.314^{a}	27.33±0.229^{a}
Normal + Extract	5.95±0.081^{a}	82.86±0.142^{a}	140.57±0.396^{a}	4.63±0.316^{a}	110.58±0.301^{a}	23.91±0.420^{a}

Values are presented as mean ± SD, n=6

a = statistically significant when compared with diabetic control (P<0.05)

4.0 Discussion

Diabetes is induced by streptozotocin a glucosamine-nitrosurea compound derived from *stretomycesachromo* genes that is used clinically as a chemotherapeutic agent in the treatment of pancreatic and cell carcinoma. Streptozotocin damages pancreatic beta-cell, resulting in hypoinsulinemia and hyperglycemia (Graham *et al*., 2011). The result of the present study confirms that the administration of *Cucumis sativus* juice possesses anti diabetic activity against streptozotocin induced diabetic rats. The finding is in accordance to research of Luka and Mohammed, (2012) which showed mark decrease in blood glucose level of rats induced with alloxan when administered with extract of *Mangifera indica* leaf. The anti-diabetic effect may be due to increased release of insulin from the existing beta-cells of pancreas.

Diabetes mellitus is also associated with hyperlipidaemia with profound alteration in the concentration and composition of lipid (Odetola *et al*., 2006). Changes in the concentration of the lipid with diabetes mellitus contribute to the development of vascular disease (Pari et al., 2007, Schofield *et al*., 2016). Fatty acid, an important component of cell membranes are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body (Rajasekaran *et al*., 2006). Lipid profiles have been shown to be the important predictors for the metabolic disturbances including dyslipidemia, hypertension, diabetes, cardiovascular disease, hyperinsulinemia etc. (Kaur, 2014). Administration of aqueous extract of *Cucumis sativus* fruit caused anti-hyperlipidaemia which have been reported in similar findings of Mohammed *et al*., (2017) which showed significant (p<0.05) decrease in serum cholesterol, triglycendes and LDL levels with corresponding increase in HDL level when rats induced with alloxan and were administered with *Cocos nucifera* oil, and Luka *et al*., (2013) also showed significant (p<0.05) decrease in serum cholesterol and triglycendes levels with corresponding increase in HDL level when rats induced with alloxan were administered with aqueous extract of *Mangifera indica* leaf.

Damage to the liver is a serious disease characterized by disturbances in normal functions of the liver. It is clinically diagnosed by determining the serum concentration of liver enzymes (ALT, AST and ALP). These

enzymes are non-plasma specific enzymes and were reported to reach higher than normal levels in the blood when there is necrosis of the parenchymal cells of the liver as in viral or toxic hepatitis, with ALT being the most specific liver injury marker and a more selective liver parenchymal enzyme (Alhassan *et al*., 2017). Alkaline phosphatase (ALP) test is also used to detect bone disorders. In conditions affecting the liver, damaged liver cells release increased amounts of ALP into the blood. This test is often used to detect blocked bile ducts because ALP is especially high in the edges of cells that join to form bile ducts. If one or more of them are obstructed, then blood levels of ALP will often be high (Sherlock and Dooley, 2002). However, the difference observed in the activities of these enzymes at the dose employed (400mg/kg body weight) showed that *Cucumis sativus* fruit extract has no toxic effect on the liver of the rats. This is similar to finding of Luka *et al*., (2013) which showed statistically significant ($P<0.05$) decrease in ALT, AST, ALP, total bilirubin and direct bilirubin levels when rats were administered with *Thymus Vulgaris* and *Xylopia Aethiopica* extract with significant ($P<0.05$) increase in Albumin level when compared with diabetic control group.

Kidneys are the major organs in metabolizing toxic compound besides liver. It receives about 1200ml of blood per minute containing a lot of chemical compounds (Dollah *et al*., 2013). Therefore, damage to the kidneys can be determined by measuring the level of urea, electrolyte and creatinine in blood as an indicator of kidney damage. Administration of 400mg/kg body weight of *Cucumis sativus* fruit extract showed electrolytes balanced, this findings support the report of Ngwen *et al*., (2018) which showed significant ($p<0.05$) increase in Na^+, Cl^- and HCO_3^- with concomitant reduction in K^+ in diabetic administered with *Buchholzia coriacae* extract. Also, diabetic administered with 400mg/kg body weight of *Buchholzia coriacae* extract showed significant ($P<0.05$) decrease in urea, and creatinine levels when compared with diabetic control group. Also similar findings in the case of [18] showed significant ($p<0.05$) increase in Na^+, Cl^- and HCO_3^- with reduction in K^+ in diabetic administered with *Curcuma longa* Linn root extracts. Also, diabetic administered with *Curcuma longa* Linn root extracts showed significant ($P<0.05$) decrease in urea, and creatinine levels when compared with diabetic control group.

5.0 Conclusion

Administration of *Cucumis sativus* fruit extract decreased the concentration of blood glucose as well as protein in streptozotocin induced diabetic rats. Therefore, the friut have hypoglycaemic effects. The study also showed that *Cucumis sativus* fruit may not have toxic effect on the liver at the employed dosage since it produced no significant effects on the enzymes activities as biochemical enzymes makers of liver damage.

6.0 Ethical approval

All authors hereby declare that Principle of laboratory animal care and ethical guidelines for investigation of experimental pain in conscious animals were observed during experimentation (Zimmermann, 1983; NIH, 1996).

7.0 Competing interests

Authors have declared that no competing interests exist.

8.0 Consent

It is not applicable.

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