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Effect of Allium Cepa Supplemented Diets on Plasma Glucose, Electrolytes and Renal Histology of Streptozotocin-Induced Diabetic Rats

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Abstract: *Allium cepa (onion) contains phytochemicals that are anti-diabetic. The anti-hyperglycemic benefits of onion and its possible effects in alleviating deleterious histological changes in kidney of streptozotocin (STZ)-induced diabetic mice was studied. 20 wistar rats were grouped into 4; A: was intraperitoneally administered a single dose of STZ in NaCl (60mg/kg body weight[BW]) + 2ml onion juice 100gBW/day), B: injected with a single dose of STZ; while groups C and D served as negative and positive controls. Fasting plasma samples were drawn, glucose and electrolytes assayed using standard laboratory procedures. Histological changes in renal cells was examined using haematoxylin and eosin stains. Plasma glucose of STZ-induced diabetic rats was reduced from 18.82 ± 0.79 to 7.30 ± 1.13mmol/L after treatment with onion juice. Potassium, sodium and chloride levels were increased in diabetic rats than in controls. Tubular injury, degenerative and atrophic changes in renal glomeruli seen in untreated diabetics were less prominent and well – improved in the treated diabetic group. A normal glomerulus was observed in the control rats.*
Results from this study showed anti-diabetic and hypoglycemic effects of Allium cepa juice and suggests its possible renoprotective role in improving renal damage in diabetic animals.
Keywords : *Allium cepa Linn, diabetes mellitus, glucose, renal histology, streptozotocin*

I. Introduction

Hyperglycemia plays a major role in long-term complications associated with diabetes mellitus which include weight loss, retinopathy and nephropathy that may lead to renal failure [1,2]. The important goal of treatment of diabetes is to keep blood glucose close to normal level resulting in a major decline in progression of complications including renal damage. Onions possess bioactive substances mostly antioxidants which could prevent renal organ damage resulting from hyperglycemia in diabetes mellitus [3]. Onion (Allium cepa. Linn), commonly used as a staple in daily diet, has been extensively studied for its therapeutic properties. Phytochemicals in Allium cepa include organosulfur compounds such as cepaenes, thiosulfinates [4,5], flavonoids including quercetin and kaempferol [6] that have been reported to possess antidiabetic, antibiotic and hypocholesterolaemic properties [7].

Plasma urea and creatinine concentrations as well as urea and creatinine clearances were reported to have been improved in onion treated rabbit with renal damage [3]. Allium cepa may enhance pancreatic beta cells for insulin production [8] and also enhance cellular response to insulin thereby making target cells more responsive to insulin [3]. Oral antidiabetic agents exert their effects by stimulating beta cells in the pancreas to produce more insulin, increasing the sensitivity of muscles and other tissues to insulin, decreasing gluconeogenesis by the liver and delaying the absorption of carbohydrates from gastrointestinal tract. These treatments are often associated with adverse, toxic effects [9]. Insulin – producing cells that do not express the GLUT 2 transporter are resistant to streptozotocin toxicity [10,11] but there is damage to a variable degree to other GLUT 2 transporter bearing cells such as renal tubular cells [12].

Disturbances in blood electrolyte balance such as hyperkaelemia, hyponatreamia and hyperchloraemia are associated with kidney problems [13,14,15].

The present study was designed to assess the effects of oral administration of onion juice on plasma glucose, electrolytes, urea and creatinine as well as renal histology in streptozotocin induced diabetic male rats.

II. Materials And Methods

2.1 Experimental Animals

20 healthy male Wistar rats weighing between 140-160g were obtained from Ladoke Akintola University of Technology animal house. The wistar rats were acclimatized for a period of one week with access to drinking water and animal feed ad libitum (Table 2.1).

Table 2.1: Experimental protocol

Group (n=5)	Treatment	Inference
A	Experimental diet*+ water + Streptozotocin + onion juice	Test + Treatment
B	Experimental diet + water + Streptozotocin	Test
C	Experimental diet + water	Negative Control
D	Experimental diet + water + onion juice	Positive Control

*Composition of experimental diet (Unit: g/100g diet):

Corn starch	-	54.7
Casein	-	20.0
Cellulose	-	5.0
Vitamins-Minerals mixture**	-	5.0
DL methionine	-	0.3
Corn oil	-	15.0

**Composition of vitamins-minerals mixture are:
Vit.A acetate (500,000 IU/g) 1.8 g, Vit.D conc. (850.000 IU/g) 0.125 g, α-Tocopherol (250 IU/g) 22.0 g, Ascorbic acid 45.0 g, Inositol 5.9 g, Choline chloride 75.0 g, Menadione 2.25 g, P-Aminobenzoic acid 5.0 g, Niacin 4.25 g, Riboflavin 1.0g, Pyridoxine hydrochloride 1.0 g, Calcium pantothenic acid 3.0 g, Biotin 0.02 g, Folic acid 0.09 g, Vit.B12 0.00135 g, Dextrose to 1 kg, cobalt 20g, copper 300g, iron 2000g, iodine 50g, zinc 2000g, manganese 1800g, selenium 5g, antioxidants 10000g.

The animals were kept in animal house with 12/12hr light – dark cycle that was maintained at 25°C. All animals were weighed before induction and weekly before and during treatment. After 7 days of acclimatization, groups A and B animals were made diabetic using a single intraperitoneal injection of 60 mg of streptozotocin /Kg BW dissolved in 0.9% NaCl solution. After 5 days, rats with fasting glucose concentration above 15mmol/L were considered diabetic. Control rats were injected with saline solution. Treatment was started on the sixth day after streptozotocin injection with 2ml onion juice/100gBW/day and this was considered the first day of treatment. The treatment continued for 30 days [16].

2.2 Extraction of Allium cepa juice

Fresh onion (Allium cepa Linn) bulb was cut into small pieces. About 250ml of distilled water per 100g of onion obtained was crushed in a mixing machine. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen and stored at a temperature of 4℃ [7].

2.3 Blood Sampling

Blood samples were collected at onset via orbital venous plexus into appropriate containers. At the end of the experiment (after 30 days) the rats were sacrificed via cervical dislocation and blood samples collected through cardiac puncture. Venous blood was withdrawn from each rat only after they had been allowed to fast overnight with access to water only. The blood sample for fasting glucose estimation was collected in fluoride oxalate bottles and the remaining volume was collected in heparinised containers. The plasma samples were obtained by centrifugation at 1000g for 15 minutes and stored at -20℃ till analysis [7,16]. The plasma samples were analyzed within 12 hours of collection.
All laboratory procedures were carried out with strict adherence to the guide for the care and use of Laboratory Animals [17].

2.4 Laboratory Analysis

Sodium and potassium ions were estimated using flame photometer [18**],** plasma bicarbonate was estimated using back titration modified method [19] while chloride concentration was determined according to the method of Schales [20]. Fasting blood glucose was estimated using Oxidase Peroxidase method [21], plasma urea estimation was done by Berthelot's reaction [22] and creatinine was estimated by modified Jaffe's method [23].

2.5 Histopathological examination

The kidneys were harvested from the animals in the four groups and then fixed in 10% formosaline and processed to paraffin wax. The specimens were dehydrated in ascending grades of alcohol, cleared in xylene, infiltrated and embedded in paraffin wax. Using rotary microtome, five microns (5µm) thickness from the tissue sections were obtained on slides and were stained with Haematoxylin and Eosin stains. Micrographs were taken under light microscope at 400x magnification [24].

2.6 Procedure for Haematoxylin and Eosin Staining:

The sections from group A, B, C, and D were dewaxed for 30 minutes in xylene, passed through absolute, 80% and 70% alcohol respectively. Then rinsed with distilled water for 1 minute and stained with Harris haematoxylin for 10 minutes. The sections were again rinsed with water and differentiated with 1% acid alcohol for 10 seconds, re-rinsed with water and the blue colour was called back by dipping in water for 10 minutes. The sections were counter-stained with 1% eosin for 2 minutes, rinsed with water and dehydrated by passing through ascending grades of alcohol that is 70%, 80% and absolute alcohol respectively. They were thereafter cleared in xylene and mounted with Dibutyl phthalate xylene (DPX).

2.7 Statistical Analysis

The data obtained from this study were expressed as mean (X) ± standard error of mean (SEM). Student t-test was used to analyze data. A probability value $P < 0.05$ was considered statistically significant using SPSS 20.0 version.

III. Results

Initial body weight of diabetic animals in group B before treatment was increased than the final body weight after treatment with STZ. This was not consistent with the pattern observed in the other groups (Table 3.1).

Table 3.1 : Body weight of all animals in each group (Mean ± SEM)

N = 5	Initial body weight (g)	Final body weight (g)
Group A	115.82 ± 4.70	121.84 ± 5.17
Group B	127.15 ± 4.90	124.50 ± 5.91
Group C	142.94 ± 3.28	160.76 ± 3.45
Group D	138.36 ± 3.52	147.54 ± 5.10

From Table 3.2, the glucose concentration of group A rats was significantly increased after induction with STZ and then decreased by about 36.2% after treatment with onion juice although this concentration was slightly higher than the initial value before induction. The highest value for glucose level was recorded in group B animals at the end of the study. At the end of the experiment, the glucose level of group C animals was reduced to 5.34±0.39. The lowest concentration of plasma glucose was recorded in group D rats (treated control) throughout the period of the experiment.

The percentage change in glucose concentration after induction and treatment was calculated thus:

$$\text{Percentage change} = \frac{\text{final value} - \text{initial value}}{\text{Initial value}} \times 100$$

Table 3.2 : Plasma Glucose concentration (mmol/L) in all groups before and after induction with streptozotocin and treatment with onion juice (Mean ± SEM)

N = 5	Initial Glucose concentration	Glucose After Induction	Glucose After Treatment	% Glucose After Induction	% Glucose After Treatment
Group A	5.36 ± 0.57	18.82 ± 0.79[a]	7.30 ± 1.13[b]	251.2%↑	36.2%↓
Group B	5.23 ± 0.35	17.15 ± 0.73	-	227.9%↑	-
Group C	5.47 ± 0.89	-	-	-	-
Group D	5.05 ± 0.12	-	4.24 ± .034	-	-16.0%↓

a*b : significant at $p<0.05$; ↑: increased glucose, ↓: decreased glucose

Table 3.3 showed the concentrations of creatinine, urea and electrolytes of all experimental animals after treatment. Plasma urea, potassium, sodium and chloride had their highest concentrations in the diabetic animals without treatment (group B) while the least values for creatinine and bicarbonate were observed in this group. In the negative control without treatment (C) the least values were recorded for plasma concentrations of potassium, sodium and chloride with group D (treated control) rats having the lowest value for urea. These differences were however not significant statistically.

Table 3.3 : Plasma Urea, Creatinine and Electrolytes concentrations in all groups (Mean ± SEM)

N = 5	Urea (mmol/L)	Creatinine (µmol/L)	HCO_3^- (mmol/L)	Potassium (mmol/L)	Sodium (mmol/L)	Chloride (mmol/L)
Group A	9.44 ± 0.86	64.82± 9.23[a]	19.00± 1.70	5.06± 0.36	147.20± 0.80	117.80± 2.00[b]
Group B	10.98 ± 0.46[c]	55.42± 0.74[a]	8.00 ± 0.71	6.53± 0.54	158.00± 6.80	139.50± 5.81[b]
Group C	10.14 ± 1.39	65.24± 7.27	15.60± 0.40	4.52± 0.18	143.20± 2.10	116.80± 1.83
Group D	7.86 ± 0.47[c]	59.98± 3.39	17.00± 0.32	5.20± 0.11	145.60± 1.72	118.40± 2.10

a*a, b*b, c*c : significant at $p<0.05$.

3.4 Histological section of kidneys in rats

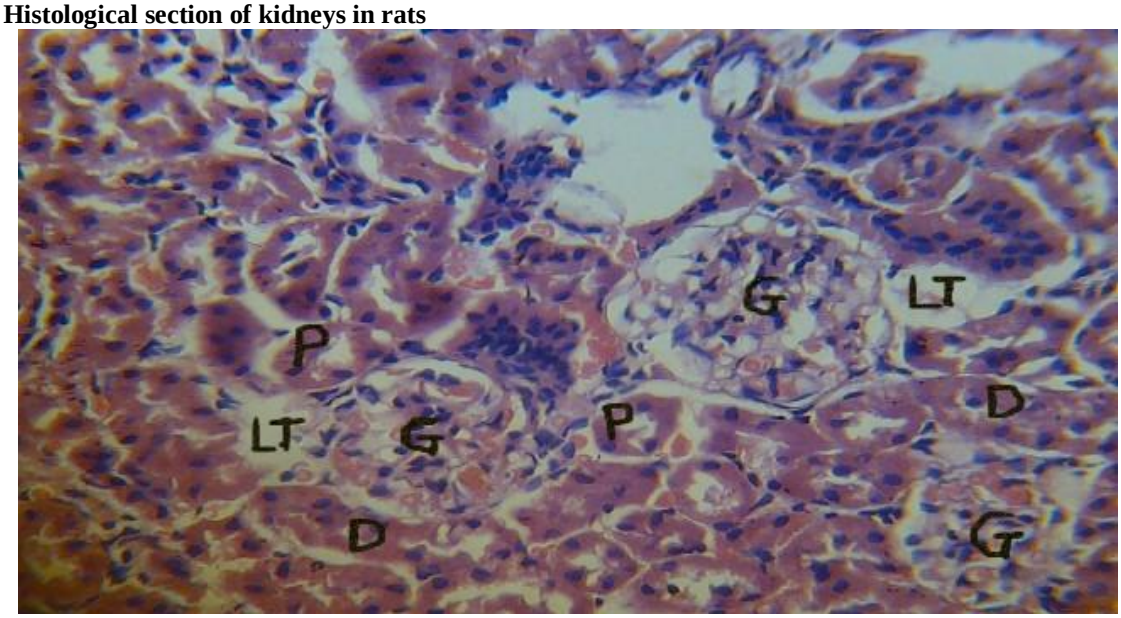

Figure 3.4.1 depicts the histological section of the kidney of group A rats after staining with haematoxylin and eosin. This showed normal glomeruli (G), proximal tubule (P) and distal tubule (D) and some degenerated renal tubules (LT).

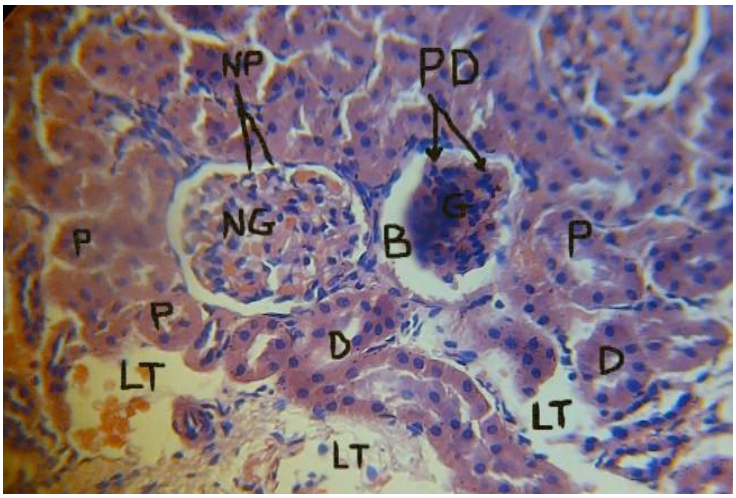

Figure 3.4.2 depicts the kidney section in the diabetic group (B). This showed normal glomerulus (NG), glomerular atrophy (G), densely packed podocytes with mild loss of cellularity (PD), increased Bowman's capsular space (B), normal proximal tubule (P) and distal tubule (D) with some degenerated tubules (LT).

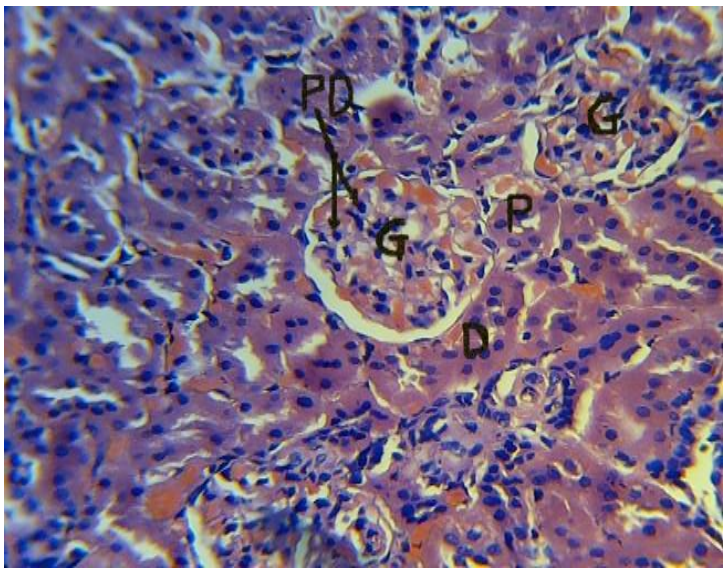

Figure 3.4.3: Kidney section of control group without treatment (Group C), showing normal glomeruli (G), proximal and distal tubules and evenly distributed podocytes (PD).

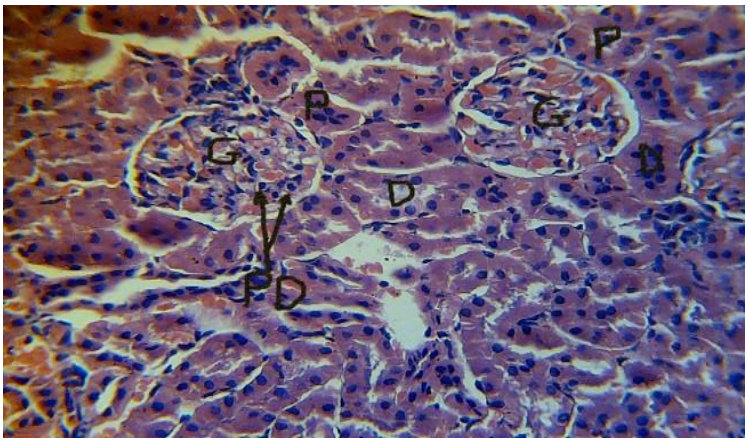

Figure 3.4.4 depicts kidney section of control group with treatment (Group D), showing normal glomeruli (G), proximal and distal tubules and evenly distributed podocytes (PD).

IV. Discussion

Data from the present study showed a slight decrease in the body weight of untreated diabetic animals in comparison with the treated diabetic and other groups. Studies have shown that weight loss is a complication of diabetes mellitus [1,2] and the onion juice might have increased the feed efficiency ratio in the treated group A rats [25].

The plasma glucose level of streptozotocin induced – diabetic rats without treatment was observed to be greatly increased than in the treated diabetic group. Streptozotocin has been reported to possess diabetogenic properties and to be selectively toxic by accumulating in beta cells as glucose analogues through uptake via the GLUT 2 glucose transporter [26]. Once the beta cells are damaged as a result of the toxic effect, insulin production will possibly be altered depending on the extent of damage and this may consequently result in increased level of plasma glucose observed in the diabetic animals in this study. This hypothesis is further supported by various works that reported that insulin – producing cells that do not express this GLUT 2 transporter are resistant to streptozotocin toxicity[26].

The effects of oral administration of onion juice brought about a reduction in plasma glucose by about 36.2% in group A animals from about 215% after induction with streptozotocin. It is also worth mentioning that the glucose concentration was lower in the treated control than in untreated control. This is a further reflection of the hypoglycemic effect of Allium cepa even in the non-diabetic group (D). Other workers also reported the hypoglycemic effect of onion juice [2,7]. This is not surprising since Allium cepa is known to contain flavonoids [6] that have been reported to possess antidiabetic, properties [7] and reduce symptoms associated with diabetes mellitus [3].

The intra-peritoneal injection of streptozotocin has possibly resulted in degenerative and atrophic changes observed in the renal glomeruli of untreated diabetic rats. The possible deduction from this result is that streptozotocin, which are largely responsible for mutagenic and diabetogenic activities [27] has been reported to rapidly and extensively degrade cellular DNA [28] by its selective toxic effect to those cells that possess GLUT 2 transporter [11]. Degenerated renal tubules that were observed in both the treated and untreated diabetic rats might also be due to the action of streptozotocin which invariably affects other GLUT 2 transporter bearing cells such as hepatocytes and renal tubules [12].

Histologically, the tubular injuries were more prominent in the untreated diabetic rats (group B) coupled with glomerular atrophy, whereas the tubular injuries were improved and less prominent in the treated diabetic rats (group A). Meanwhile, there were no observable changes in renal histology in both negative and treated control groups respectively. This is consistent with a recent work that showed that the Allium cepa has protective effect against renal damage [3].

The healing effect of onion juice might be due to the activities of phytochemicals organosulfur and flavonoids [29]. Organosulfur are largely responsible for the taste and smell of onions [30] and were shown to possess antidiabetic properties and reduce symptoms associated with diabetes mellitus [2,7]. Flavonoids have the capacity to act as antioxidants [31] and interfere with inducible nitric – oxide synthase activity [32].

Increase in Na^+ concentration recorded in the untreated diabetic group may be caused by dehydration [15]. Excessive urination was observed in the untreated diabetic rats than what obtains in other groups. This condition improved and eventually stopped in group B animals as the treatment with onion juice began after five days and continued. It was reported that in glomerular disease, there is a marked decrease in the amount of Na^+ filtered without a corresponding decrease in Na^+ reabsorption which consequently leads to decreased amount of Na^+ reaching the distal tubule and may result in an increase plasma sodium ion (Na^+). Degenerated renal tubules (nephrosis) result in an increased aldosterone secretion which consequently contributes to the salt retention [33]. This was corroborated by the histological changes seen in the untreated diabetic group in the present study.

Increased potassium ion (K^+) seen in the untreated diabetic may be a sign of kidney problem [14]. This is probably due to the fact that K^+ excretion is mainly dependent on the amount of Na^+ reaching the distal tubule which is usually low in glomerular disease with the consequent increase in plasma K^+ [33].

It could be postulated from the present experiment that damage to renal tissue may have resulted in the recorded increases in plasma urea, potassium, sodium and chloride ions in untreated diabetic group when compared with other groups. The present finding, therefore, suggests the relevance of these plasma electrolytes in early detection of renal damage. The reduction in plasma bicarbonate that was observed in this present work is possibly due to buffering action of bicarbonate. This is because as ketone body loses a proton (in diabetic condition) it circulates in the blood which lowers the pH of the body. The proton (hydrogen ions) released from ketone are buffered by plasma bicarbonate [34]. The plasma chloride level was higher in the untreated diabetic group compared with other groups . This observable high level may be a fallout of the renal damage seen [15].

The plasma creatinine in all the groups were slightly, insignificantly increased than that of the untreated diabetic group (B). This could probably be attributed to the decreased body weight observed in the group B wistar rats. A low level of plasma creatinine is associated with reduced muscle mass [14].

Since the plasma urea and creatinine of the untreated diabetic group B were not significantly different from that of the negative control group C, it could be suggested that the extent of the renal dysfunction was not sufficient to shut down renal functions. Consequently it could be hypothesized that plasma urea and creatinine, although widely used routinely as markers of renal functions, are not independent sensitive markers of renal damage. Peiris et al [35] reported that subjects with moderately impaired renal function are most likely to be asymptomatic. It was also reported that serum creatinine and blood urea concentrations increase when approximately 75% of the renal mass is lost [36].

Conclusively, this research outcome showed that Allium cepa juice possesses hypoglycemic and anti-diabetic effects and capable of restoring renal tissue damage to an appreciable extent in streptozotocin-induced diabetic rats.

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Authors Disclosure Statement

We wish to state categorically that there is no existing competing financial interest with respect to this study.

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