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Original Article

Hypo-glycaemic and hypo-lipidemic effects of *citrus aurantifolia* (lime) juice in alloxan-induced diabetic rats

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Abstract

Diabetic dyslipidemia causes cardiovascular-related death. Due to the complexity of this disorder, management may be complicated. We evaluated the effects of *Citrus aurantifolia* juice plasma glucose and lipids on diabetic Wister rats. Twenty-eight female rats were grouped into four of seven rats per group: group I (control), group II (non-diabetic rats treated with lime juice), group III (diabetic rat treated with lime juice) and group IV (diabetic rats not treated with lime juice). Diabetes was induced after an overnight fast by intraperitoneal injection of 120 mg/kg alloxan dissolved in 0.9v/v normal saline. The animals of groups I and IV were given daily treatment of 5 ml distilled water, while groups II and III were administered 5 ml lime juice for seven days. The plasma glucose and lipids were determined using the spectrophotometric method. Pre-lime treatment, glucose, total cholesterol (T. Chol), triglycerides (TG), and low-density lipoprotein (LDL) were significantly higher in diabetic rats compared to non-diabetic rats (p=0.000, 0.010, 0.002 and 0.012) while high-density lipoprotein (HDL) (p=0.008) was lower. Post-lime treatment, there was no significant difference in the concentrations of glucose, T. Chol, TG, HDL and LDL of group III compared to group I rats (p=0.010, 0.643, 0.653, 0.616 and 0.429, respectively). Administration of lime juice may be able to control glycaemic and dyslipidaemia states. We suggest that its application in the management of diabetes and its associated dyslipidaemia be explored.

Keywords: diabetes, dyslipidemia, *citrus aurantifolia*, hypoglycaemic, hypo-lipidemic.

Introduction

Diabetes mellitus (DM) is a chronic progressive disease characterized by elevated concentrations of plasma glucose with typical symptoms of polydipsia and polyuria [1]. DM results from insulin insufficiency or defects in its actions [2]. Urbanization, increasing age, obesity and lifestyle are risk factors for developing diabetes [3]. Depending on the population of interest, the prevalence of diabetes may vary. Various studies have reported different prevalence of diabetes among various populations [3–5].

Dyslipidaemia, defined as a high plasma concentration of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and a low concentration of high-density lipoprotein cholesterol (HDL-C), is highly prevalent among diabetic patients [6, 7]. The screening and management of dyslipidemia among persons with diabetes are pertinent and of a public health concern, as dyslipidemia is a major and independent predictor of cardiovascular-related mortality and morbidity in DM patients [5]. Hyperglycaemia, insulin resistance or insufficiency and adipocytokines may contribute to the initiation and progression of dyslipidaemia among diabetics [5].

Diabetic dyslipidaemia is a major cause of progressive cardiovascular complications in diabetic patients. A major feature of diabetic dyslipidaemia is

the presence of hypertriglyceridemia [6]. Strategies employed in the management of diabetic dyslipidemia include lifestyle modifications and the use of pharmacologic agents (Statin) to normalize blood glucose and lipid concentrations. The current approach may not produce optimum results either due to non-compliance of patients to lifestyle modifications or following slow implementation and/or escalation of antidiabetic pharmacologic therapy [6]. Also, a combination of lipid-reducing agents may be required in diabetes with mixed lipid abnormalities [7]. A combination of various drugs may result in poor adherence to medication and significant drug-disease and/or drug-drug interactions [8]. This may reduce the effectiveness of the treatment strategy. Also, most synthetic agents used in the management of diabetes may not provide a balanced therapeutic effect that addresses the cascade of metabolic abnormalities associated with diabetes [9]. There is, thus, the need to develop an effective and safe approach to managing diabetes and its associated complications.

Citrus aurantifolia, commonly known as lime, is a fruit rich in flavonoids with antioxidant properties, citric acid and vitamin C [10]. Due to the availability and cost-effectiveness of C. *aurantifolia*, it is often used among Africans either in its natural state or in combination with palm oil, honey or sugar to manage health conditions [11]. Lime juice extract has been demonstrated to have ameliorative effects on diabetic complications when administrated in combination with *Cinnamomum burmanni* [10]. Also, its effects on the lipid concentrations of rats fed with a cholesterol-rich diet have been demonstrated in previous studies [12]. Not much research has been carried out on the effect(s) of the administration of *Citrus aurantifolia* juice on dyslipidemia secondary to diabetes. We aimed to study the effects of short-term administration of fresh lime juice on the blood lipids of Wister rats with diabetic dyslipidemia.

Material and methods

Study animals

Twenty-eight female Wistar rats weighing between 100 g and 150 g were used for the experiment. They were procured from the animal house, Department of Animal Science, Faculty of Agriculture, Ebonyi State University Abakaliki. The animals were housed in the Animal House, College of Health Sciences, Presco campus, Ebonyi State University, in well-ventilated clean cages at an ambient temperature of temperature 27°C±3°C with a 12-hour light-dark cycle. The rats were allowed to acclimatize for one week prior to the experiment and had access to standard finisher feed and clean water ad libitum.

Study design

The rats were randomly grouped in to four, each group with seven rats. The base line values of the studied biochemical parameters were obtained after the period of acclimatization.

The rats were grouped and treated as described:

- Group I: Normal control: These are given distilled water;
- Group II: Non-diabetic rats: Received 5 ml/kg body weight of fresh lime juice for 7 days;
- Group III: Diabetic rats: Received 5 ml/kg body weight of fresh lime juice for 7 days post-induction of diabetes;
- Group IV: Diabetic rats received distilled water for 7 days post-induction of diabetes.

Chemical (Alloxan)

Alloxan (5,5-dihydroxyl pyrimi-dine-2,4,6-trione), an organic chemical compound and a cytotoxic glucose analog with a brownish-red color and powdery from Sigma Co St. Louis, Missouri, USA, was purchased from Clanol pharmaceutical stores, Abakaliki, Ebonyi state. It induces diabetes by selectively destroying insulin-producing cells (Beta cells) of the pancreas [13].

Collection of lime fruits and extraction of juice

Fresh lime fruits used for this study were obtained from a local market in Ebonyi state, Nigeria. The fruits were properly washed, and each was sliced into halves using a knife. The juice was extracted by gently squeezing the halves. The resulting juice was then strained using a clean sieve cloth and the residual pulp and seeds were discarded. The preparation of the lime juice was freshly done whenever needed.

Induction of diabetes

Diabetes was induced in overnight-fasted rats (12 hours) by intra-peritoneal injection of 120 mg/kg body weight of alloxan dissolved in 0.9v/v normal saline immediately before use, as adapted by [13]. Seventy-two (72) hours after Alloxan administration, blood

samples were withdrawn from the rat tails, and glucose levels were determined to confirm the development of diabetes. The rats exhibiting blood glucose levels above 10 mmol/L were considered diabetic and were used for further experimentation.

Administration of lime juice

After diabetes was induced, the rats in groups II and III were treated with 5 mls of freshly prepared lime juice, while those in groups I and IV were treated with 5 mls of distilled water. The treatment lasted for seven days post-induction of diabetes.

Laboratory, anthropometrics and clinical data collection

After 7 days of treatment with lime juice, the rats were anesthetized using chloroform after 12hour period of fasting and blood samples were collected via cardiac puncture. The blood samples were dispensed into respective sample containers, lithium heparin sample container for lipid profile and fluoride oxalate sample container for glucose estimation.

The concentrations of the studied variables were determined before induction of diabetes (day 1), after induction of diabetes (day 3) and after lime treatment (day 10).

The laboratory determination of glucose, triglycerides, total cholesterol and high density lipoproteins were done using RANDOX diagnostic test kits (Randox Laboratories, UK. The procedures and instructions for the manufacture of the kits were strictly adhered to.

Low-density lipoprotein was determined using the Friedewald equation [14].

Statistical analysis

Data generated from this experiment was analyzed using Statistical Package for Social Sciences (SPSS) version 23 (IBM Corp., Chicago, Illinois, United States). The data are presented in terms of means±SD, and ANOVA was used to arrive at the p-value. The level of significance was established at $p<0.05$ with a 95% confidence interval.

Results

Samples collected from the entire animal in the various groups at the end of different stages of the study were analyzed to determine the level of glucose, Total Cholesterol (Chol), Triglycerides (TG), High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL). The results of data analysis are shown in Tables 1–6.

Table 1 compares the mean±SD of the experimental animals in which diabetes was not induced, and lime treatment was not administered (group I). The mean±SD plasma concentrations of glucose, total cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein on days 1, 3 and 10 of the experiment for the animals not induced with alloxan and not treated with lime juice (group I) are presented in Table 1. The mean concentrations of glucose, total cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein on day 1 of the experiments are 3.83±0.75 mmol/L, 2.93±0.57 mmol/L, 1.33±0.15 mmol/L, 1.33±0.06 mmol/L and 1.00±0.46 mmol/L, respectively, 3.70±0.46 mmol/L, 2.73±0.35 mmol/L, 1.26±0.15 mmol/L, 1.13±0.12 mmol/L and 1.03±0.23 mmol/L respectively for day 3 and 3.87±0.2546 mmol/L, 3.37±0.7546 mmol/L, 1.40±0.1046 mmol/L, 1.77±0.4246 mmol/L and 0.93±0.4046 mmol/L respectively on day 10.

There was no significant difference in the plasma concentrations of glucose and T.chol. TG and LDL when the concentrations of the studied parameters of the different days of the experiments were compared p=0.92, 0.44 and 0.53 respectively. The results showed statistical significant difference in the HDL concentrations when the values of the various days were compared p=0.05.

Table 2 compares the mean±SD of the experimental animals in which diabetes was not induced but received lime treatment (group II). The result from the experiment showed no statistically significant difference in the plasma concentrations of glucose, total cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein when the concentrations on day 1 (3.83±0.31 mmol/L, 3.37±0.67 mmol/L, 1.17±0.21 mmol/L, 1.67±0.35 mmol/L and 1.17±0.35 mmol/L), day 3 (3.73±0.38 mmol/L, 3.53±0.64 mmol/L, 1.30±0.10 mmol/L, 1.67±0.25 mmol/L, 1.30±0.34 mmol/L) and day 10 (3.70±0.30 mmol/L, 3.33±0.55 mmol/L, 1.27±0.06 mmol/L, 2.13±0.47 mmol/L and 0.63±0.15 mmol/L) were compared, p=0.878, 0.916, 0.506, 0.279 and 0.072 respectively. When the plasma concentrations of the studied biochemical parameters were compared on day I and day 3, there was a significant increase in the concentration in the total cholesterol (3.37±0.67 mmol/L and 3.53±0.64 mmol/L, respectively), p=0.038.

The mean±SD of the plasma concentrations of glucose, T.Chol, TG, HDL and LDL before induction of

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Table 1: Mean±standard deviation (SD) of the plasma concentrations of glucose, T.Chol, TG, HDL and LDL of group I experimental animals.

Variables	Day 1	Day 3	Day 10	f-value	p-value
Glucose (mmol/L)	3.83±0.75	3.70±0.46	3.87±0.25	0.840	0.924
TChol (mmol/L)	2.93±0.57	2.73±0.35	3.37±0.75	0.933	0.440
TG (mmol/L)	1.33±0.15	1.26±0.15	1.400.10	0.712	0.535
HDL (mmol/L)	1.33±0.06	1.13±0.12	1.77±0.42	4.920	0.051
LDL (mmol/L)	1.00±.46	1.03±0.23	0.93±0.40	0.050	0.955

Note: TChol – Total Cholesterol; TG – Triglyceride; LDL – Low Density Lipoprotein; HDL – High Density Lipoprotein; Day 1 – Before induction of diabetes; Day 3 – Before lime treatment; Day 10 – After lime treatment.

Table 2: Mean±standard deviation (SD) of on the plasma levels of glucose, T.Chol, TG, HDL and LDL before and after lime treatment of group II experimental animals (diabetes not induced but treated with lime juice).

Variables	Day 1	Day 3	Day 10	f-value	p-value
Glucose (mmol/L)	3.83±0.31	3.73±0.38[a]	3.70±0.30[a]	0.133	0.878
TChol (mmol/L)	3.37±0.67	3.53±0.64[b]	3.33±0.55[a]	0.089	0.916
TG (mmol/L)	1.17±0.21	1.30±0.10[a]	1.27±0.06[a]	0.765	0.506
HDL (mmol/L)	1.67±0.35	1.67±0.25[a]	2.13±0.47[a]	1.593	0.279
LDL (mmol/L)	1.17±0.35	1.30±0.34[a]	0.63±0.15[a]	4.200	0.072

Note: TChol – Total Cholesterol; TG – Triglyceride; LDL – Low Density Lipoprotein; HDL – High Density Lipoprotein; Day 1 – Before induction of diabetes; Day 3 – Before lime treatment; Day 10 – After lime treatment; [a] – Not statistically significant when compared to day 1 on the same row ($p>0.05$); [b] – statistically significant when compared to day 1 on the same row ($p<0.05$).

diabetes (day 1), after induction of diabetes (day3) and after treatment with lime juice is presented in Table 3. The plasma concentrations of glucose on day 1, day 3, and day 10 were 3.97±0.64 mmol/L, 10.60±0.44 mmol/L and 6.43±0.61 mmol/L, respectively. T. Chol concentrations were 3.47±0.45 mmol/L, 5.20±0.62 mmol/L and 3.70±0.87 mmol/L, respectively. TG concentrations were 1.37±0.12 mmol/L, 2.67±0.51 mmol/L and 1.33±0.21 mmol/L, respectively. The concentrations of HDL were 1.80±0.30 mmol/L, 1.10±0.10 mmol/L and 2.03±0.72 mmol/L respectively and LDL values were 1.30±0.53 mmol/L, 2.73±0.37 mmol/L and 1.67± 0.15 mmol/L respectively. There was a statistically significant difference when the concentrations of glucose,

Table 3: Mean±standard deviation (SD) of on the plasma levels of glucose, T.Chol, TG, HDL and LDL before and after lime treatment of group III experimental animals (alloxan induced diabetic rats also treated with lime juice).

Variables	Day 1	Day 3	Day 10	f-value	p-value
Glucose (mmol/L)	3.97±0.64	10.60±0.44[b]	6.43±0.61[ac]	103.58	0.000
TChol (mmol/L)	3.47±0.45	5.20±0.62[b]	3.70±0.87[ad]	5.884	0.039
TG (mmol/L)	1.37±0.12	2.67±0.51[b]	1.33±0.21[ac]	16.260	0.004
HDL (mmol/L)	1.80±0.30	1.10±0.10[a]	2.03±0.72[ad]	3.406	0.103
LDL (mmol/L)	1.30±0.53	2.73±0.37[b]	1.67±0.15[ac]	15.201	0.004

Note: TChol – Total Cholesterol; TG – Triglyceride; LDL – Low Density Lipoprotein; HDL – High Density Lipoprotein; Day 1 – Before induction of diabetes; Day 3 – Before lime treatment; Day 10 – After lime treatment; [a] – Not statistically significant when compared to day 1 on the same row ($p>0.05$); [b] – statistically significant when compared to day 1 on the same row ($p<0.05$); [c] – statistically significant when compared to day 3 on the same row ($p<0.05$); [d] – Not statistically significant when compared to day 3 on the same row ($p>0.05$).

T.Chol, TG and LDL were compared (p=0.000, 0.039, 0.004 and 0.004, respectively).

There was a statistically significant increase in the concentrations of glucose, TChol, TG and LDL when the concentrations were compared between day3 and day1 (p=0.000, p=0.005, p=0.035 and p=0.016 respectively) while the difference in LDL concentration was not statistically significant (p=0.094).

After treatment, that is, on day 10, there was no statistically significant difference in the concentrations of TChol, TG, HDL, and LDL (6.43±0.61 mmol/L, 3.70±0.87 mmol/L, 1.33±0.21 mmol/L, 2.03±0.72 mmol/L and 1.67±0.15 mmol/L respectively) when compared to the concentrations of day 1 (3.97±0.64 mmol/L, 3.47±0.45 mmol/L, 1.37±0.12 mmol/L, 1.80±0.30 mmol/L, 1.30±0.53 mmol/L respectively) p=0.053, p=0.593, p=0.742, p=0.506 and p=0.716 respectively for glucose, TChol, TG, HDL, and LDL. Also, on comparing the difference in the mean concentration of the studied biochemical parameters on days three and ten, a significant decrease in glucose (p=0.01), TG (p=0.032) and LDL (p=0.01) was observed while there was no significant difference in the concentration of T.Chol and HDL (p=0.07 and 0.09 respectively) when the concentrations were compared on day 10 and day 3.

Table 4 shows the serum levels of glucose, TChol, TG, HDL and LDL in alloxan induced diabetic animals. The serum level of glucose, TChol, TG, HDL and LDL before the inducement of diabetes were 4.03±0.2 mmol/L5, 3.50±0.62 mmol/L, 1.40±0.10 mmol/L, 1.47±0.38 mmol/L and 1.40±0.53 mmol/L respectively. At the end of the experiment, the values were 13.43±1.12 mmol/L, 5.57±1.23 mmol/L, 2.37±0.32 mmol/L, 0.70±0.10 mmol/L and 3.50±1.57 mmol/L for glucose, TChol, TG, HDL and LDL respectively. The difference in the serum levels of the parameters except for TChol and LDL were statistically significant (p=0.000, p=0.108, p=0.015, p=0.019 and p=0.145 respectively for glucose, TChol, TG, HDL and LDL). Comparative analysis of the difference in the levels of glucose, TChol and TG between day 3 and day 1 were statistically significant (p=0.001, p=0.040 and p=0.049), while the difference in HDL and LDL were not statistically significant (p=0.369 and p=0.113). Comparing the difference between day 10 and day 1, glucose, TChol, TG and HDL showed statistically significant differences (p=0.006, p=0.043, p=0.022 and p=0.049), while the difference in LDL was not statistically significant (p=0.126).

Table 5 compares the concentration of plasma glucose and lipids in Wister rats before lime treatment. Animals in the group III and IV (induced diabetes) had higher levels of glucose (10.60±0.44 mmol/L and 10.80±0.30 mmol/L respectively), TChol (5.20±0.62 mmol/L and 4.67±1.01 mmol/L respectively), TG (2.67±0.51 mmol/L and 2.03±0.35 mmol/L respectively), LDL (2.73±0.38 mmol/L and 2.50±0.95 mmol/L respectively) and HDL level of 1.10±0.10 mmol/L and 1.23±0.12 mmol/L compared to animals in group I and II (not induced) with glucose levels of 3.70±0.45 mmol/L and 3.73±0.37 mmol/L respectively. TChol, 2.73±0.35 mmol/L and 3.53±0.64 mmol/L respectively. TG, 1.27±0.15 mmol/L and 1.30±0.10 mmol/L respectively. HDL concentrations were 1.13±0.12 mmol/L and 1.67±0.25 mmol/L respectively for groups I and II and LDL, 1.03±0.23 mmol/L and 0.30±0.34 mmol/L respectively. The difference in the values of all the parameters among the various groups was statistically significant (p=0.000, p=0.010, p=0.002, p=0.008 and 0.012, respectively, for glucose, TChol, TG, HDL and LDL). The difference between group I and II were not statistically significant except for HDL (p=0.927, p=0.152, p=0.770, p=0.049 and p=0.338 for glucose, TChol, TG, HDL and LDL, respectively). Comparing the difference between group I and III were statistically significant

Table 4: Serum levels of glucose, TChol, TG, LDL and HDL among animals induced but not treated (group IV).

Variables	Day 1	Day 3	Day 10	f-value	p-value
Glucose (mmol/L)	4.03±0.25	10.80±0.30[b]	13.43±1.12[b]	149.381	0.000
TChol (mmol/L)	3.50±0.62	4.67±1.01[b]	5.57±1.23[b]	3.291	0.108
TG (mmol/L)	1.40±0.10	2.03±0.35[b]	2.37±0.32[b]	9.169	0.015
HDL (mmol/L)	1.47±0.38	1.23±0.12[a]	0.70±0.10[b]	8.340	0.019
LDL (mmol/L)	1.40±0.53	2.50±0.95[a]	3.50±1.57[a]	2.713	0.145

Note: TChol – Total Cholesterol; TG – Triglyceride; LDL – Low-Density Lipoprotein; HDL – High-Density Lipoprotein; Day 1 – Before induction; Day 2 – Before treatment; Day 3 – After treatment; [a] – Not statistically significant when compared to day 1 on the same row (p>0.05); [b] – Statistically significant when compared to day 1 on the same row (p<0.05).

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Table 5: Comparative analysis of mean difference in the levels of glucose, TChol, TG, LDL and HDL among alloxan-induced diabetic animals before treatment with lime juice.

Variable	Group I	Group II	Group III	Group IV	f-value	p-value
Glucose (mmol/L)	3.70±0.45	3.73±0.37[a]	10.60±0.44[b]	10.80±0.30[b]	3038.132	0.000
TChol (mmol/L)	2.73±0.35	3.53±0.64[a]	5.20±0.62[b]	4.67±1.01[b]	7.594	0.010
TG (mmol/L)	1.27±0.15	1.30±0.10[a]	2.67±0.51[b]	2.03±0.35[b]	12.751	0.002
HDL (mmol/L)	1.13±0.12	1.67±0.25[b]	1.10±0.10[a]	1.23±0.12[a]	8.222	0.008
LDL (mmol/L)	1.03±0.23	1.30±0.34[a]	2.73±0.38[b]	2.50±0.95[a]	7.061	0.012

Note: TChol – Total Cholesterol; TG – Triglyceride; LDL – Low-Density Lipoprotein; HDL – High-Density Lipoprotein; [a] – Not statistically significant when compared to Group 1 on the same row ($p>0.05$); [b] – Statistically significant when compared to Group 1 on the same row ($p<0.05$).

except for HDL (p=0.000, p=0.008, p=0.033, p=0.725 and p=0.005 for glucose, TChol, TG, HDL and LDL, respectively). Comparing the difference between group I and IV was statistically significant except for HDL and LDL (p=0.000, p=0.035, p=0.047, p=0.349 and p=0.061 respectively).

Table 6 compares the studied parameters of diabetic rats and non-diabetic rats after treatment with lime juice. Animals in group I and II (not induced) had glucose, TChol, TG, HDL and LDL level of 3.87±0.25 mmol/L, 3.37±0.75 mmol/L, 1.40±0.10 mmol/L, 1.77±0.42 mmol/L, 0.93±0.40 mmol/L and 3.70±0.30 mmol/L, 3.33±0.55 mmol/L, 1.27±0.06 mmol/L, 2.13±0.47 mmol/L and 0.63±0.15 mmol/L respectively. Animals in group III (induced and treated) had 6.43±0.61 mmol/L, 3.70±0.87 mmol/L, 1.33±0.21 mmol/L, 2.03±0.72 mmol/L and 1.17±0.15 mmol/L respectively and animals in group IV (induced and not treated) had 13.43±1.12 mmol/L, 5.57±1.23 mmol/L, 2.38±0.32 mmol/L, 0.70±0.10 mmol/L and 3.47±1.53 mmol/L respectively. The differences in all the parameters among the various were statistically significant (p=0.000, p=0.044, p=0.000, p=0.023 and p=0.012 for glucose, TChol, TG, HDL and LDL respectively). The difference in the levels of all the parameter between group I and II were not statistically significant (p=0.503, p=0.954, p=0.134, p=0.371 and p=0.328 for glucose, TChol, TG, HDL and LDL respectively). The difference between group I and III were not statistically significant except for glucose (p=0.010, p=0.643, p=0.653, p=0.616 and p=0.429 for glucose, TChol, TG, HDL and LDL respectively). The difference between group I and IV were statistically significant except for LDL (p=0.003, p=0.070, p=0.026, p=0.041 and p=107 for glucose, TChol, TG, HDL and LDL respectively). The difference in the concentration of glucose (p=0.002), TG (p=0.01), HDL (0.034) were significant and differences in LDL, T. Chol p=0.12 and 0.07 respectively were not significant when the concentration on day 10 were compared between groups III and IV.

Discussion

A high prevalence of diabetes has been reported among Nigerians. The risk factors may include rapid urbanization and an increase in age [3]. Cardiovascular

Table 6: Comparative analysis of mean difference in the levels of glucose, TChol, TG, LDL and HDL among the animals after treatment with lime juice.

Variable	Group I	Group II	Group III	Group IV	f-value	p-value
Glucose (mmol/L)	3.87±0.25	3.70±0.30	6.43±0.61	13.43±1.12[b]	139.299	0.000
TChol (mmol/L)	3.37±0.75	3.33±0.55	3.70±0.87	5.57±1.23[b]	4.304	0.044
TG (mmol/L)	1.40±0.10	1.27±0.06	1.33±0.21	2.38±0.32[b]	20.243	0.000
HDL (mmol/L)	1.77±0.42	2.13±0.47	2.03±0.72	0.70±0.10[b]	5.576	0.023
LDL (mmol/L)	0.93±0.40	0.63±0.15	1.17±0.15	3.47±1.53	7.042	0.012

Note: TChol – Total Cholesterol; TG – Triglyceride; LDL – Low Density Lipoprotein; HDL – High Density Lipoprotein; [b] – Statistically significant when compared to Group 1 on the same row ($p<0.05$).

mortality associated with diabetic dyslipidemia is high among diabetic persons [15–18]. Available antidiabetic agents are relatively expensive and have adverse side effects [16], hence the need for alternative methods of managing diabetes and its associated complications. Although works have been previously done on the use of lime juice in the control of isolated cases of hyperglycemia and dyslipidemia, not many researchers have explored its use in managing diabetic dyslipidemia. We assessed the effects of lime juice on the plasma lipid concentrations of alloxan-induced diabetic Wister rats with diabetic dyslipidemia.

The results of this study showed a significant increase in the plasma concentration of glucose among the various groups of animals treated with alloxan on the third day of the experiment, that is, after induction of diabetes compared to the glucose concentration on day 1 ($p=0.000$ and $p=0.000$ for group III and IV respectively). Alloxan is a known diabetogenic agent used to assess the antidiabetic properties of plant extracts and pure compounds [17]. It induces diabetes by exerting selective cytotoxic effects on the β cells of the pancreas, resulting in the destruction of the cells and resultant diabetes [18]. This finding confirms the diabetogenic efficacy of alloxan.

We also noted significant dyslipidemia (high T.Chol, TG, LDL and low HDL) among diabetic rats compared to the non-diabetic groups before lime juice intervention ($p<0.05$). This is similar to past reports of dyslipidemia in alloxan-induced diabetic rats [15, 18–22]. Dyslipidemia is a common complication and cause of cardiovascular-related mortality among diabetic persons [20]. Due to the insufficiency of insulin following alloxan-induced destruction of the pancreas, the activity of the hormone-sensitive enzyme lipase may be deregulated. It is known to convert triglycerides to free fatty acids and glycerol in the adipose tissues and may be disturbed, resulting in dyslipidemia among diabetic rats. In excess of plasma circulating fatty acids, the hepatic conversion of fatty acids to triglycerides, phospholipids and cholesterol and resultant increase of these lipids in the peripheral circulation is favored [19].

Previous researchers have demonstrated the effects of lime juice on the lipid profile of normoglycemic rats [12] and the effects of lime juice on blood glucose concentration. Our research agrees with their finding on the glucose reduction and hypolipemic effects of lime. Our study design differs from those of previous studies in that we studied the effect of lime in comorbid hyperglycemic and dyslipidemic states and observed the potential of lime as a useful agent in the management of diabetic dyslipidemia. We observed a significant decrease in the concentration of glucose, TG and LDL ($p<0.05$) after treatment with lime juice (day 10) when compared to day 3 of the experiment. There was also observed increase in HDL and a decrease in TChol on day 10 (2.03±0.72 mmol/L and 3.70±0.87 mmol/L respectively) compared to day 3 (1.10±0.10 mmol/L and 5.20±0.62 mmol/L respectively) though not statistically significant ($p>0.05$) (Table 3). The cholesterol-reducing potentials of lime juice may be attributed to the presence of phytochemicals and antioxidants. These phytochemicals and antioxidants are able to prevent oxidation of LDL particles, thus providing protection to the arterial walls [12].

Hesperidin, naringenin, eriocitrin [21], as well as ascorbic acid richly contained in Lime juice, have been suggested to have some preventive properties against atherosclerosis [23]. Phytochemical contents of *C. aurantifolia* such as the flavonoids and phenolic contents act as hypoglycemic agents [23]. These bioflavonoids are able to alter the activity of glucokinase and decrease the level of G6pase, hence reducing the plasma concentration of glucose [22]. In the diabetic rats that were not treated with lime, a progressive increase in glucose, T.Cho, TG and LDL with a progressive decrease in HDL was observed. The result of this study thus suggests that the use of fresh lime juice may be effective in the management of hyperglycemia and its associated dyslipidemia (diabetic dyslipidemia).

Interestingly, we observed no significant difference in the glucose concentration and lipid profile of non-diabetic rats that received lime treatment (group II). This suggests that lime juice may not affect the metabolic state in normoglycaemic and normolipidemic individuals. The high phenolic and flavonoid components of the fruit possibly play a role in maintaining glucose homeostasis [23].

Conclusion

Freshly prepared lime juice has hypolipemic and hypoglycemic potentials and may be a useful tool in the management of hyperglycemia and its associated dyslipidemia. More research is therefore encouraged to explore methods to safely use lime juice in the management of metabolic disorders among humans.

The short-term duration of the research is a limitation as possible harmful effects of long term administration of lime juice was not studied.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Mekala KC, Bertoni AG. Epidemiology of diabetes mellitus. Transplantation, Bioeng Regen Endocr Pancreas, 1:49–58, 2019.
2. Emos ET, Ismaeel Y, Judith H H, Ibrahim A C, Victor B O. Prevalence, Contributing Factors and Management Strategies (Self-Management Education) of Type 2 Diabetes Patients in Nigeria: A Review. Int J Diabetes Clin Res, 8(3): 148, 2021.
3. Adebimpe WO, Faremi AO, Hassan AWO. Prevalence and knowledge of Salmonella infections among food handlers: Implications for school health in Southwestern Nigeria. Sahel Med J, 21(2):99–103, 2018.
4. Kalu ME, Ude UA, Usanga VU, Arihilam oluchi M.. Original Article Dyslipidemia among students of a tertiary institution in Ebonyi State : a cross sectional prevalence study. IASR J Med Pharm Sci, 9343:1–5, 2021.
5. Abdissa D, Hirpa D.. Dyslipidemia and its associated factors among adult diabetes outpatients in West Shewa zone public hospitals, Ethiopia. BMC Cardiovasc Disord, 22(1):1–8, 2022.
6. Monnier L, Schlienger J-L, Colette C. Updated management of dyslipidaemia in diabetes: A new step towards more stringent targets. Diabetes Epidemiol Manag 1:100001. 2021.
7. Filippatos TD, Florentin M, Georgoula M, Elisaf MS. Pharmacological management of diabetic dyslipidemia. Expert Rev Clin Pharmacol, 10(2):187–200. 2017.
8. Zaman Huri H, Chai Ling L. Drug-related problems in type 2 diabetes mellitus patients with dyslipidemia. BMC Public Health, 13(1): 1192, 2013.
9. Anioke I, Okwuosa C, Uchendu I. *et al*. Investigation into hypoglycemic, antihyperlipidemic, and renoprotective potentials of dennettia tripetala (Pepper Fruit) seed in a rat model of diabetes. Biomed Res Int. Article ID 6923629, 2017.
10. Mawarti H, Khotimah, As'ad MZ, Rajin M. Ameliorative effect of *Citrus aurantifolia* and *Cinnamomum burmannii* extracts on diabetic complications in a hyperglycemic rat model. Trop J Pharm Res, 17(5):823–829, 2018.
11. Aibinu I, Adenipekun T, Adelowotan T, Ogunsanya T, Odugbemi T. Evaluation of the antimicrobial properties of different parts of *citrus aurantifolia* (lime fruit) as used locally. African J Tradit Complement Altern Med, 4(2):185–190, 2007.
12. Idoko A, Ikpe VPO, Nelson NO *et al*. Effects of lime juice and honey on lipid profile of cholesterol-enriched diet fed rat model. Annu Res Rev Biol, 20 (3): 1-10, 2017.
13. Cheekati RR, Rao AS, Vijayaraghavan R. A histological study of alloxan-induced diabetes on experimental male Wistar rats. National Journal of Physiology, Pharmacy and Pharmacology, 7(12):1329–34, 2017.
14. Friedewald W, Levy R, Fredrickson D.. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 18(6):499–502, 1972.
15. Sarfraz M, Sajid S, Ashraf MA. Prevalence and pattern of dyslipidemia in hyperglycemic patients and its associated factors among Pakistani population. Saudi J Biol Sci, 23(6):761–6, 2016.
16. Nduka FO, Nwigwe JO, Nwaso CB, Okolo IO, Okeke ES, Ogugua VN. Lipid Profile of Alloxan-Induced Diabetic Wistar Rats Treated With Ethanolic Leaf Extract of Eriosema Psoraleoides. Int J Sci $ Eng Res, 10(6):1619–29, 2019.
17. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina, 53(6):365–74, 2017.
18. Yin P, Wang Y, Yang L, Sui J, Liu Y. Hypoglycemic Effects in Alloxan-Induced Diabetic Rats of the Phenolic Extract from Mongolian Oak Cups Enriched in Ellagic Acid, Kaempferol and Their Derivatives. Molecules, 23, 1046, 2018.
19. Waziri PM, Bulus T, Gwarzo MY. Lipid Profile of Alloxan-Induced Diabetic Wistar Rats Treated With Methanolic Extract of Adansonia Digitata Fruit Pulp. Sci World J, 9(2):19–24, 2014.
20. Krishnamurthy V, Kerekoppa AR, Prabhakar B. Cross-sectional study of pattern of Dyslipidemia and Prevalence of Atherogenic Diabetic Dyslipidemia in newly detected Diabetic patients. Asian Journal of Medical Sciences, 10(6): 45-49, 2019.
21. Boshtam M, Asgary S, Moshtaghian J, Naderi G, Jafari-dinani N. Impacts of fresh lime juice and peel on atherosclerosis progression in an animal model. ARYA Atheroscler, 9(6): 357-362, 2013.
22. Karimi A, Nasab NK. World Journal of Pharmaceutical Sciences Effect of garlic Extract and *Citrus aurantifolia* (lime) juice and on Blood Glucose level and Activities of aminotransferase Enzymes in streptozotocin-induced diabetic rats Akbar Karimi, Nahid Khayam Nasab Depart. World J Pharm Sci, 2(8):821–827, 2014.
23. Ezeigwe O, Ani ON. Toxicological Studies of *Citrus aurantifolia* Fruit Juice in Wistar Rats Toxicological Studies of *Citrus aurantifolia* Fruit Juice in Wistar Rats. Asian J Biochem Genet Mol Biol. 10(4):38–47. 2022.