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L-Arginine supplementation moderates some biochemical parameters and detoxifying enzyme activities in alloxan-induced diabetic rats.

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ABSTRACT

Diabetes mellitus (DM) is associated with some biochemical changes and preventable complications which call for therapeutic supplementations that guarantee meaningful life spending among diabetics. This study investigated the effect of different doses of L-arginine on biochemical parameters and detoxifying enzymes in diabetic rats.

Twenty five adult rats, divided into five groups (five rats each), were induced using alloxan and different doses of L-arginine administered orally for 21 days. Fasting blood glucose concentration was determined daily using tail vein. On day 21, fasting blood samples were collected for biochemical studies and the liver harvested for determination of detoxifying enzymes activities. Atherogenic and coronary risk indices were computed.

Results showed that arginine significantly ($p<0.05$) lowers plasma blood glucose, total cholesterol, LDLC, triglyceride, AST, atherogenic and coronary risk indices while it significantly ($p<0.05$) increases plasma HDLC and ALT levels in L-arginine treated groups. A significant ($p<0.05$) decrease in the activities of arginase and rhodanese and a significant ($p<0.05$) increase in the activities of 3-MST was observed in L-arginine treated and control groups.

In conclusion, L-arginine supplementation modifies biochemical parameters and activities of detoxifying enzymes in alloxan diabetes.

Key word: Arginine, atherogenic index, coronary risk index, detoxifying enzyme, diabetes mellitus.

INTRODUCTION

The prevalence of diabetes mellitus {DM} and its morbidity and mortality pattern is increasing worldwide with 80% of affected people living in low-income and middle-income countries[1,2]. The incidence of DM varies from one geographical area to another due to environmental and lifestyle risk factors[3]; dietary modifications resulting in deviant from high fiber diet to fast foods intake and economic-induced stress among low-income and middle-income earners.

Various therapeutic agents such as insulin, sulfonylureas, biguanides, α-glucosidase inhibitors, and α-amylase inhibitors[4] have been employed to ameliorate this condition and prevent associated complications but with varying degrees of side effects. Efforts to prevent the side effects involve supplementation with various products showing appreciable improvement in the condition.

L-arginine is a basic and semi-essential amino acid found abundantly in body fluids[5]. Endogenous source include denovo synthesis from L-citrulline (5-15%) and total body protein turnover (85-95%) while its exogenous source is through dietary intake which mainly determines plasma arginine levels and homeostasis disturbances[6,7]. It regulates a man cellular functions and metabolism, scavenges free radicals[8], and protects against some chronic diseases where oxidative stress is of etiological importance.

The rising prevalence of DM in the world, high cost of diabetes management, development of various complications, side effects of anti-diabetic drugs and the socio-economic impact of the disease results in the search for a relatively non-toxic supplements to further give hope of living normal life to diabetic patients. This research thus focused on determining the effect of L-arginine supplementation on the management of DM.

MATERIALS AND METHOD

Experimental design

The study was carried out using twenty five (25) male Wistar Albino rats weighing between 150-200g and randomly divided into five groups consisting of five (5) rats each. The rats were kept at room temperature and under natural cycle of daylight and night darkness. The various L-arginine doses were freshly prepared and administered orally as a single dose once daily for 21days.The rats were fed with rat chow and water ad libitum, and taken care of in accordance with the US Public Health Service Guidelines[9].

Animal grouping

The animals used were grouped into five namely:

Control group: The rats in this group were not induced to have diabetic and were only given water in place of

the treatment with L-arginine.

200mg/kg group: The rats in this group were induced to have diabetes and were each treated orally with 200mg/kg body weight L-arginine for twenty one (21) days.

400mg/kg group: The rats in this group were induced to have diabetes and were each treated orally with 400mg/kg body weight L-arginine for twenty one (21) days.

800mg/kg group: The rats in this group were induced to have diabetes and were each treated orally with 800mg/kg body weight L-arginine for twenty one (21) days.

Diabetic untreated group: The rats in this group were induced to have diabetes but were not treated with L-arginine. They were only given water in place of the treatment.

Specimen collection, storage and processing

At the end of 21days treatment period, the rats were fasted overnight and blood collected into heparinized bottles by cardiac puncture under diethylether anesthesia. Blood samples were then centrifuged at 5000 rpm for 5 min to obtain the plasma which was stored at in a refrigerator till assayed.

Biochemical studies

Whole blood glucose was determined using glucometerby applying a drop of fasting capillary blood sample {obtain from tail} on to accuchek glucose strips. Plasma triglycerides, total cholesterol, HDL-C, total protein, ALT and AST were assayed using Randox commercial test kits based on standard methods {10, 11, 12, 13}. The LDL-C, atherogenic index (LDL-C/HDL-C ratio) and coronary risk index (total cholesterol/HDL-C ratio) were calculated for all samples using the standard methods {14, 15, 16}. Arginase, rhodanese and 3-Mercaptopyruvate sulfur transferase {3-MST} activities were also determined {17, 18, 19}.

Data Analysis

Data was analyzed using SPSS version 21 statistical package. The variables were all expressed as mean and standard error of mean {Mean ± SEM} and levels of statistical significance was set at p<0.05. Bar chart and one way ANOVA were also used to describe variables and compare mean differences between the groups respectively.

RESULTS

Result of plasma glucose shows that oral administration of L-arginine supplement significantly (p<0.05) reduced plasma glucose levels in alloxan induced diabetic rats. This reduction was observed to be dose-dependent (Figure 1). Result of lipid profile shows a significant decrease (p<0.05) in the plasma Total cholesterol, LDL-C and triglycerides and a significant (p<0.05) increase in plasma HDL-C in the L-arginine treated groups and control groups when compared to the diabetic untreated group. The concentration of ALT was significantly in the L-arginine treated groups and control groups when compared to the diabetic untreated group. Also, a non-significant difference (p<0.05) was observed in the plasma AST, urea, creatinine and total protein in the L-arginine treated groups and controls (Table 1).

The atherogenic and coronary risk indices were also observed to reduce in L-arginine treated and control groups (Table 1). Result of detoxifying enzymes shows an increase in the activities of arginase, 3-MST and rhodanese in all the L-arginine treated and control groups, although only 3-MST reduction was significant (Table 2).

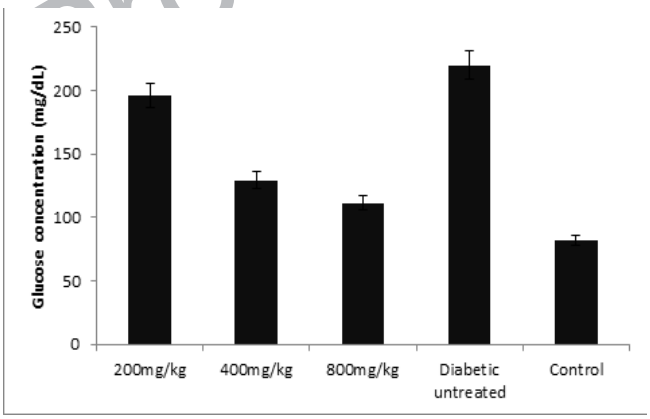

Figure 1: Plasma glucose levels amongst arginine treated, diabetic untreated and control groups

Table 1: Plasma biochemical parameters among L-arginine treated, diabetic untreated and control groups

Dose/Parameters	200 mg/kg	400 mg/kg	800 mg/kg	Diabetic untreated	Control
Tprot{mg/dl}	67.4 +5.5	75.8+ 6.6	73.2+ 7.7	53.2+ 2.5	62.8+ 7.5
Trig{mg/dl}	95.8+ 2.9*	68.4+ 7.9*	106.0 +4.7	123.4+ 8.3	78.6 +5.4*
Tchol{mg/dl}	84.4 +5.8*	93.6 +3.9*	87.2+ 4.7*	130.2+ 6.9	86.8 +4.9*
HDLC{mg/dl}	43.0 +2.3*	21.0 +1.5	32.4+ 1.9	22.0 +1.8	44.2 +7.9*
LDLC{mg/dl}	34.4 +4.8*	58.9 +3.1*	33.6 +4.2*	83.5 +6.3	26.9 +6.4*
Creatinine{mg/dl}	1.1 +0.1	1.2 +0.1	1.3 +0.1	1.3 +0.1	1.2 +0.1
Urea{mg/dl	24.3 +8.8	31.6 +9.6	32.6 +12.0	21.4 +3.9	37.2 +11.5
ALT{IU/l}	28.2+ 12.5*	17.2+ 2.7*	21.2 +3.9*	52.8 +15.8*	26.8 +3.0*
AST{IU/l}	46.0 +11.2	49.2 +10.5	48.0 +6.2	64.0 +9.3	44.0 +10.5
Atherogenic Index	1.1+ 0.1*	0.8 +0.1*	1.1 +0.1*	3.9 +0.7	0.8+ 0.3*
Coronary risk index	2.7 + 0.2*	2.0+ 0.2*	2.7+ 0.2*	6.0+ 0.8	2.2 +0.4*

Values are Mean ± Standard error of mean. * Represent statistically significant difference at p< 0.05

Table 2: Activities of detoxifying enzymes in the liver of L-arginine treated, diabetic untreated and control groups

Dose/Parameters	200 mg/kg	400 mg/kg	800 mg/kg	Diabetic untreated	Control
Arginase{µmol/ml/min}	1.04 +0.12	1.56+ 0.10	1.79+ 0.00	0.57+ 0.12	1.57+ 0.49
3-MST {MU}	1.61+ 0.15*	6.71+ 1.06*	7.03 +0.01*	0.77+ 0.28*	10.45 +1.43*
Rhodanese{RU}	9.45 +0.25	9.13 +0.05	9.68+ 0.83	6.56+ 0.95	10.74 +0.43

Values are Mean ± Standard error of mean. * Represent statistically significant difference at p< 0.05.

DISCUSSION

Glucose and fat are the main nutrients for basal energy metabolism while glucogenic amino acids obtainable from protein hydrolysis are convertible nutrients. They are all of particular interest in metabolic disorders such as obesity and DM. L-arginine and its metabolites have been reported to play a complex role in the regulation of body energy homeostasis and thus dietary supplementation could have multiple beneficial effects in the management of DM[7].

The result obtained from this study revealed that L-arginine significantly (p<0.05) reduce plasma glucose levels in alloxan induced diabetics rats (Figure 1). This finding corroborates that of Tan et al.[2012] who reported antihyperglycemic effect of L-arginine[20]. The mechanisms involved could be by stimulating insulin secretion [21]; improving peripheral and hepatic insulin sensitivity[22]; increasing antioxidant enzyme activities [23]; and attenuating pancreatic damage [24].

Result of hepatotoxic markers showed a significant (p<0.05) decrease in plasma ALT activities in L-arginine treated groups. There was also a reduction in AST but not significant (p<0.05). The observed decrease in ALT and AST activities implies that L-arginine is hepatoprotective against oxidative damage from the administered alloxan. L-arginine supplementation exhibit antioxidant activities and act to mop up alloxan and hyperglycemia-induced free radicals generated in alloxan diabetes. The high plasma activities of ALT and AST enzymes in diabetic untreated group indicate characteristic hyperglycemia-induced hepatocellular injury and hepatotoxic effect of the administered alloxan. Similar increases in ALT and AST activities had earlier been reported in alloxan diabetes[25]. Also, no significant change was observed in plasma creatinine level among L-arginine treated groups, indicating that L-arginine is nephroprotective as well.

Result of plasma total protein and urea revealed an increase in plasma concentration of both in L-arginine treated groups. Similar increase in total protein and urea was reported in rats fed on high-fat diet with L-arginine supplementation {26} the fact that L-arginine participates in protein synthesis[27].

Results of lipid showed a significant (p<0.05) decrease in total cholesterol, LDL-C, and triglycerides in a dose-dependent manner and a significant (p<0.05) increase in HDL-C in L-arginine treated groups (Table 1). These reflect improvements in characteristic diabetic dyslipidemia and thus may prevent cardiovascular complications in DM.

Studies had shown that patients with DM have high atherogenic index value which increases with increasing atherogenic risk[28] and also increase the chances of developing cardiovascular disease[29]. Results from this work showed that atherogenic and coronary risk indices in diabetic untreated group were higher than those of the L-arginine treated groups (Table 1). The observed reduction of atherogenic and coronary risk indices by L-arginine supplementation thus implies that L-arginine may also be cardioprotective in DM.

Detoxification systems in the body functions generally to convert lipid-soluble xenobiotics {toxins, pharmaceuticals and food components} to water-soluble products for excretion. Diabetes mellitus is associated with increase oxidative stress markers (carbonyls and TBARS) and oxidative stress-induced tissue damage {30}. In this instance, the detoxifying enzyme system functions to reduce high free radicals generated during increased mitochondrial glucose oxidation. Secondary tissue damage from hyperglycemia-induced excess free radicals is naturally prevented by rapid conversion of intermediary metabolites to less toxic products which are easily excreted through the bile and urine. This is achievable by high levels of plasma amino acids (arginine, glycine, taurine, ornithine and glutamine) that facilitate conjugation of the intermediary metabolites[31]. Among the detoxifying enzymes are cytochrome P450, epoxide hydrolase, glutathione transferases, amino acid transferases, N-acetyl transferases, N- and O-methyl transferases, glucuronyltransferases, arginase, rhodanese and 3-Mercaptopyruvate sulfotransferase (3-MST)[31].

Arginase, largely confined to the liver cytosol, hydrolyzes L-arginine to urea and L-ornithine and eliminates excessive nitrogen generated primarily during metabolism of amino acids[32,33,34]. This study shows increased plasma arginase activities in the L-arginine treated groups (Table 2). This finding corroborates the earlier reported increase in pancreatic arginase activity in alloxan diabetes rats supplemented with L-arginine[35]. The administered L-arginine after absorption and transportation to the liver is used for both hepatic urea cycle and as a substrate for NO production [36]. Thus, improvement in the biochemical parameters in L-arginine treated diabetic rats is largely a function of the gastrointestinal tract.

3-Mercaptopyruvate sulfotransferase (3-MST) is both a cytoplasmic and mitochondrial enzyme found in cells that catalyzes transfer of sulfur-containing groups from a donor molecule to a nucleophile acceptor[37]. However, the natural sulfur donors and acceptors and the physiological functions of most sulfotransferases

remain uncertain[38]. From this study, a significant ($p<0.05$) increase in the activities of 3-MST was observed in all the L-arginine treated groups (Table 2). This finding reflects the effect of hyperglycemia-induced oxidative stress which results in conformational changes at the catalytic site arising from the conversion of cysteine to a sulfonate. The formed sulfonate inhibits the enzyme activity and this explains the reduced activities of the enzyme observed in diabetic condition. Similar finding was reported by Módis and co-workers[39] who demonstrated that oxidative stress inhibits 3-MST activity and interferes with the positive bioenergetic role of the 3-mercaptopyruvate sulfurtransferase/hydrogensulfide pathway. Increase in 3-MST activity in the L-arginine treated groups showed that L-arginine supplementation reduces oxidative stress which is a major problem in DM.

Also, an increase in the activities of rhodanese was observed in the L-arginine treated groups (Table 2). The observed reduction in rhodanese activity in the diabetic untreated group could be as a result of existing hyperglycemia-induced chronic intra-mitochondria oxidative stress in the diabetic rats. The reduction of rhodanese expression indicates an increase of oxidative oxygen species as well as higher mitochondrial superoxide[40].There is a compensatory rhodanese induction which acts in synergy with 3-MST to promote sulfane sulfur, GSH, or thioredoxin regulations, resulting in activation of anti-oxidative stress functions in other to annul the oxidative stresses[40].

In conclusion, L-arginine supplementation increases activities of detoxifying enzymes {arginase, 3-MST and rhodanese}; reduces fasting blood glucose level, atherogenic and coronary risk indices; and improves lipid profiles in alloxan induced diabetic rats. These may be of physiologic or compensatory importance in oxidative stress conditions like diabetes mellitus.

Conflict of Interests

The authors declare that there is no conflict of interests.

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