Original article

The hyperglycemic and dyslipidemic effects of L-arginine in streptozotocin-induced diabetic rats

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ABSTRACT

Background: Diabetes is a serious disease which is posing a great risk to the overall health care all around the globe. Major factor in its systemic adverse effects is the enhancement of lipolysis by prolonged hyperglycaemia ultimately leading to dyslipidemia. This dyslipidemia is a big risk feature for all cardiovascular diseases related to most of the diabetic patients. L arginine blessed with antioxidant properties can play a substantial role in the prevention, and management of diabetes and related complications. The study was aimed at finding the novel uses of L-Arginine in diabetes.

Subject and method: 15 adult Sprague Dawley rats , only female (to avoid pregnancy) weighing 250± 50 g were used in this study which were divided equally (five rats each) into three groups randomly after ensuring that their lipid profiles and blood sugar were normal. After keeping one group as Normal Control Group (NC), the remaining two groups were made quasi-analogue Type II diabetic model through administration of Streptozotocin (35 mg/kg) in a single dose intra-peritoneal (IP). After lapse of 48 hours, all the rats of two groups had shown blood sugar levels over 300 mg/dl and therefore were taken as diabetic. One of the two diabetic groups was taken as "Positive Control" with DM rats (DM), while the other was taken as treatment group DM+L-ARG Group". The NC and DM groups were fed on standard diet and clean drinking water, while DM+L-ARG group was treated with L-arginine (200 mg/kg per day) administered by oral gavage. After four weeks treatment, blood tests were taken to conduct comparison of their fasting blood sugar and lipid profile.

Results: With regards to fasting blood sugar (BSF), total cholesterol (T-Chol), triglyceride (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL), Group III (DM+L-ARG) showed significant improvement with the p-value of less than 0.001.

Conclusion: After conducting this experimental animal work, we are able to conclude that in Streptozotocin induced diabetic model, the novel use of antioxidant L-Arginine with good safety profile and minimal adverse effects also has significant anti-hyperglycemic and anti-lipidemic activity.

Keywords: L-Arginine; Diabetes Mellitus; Dyslipidemia; Hyperglycemia; Streptozotocin

INTRODUCTION

A number of heterogeneous collections of chronic metabolic conditions and disorders are associated with Diabetes Mellitus (DM) especially Type II DM. Type II DM is one of the leading cause of global deaths these days. It is depicted by persistent hyperglycemia rising from compromised insulin secretion or actions linked with protein, carbohydrate and lipid metabolism abnormalities. Currently, around 171 million people are impacted with this disease and the number is likely to grow over to 366 million mark by year 2030[1]. Glucose is the primary oxidizable substrate in various cell categories. Gluconeogenesis and glycogenolysis are the catabolic pathways in a diabetic state which are activated to keep homeostasis. In this case, dyslipidemia/ atherogenic index of plasma (AIP) has an important role in the prognosis of Type 2 DM and this counts for around 97% of diabetic patients[2].

The time span of diabetes, level of dyslipidemia and hyperglycemia, are the main risk factors for chronic complications of DM. Diabetic dyslipidemia may increase due to relative increase in "Reactive Oxygen Species" (ROS) and reduction in "Nitric Oxide" (NO) bioavailability due to higher levels of plasma glucose[3].

Chronically and persistently increased blood glucose in Type II DM is generally associated with abnormalities in fat, carbohydrates and protein metabolism, which may then lead to various ailments like neuropathy, nephropathy, retinopathy, metabolic disorders, weakened antioxidant defence system,

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dyslipidemia and atherosclerotic cardiovascular disease (ASCVD)[4].

Antioxidants have been proving a good alternative to diabetic complications including dyslipidemia, hence exploring of new agents and the related strategies to treat DM related hyperglycemia, dyslipidemia, and allied complications. Natural substances which have hypoglycemic and anti-oxidant properties have helped in improving degenerative diseases like diabetes and its complications[5].

L-Arginine is considered as a crucial amino acid for functioning of immune cells, protein synthesis, urea cycle and the tissue repairing. The antioxidant effects of L-Arginine's can be attributed to the dropping radical reactions or may be due to the reduced superoxide anion which are usually released from the endothelial cells which leads to the reduction in the oxidative stress[6].

L-Arg is nitric oxide's (NO) precursor which affects in the release of insulin as concluded in the studies of animals in vivo, vitro and also amongst the diabetic and healthy humans. Various mechanisms of NO on glucose homeostasis have also been proposed in numerous studies[7]. The major effects of L-Arg on lipid metabolism demonstrated an increase in NO level and also regulated the expression of lipid metabolic genes. L-Arg has multiple valuable effects on cardiovascular system. Therefore provision of L-Arg may improve endothelium in atherosclerotic disorder and also lead to development of NO[8].

L-Arg endowed with antioxidant features also have the ability to maintain glucose homeostasis, reduce oxidative stress and manage dyslipidemia[9]. It is in this purview that the study has been aimed at discovering the novel uses of these drugs and thus helps medical professionals to reconstruct the diabetic regime in a more valuable manner.

SUBJECTS AND METHODS

The study was undertaken in Department of Pharmacology & Therapeutics, Army Medical College (AM College) Rawalpindi in association with the National Institute of Health (NIH). The study and research were undertaken in the animal house of National Institute of Health (NIH), Islamabad. The biochemical analysis of rats' serum was undertaken at Armed Institute of pathology.

The ethics committee of Centre for Research in Experimental and Applied Medicine (CREAM), AM College, Rawalpindi provided the approval of the study protocol. The duration of the study was one year. The study design was a laboratory based randomized controlled trial.

Fifteen adult Sprague Dawley rats (Fifteen (female only to avoid pregnancy) weighing about 250±50 g, were brought from National Institute of Health (NIH), Islamabad and were kept in its animal house during the whole course of study period.

Lab conditions which were maintained in the animal house were the standard ones. Well ventilated rooms maintained at room temperature of 22-24 degree Centigrade were maintained with twelve hourly cycles of light and dark. The animals were provided with free access to clean drinking water and standard diet. Acclimatization of the rats had already been carried out for a period of one week before induction of DM.

Freshly prepared Streptozotocin mixed in citrate - phosphate buffer at pH 4.5 administered in a single dose of 35 mg/kg $^{-1}$ body weight; intra-peritoneal (IP) was used for inducing diabetes in the rats. After 48 hours of Streptozotocin injection, the fasting blood glucose levels of the rats were taken from the tail vein with glucometer to study if the animals had developed diabetes or otherwise. The rats with fasting blood glucose level ≥300 mg/dl were considered as diabetic and were utilized in the experiment. Animal grouping was carried out through Non probability convenience method and randomly divided into 3 groups (n=5).

Blood samples were obtained for biochemical assessment at the start and end of the experimental period. Initially the samples were taken from the tail vein of the rats after keeping them fasted for 8 hours and before the induction of DM. Later, at the end of experimental period (after 28 days), the animals were kept fasted for twelve hours. The animals were then sacrificed with chloroform anaesthesia. The sample was then obtained via cardiac puncture. The serum was separated from the collected blood and used for biochemical parameters.

Data was studied and analysed through "Statistical Package for Social Sciences (SPSS) Version 25". The quantitative variables were summarized using mean ± SD. *Paired sample t-test* was applied to find out significance at different times within group. One-Way

Table 1: The Intervention protocols

Group	Intervention Protocol
Group I: Control Group (NC)	Rats given clean drinking water and fed on standard diet
Group II: DM Group (DC)	DM rats given clean drinking water and fed on standard diet
Group III: DM+ L-ARG Group	DM rats treated with L-arginine (200 mg/kg/day) given by oral gavage for 28 days.

Analysis of Variance (ANOVA) was used to determine the difference between all the groups, which was followed by Tukey's Post Hoc correction for multiple comparisons.

RESULTS

Comparison of Groups Based on Variables/ Parameters: The behavior of various groups in each category of parameters can be summarized in terms of their mean values as under:

Blood Fasting Sugar (BSF): With regards to the BSF test, the results show significant improvement, in L-Arginine Group (L-Arg). The results were significant statistically with value of p less than 0.001. The tabulated data and graphical charts are appended below:-

Table 2: Comparison of mean values of BSF*

Comparative Analysis	(Mean Values)		
Parameters	**NC**	**DC**	**DM+L-Arg**
Pre STZ Infusion	102.8	103.6	105.2
Post Treatment (mg/dL)	109	379.4	147.8
p value <0.001*			

* Significant p value ≤ 0.05

Lipid Profile: With regards to the lipid profile, T-Chol, TG, HDL and LDL tests results showed significant improvement in L-Arg group. The results were significant statistically with value of p less than 0.001. The tabulated data and graphical charts are appended below:-

Table 3: Comparison of mean values of lipid Profile

Comparative Analysis	(Mean Values)		
Parameters	**NC**	**DC**	**DM+L-Arg**
Pre STZ Infusion T Chol.	1.25	1.30	1.34
Post treatment T – Chol. (mmol/L)	1.32	3.56	1.52
Pre STZ Infusion TG	0.64	0.61	0.63
Post treatment TG (mmol/L)	0.66	2.64	0.70
Pre STZ Infusion HDL	1.34	1.29	1.25
Post Treatment HDL (mmol/L)	1.38	1.75	1.09
Pre STZ Infusion LDL	0.15	0.14	0.15
Post treatment LDL (mmol/L)	0.14	0.71	0.29
p value <0.001*			

* Significant p value ≤ 0.05

DISCUSSION

The world today is deeply affected by ever increasing prevalence of Diabetes Mellitus (DM) spreading viciously all across the globe. Presently over 150 million people are suffering from diabetes and the figure is likely to double by year 2025 as per the estimates of World Health Organization, i.e. an alarming 300 million cases. These complications include neuropathy, nephropathy, metabolic disorders, retinopathy, weakened defence system and discrepancies in lipid profile and the atherosclerosis, most of them due to the abnormal blood glucose levels[10]. Intricate aetiology of diabetes has thus impacted upon patterns and techniques of management and its medication; from monotherapy to a set of composite and combination therapies. Based on number of empirical studies, it has been established that combined therapy of synthetic and antioxidant drugs performs better than any single therapy[11].

DM is related to oxidative stress and ROS (Reactive Oxygen Species) causing variation in the cellular redox state in case of chronic hyperglycaemia. It also reduces the ability of tissues to use carbohydrates thus leading to various disorders and complications in metabolism of proteins and fats[12]. In this regard, empirical evidence show that extended higher levels of blood glucose do contribute in the production of ROS and worsen oxidative stress through oxidation of proteins and glucose autoxidation[13]. Recently many studies have shown that augmentation of antioxidants and nutritional supplements reduce the oxidative stress and protect tissues from ROS damage thus dropping the occurrence of numerous degenerative diseases like diabetes and related complications[14].

This study was designed to assess the effects of L-Arginine (L-ARG) in STZ induced diabetic model based on the fact that L-arginine possesses antioxidant hypoglycemic and dyslipidemic properties. Results of the study exhibited that the hyperglycaemia in diabetes was lowered by the administration of antioxidants consistent with the studies of Yousefi[15] and Kumawat[16]. While the rise in HDL is primarily linked to enhanced insulin functions promoted by antioxidants, the improved glycaemic control may be related with the reduction in both TG and LDL levels. The efficacy of antioxidant with regards to lowering of serum LDL and TG could be related to protective membrane-bound lipoprotein lipase against lipid peroxide[17]. Decline in the levels of VLDL and LDL, could be due to anti-hyperglycaemic effects of antioxidants leading to better diabetic function[18]. The defence systems of the antioxidant removed sufficient quantity of ROS under normal conditions meanwhile the additionally created ROS lead to enhance lipid peroxidation and reduced Super Oxide Dismutase (SOD) and Glutathione per oxide activity (GSH-Px). This all accentuates oxidative stress managed mainly by hyperglycaemia[19]. The lowering of antioxidant enzyme activity under diabetic conditions may be related to glycation of the enzyme, happening at continued raised blood glucose levels. Glycation of SOD lowers its motion, leading to

insufficient dismutation of superoxide anions (O^{-2}), formed a correlation between the improved glycemic control vis-à-vis inhibition of protein glycation affecting into a rise in SOD activity as already stated by Teixeira and Alves[20].

Redox disparity between the growth of ROS and the compensatory response from network of endogenous antioxidant leads to the oxidative stress. It is worth mentioning that the consensus is yet to be reached amongst the academics regarding the disparities in the actions of antioxidant enzymes in diabetic rats[21]. Some studies on the activities of SOD in diabetes showed decline in the levels of these enzymes, while findings buy few others reported increased activity in STZ-induced DM rats. This increase in SOD activity may well be attributed to correspondingly enhanced production of superoxide and H_2O_2 acting as an inducer of tissue SOD[22]. Since diabetes is associated with enhanced oxidative stress due to persistent hyperglycaemia, supplementing antioxidant to regulate glycaemia had a shielding impact against lipid peroxidation in DM, an important conclusion which is in line with the findings of Aldini[23]. To conclude the discussion stated above, it is to state that the administration of antioxidant ameliorated hyperglycaemia and dyslipidemia in the STZ-induced diabetic rats. Thus, there is a need to control and manage the imbalance between the generation of ROS and enzyme activity in the diabetic rats.

CONCLUSION

After conducting this experimental animal work, we are able to conclude that in Streptozotocin induced diabetic model, the novel use of antioxidant L-Arginine with good safety profile and minimal adverse effects also has significant anti-hyperglycemic and anti-lipidemic activity.

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