ORIGINAL ARTICLE

Protective Effect of L-Arginine on Streptozotocin-Induced Diabetic Nephropathy in Albino Rat

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ABSTRACT:

Objective: This study was designed to evaluate the protective role of L-arginine on body weight, and absolute and relative kidney weight of Streptozotocin (STZ)-treated albino rats.

Methodology: This experimental study was conducted in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, from February to March, 2010. In this study, 30 male albino rats were divided into 3 groups, containing 10 animals each. Group-A was treated as control. Group-B animals received STZ in a dose of 37 mg/kg intraperitoneally (I/P) only once at the start of experiment. Group-C received L-arginine orally in a dose of 0.3 mg/100 gram (G)body weight/day a week before STZ treatment. Body weight of animals was calculated at start and end of the study period, along with absolute and relative kidney weight and serum glucose level.

Results: There was a highly significant increase in serum glucose level in group B animals when compared to the control group A. In group C, the serum glucose levels returned near to control. The final body weight of group B animals decreased significantly when compared to their initial weight, as well as when compared to control. The data also showed that there was a significant decrease in absolute kidney weight whereas, significant increase in relative kidney weight in group B animals when compared to group A animals respectively. There was significant restoration of body weight, and absolute and relative kidney weight in group C animals receiving L-arginine along with STZ.

Conclusion: Our findings conclude that L-arginine as a nitric oxide donor and as an antioxidant, plays a significant role in preserving renal morphology in streptozotocin-treated hyperglycemic rats.

Keywords: Streptozotocin, Kidney, L-arginine, Hyperglycemia.

INTRODUCTION:

Diabetes mellitus is a metabolic syndrome with chronic hyperglycemia due to insulin deficiency.[1] Diabetic nephropathy is an important cause of end-stage renal failure. The pathogenesis of diabetic nephropathy involves vasodilatation in the pre- and post-glomerular arterioles and then, final irreversible vasoconstriction of the glomerular arterioles, which results in reduced blood flow and glomerular filtration rate.[2] Hyperglycemia is nephrotoxic. Diabetes mellitus affects more than 120 million people worldwide, and it is estimated that it will affect nearly 400 to 500 million people by year 2030.[2,3] STZ results in hyperglycemia in rats within 72 hrs.[4] It is a pancreatic ß-cell toxin which induces rapid and irreversible necrosis of these cells. The mechanism of STZ-induced ß-cell injury involves excessive reactive oxygen species (ROS) production, lipid peroxidation, protein oxidation and DNA damage leading to ß-cell death.[5] Formation of ROS is thought to be a mediator of cytotoxic actions of STZ leading to oxidative stress, which may be one of the stresses influencing the morphology of kidney.[6] Most previous studies have shown that in rodents, STZ-induced hyperglycemia results in a reduced response to insulin, despite increased numbers of insulin receptors.[7] Studies in animal models of STZ-induced hyperglycemia indicate that antioxidants improve insulin sensitivity[8]. L-arginine is the substrate for the synthesis of nitric oxide (NO), and it has direct anti-oxidant activity.[9] It is an essential amino-acid which participates in many important biochemical reactions associated with normal physiology of the organism. Exogenous L-arginine increases NO production in a variety of cells. It is both a NO precursor and donor.[10] Previous studies have demonstrated that endogenously generated NO is involved in the modulation of corticosterone production and that adrenal NO synthase activity is dependent on extracellular L-arginine.[11] The purpose of this study was to evaluate the protective role of L-arginine on the morphological changes on the kidney along with improvement of insulin resistance in STZ-induced albino rats.

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Received: 20-11-2016
Revised: 02-12-2016
Accepted: 19-12-2016

METHODOLOGY:

This study was conducted in the department of Anatomy, BMSI, JPMC, Karachi, for a period of 6 weeks, after obtaining ethical approval from Feb 2010 to March 2010. In this study, 30 young, healthy male albino rats, weighing around 250-300 G, were obtained from the animal house of BMSI and divided into 3 groups, each containing 10 animals. All the animals were kept under observation for one week prior to the beginning of the study for the evaluation of their health status. Food and water were supplied ad libitum. Group-A was taken as control. Groups-B and C animals were fasted overnight and administered STZ intraperitoneally in a dose of 37

mg/kg body weight.[12] dissolved in freshly prepared 1ml of Citrate buffer at 4 pH only on the first day of the experiment. Group-C received L-arginine orally in a dose of 0.3mg/100 G body weight/day[13] dissolved in 5cc of distilled water, one week before administering STZ. The serum glucose of the rats was measured at the start of the experiment and then twice weekly by glucose oxidase method from the tail vein by using a glucometer.[14] The animals were weighed and sacrificed at the end of the treatment period by using ether anaesthesia. Abdomen was opened by midline incision and both the kidneys were exposed and carefully dissected out. The absolute weight of the kidneys was recorded on Sartorius balance. The relative weight of kidney was calculated with the help of formula.[15] The results were evaluated by student "t" test. P-value was considered for significant differences.

RESULTS:

The mean values of serum glucose level in control group A at the start and the end of the treatment were 118.3±4.1 and 121.3±4.1 mg/dl respectively. In STZ-treated group B animals, the values of serum glucose level were 117.3±4.2 and 702.0±48.21 mg/dl respectively. The data showed that there was highly significant increase ($P<0.001$) in serum glucose in group B when compared to the corresponding control group A. In L-arginine with STZ-treated group C, the serum glucose levels were 115.1±3.3 and 195.3±7.4mg/dl respectively, which showed highly significant decrease in final blood sugar when compared with final blood sugar level of group B animals (Table-1). The animals of group A showed insignificant change in weight at the end of experimental study. The mean values of body weight in STZ-treated groups B were 276.4 and130.3 G respectively. The data showed a significant decrease in body weight ($P<0.001$) when group B final body weight was compared to its initial body weight as well as group A final body weight (Table-2). In STZ and L-arginine treated group C, the mean body weight at the end of study was 202.6G, which showed significant increase as compared to final body weight of STZ-treated group B animals (Table-2). The mean absolute and relative weight of kidney in control group A animals were 0.563mg and 0.249 mg respectively, while absolute and relative kidney weight in group B were 0.491 mg and 0.378 mg respectively (Table-3). There was insignificant decrease in absolute kidney weight in group B animals, whereas a significant increase in relative kidney weight in diabetic rats of group-B when compared with corresponding control group-A. The mean absolute and relative kidney weight in group C animals were 0.513 mg and 0.216 mg respectively (Table-3) which showed significant decrease in relative kidney weight when compared to STZ-treated group B animals depicting protective effect of L-arginine.

Table-1
Mean Serum Glucose (mg/dl) In Different Groups of Albino Rats

Groups	Initial Serum glucose (mg/dl)	Final Serum glucose (mg/dl)
A (control)	118.3±4.1	121.3±4.1
B (STZ-treated)	117.3±4.2	702.0±48.21
C (STZ+L-arginine)	115.1±3.3	195.3±7.4

Table-2

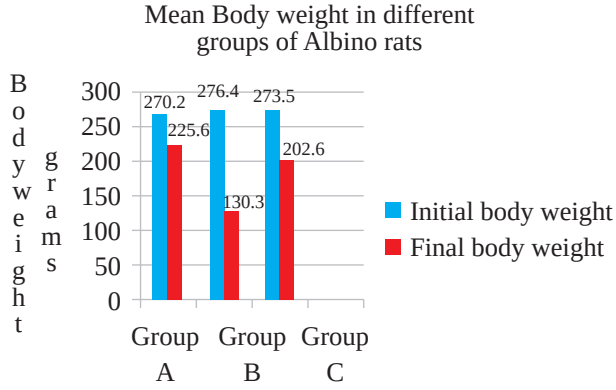
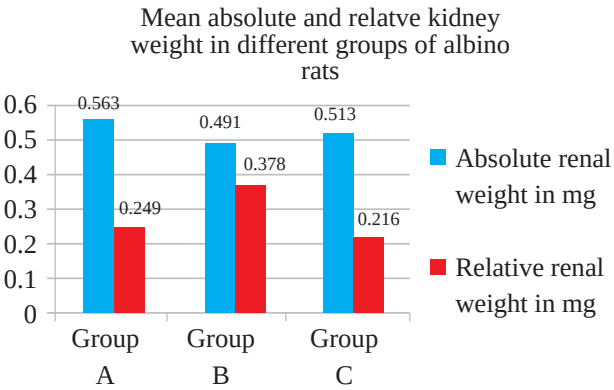


Table-3



DISCUSSION:

Different approaches have been planned to reduce diabetes-induced nephrotoxicity. L-arginine, a nitric oxide precursor, is found to exert a protective effect and improves renal functions in various forms of acute and chronic renal injury.[16]

In the present study, we have investigated the ability of L-arginine to prevent STZ-induced nephrotoxicity. Streptozotocin administration to rats increased blood glucose. L arginine-treated streptozotocin-diabetic rats exhibited a decrease in plasma glucose. L-arginine by its ability to scavange free radicals and to inhibit lipid peroxidation, prevents streptozotocin-induced oxidative stress and protects ß-cells resulting in increased insulin secretion and decreased blood glucose levels.[17] Studies have proved that L-arginine has protective effect on renal hypertrophy induced by STZ-treatment.

Hyperglycemia due to STZ result in nephrotoxicity.[18] STZ induced hyperglycemia caused increase in both absolute and relative renal weight.[19] Studies have proved long term consequences of high glucose levels on the morphology and function of different cell types.[20] This finding was in agreement to the present study. The increase in the absolute weight of kidney was due to hypertrophy of the organ.[21] Whereas, the relative renal weight gain was affected by the total body weight loss. Many reports demonstrated the inhibitory effect of exogenous NO on the nephrotoxicity.[22] This observation was in accordance with the present study demonstrating the significance of L-arginine in reducing the severity of renal damage in animals exposed to nephrotoxic drugs like STZ.[23,24] Group B animals given STZ showed marked body weight reduction.[25] There was an increase in absolute and relative renal weight which was due to STZ induced hyperglycemia.[26]

In Group C L-arginine was used to reverse the damage by STZ induced hyperglycemia; it also prevented absolute and relative renal weight gain. The ability of L-arginine to reduce the body weight loss resulted from increase in skeletal muscle protein synthesis and reduction of skeletal muscle protein degradation.[24]

CONCLUSION:

L-arginine as nitric oxide donor and antioxidant plays substantial role in preventing body weight loss and preserving renal morphology in STZ-treated hyperglycemic experimental animals. Further experimental and clinical studies are required before L-arginine could be used as a supplement for the treatment of diabetes mellitus and its complications.

ACKNOWLEDGEMENT:

I am thankful to Dr. Aisha Qamar, my senior colleague who supported me to write this article.

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