

Restorative Effect of L-Arginine on Gross Morphology and Adrenal Weight in Streptozotocin Induced Diabetic Rats

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ABSTRACT:

Objective: To observe the restorative effect of L-arginine on body weight and weight of adrenal glands in streptozotocin (STZ) induced diabetic rats.

Methodology: This experimental study was undertaken at the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Post graduate Medical Centre (JPMC), Karachi. Fifty healthy albino rats were equally divided into five groups. Group-A was taken as control. Group-B was treated with STZ. Group-C was given STZ with insulin. Group-D received STZ with L-arginine and Group-E was administered STZ along with both L-arginine and insulin. After the confirmation of diabetes by STZ, treatment was continued for a period of 6 weeks. The animals were sacrificed at the end of 6 weeks. At the start and end of the study period mean body weight of animals was calculated. Effect of L-arginine was observed on body weight of animals and weight of adrenal glands and compared with insulin.

Results: The decrease in mean body weight of animals due to STZ was less significant when L-arginine and insulin were added to the therapy. Whereas the increase in adrenal glands weight was also less marked on administering L-arginine and insulin.

Conclusion: L-arginine has restorative effect on gross morphology and weight of adrenal glands which is comparable to insulin.

Keywords: L-arginine, Adrenal glands, Streptozotocin, Restorative

INTRODUCTION:

L-arginine is one of the 20 most common natural amino acids, also considered as a semi-essential amino acid¹. In general, there is no need to take arginine supplements as human body usually produces it in sufficient amount^{2,3}. However, Individuals having in sufficient nutrition or certain physical illnesses may be recommended to increase arginine intake by taking food rich in l-arginine. Food sources include cottage

cheese, milk, yogurt, protein drinks, beef, chicken and sea foods. Plant sources include wheat, cashew, almond, coconut, walnut, pumpkin and water melon³. L-arginine is involved in synthesis of Nitric oxide (NO) by the enzyme NO synthase (NOS) which is important in regulation of a wide variety of biological functions⁴. A lot of activities of arginine are reported in literature. Its role is important in reducing the healing time of injuries mainly bone, and quickens repair time of damaged tissue.⁵ It has a novel role in the management of diabetes and obesity by reducing the mass of adipose tissues and promoting the body weight loss in zucker diabetic rats⁶. It decreases the risk of atherosclerosis by preventing endothelial dysfunction and reducing blood pressure, and effects early β -cell maturation and lymphoid organ development⁷.

Streptozotocin (STZ) was found to be selectively toxic to the β -cells of pancreatic islets in mid-1960. This suggested its use as diabetes-inducing agent in animals⁸. Rakieten et al were the first to report its diabetogenic activity in rats, mice, hamsters, monkeys and dogs⁹. It has been used in patients with irresectable hormone secreting pancreatic islet cell carcinoma, in whom STZ not only reduced tumor size, but also decreased symptoms of hypoglycemia due to excessive insulin secretion by insulinomas¹⁰.

STZ affects the DNA of pancreatic β -cells and activates various pathways that involves protein kinase C poly (ADP ribose) polymerase and NADPH oxidase activation, with production of reactive oxygen species and advanced glycation¹¹. Hyperglycemia produced by STZ generates oxidative stress leading to activation of hypothalamopituitary (HPA) axis resulting in activation of corticotropin releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) secreting cells

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in STZ induced diabetic rats¹².

This study was specifically planned to observe the role of L-arginine on gross morphology and weight of adrenal glands in STZ induced diabetic rats and associate it with simultaneous administration of insulin with streptozotocin.

METHODOLOGY:

This experimental study was undertaken at the Department of Anatomy, BMSI, JPMC, Karachi for 06 weeks from February to March 2010. Fifty healthy male albino rats, weighing from 250-320gram, 90-120 days old were taken for the study.

Rats were randomly distributed into five groups comprising of 10 rats each. Group-A was taken as control and was given only normal saline. On the first day of experiment study, Group-B was given STZ (Sigma Aldrich, USA) in a dose of 37 mg/kg dissolved in 1 ml of citrate buffer at 4 pH intraperitoneally¹³. Group-C was administered STZ in the same dose as in group B along with insulin 70/30 (Eli Lilly, USA) once daily subcutaneously (1 unit/100g body weight) three days after the administration of STZ¹⁴. Group-D received same dose of STZ along with L-arginine as Arginine GNC, USA 0.3mg/g body weight in drinking water¹⁵. Group-E was given the same doses of STZ and arginine, and insulin three days after the administration of STZ as in other corresponding groups.

The experimental animals were observed preceding the beginning of study for a week, for the assessment of their well-being. Food and water were available to them as per requirement. STZ was prepared freshly. Animals were fasted overnight before injecting STZ and were given 5% glucose in drinking water for the first three days. Tail vein was selected for collection of blood

sample. For the confirmation of diabetes, Accu-check Active one touch Glucometer was used (Roche Diagnostics, USA). Treatment was started in all groups which were continued for six weeks. The weight of rats was checked again at the end of experimental period and they were sacrificed by using ether anaesthesia. Sartorius balance was used to calculate the absolute weight of each adrenal gland and its relative weight was calculated¹⁶. Statistical analysis was done using SPSS version 20 through Microsoft excel. The statistical significance of differences of results between groups was evaluated using student's t test, and considered statistically significant if P-value ≤ 0.05.

RESULTS:

The appearance of adrenal gland regarding color, size, shape, surface and contour was observed. It was found to be similar to control group. A in groups C, D and E. Some changes like darker color, increased size and slight irregular surface was observed in STZ treated group B animals, however haemorrhage was not seen; indicating toxic effect of STZ on morphology of adrenal gland. The appearance of adrenal gland in group C and D given insulin and arginine respectively along with STZ indicated their restorative effect on morphology of adrenal gland.

The study showed highly significant decrease in mean body weight of STZ -treated group B animals in comparison to control group A animals. However, reduction became less significant when insulin and arginine were also administered along with STZ in group C and D animals respectively. When the mean body weight of group C, D, and E animals were compared to group B animals, there was highly significant increase suggesting restorative effect of insulin and arginine on mean body weight of animals (Table-1).

Table -1: Variations in Mean Body Weight between Different Groups of Albino Rats

Groups	Treatment Given	Body weight at start of study(gram)	Body weight at the time of sacrifice (gram)
A	Control	295.3±-1.327	308.93±235
B	STZ treated	269.0±107	217.5±1.341**
C	STZ + insulin	303.8±1.237	296.8±397*
D	STZ+ L-arginine	298.7±1.236	270.3±1.345*
E	STZ+Insulin+L-arginine	305.4±1.324	308.90±1.345*

Significant difference* Highly significant difference**

The absolute weight of both adrenal glands was recorded in different groups at the time of sacrifice. The results have revealed that there was a highly significant increase in absolute weight of adrenal glands of STZ treated group-B animals in comparison to control. When insulin

and L-arginine treated groups were compared to group B animals, there was significant decreased absolute adrenal weight in group C and D animals, whereas highly significant decrease was observed in group-E animals which was comparable to control group-A (Table-2).

Table-2: Variations in Absolute Adrenal Gland Weight between Different Groups of Albino Rats

Groups	Absolute weight of Right adrenal gland	Absolute weight of Left adrenal gland
A	38.84±0.16	39.16±0.82
B	72.24±1.74**	73.39±1.03**
C	69.25±1.26*	69.99±10.2*
D	53.41±0.62*	54.45±0.76*
E	26.91±0.90**	27.21±0.32**

Significant difference* Highly significant difference**

The study showed a highly significant increase in relative weight of both right and left adrenal glands in STZ treated group-B animals as compared to control group-A, when calculated with the help of body weight and absolute adrenal weight of animals. When the relative adrenal gland weight (both) of insulin receiving group-C animals was compared to group-B, there was

significant decrease, which was more marked in group-D animals having L-arginine. A highly significant decrease was observed in relative adrenal weight in group-E, treated with STZ along with both insulin and L-arginine in comparison to group B animals, which was similar to control group A. All these results suggested significant restoration by insulin and arginine (Table-3).

Table-3: Effects of L-Arginine on Relative Adrenal Gland Weight

Groups	Relative weight of Right adrenal gland	Relative weight of Left adrenal gland
A	12.57±0.89	12.98±0.82
B	33.18±0.82**	34.18±0.67**
C	23.33±0.47*	23.43±0.42*
D	18.158±0.42*	18.58±0.42*
E	8.811±0.52**	8.811±0.52**

Significant difference* Highly significant difference**

DISCUSSION:

In this study, diabetes in animals was generated by using STZ. Diabetogenic effect of streptozotocin is the direct effect of permanent loss of pancreatic β -cells causing degranulation and loss of capability to secrete insulin. The probable mechanism for the ability of STZ to specifically damage pancreatic β -cells is its ability to be transported by GLUT2 (Glucose transport protein) inside the β -cells. STZ is similar enough to glucose to be transported via these proteins but it is not recognized by other glucose transporters. This explains its relative toxicity to β -cells, since these cells have relatively high levels of GLUT2¹⁷.

Adrenal gland is an essential stress responsive organ that is part of both HPA-axis and sympatho-adrenomedullary system¹⁸. It is also the most common endocrine organ associated with chemically induced lesions¹⁹. STZ causes morphological and microvascular changes in the adrenal gland²⁰. In this study, L-arginine and insulin were used for the morphological protection of adrenal gland affected by STZ-induced diabetes.

This study demonstrated highly significant decrease in mean body weight of STZ-treated group B animals as compared to control group. Howarth et al²¹ reported marked increase in blood glucose levels in STZ-induced diabetic rats with a marked reduction in the mean body

weight. The decrease in body weight was less significant when insulin and arginine were added with STZ in group C and D animals suggesting restorative effect of insulin and arginine, as arginine improves peripheral and hepatic insulin sensitivity effected by diabetes²².

The absolute and relative weights of both adrenal glands were considerably increased in group B animals. Another study¹⁵ also demonstrated increase in organ weight in proportion to mean body weight in STZ-treated animals when compared with control animals despite the fact that the mean body weight of all the animals in STZ-treated group was decreased. Hyperglycemia as a result of STZ-induced diabetes stimulates the stress system specially the HPA-Axis and generates reactive oxygen species (ROS) which are responsible to cause stress. ROS induced stress causes increased secretion of ACTH and corticosterone leading to hyperplasia and hypertrophy of adrenal gland¹⁴. The increase in weight of adrenal glands is most likely due to their hypertrophy.

STZ causes adrenal toxicity by accumulating excess lipids in cells resulting in secretion of excess steroid precursors²³. It causes atrophy of parenchymal cells in zona glomerulosa resulting in decreased aldosterone synthesis, while it causes hypertrophy of cells in zona fasciculata and increases plasma corticosterone secretion²⁴.

In present study, the increase in absolute and relative weight of adrenal glands was less marked when L-arginine and insulin were added to the therapy. L-arginine has a key role for the synthesis of NO (Nitric oxide) and it has direct antioxidant activity²⁵. It is reported that endogenously generated NO is involved in the modulation of corticosterone production²⁴. Another study also reported that insulin treatment not only normalizes blood glucose levels but also ACTH and corticosterone secretion²⁶. These findings support the recuperative effect of L-arginine and insulin.

CONCLUSION:

The present study concluded that L-arginine has restorative effect on gross morphology and weight of adrenal glands which is comparable to insulin. However, experiments on large number of animals should be conducted to reach at a definite conclusion.

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