Original Article

Protective Effect of L-Arginine on Streptozotocin (STZ)-Induced Adrenal Cortical Damage

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ABSTRACT

Objective: To assess the extent of restorative potential of L-arginine on streptozotocin (STZ)-induced adrenal gland injury in albino rats, and to compare its effectiveness to that of insulin.

Study Design: Prospective experimental study.

Place and Duration of Study: This study was conducted at the Basic Medical Sciences institute (BMSI), Jinnah postgraduate medical center (JPMC), Karachi from February to March 2018.

Materials and Methods: Forty male, healthy albino rats, 90-120 days of age were divided into 4 groups. Group A was taken as control, Group B was administered STZ, Group C and D were given STZ along with insulin and L-arginine respectively. Tissue from right adrenal gland was processed for paraffin embedding and tissue slides were stained with haematoxylin and eosin for morphometry, and Mallory's Trichrome stain to examine blood vessels in sections from control and treated groups.

Results: Mallory's Trichrome stained sections of group B showed hypertrophy of zona fasciculata with large number of dilated blood vessels as compared to control Group-A. Mallory's Trichrome stained sections of Group C showed slight recovery as there were numerous dilated vessels in hypertrophied zona fasciculata, whereas tissue sections from Group D displayed marked recovery with normal arrangement of blood vessels in zona fasciculata similar to control.

Conclusion: STZ- induced adrenal cortex injury was protected by simultaneous use of insulin and L-arginine. The improvement was more pronounced with L-arginine when compared to insulin.

Key Words: Adrenal gland, L-arginine, Insulin, Dilated blood vessels.

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INTRODUCTION

Homeostasis is essential for survival. This capability to respond and adjust to different physical and emotional threats is regulated by integration between nervous, endocrine and immune systems, also called stress system. This consists of hypothalamic-pituitary-adrenal (HPA) axis and sympathetic-adrenal medullary system, and its activation results in enhanced production of glucocorticoids and catecholamines from adrenal cortex and medulla, respectively[1].

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Cortisol is crucial glucocorticoid in human body. After its release, cortisol is placed on GLUT-4 receptors and does not allow blood glucose to enter into cells, resulting in enhanced levels of blood glucose. As a consequence, cortisol counterbalances action of insulin[2]. In addition to secretion of corticosteroids, stress stimulates free radical formation and oxidative tissue damages[3]. The activity of HPA axis is also altered in diabetic and obese individuals[4]. Thus function of adrenal gland is compromised in experimental model of diabetes[5].

Streptozotocin (STZ) is 2-deoxy-D-glucose derivative of N- methyl- N- nitroso urea. It has antibacterial, antitumor and mutagenic effects6. Its toxic effects lead to irreversible injury to β cells of endocrine pancreas7. It causes oxidative stress and DNA damage resulting in β cell death[8,9].

Antioxidants are substances which prevent harm to body cells caused by unstable molecules known as free radicals. L-arginine is one of semi- essential amino acid, which has a strong anti-oxidant potential. Normally sufficient quantities are produced in human body, but in certain cases, such as heavy metal poisoning, insufficient intake or physical illnesses, its supplementation is required[10,11]. It is precursor of nitric oxide and its biological effects are attributed to it[12].

Nitric oxide causes smooth muscle relaxation leading to improvement in blood flow to organs[13].

With this background, this histological study was designed to evaluate improvement provided by concomitant use of L-arginine on STZ- induced adrenal cortical injury and to compare its therapeutic potential to that of insulin.

MATERIALS AND METHODS

Forty male albino rats were acquired from institute's animal house. Only healthy rats with ages ranging from 90-120 days and body weight of 250-300 grams were included. Those less than 90 days, or with weight less than 250grams, or with age more than 4 months or weighing more than 300 gram were excluded from the study. Unhealthy, or inactive rats were also part of the exclusion criteria. The animals were separated into four groups, with 10 animals each on basis of experimental treatment.

Group A animals were taken as control. They were provided standard laboratory diet only. Group B was given 37 mg/kg intraperitoneal STZ (Sigma Aldrich, USA) dissolved in 1ml of citrate buffer at 4 pH on day one of experiment[15]. Group C was administered STZ in same dose as in group B, along with 1 unit/ 100 gram body weight insulin 70/30 (Eli Lily, USA) once daily subcutaneously[14] three days after administration of STZ. Group D was given same dose of STZ as in group B and L-arginine as Arginine GNC, USA 0.3 mg/gm body weight in drinking water[15].

The animals were assessed for their health status before start of study and kept on 12 hour-light and dark cycle. Standard laboratory diet was given. They were weighed, numbered and kept in propylene cages. Their serum glucose level was determined before and after giving STZ, for confirmation of diabetes, and then twice weekly, by obtaining blood samples from tail vein. Estimation was carried out with glucometer (Accu-Check Active one touch, Roche Diagnostics, USA). After an overnight fast, animals were administered STZ. Hypoglycemia was prevented by adding 5% glucose in their drinking water for initial 72 hours after giving STZ. L-arginine was freshly prepared daily and supplied in clean water bottles. The animals were sacrificed with ether anaesthesia at end of study period. A midline xiphipubic laparotomy was done. Adrenal gland was carefully removed using a blunt probe and washed with normal saline. They wereplaced overnight in 10% buffered neutral formalin for fixation[16]. The right adrenal glands were processed for paraffin block formation and tissue sections were placed and fixed to glass slides[16]. Staining was done with Haematoxylin & Eosin and Mallory Trichrome stain[16]. H & E stained tissue sections were used for morphometry, which was done with help of stage micrometer scale and ocular counting reticule under light microscope. Twenty observations were taken for each animal under 100X objective to observe cell diameter of adrenal cortical zones.

Mallory's Trichrome stained sections were observed regarding state of blood sinusoids under 40X objective. Dilatation of blood vessels was assessed assuming that cords were separated by capillary sinusoids and enlarged gaps between cords indicated dilatation of blood vessels.

The statistical significance of differences in diameter of cells in all zones of adrenal cortex between control and treated groups was assessed by student 't' test. Difference was considered statistically significant with p value ≤ 0.05. Computer software SPSS version 20 was used for calculations.

RESULTS

Table No. 1: Mean cell diameter (µm) in three adrenocortical zones of control & treated groups of albino rats.

G	Treatment	Zona Glomerulosa	Zona Fasciculata	Zona Reticularis
A	Control	13.81± 1.71	12.10± 0.18	7.9± 0.77
B	STZ	11.91± 1.51	15.59± 1.06*	8.01± 1.17
C	STZ + Insulin	13.4± 1.12	12.51± 1.05*	7.6± 1.18
D	STZ+L-arginine	13.5± 0.51	12.4± 1.42*	7.14± 1.14

KEY: Test applied: paired student "t" test. $p<0.05$: statistically significant difference*

Mallory's Trichrome stained tissue section of control group-A showed normal arrangement of blood vessels in the cortical zones (Figure No. 1).

Mallory's Trichrome stained sections of group-B displayed hypertrophy of zona fasciculata with abundance of dilated blood vessels (Figure No. 1).

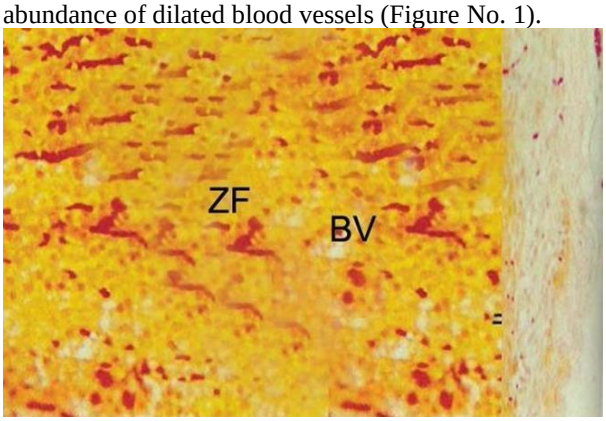

Figure No. 1: Mallory's Trichrome stained, 4 µm thick section of rat adrenal cortex from group-B showing numerous dilated blood vessels (BV) in zona fasciculata (ZF) after 6 weeks treatment with streptozotocin. Photomicrograph X400.

Mallory's Trichrome stained section of group C showed numerous dilated vessels in hypertrophied zona fasciculata (Figure-2), although dilatation of blood vessels was less as compared to STZ- treated group B.

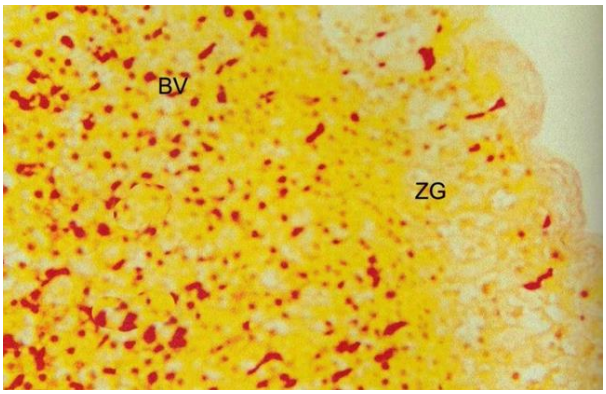

Figure No. 2: Mallory's Trichrome stained 4 µm thick section of rat adrenal cortex from group-C showing ZG and ZF. The blood vessels (BV) are dilated but seen mainly in hypertrophied zona fasciculata after 6 weeks treatment with Streptozotocin andInsulin. Photomicrograph X400

Tissue sections stained with Mallory's Trichrome from group D adrenal glands showed normal arrangement of blood vessels in zona fasciculata and reticularis same as that of control Group A.

DISCUSSION

The aim of this experimental study was to estimate the protective effect of L-arginineon STZ- induced adrenal cortical damage, and to compare its efficacy with that of insulin.

The diameter of cells in zona fasciculata showed significant increase in STZ- treated group B as compared to control. This was most likely due to accumulation of fat globules in cells because of decreased insulin secretion due to damage to beta cells of pancreas by STZ. This decreased insulin levels resulted in hyperglycemia, causing direct oxidative stress as well as stimulation of HPA axis. Similar results were seen by El- Helbawy et al (2017)[17] who observed distortion of cellular architecture with enlarged, swollen cells, filled with lipid vacuoles in zona fasciculata of monosodium glutamate (MSG) treated albino rats after two weeks of treatment. The results also demonstrated restoration of diameter of adrenal cortical cells in all three zones of adrenal cortex in group C and D when insulin and L-arginine were used simultaneously with STZ, respectively. It has been observed in a previous study[14] that co-administration of insulin with STZ in rats markedly improved histological architecture of proximal convoluted tubules of kidney as decrease in blood sugar levels mediated by insulin, also reduce oxidative stress, hence improving morphological structure[14]. In L-arginine treated group D, it was most likely due to enhanced synthesis of nitric oxide by L-arginine, as suggested by another study[18]. They observed significant improvement in architecture of liver and kidney damage when L-arginine was given simultaneously. L-arginine has an effective antioxidant activity by generation of nitric oxide, which prevents oxidative stress in tissues by disrupting chain reaction of lipid peroxidation by generating nitrogen-containing lipid molecules. Also it initiates expression of nitrosative stress resistance genes and antioxidant enzymes. It also improves antioxidant potential of glutathione (GSH) by developing S-nitrosoglutathione which is about 100 fold more effective than GSH[18]. The results also determined an insignificant decrease in cellular diameter in all zones of adrenal cortex in group D as compared to group C, highlighting role of L-arginine in accentuating nitric oxide synthesis, which in tun leads to reduction of oxidative stressas mentioned above[19].

The Mallory's trichrome stained adrenal cortical tissue sections from group B demonstrated visibly dilated blood vessels in zona fasciculata. This could be consequence of excessive ACTH stimulation and increased functional activity of cortical cells. Komolafe et al (2018)[20] also observed distorted histology of adrenal glands in STZ treated Wistar rats with dilated sinusoids in adrenal cortex. This was also in accordance to another study[21] which demonstrated widened spaces between cellular cords in tissue sections, depicting dilated sinusoids in albino rats subjected to restraint stress.

Sections stained with Mallory's trichrome stain of group C animals showed dilatationof blood vessel with hypertrophy of zona fasciculata, but it was less than that seen in group B. This improvement was most likely due to effects of insulin as mentioned earlier[18]. The tissue sections from group D animals revealed near to normal vascular

pattern in all three cortical zones of adrenal gland. This was likely due to localized effects of L-arginine on steroidogenesis and also by decreasing amount of circulatory lipids thus lowering levels of ACTH. Results were similar to another study[22] who observed amelioration of dilated and congested blood sinusoids between distorted cords in zona fasciculata of STZ - treated albino rats after administration of barley grain.

The results were also in accordance to Quddus et al[23] (2018), who demonstrated improvement in vascular pattern as less numerous and less dilated blood vessels were seen after adding L-arginine to diet in rats receiving butter and corn oil as compared to butter and corn oil only. This was likely because in addition to anti-oxidant activity, nitric oxide has direct effect on vascular smooth muscles, thus improving blood flow to organs[18].

The present study thus demonstrated positive correlation between diameter of cells in all zones of adrenal cortex and histological findings revealed by Mallory's trichrome stained tissue sections in treated and protected groups.

CONCLUSION

STZ- induced adrenal cortex injury was protected by simultaneous use of insulin and L-arginine. The improvement was more pronounced with L-arginine when compared to insulin.

Author's Contribution:

Concept & Design of Study:	Yasmeen Mahar, Aisha Qamar
Drafting:	Ayesha Mehwish
Data Analysis:	Ayesha Mehwish, Surriyya Sarwat
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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