Ameliorating Effect of L-arginine and Insulin on Streptozotocin-induced Adrenal Gland Injury in Albino Rats

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ABSTRACT

Background: Use of dietary supplements to treat illnesses has increased tremendously in recent years. Adrenal gland is one of the most commonly damaged endocrine gland in the body, not only by chemical or radiation injuries, but also as a result of different types of stress. Search is underway for use of natural foods for protection of adrenal gland from different types of insults.

Objective: To determine the protective effects of L-arginine on streptozotocin (STZ)-induced adrenal gland injury in albino rats, and to compare its efficacy to insulin.

Material and Methods: This prospective experimental study was done at BMSI, JPMC, Karachi. Forty male, healthy albino rats, 90-120 days old were segregated into 4 groups. Group A was marked as control, group B was administered STZ, group C and D were treated with STZ along with insulin and L-arginine respectively. At the end of study period, i.e., 6 weeks, animals were sacrificed under ether anaesthesia. Tissue from the left adrenal gland was processed for frozen sectioning to observe fat content of the adrenal cortex by applying Oil Red O stain.

Results: Oil Red-O stained frozen sections revealed closely aggregated fat globules in adrenal cortex of STZ treated group B as compared to control. Moderate betterment was seen in group C and in group D Oil Red O stained frozen sections as compared to STZ treated group B.

Conclusion: The results of the study demonstrated adrenal cortex injury by STZ which ameliorated with concomitant use of insulin and L-arginine. The protection was more pronounced with L-arginine as compared to insulin.

Key words: STZ, adrenal gland, insulin, L-arginine

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INTRODUCTION

The adrenal gland consists of two embryologically different parts, the adrenal cortex, derived from the mesoderm, and the adrenal medulla, which is neural crest in origin. The adrenal cortex synthesizes steroid hormones, essential for life that, is, mineralocorticoids and glucocorticoids¹. It is one of earliest and fastest developing gland².

Adrenal gland is considered as the endocrine organ which is most frequently effected by chemically induced lesions. It responds to different types of stress by synthesizing cortisol, stimulated by the release of adrenocorticotrophic hormone (ACTH) by the pituitary gland, which is itself released by the activation of hypothalamus-pituitary-adrenal (HPA) axis^{3,4}. Elevated levels of serum glucocorticoids are helpful during times of stress, as they cause mobilization of glucose stores from liver. At the same time, adipose tissue liberates free fatty acids, and further activation of HPA axis is subdued. However, prolonged exposure to raised glucocorticoid levels is

injurious, because it suppresses glucose uptake in adipocytes and fibroblasts, and decreases glucose utilization by the brain in addition to the other harmful effects⁵.

Streptozotocin (STZ) is a nitrosourea which is commonly utilized to cause insulin-dependent diabetes mellitus in research animals due to its damaging effects on insulin secreting beta cells of pancreas. In addition to this, it has harmful effects on other organs such as kidney, liver and stomach. These effects are because of its biological actions, such as development of acute and chronic injury to the cells, teratogenesis, carcinogenesis, and mutagenesis⁶. Formation of reactive oxygen species (ROS) is one of the mediator of the cytotoxic effects of STZ⁷.

Search has been made to utilize commonly used food items for the prevention of diseases. Stress being so common in today's life style, calls for dietary supplements to protect the major body organs and adrenal glands are among them. L-arginine, a semi-essential amino acid, is one of the chief component of the human diet. It is ample in natural foods such as dairy products, wheat flour, nuts and seeds⁸. It functions as a precursor of nitric oxide (NO) by NO synthase (NOS)⁹. It is also involved in the synthesis of other biologically necessary compounds involved in cellular homeostasis. It can be correlated with different

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physiological benefits including reduction of the stress response, augmentation of the immune function, prevention of atherogenesis and decrease in blood pressure¹⁰. L-arginine also stimulates the secretion of growth hormone, insulin, glucagon and prolactin. Its antioxidant property has been documented by several researchers¹¹.

Keeping this knowledge in mind, this study was designed to appraise the effects of STZ on adrenal cortex, and to observe prevention provided by L-arginine in this injury, and also to compare the effects of L-arginine with that of insulin on STZ induced adrenal cortical damage.

MATERIAL AND METHODS

This experimental study was done in anatomy department of BMSI, JPMC, Karachi, after obtaining ethical approval from ethical review committee of BMSI (23-11-2015), from December 2015 to January 2016 for duration of 60 days. Forty male, healthy albino rats, with ages from 90-120 days, and weight from 250-320 gram were obtained from the animal house of the institute. The animals were divided into four groups on the basis of the experimental treatment.

10 animals were assigned to each group. **Group A** animals were assigned as control. They were provided standard laboratory diet only. **Group B animals** were administered 37 mg/kg intraperitoneally STZ (Sigma Aldrich, USA) dissolved in 1ml of citrate buffer at 4 pH on the day one of the experiment⁷. **Group C** was administered STZ in the same dose as in group B, along with 1 unit/ 100 gram body weight insulin 70/30 (Eli Lilly, USA) once daily subcutaneously¹² three days after administration of STZ. **Group D** was treated with same dose of STZ as in group B and L-arginine as Arginine GNC, USA 0.3 mg/gm body weight in drinking water¹³.

The animals were observed for their well being by the level of their activity, and their food and water intake for a week before the commencement of the study and kept on 12 hours light and dark cycle. Standard laboratory diet was given to them. They were numbered, weighed, and kept in propylene cages. The serum glucose level of all the animals was recorded before and after giving STZ, for the verification of diabetes, and then twice weekly. Tail vein was used for drawing blood samples, and estimation was carried out with the help of glucometer (Accu-Check Active one touch,

Roche Diagnostics, USA). After an overnight fast, animals were administered STZ. In order to prevent hypoglycemia, they were provided 5% glucose in drinking water for initial 72 hours after giving STZ. Freshly prepared L-arginine was given each day in clean water bottles. At the end of the study period, that is, 6 weeks, animals were sacrificed by ether anaesthesia. The adrenal glands were exposed by a midline abdominal incision (Figure-1). They were removed from the abdomen and washed with normal saline. Both adrenal glands were placed overnight in 10% buffered neutral formalin for fixation¹⁴.

Frozen sections were prepared by using left adrenal gland. 2mm sized tissue was placed on the chuck of the cryotome. Freezing liquid was poured on the tissue and it was allowed to freeze at -35°C in the chamber. Sectioning was done within the cryostat chamber. 10µm thick sections were taken on gelatinized glass slides. Sections obtained were stained with Oil Red O stain¹⁴ to observe lipid globules within the tissue.

RESULTS

The Oil Red O stained frozen sections from control group A animals revealed normal architecture of adrenal cortex showing innermost zona reticularis, middle zona fasciculata and outer most zona glomerulosa, surrounded by a connective tissue capsule. Mild accumulation of fat globules was seen in all the zones of adrenal cortex, but mainly in zona glomerulosa (Figure-2). Oil Red O stained frozen sections from STZ- treated group B rat adrenal cortex showed hypertrophied zona fasciculata, with fat accumulation in the form of closely aggregated fat globules in fasciculata and reticular zones (Figure-3) when compared with control group A (Figure-2). Oil Red O stained frozen sections from group C (STZ and insulin-treated) rat adrenal cortex showed dense fat accumulation in the form of fat globules in hypertrophied zona fasciculata, but moderate fat accumulation in zona reticularis (Figure-4) when compared to group B. Oil Red O stained frozen sections from group D (STZ & L-arginine-treated) rat adrenal cortex showed mild to moderate fat accumulation in the form of globules in zona glomerulosa and zona fasciculata (Figure-5) when compared to group B.

Figure-1: A control group rat with exposed abdomen revealing normal adrenal glands is shown in above photograph.

Figure-2: Oil Red O stained frozen tissue section from control group A rat adrenal cortex, revealing mild fat accumulation (FA) in ZG (zona

glomerulosa), ZF (zona fasciculata) and ZR (zona reticularis). Photomicrograph X 400

Figure-3: Oil red-O stained, frozen tissue section from 6 week STZ- treated group B rat adrenal cortex, revealing densely aggregated fat

accumulation (FA) in hypertrophied ZF (zona fasciculata) and ZR (zona reticularis). Photomicrograph X 400

Figure-4: Oil red O stained, frozen tissue section from 6 week STZ with insulin treated group C rat adrenal cortex, revealing moderate fat

accumulation (FA) in ZF (zona fasciculata) and ZR (zona reticularis). Photomicrograph X 400

Figure-5: Oil Red-O stained frozen tissue section from 6 week STZ and L-arginine treated group D rat adrenal cortex, revealing mild fat accumulation

(FA) in ZG (zona glomerulosa), and ZF (zona fasciculata). Photomicrograph X 400

DISCUSSION

Drugs and diseases act as stressor and stimulant to hypothalamo- pituitary adrenal axis¹⁵. Lai et al (2006) stated that the adrenal gland is indispensable to stress response, being part of both the HPA- axis and sympathetic nervous system¹⁶. The cells of adrenal cortex possess ample amount of lipids used as substrate for steroid hormone synthesis. Many substances which are injurious for the adrenal cortex are lipophilic and store in these lipid rich cells, leading to impaired function resulting in abundant steroid precursors and vacuolation in different zones of adrenal cortex¹⁷. It has been known by the study of Repetto et al (2010) that diabetes mellitus is related with hyperfunction of the adrenal gland¹⁸. Thus the purpose of this study was to determine the protective potential of L-arginine on the cortex of the adrenal gland in diabetes mellitus induced by streptozotocin (STZ) in rats, and to equate the results obtained with simultaneous use of insulin.

STZ is highly injurious to the β -cells of pancreas. Raza & John (2012) also used it to induce experimental diabetes. STZ retards the synthesis of DNA in bacteria as well as in cells of mammals¹⁹. Sharma (2010) described the mechanism of

action of STZ by which it caused cell death²⁰. Fat accumulation demonstrated by Oil Red O stained sections was pronounced in STZ- treated group-B animals due to stimulation and release of ACTH by toxic effect of STZ and hyperglycemia caused by the drug which acted on hypothalamic nuclei. ACTH enhances synthesis of steroid hormones produced by adrenal cortex by raising number of receptors on adrenocortical cells for LDL and augments the activity of enzymes which release cholesterol from LDL²¹.

Another study emphasized that there could be a proportionate increase in cholesterol in adrenal gland with increased ACTH release⁵. Diabetic stress increases glucocorticoid secretion and produces insulin resistance. Cummins et al (2006) worked on cholesterol transport receptor genes and demonstrated large amount of free cholesterol in adrenal gland in response to stress²². El-feky et al (2010) also observed that fat entered in adrenal cortex caused increase in the size and number of lipid rich cells in zona fasciculata and in zona reticularis²³.

The present study demonstrated decrease in fat accumulation in group C and group D, which were

given insulin and L-arginine respectively, along with STZ as compared to group B Oil Red O stained frozen sections. The decrease was more marked in group D (figure-5) than Group C (figure-4), as L-arginine supplementation augments vasodilation in humans with increased HDL cholesterol levels, and, in animal models, reduces oxidative stress, platelet aggregation, monocyte adhesion and the formation of intimal lesions, thus improving blood flow²⁴. Abdel Malak and Amin (2018) noticed significant improvement in the adrenal cortical architecture of suprarenal glands and marked decrease in the extent of vacuolar degeneration in all three zones of adrenal cortex when L-arginine was used in restraint stress in experimental animals¹¹. These results were also in accordance to Quddus et al (2018) who also found significant decrease in fat deposition in Oil Red O stained adrenal cortical sections in L-arginine receiving group as compared to those animal sections receiving butter or corn oil only. The improvement was more marked in animals receiving L-arginine with corn oil as compared to those which received butter along with L-arginine²⁵. The increased effectiveness of L-arginine in preventing fat deposition was most likely because of synthesis of nitric oxide, and its effectiveness in reducing oxidative stress.

CONCLUSION

The results of this study have emphasized that STZ caused damage to the adrenal gland morphology as shown by excessive deposition of fat in the adrenal cortex, where as its harmful effects were prevented by the concomitant use of L-arginine, which were more effective than insulin. Therefore it is recommended that diabetic patients should be advised to include L-arginine supplements in their diet for protection of adrenal gland damage, and to improve blood glucose levels.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Mahar Y, Qamar A:	Concept and design of study, Collection of data, statistical analysis
Khan I, Fatimee S:	Writing of manuscript, critical review of manuscript
Saeeduddin MF:	Analysis and interpretation of data, statistical analysis
Chaudhry S, Salman S:	Data collection, bibliography

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.