ORIGINALARTICLE

EFFECT OF L-ARGININE AND INSULIN ON ADRENAL GLAND DAMAGED BY STREPTOZOTOCIN IN ALBINO RATS

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Background: The adrenal gland is one of the major endocrine glands involved not only in different physiological functions, but also in response to stress This study was done to asses the effects of L-arginine and insulin on streptozotocin (STZ) induced adrenal gland damage in albino rats. **Method**: This laboratory based experimental study on animals was undertaken in the Anatomy department of Basic Medical Sciences Institute (BMSI), Jinnah postgraduate Medical Center (JPMC), Karachi, from February to March 2018. Forty adult, healthy male albino rats were placed into 4 groups (10 each). Group A was taken as control. Group B was given STZ. Group C and D were given STZ as in group B with insulin and L-arginine respectively. Absolute and relative weight of adrenal glands was measured at the end of the study. Tissues from adrenal glands were processed and stained with Haematoxylin and Eosin for the morphometric study. **Results:** The absolute as well as relative adrenal weight of animals was significantly raised in group B in comparison to control, although showed a significant recovery in group C and D animals when insulin and L-arginine were added to STZ. Haematoxylin and Eosin (H&E) stained sections of adrenal cortex of STZ treated Group B showed reduced width of zona glomerulosa, with increased width of zona fasciculata and zona reticularis when compared to control. The width of these zones of adrenal cortex recovered to a significant extent when group C and D tissue sections were compared with STZ- treated group B tissue sections. **Conclusion:** This study highlighted the protective effect of L-arginine on adrenal gland weight and histology in streptozotocin induced adrenal gland damage, which was comparable to insulin.

Keywords: Streptozotocin; L-arginine; Adrenal gland; Insulin

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INTRODUCTION

The adrenal gland is one of the major endocrine gland involved not only in different physiological functions, but also in response to stress.[1] The adrenal glands respond to different types of stress by production of several molecules such as nitric oxide (NO), cytokines and prostaglandins.[2] In the hypothalamic-hypophyseal-adrenal (HPA) axis, adrenocorticotropic hormone (ACTH), secreted by the anterior pituitary gland, induces the release of glucocorticoids from the zona fasciculata of the cortex of the adrenal gland. In chronic stress, it can cause hyperplasia of zona fasciculata resulting in permanent injury.[3,4]

Streptozotocin has been used in experimental studies to develop animal models of diabetes. It effects pancreatic ß- cell DNA and augments different pathways involved in activation of protein kinase C (ADP ribose) polymerase and NADPH oxidase, with consequent excessive production of reactive oxygen species. This oxidative stress results in activation of hypothalamic-hypophyseal-adrenal (HPA) axis with subsequent enhanced production of corticotropin releasing hormone (CRH) from the hypothalamus, and ACTH from the pituitary gland, causing increased activity of adrenal cortex, specially fasciculate.[5,6]

L-Arginine is a semi-essential amino acid, which is abundant in natural foods, such as, as dairy products, nuts, seeds, beef, chicken, wheat flour and sea foods. It is a precursor of nitric oxide (NO) synthesis by NO synthase.[7,8] Studies have shown that nitric oxide is involved in the regulation of steroid synthesis.[9] Nitric oxide is also involved in the modulation of adrenal blood flow.[10] It causes relaxation of endothelial smooth muscles leading to increased blood flow.[11]

As adrenal cortex is involved in maintaining water and electrolyte balance as well as carbohydrate metabolism, it is vital for the human body[12]. Therefore, efforts should be focused on agents which can prevent adrenal gland damage, and readily available at the same time. With this background, this study was planned to assess the protection provided by L-arginine on STZ treated adrenal gland, and it was compared with that provided by the insulin.

MATERIAL AND METHODS

This laboratory based experimental study on animals was carried out in the Anatomy Department of BMSI,

JPMC, Karachi, after obtaining ethical approval from ethical review committee of the institute, for 60 days from February to March 2018. Forty male, healthy albino rats, with ages from 90-120 days, and weight ranging between 250–320 gram were procured from institute's animal house. The animals were segregated into four groups on the basis of the experimental treatment.

There were 10 male Wistar rats in each group. Group-A animals were taken as control. They were given standard laboratory diet only. Group-B animals was administered STZ (Sigma Aldrich, USA) in a dose of 37 mg/kg intraperitoneally dissolved in 1ml citrate buffer at a pH of 4 on the first day of the experiment.[13] Group-C was given STZ in similar dose as in group B, along with insulin 70/30 (Eli Lily, USA) once daily subcutaneously in a dose of 1 unit/100gram body weight[14] three days after treatment with STZ. Group-D was treated with STZ in a similar way to group B and L-arginine[15] was added as Arginine GNC, USA in drinking water at 0.3 mg/gm body weight.

The animals were observed for their food and water intake and weight gain for one week before the start of the experimental study and maintained on 12 hours light and dark cycle to ensure their health. They were provided standard laboratory diet. They were weighed, numbered by ear punching, and placed in propylene cages with wood chip floor bedding. STZ was freshly prepared. After overnight fasting, animals were injected STZ. The animals were provided 5% glucose in drinking water for initial 72 hours after the injection. For confirmation of diabetes, blood was drawn from tail vein and Accu-Check Active one touch Glucometer (Roche Diagnostics, USA) was used. Clean water bottles and freshly prepared L-arginine was provided each day. The animals were sacrificed by using ether anaesthesia at the end of the study after recording their weight. A midline incision was used to open the abdomen, and adrenal glands were exposed. The weight of each adrenal gland was measured on a Sartorius balance and then relative weight was determined.[16]

Both adrenal glands were placed in buffered neutral formalin overnight for fixation. The right adrenal glands were further processed by overnight immersion in 70% alcohol, and then hourly changes were made in ascending grades of alcohol for dehydration.[17] Then the tissue was cleared in two changes of xylene for one hour each. Infiltrated was done in two changes of paraffin for one hour each in the laboratory oven at 58 ^{o}C. The paraffin blocks were made on paraffin embedding system and 4µm thick sections were sliced on a rotary microtome. Tissue sections were placed in water bath, and then taken on albumenized and labelled glass slides. Mounting was done with DPX. Tissue was fixed on hot plate at 32 ^{o}C. Staining was done with Haematoxylin & Eosin.[17]

Morphometric study was done under light microscope with the help of stage micrometer scale and ocular counting reticule. Twenty observations were taken for each animal under 10X ocular and 40X objective to observe morphometric parameters, i.e., radial width of the zona glomerulosa, fasciculata and reticularis of adrenal cortex in control and treated animals. Data was analysed using SPSS 20.0. The statistical significance of differences in absolute and relative adrenal weight, and differences in width of cortical zones between control and treated groups was assessed by student 't' test. The difference was considered significant statistically if the '*p*' value was equal to or <0.05.

RESULTS

The groups A, C, and D animals remained healthy and active and reacted well to the extrinsic stimulation (Figure-1).

The animals of the group-B became inactive with the passage of time. Their appetite had increased initially towards the end of the first week. By the end of third week, they showed sluggish response to external stimuli. By the end of sixth week, the animals further lost all interest in their surroundings (Figure-2).

The absolute weight of right and left adrenal glands from all the experimental animals was measured in milligram (Figure-3). The results demonstrated a highly significant (p<0.001) increase in the adrenal gland weight of group B animals in comparison to group A. The data also revealed a significant decrease (p<0.01) in absolute adrenal gland weight on both right and left sides in group C and group D animals in comparison to STZ treated group B. There was an insignificant decrease in the weight of both adrenal glands in group D when compared to group C animals suggesting the efficacy of L-arginine treatment (Figure-3) .

The relative weight of adrenal glands belonging to animals in different groups were calculated (Figure-4). The results showed a highly significant increase (p<0.001) in both right and left relative adrenal gland weight in group B animals in comparison to control, whereas a significant decrease (p<0.01) in group C and D animals was discovered in comparison to STZ treated group B. In comparison between treated groups, group D which was given L-arginine showed insignificant

decrease in relative adrenal weight as compared to insulin receiving group C (Figure-4).

The mean width of the three zones of adrenal cortex was noted after staining tissue section with H & E stain from the control as well as treated groups. H & E tissue section from the control group A showed regular arrangement of the cells in three zones of the adrenal cortex (Figure-5). The data showed an insignificant decrease in the thickness of the zona glomerulosa, whereas, thickness of zona fasciculata and zona reticularis showed raised thickness (p<0.001) in tissue sections from group B animals in comparison to control (Figure-6 & 7). There was marked hypertrophy of zona fasciculata, with abundance of fatty vacuoles, giving empty or sponge-like appearance to the cells, as well as pyknosis of nuclei in the zona fasciculata (Figure-6). The mean width of zona glomerulosa showed insignificant change in group C when compared to group B, however, fasciculata, and reticular zones showed significant decrease in the comparison with group B. The mean width of zona glomerulosa showed an insignificant decrease, where as, fasciculata and reticular zones demonstrated highly significant decrease in width when group D tissue sections were compared with STZ treated group B (Figure-7).

Figure-1: Photograph of healthy and active albino rats of control group A

Figure-2: Photograph of STZ - treated group B albino rats after 6 weeks of treatment, showing loss of interest in their surroundings

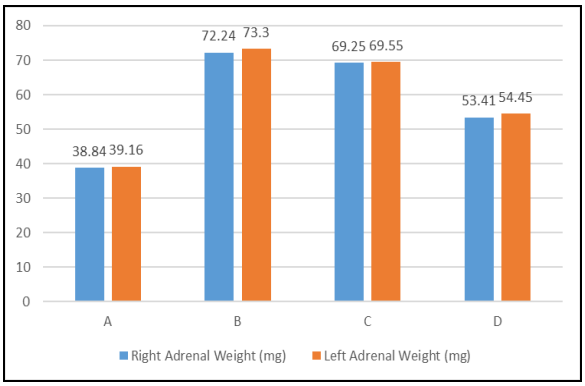

Figure-3: Mean Absolute Weight of Adrenal Glands in treated and control groups of Albino Rats (in milligrams). The groups were assigned as; Group A: Control Group B: STZ- treated. Group C: STZ and insulin treated. Group D: STZ with L-arginine

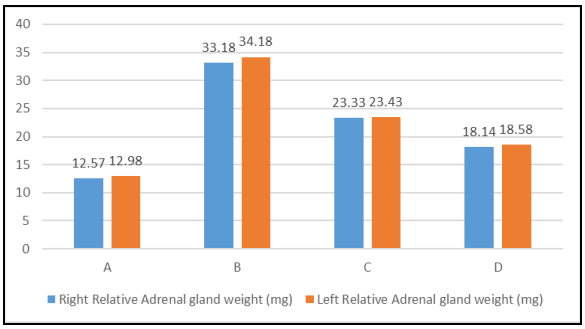

Figure-4: Mean Relative Weight of Adrenal Glands in treated and control groups of Albino Rats Key: The relative adrenal weight of both adrenal glands was taken in milligram. The groups were assigned as: Group A: Control . Group B: STZ- treated. Group C: STZ and insulin treated.Group D; STZ and L arginine treated.

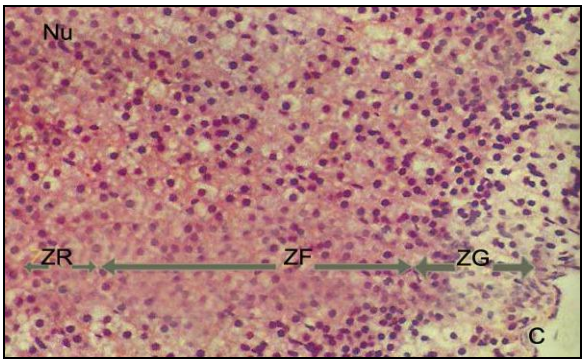

Figure-5: 4µm thick tissue section stained with haematoxylin & eosin, from control rat adrenal cortex showing adrenocortical zones, that is, glomerulosa (ZG), fasciculata (ZF) and reticular zones (ZR) along with capsule C and nuclei (Nu). Photomicrograph 400X

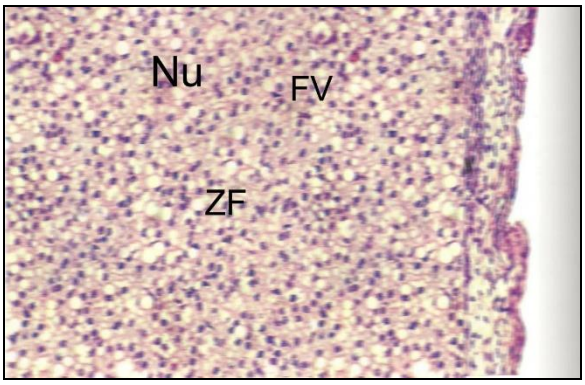

Figure-6: **4µm thick tissue section stained with haematoxylin & eosin, from 6 week STZ- treated rat adrenal cortex showing hypertrophied zona fasciculata (ZF) with abundance of fat vacuoles (FV) and aggregations of pyknotic nuclei (Nu) in vacuolated cells. Photomicrograph 400X**

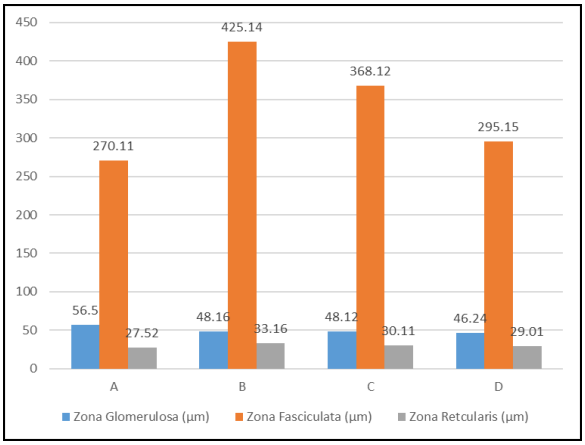

Figure-7: Mean Width (µm) of Adrenocortical Zones in Different Groups of Albino Rats

DISCUSSION

This study was planned to assess the protective effects of L-arginine on the STZ treated adrenal gland as adrenal gland is most frequently involved endocrine organ by chemicals and stress[18] and to compare it with insulin.

The animals of the control group A and insulin and L-arginine treated groups C and D stayed healthy and active throughout the study period. The animal of STZ treated group B became lethargic with the passage of time and consumed more water. These findings were similar to Zafar and Naqvi (2010)[19] who also recorded analogous findings in control and STZ treated rats.

The results of the study reported highly significantly raised absolute and relative adrenal weight in STZ treated group B, which was in concordance to other studies, which had also showed increased organ weight after treatment with STZ.[13,19] STZ causes cellular damage by DNA fragmentation and evokes other harmful changes in the cells.[13] It causes damage to ß- cells of pancreas, due to formation of excessive reactive oxygen species (ROS), lipid peroxidation, and DNA injury resulting in ß- cell death.[20] The resulting hyperglycemia stimulates HPA axis, causing enhanced secretion of ACTH[3], which in turn stimulates hypertrophy and hyperplasia of zona fasciculata and reticularis, leading to increased absolute and relative organ weight. The results also revealed restoration of adrenal weight in animals of group C and D, receiving insulin and L-arginine respectively. It has been reported in literature[21] that treatment with insulin normalizes serum glucose level as well as decreases release of ACTH and corticosterone, thus resulting in decrease of oxidative stress and reduced organ weight. The decrease was more marked in animals with L-arginine treatment, emphasizing its role in increasing nitric oxide synthesis, which in tun leads to reduction of oxidative stress.[9]

The results of the present study showed differential effects on the three zones of adrenal cortex. The width of glomerulosa zone was reduced, whereas it was raised in fasciculata and reticular zones in STZ- treated group B animals when compared to control. STZ causes damage to the adrenal gland by accumulation of excess lipids in the cells, leading to excess steroid precursor formation[6]. This was in agreement to Gawad *et al*[22] who found degenerative changes in zona glomerulosa in prepubertal male albino rats exposed to restraint stress. Addition of insulin and L-arginine to group C and D in addition to STZ resulted in decreased width of glomerulosa and fasciculata zones, with insignificant change in zona glomerulosa, more marked in group D when compared to group C, as arginine augments peripheral and hepatic insulin sensitivity in individuals suffering from diabetes.[23] This finding was similar to another study[24] in which significant improvement was seen in histology of the liver tissue on addition of L-arginine, after experimental ischemia and reperfusion in liver of adult Wistar rats. Quddus *et al*[25] also noticed significant decrease in thickness of zona fasciculata when L-arginine was added to the high-fat diet induced stress in albino rats.

CONCLUSION

The observations of the present study have highlighted that STZ lead to the damage to the adrenal gland structure, but its toxic effects were ameliorated to a significant extent by the concomitant use of L-arginine, which were comparable to the effects of insulin in STZ induced adrenal gland

toxicity. Thus dietary supplementation of L-arginine could be an easy and inexpensive method of protecting diabetic patients from adrenal gland injury. The results of this study provide a foundation for advising diabetic patients to add L-arginine supplementation in their diet for better control of blood sugar as well as protecting organ damage.

AUTHORS' CONTRIBUTION

AQ, YS: Conceived, design and did the statistical analysis. MH: participated in the editing along with reviewing it in the end. SS, SC, MFS: Data collection, write-up.

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