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ORIGINAL ARTICLE

ANTI-HYPERGLYCEMIC AND ANTI-OXIDATIVE EFFECTS OF L-CARNITINE ADMINISTRATION IN ALLOXAN INDUCED DIABETIC ALBINO WISTAR RATS

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ABSTRACT

OBJECTIVE: To evaluate anti-hyperglycemic and anti-oxidative effects of L-carnitine in alloxan induced diabetic albino wistar rats.

METHODS: This quasi-experimental study was conducted at Isra University, Hyderabad from June 2017 to August 2017. Thirty-six albino wistar male rats were equally divided into 3 groups (n=12/group); group A (control), group B (alloxan 150mg/kg intraperitoneally) and group C (alloxan 150mg/kg intraperitoneally + L-carnitine 500mg/kg orally for 21 days). Diabetes was induced in group B and C by single intraperitoneal dose of alloxan 150mg/kg body weight and rats having blood glucose >200mg/dl were labeled as diabetic rats and included in study. Biochemical (blood glucose, serum insulin and glutathione peroxidase) and histopathological analysis of pancreas was performed in all three experimental groups.

RESULTS: Post-experimental body weight in groups A, B and C were noted as 249.58±6.63, 199.08±12.18, 210.58±5.14 grams respectively. The fasting blood glucose in groups A, B and C were noted as 104.58±7.05, 221.25±8.22, 110.17±12.85 mg/dl respectively (P<0.001). Serum insulin in groups A, B and C was noted as 1.45±0.083, 0.31±0.16, 1.74±0.23 ηg/ml respectively (P<0.001). Glutathione peroxidase levels in groups A, B and C were noted as 1.45±0.17, 0.93±0.11, 1.74±0.17 ηg/ml respectively (P<0.001). Histopathology of pancreas showed reduction in size (mean islet diameter 157±1.5 μm) and number of islets of Langerhans in diabetic rats, while L-carnitine treated rats have shown compensatory increase in size of islets of Langerhans (mean islet diameter 210±6.3 μm).

CONCLUSION: L-carnitine therapy is a potent anti-hyperglycemic and anti-oxidative regimen capable of reducing blood glucose and increasing plasma anti-oxidant levels.

KEYWORDS: L-Carnitine (MeSH); Carnitine (MeSH); Diabetes (Non-MeSH); Oxidative Stress (MeSH); Pancreas (MeSH); Islets of Langerhans (MeSH); Reactive Oxygen Species (MeSH); Blood Glucose (MeSH); Insulin (MeSH); Glutathione Peroxidase (MeSH).

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorders defined as hyperglycemia due to insufficiency of secretion of insulin, action of insulin or combination of both. It is classified as diabetes mellitus type 1 and 2. Type 1 diabetes is caused by destruction of β-cells of pancreas which leads to absolute deficiency of insulin. Type 2 diabetes is caused by defect in secretion of insulin with insulin resistance.[1] Alloxan is a toxic analogue of glucose which enters the β-cell using glucose transporter type 2 (GLUT-2). It induces diabetes through excessive formation of reactive oxygen species (ROS) which are highly toxic to β-cells and selectively destructs the insulin-producing β-cells in rodents and other animals. Glucokinase activity is also restricted by the alloxan. Alloxan diabetes is an insulin-dependent diabetes with characteristics features resembling the type 1 diabetes in humans.[2,3] L-carnitine is a naturally occurring vitamin-like compound, is an ubiquitous constituent of human plasma and body tissues. L-carnitine performs a variety of essential intra-cellular and metabolic functions, like transport of fatty acid across the inner mitochondrial membrane into mitochondrial matrix for β-oxidation, detoxification of potentially toxic metabolites, regulation of mitochondrial acyl-CoA/free CoA ratio and stabilization of the cell membrane.[4] In diabetic animal experimental models, pancreatic carnitine levels have been found decreased both in early and advanced stages of diabetes mellitus and excretion of carnitine in urine to increase.[5] L-carnitine short-circuit the Randle cycle by binding inhibitory acetyl-CoA units forming acetyl-carnitine and concomitantly releasing free CoA. Lowering of mitochondrial acetyl-CoA : free CoA ratio is than favorable for glucose oxidation.[6] L-carnitine have got antioxidant properties by which it suppresses immune mediated responses by extinguishing ROS, as a result of that it inhibits the signaling for T cell activation, this is how pro-inflammatory cytokine production is suppressed including IL-6.[7] L-carnitine is recommended at a dose of 1-3 gram per

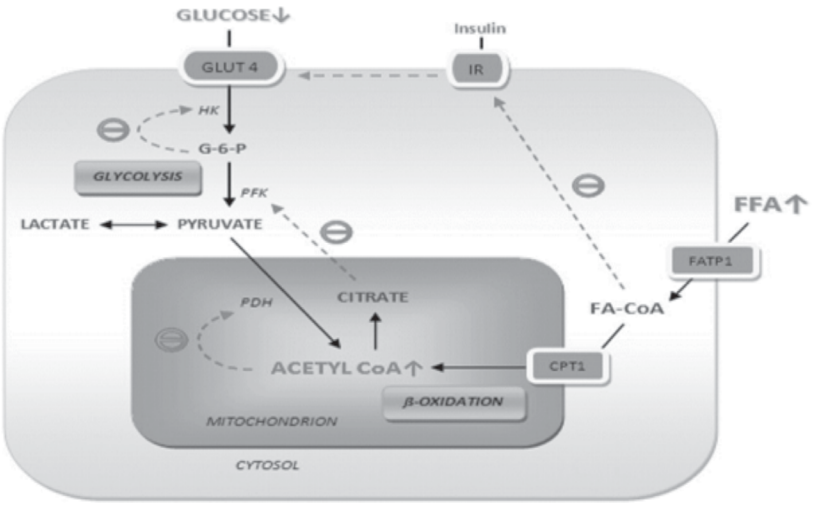

Figure 1: Randle cycle showing inhibition of cellular glucose uptake[8]

day to adult individuals, supplementation with 2 gram L-carnitine to hypothyroid patients have shown its effects associated with reduction in fatigue and better body composition.[9] Dosage upto 3000mg/day have been shown free of any side effect.[10] Sensitivity of insulin can be enhanced with supplementation of L-carnitine in over weight persons and in those who have impaired glucose tolerance, such as pre-diabetic individuals or those with metabolic syndrome.[11] Serum carnitine level appears to be regulated in range between 23μmol/L to 73μmol/L while acetyl-L-carnitine appears to be in range between 3μmol/L to 14μmol/L, both are present in serum independently of their dietary supplementation.[12] Studies have suggested worthwhile effects of L-carnitine supplements on inflammatory parameters, in secondary prevention of cardiovascular diseases, in management of diabetes mellitus, on control of serum lipid profile in patients on hemodialysis and for adult patients with end-stage renal disease on hemodialysis.[13]

The main objective of this study was to evaluate the anti-hyperglycemic and anti-oxidant effects of L-carnitine in alloxan induced diabetic albino wistar rats, evaluated by body weight, blood glucose, serum insulin, glutathione peroxidase (GPX) levels and histopathology of pancreas. Findings of this study will be significant in studying the effects of diabetes related metabolic disturbances and possible protective effects of L-carnitine in human populations.

METHODS

This quasi-experimental study was conducted at Isra University, Hyderabad from June 2017 to August 2017 after taking approval from Isra University Ethical Review Board. Sample of 36 albino wistar male rats were selected by non-random purposive sampling. The sample size was calculated using animal study sampling formula as described in previous studies.[14] Inclusion criteria was healthy non-diabetic male rats weighing 200 to 250 grams.

Animals were housed in the stainless steel cages (with saw dust bedding). The cages were fully equipped with stainless steel feeding containers and plastic drinking bottles with stainless steel nozzles. The animals were housed in a hygienic and well ventilated environment. Rats were provided food (lab chow diet) and tap water *ad libitum*. The light/dark cycle was maintained at 12 hour intervals. Rats were equally divided into three groups. Group A was control group, group B was diabetic control group (alloxan 150mg/kg i.p.) and group C was experimental group (alloxan 150mg/kg i.p. + L-carnitine 500mg/kg orally for 21 days). Diabetes was induced in rats of group B and C by single intraperitoneal dose of alloxan 150mg/kg body weight and diabetes induction was confirmed by serial blood glucose monitoring at 24, 48 and 72 hour interval after induction dose. Rats having blood glucose >200mg/dl were labeled as diabetic rats and included in study.[15] The level of the orally administered dosage of L-carnitine (500 mg/kg bwt) and intraperitoneal dose of alloxan (150mg/kg bwt intraperitoneally) was based on the previous works.[16,17] Experimental work was conducted as per rules maintained by Ethical Committee of Isra University, Hyderabad. Body weights of all group rats were recorded before and after the experiment.

At the end of experiment all the rats were given anesthesia (chloroform soaked cotton) and sacrificed by cervical dislocation. Blood samples for biochemical analysis were collected by cardiac puncture. The samples were transferred to gel tubes and centrifuged at 3000 rpm for 15 min at 4°C to separate serum which was stored in serum cups for biochemical analysis. Incision was given from the suprasternal notch to lower abdomen and the pancreas was removed and preserved in 10% formalin. Tissue samples were passed through ascending grades of ethyl alcohol (70%, 80%, 90% and 100%) for dehydration purpose. After passing the samples through xylene for clearing, they were embedded in paraffin wax and 4 micron thick sections were obtained by microtome. Slides were stained with hematoxylin and eosin (H&E) and observed under light microscope for morphological changes in pancreatic islets of Langerhans.

Blood glucose was measured using AccuCheck® advantage blood glucose monitor. Serum insulin was analyzed using DIAsource ImmunoAssay, Belgium ELISA kit (Ref no: KAP1251) and GPX was analyzed using Bioassay Technology Laboratory, Shanghai, China ELISA kit (catalogue no: E1242Ra) at Isra University diagnostic laboratory. SPSS (Statistical Packages for Social Sciences) version 22.0 was used for data analysis using ANOVA and student t-test at 95% confidence interval. Statistical significance was taken at p value ≤ 0.05.

RESULTS

Blood glucose, serum insulin, GPX and body weight were measured and histopathology of pancreas was done in all rats. Mean body weight in control group was noted as 249.58±6.63 grams, it was decreased in diabetic group to 199.08±12.18 grams while in L-carnitine treated group it was 210.58±5.14 grams.

TABLE I: BODY WEIGHT DISTRIBUTION OF THE EXPERIMENTAL GROUPS (n=12/GROUP)

Groups	Mean ± SD (grams)	Minimum	Maximum
Control Group	249.58 ± 6.63	239	259
Diabetic Group	199.08 ± 12.18	181	199
L-carnitine Treated	210.58 ± 5.14	201	218

TABLE II: DIFFERENCE IN BLOOD GLUCOSE, SERUM INSULIN AND GLUTATHIONE PEROXIDASE IN EXPERIMENTAL GROUPS

Parameter	Groups	Mean ± SD	P-Value
Blood Glucose Level (mg/dl)	Control	104.58 ± 7.05	<0.001
	Diabetic	221.25 ± 8.22	
	L-carnitine treated	110.17 ± 12.85	
Serum Insulin Level (ηg/ml)	Control	1.45 ± 0.083	<0.001
	Diabetic	0.31 ± 0.16	
	L-carnitine treated	1.74 ± 0.23	
Serum GPX Level (ηg/ml)	Control	1.45 ± 0.17	<0.001
	Diabetic	0.93 ± 0.11	
	L-carnitine treated	1.74 ± 0.17	

GPX = Glutathione Peroxidase

TABLE III: INDIVIDUAL COMPARISON OF BLOOD GLUCOSE, SERUM INSULIN AND GLUTATHIONE PEROXIDASE LEVELS IN DIABETIC GROUP AND L-CARNITINE TREATED GROUP

Parameter	Group-B	Group-C	T-Score	P-Value
Blood Glucose Level (mg/dl)	221.25 ± 8.22	110.17 ± 12.85	26.34	<0.001
Serum Insulin Level (ηg/ml)	0.31 ± 0.16	1.74 ± 0.23	20.4	<0.001
Serum GPX Level (ηg/ml)	0.93 ± 0.11	1.74 ± 0.17	11.6	<0.001

GPX = Glutathione Peroxidase

Decreased weight loss was noticed in L-carnitine treated rats as compared to diabetic rats (Table I).

Statistically significant difference in mean blood levels of glucose was found in all groups ($p<0.001$). Mean blood glucose was found to be markedly increased in alloxan (diabetic) group but was near normal in L-carnitine treated group (Table II). Similarly, statistically significant difference in mean blood levels of serum insulin and GPX levels was also seen in all groups ($p<0.001$). Both serum levels of Insulin and GPX were reduced in alloxan (diabetic) group but were comparatively higher in experimental animals receiving L-carnitine administration (Table II).

Student t-test results has also shown statistically significant difference in blood glucose, serum insulin and GPX levels between alloxan induced diabetic groups and L-carnitine treated groups ($p<0.001$) (Table III).

Pancreatic β cell protective effects of L-carnitine are also evident by histopathology of pancreas in which alloxan induction caused shrinkage in size (mean islet diameter 157±1.5 μm) and L-carnitine treatment in diabetic rats caused compensatory increase in size (mean islet diameter 210±6.3 μm). Similarly, on observation there also a marked reduction in number of islets of Langerhans of pancreas in alloxan treated diabetic rats as compared with L-carnitine group (Figure 2).

DISCUSSION

Diabetes mellitus is a multifactorial error of metabolism characterized by hyperglycemia due to insufficiency of secretion of insulin, action of insulin or combination of both.[1] Randle cycle is involved to cause hyperglycemia by accumulation of fatty acids in cytoplasm which directly inhibit the insulin receptor and inhibitory acyl-CoA in mitochondria which inhibit the action of enzymes phosphofructokinase and pyruvate dehydrogenase. Improper beta oxidation of fatty acids leads to excessive formation of reactive oxygen species and disturbed homeostasis due to hyperglycemia severely affects pancreatic β cells of islets of Langerhans as they have got low antioxidant levels compared to other cells of body.[18] L-carnitine short-circuit the Randle cycle by reacting inhibitory acyl-CoA making acyl-carnitine and increasing free CoA level that favors glucose oxidation ultimately hyperglycemia subsides and quenches ROS through its anti-oxidant properties leading to improvement in pancreatic β cell mass and insulin levels.[6]

In our study we found that alloxan diabetes induced in albino wistar rats in characterized by raised blood glucose levels, reduced serum insulin levels and reduced GPX levels. Treatment of alloxan induced diabetic rats with L-carnitine led to decrease in blood glucose levels near to normal and improvement in serum insulin and GPX levels. Our findings are consistent with findings of Samir, et al and Sasaninejad, et al who also reported reduction in blood glucose level in Sprague Dawley rats. However we differs with the study conducted by Samir, et al in that he used high fructose diet and single dose of streptozosin 30mg/kg intraperitoneally.[19,20] Rise in serum insulin level was also found in a recent study conducted by Sadighara, et al in which L-carnitine administration prevented diabetogenic activity in diabetic rats with concomitant decrease in blood glucose levels.[21] Abuzahra, et al also found results consistent with our study where L-carnitine has shown antioxidant properties by quenching ROS with significant decrease in malondialdehyde and significant increase in GPX, superoxide dismutase and catalase.[22] Hall, et al found consistent finding with positive correlation between lean body mass and L-carnitine content of the body.[23]

Our results were inconsistent with a recent study conducted by Amiri-Moghadam, et al who found weight loss in diabetics with administration of L-carnitine therapy in a randomized double blind placebo-controlled clinical trial, while our study shows preventive effects of L-carnitine from weight loss.[24] This deviation of the results from our study might be due to long duration of study for 3 months with calorie restricted diet. Nagy, et al found consistent histopathologic changes in diabetes induction by alloxan with decrease in size and number of islets of Langerhans of pancreas of rats.[14,25] L-carnitine treatment

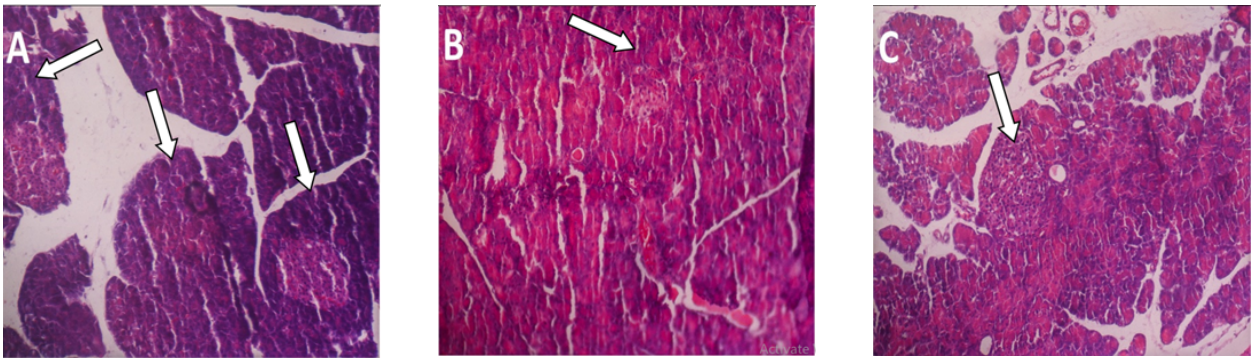

Figure 2: Section of pancreas of Experimental animals x100 magnification. **(A)** Control group: showing normal architecture of beta cells of islets of Langerhans of pancreas. **(B)** Diabetic group: showing degenerative changes with shrinkage of size and reduced number of islets of Langerhans of pancreas. **(C)** L-carnitine treated diabetic group: showing regenerative changes by increase in size of islets of Langerhans of pancreas as compared to diabetic group.

in diabetic rats has shown its pancreatic β cell protective effects by increase in size of islets of Langerhans of pancreas. This effects could be compensatory hypertrophy of islets of Langerhans of pancreas due to damage to the β cell by alloxan induction.

However, there were certain limitations in the current study. Such as, limited availability of time, monetary resources due to which certain other parameters could not be included in the study protocol i-e inflammatory markers(C-reactive protein, transforming growth factor, tumor necrosis factor etc.) and variable anti-oxidants (malondialdehyde, superoxide dismutase etc).

CONCLUSION

This study concludes that L-carnitine exerts an anti-hyperglycemic and anti-oxidative effect against alloxan-induced diabetes mellitus. Additionally, L-carnitine also shows cyto-protective effects on pancreatic β cells.

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AUTHORS' CONTRIBUTIONS

Following authors have made substantial contributions to the manuscript as under:

RAT: Conception and design, acquisition, analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

KAM & SQ: Acquisition of data, drafting the manuscript, final approval of the version to be published

AAU & GSN: Analysis and interpretation of data, drafting the manuscript, final approval of the version to be published

NK: Acquisition of data, drafting the manuscript, critical review final approval of the version to be published

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.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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