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Research Article

Investigation on the role of alpha lipoic acid in glipizide treatment in diabetic rats

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ABSTRACT

Glipizide is a second generation sulfonylurea employed for the management of diabetes. Prolonged use of glipizide as monotherapy can cause insulin resistance. Our study aimed at ascertaining the effect of glipizide and alpha lipoic acid on alloxan induced diabetes in rats and its impact on hepatic function. Diabetes was induced by single intraperitoneal injection of 100 mg/kg/b.w. of alloxan. After the confirmation of diabetes, animals were assigned into groups and treated with 2.5 mg/kg of glipizide followed by alpha lipoic acid at two dose levels (100 mg/kg and 50 mg/kg) for 4 weeks. Body weight was monitored at regular intervals. Biochemical parameters such as blood glucose, glycosylated haemoglobin, cholesterol, triglycerides, serum glutamate oxaloacetate transaminase, serum glutamate pyurvate transaminase, alkaline phosphatase, total, direct bilirubin and creatinine was determined using standard kits. Data was analyzed by one way ANOVA followed by Dunnett multiple comparison test. A significant improvement was observed in blood glucose, total cholesterol and triglycerides levels compared with the positive control group. Glipizide and alpha lipoic acid protected against hepato cellular toxicity induced by alloxan. These results were statistically significant compared with positive control ($p<0.05$). Glipizide and alpha lipoic acid in combination did not alter liver function when compared to the alloxan treated group ($p<0.05$). Our study revealed that the combination of alpha lipoic acid with glipizide was capable of maintaining a normoglycemic state and capable of reversing the hepatocellular manifestations induced by alloxan.

Keywords: Diabetes mellitus; glipizide; alpha lipoic acid.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases occuring due to defects in secretion of insulin (Preet, 2005). Type 1 Diabetes mellitus (T1D) develops as a result of genetic, environmental and immunologic factors that ultimately destroy the pancreatic beta cells. Type 1 diabetes mellitus produces autoimmune beta cell destruction leading to insulin deficiency (Ahmaadi, 2009). Type 2 diabetes mellitus has a complicated pathogenesis resulting in insulin resistance in liver and peripheral tissues (Srinivasan, 2007). Diabetes has attained epidemic proportion in developing countries of the world, with a total of 171 millions affected in 2000 to 366 millons in 2030. Indians have a greater degree of insulin resistance with a higher genetic predisposition to diabetes (Jali, 2009). The probability of damage to organs is high in chronic hyperglycemia.

Biological compounds with antioxidant properties are capable of protecting cells and tissues against deleterious effect of reactive oxygen speices generated in diabetes. Alpha lipoic acid (α-lipoic acid) functions as metaboloic antioxidant which can scavenge a number of free radicals both in hydrophilic and lipophilic environments (Addel-Zaher, 2009). Alpha lipoic acid consists of a five- membered cyclic disulphide and hydrocarbon tail ending with a carboxylic acid group (Akkas, 2007). It occurs naturally in most types of prokaryotic and eukaryotic cells and serves as a co-factor for several enzymes involved in essential steps of energy metabolism (Cremer, 2006). α-lipoic acid has been proposed for treatment or prevention of diabetes, polyneuropathy, cataract, neurodegeneration, and nephropathies, and has been studied mainly by its antioxidant properties (Alegre, 2010). α-lipoic acid has been shown to be beneficial in various forms of oxidative stress and is of interest as a therapeutic agent in ischemia-reperfusion injury, diabetic complications, HIV activation, neurodegenerative disorders, and radiation injury (Alleva, 2005)

Glipizide is a "second generation" sulfonylurea categorized as an oral hypoglycemic agent employed for the management of non-insulin dependent diabetes mellitus. It appears to act principally by stimulating insulin secretion from pancreatic beta-cells. Glipizide reduces blood glucose by stimulating insulin secretion and altering insulin sensitivity (Goyal, 2010).

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α-lipoic acid is a powerful anti-oxidant and blood glucose lowering agent. Both alpha lipoic acid and glipizide used as monotherapy are effective in diabetes. The impact of combination of alpha lipoic acid with glipizide on controlling the progression of the disease and its hepatocellular safety was investigated in this study.

Material and Methods

Chemicals

α-Lipoic acid was received as a gift sample from Culcreuch Exports Pvt Ltd, Hyderabad (Batch No: 051106), Glipizide was purchased from Oceanic Laboratories, Mumbai, Alloxan was procured from Rolex Chemical Industries, Mumbai (Batch No: 1498). Glucometer (Glucocard 1 min) was purchased from Arkray Piramal. Biochemical estimation kits were procured from Erba Diagnostics (Transia India, Excel Diagnostics Pvt. Ltd, Hyderabad). All other reagents and chemicals were of analytical grade.

Animals

Five groups of approximately 3-month old male and female inbred Wistar rats, weighing 250-270 g were used. Animals were procured from the National Institute of Nutrition, Hyderabad. Permission has been obtained from the institutional animal ethical committee to conduct the experiment and has been done according to the committee for the purpose of control and supervision of experiments on animal (CPCSEA) guidelines (NCOP/IAEC/Approved/ 17/2010: 10/4/2010). The rats were fed with pelleted food procured from National Institute of Nutrition, Hyderabad and tap water during the experiment.

Induction of diabetes

Animals were fasted for 16 h prior to the i.p. injection of alloxan (100 mg/kg body weight). Alloxan was dissolved in freshly prepared 0.9 % sodium chloride (NaCl) solution and was administered by the intra-peritoneal route within 10 min after dissolving in saline. 5% dextrose in 0.9 % NaCl solution was provided to the animals for 8-9 h to overcome hypoglycemia (Etuk, 2010, Gangagobinda, 1956)

Blood glucose levels were monitored regularly. 4 days after intraperitoneal injection of alloxan, blood glucose level of 200 mg/dl was observed. Animals which exhibited a blood glucose of 200 mg/dl and more were included in the study. Body weight of the animals were monitored during the experiment.

Experimental design

The rats were divided into 5 groups with each group consisting of six rats

Group I: Normal rats treated with saline daily for 4 weeks and served as the negative control

Group II: Animals treated with single dose of alloxan (100 mg/kg) by the i.p. route to induce diabetes and served as a positive control

Group III: Diabetic rats were treated with 2.5 mg/kg b.w. of glipizide

Group IV: Diabetic rats were treated with 50 mg/kg b.w. of alpha lipoic acid and 2.5 mg/kg b.w. of glipizide

Group V: Diabetic rats were treated with 100 mg/kg b.w. of alpha lipoic acid and 2.5 mg/kg b.w. of glipizide

Groups III, IV and V received the treatment daily for 4 weeks by the oral route.

Biochemical estimations

1) Blood glucose

Estimation of blood glucose was carried out by using glucometer at the end of 2^{nd} and 4^{th} week of treatment.

2) Glycosylated haemoglobin (HbA1c)

Estimation of Glycosylated haemoglobin was carried out by using Ion Exchange Resin Method (Trivelli,1971)

3) Cholesterol

Estimation of Plasma cholesterol was carried out by using CHOD-PAP method at the end of 2^{nd} and 4^{th} week of treatment (Carpenter, 1957)

4) Triglycerides

Estimation of triglycerides was carried out by using GPO/PAP method at the end of 2^{nd} and 4^{th} week of treatment (Werner, 1981)

5) Liver marker enzymes

Estimation of serum glutamic oxaloacetic transaminase was determined by using SGOT, Modified IFCC method at the end of 2^{nd} and 4^{th} week of treatment, serum glutamic pyruvic transaminase was carried out by using SGPT, Modified IFCC method at the end of 2^{nd} and 4^{th} week of treatment (Lorentz, 1986) alkaline phosphatase was carried out by using ALP, pNPP method at the end of 2^{nd} and 4^{th} week of treatment, total and direct bilirubin was carried out by Diazo method at the end of 2^{nd} and 4^{th} week of treatment.

6) Kidney marker enzymes

Estimation of creatinine was carried out by using Modified Jaffe's Kinetic Methodat the end of 2^{nd} and 4^{th} week of treatment, were measured using standard kits on a semi auto analyzer (Erba Chem-5 plus v2).

Statistical analysis

Data are expressed as mean ± SEM. Analysis of data was done by One-Way ANOVA followed by Dunnett comparison test using GraphPad InStat version 3.10 for Windows 2009 (GraphPad Software). The statistical significance was set at 0.05 level ($P<0.05$).

Table 1: Effect of combination of glipizide and α-lipoic acid on body weight in diabetes rats

S.NO	Group	Body weight (g)			
		Initial	2nd week	4th week	Mean change
1	Group I (Negative control)	274.54±1.02	283.65±1.13	285.24±0.85	↑10.18
2	Group II (positive control)	270.13±1.40	260.26±2.32	256.77±2.28	↓13.35
3	GroupIII (standard glipizide 2.5mg/kg)	270.65±0.82	266.31±3.27*	257.99±0.72	↓12.66
4	GroupIV (Glipizide 2.5mg/kg)+ α-lipoic acid 50mg/kg)	270.09±0.87	263.95±1.20	259.69±0.79*	↓ 10.4
5	Group V (Glipizide 2.5mg/kg)+ α-lipoic acid 100mg/kg)	268.86±1.24	265.70±1.43*	259.64±1.14*	↓ 9.22

Values are mean ± SEM, n=6, *p<0.05 compared with positive control

Table 2: Effect of combination of glipizide and α-lipoic acid on blood glucose levels in diabetes rats

S.NO	Group	Blood glucose (mg/dl)		
		Initial	2nd week	4th week
1	Group I (Negative control)	107.024±0.54	104.534±0.57	105.132±0.56
2	Group II(positive control)	303.39±1.42	302.972±0.70	302.268±0.81
3	GroupIII(standard glipizide 2.5mg/kg)	302.39±1.14	127.882±1.14*	122.042±1.36*
4	GroupIV(Glipizide 2.5mg/kg+ α-lipoic acid 50mg/kg)	298.70±1.35	123.046±1.11*	120.43±1.82*
5	Group V(Glipizide 2.5mg/kg+ α-lipoic acid 100mg/kg)	299.90±2.83	121.70±1.51*	119.85±0.79*

Values are mean ± SEM, n=6, *p<0.05 compared with positive control

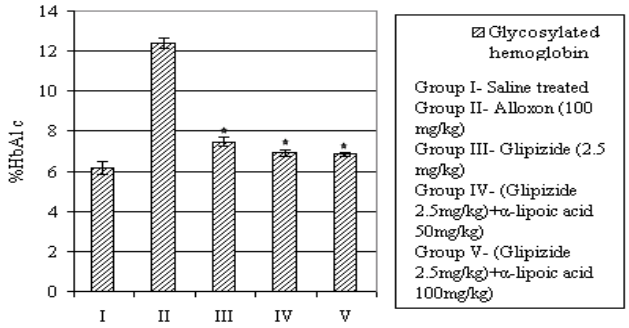

Figure 1: Effect of combination of glipizide and α-lipoic acid on Glycosylated haemoglobin levels in diabetes rats

RESULTS

Effect of combination of glipizide and alpha lipoic acid on body weight (Table 1) in diabetic rats

A decrease in body weight was observed in diabetic rats in relation to the control. Treatment of diabetic rats with glipizide (group-III) caused reduction in body weight. Alpha lipoic acid in higher doses (100 mg/kg) along with glipizide (2.5 mg/kg) exerted a prominent decline in body weight compared with positive control (Table-1).

Effect of combination of glipizide and alpha lipoic acid on blood glucose levels and glycosylated haemoglobin (HbA1c) in diabetic rats

Elevation in blood glucose was seen in 4 days after alloxan administration. Treatment with glipizide alone produced a normoglycemic state within a week of treatment and the levels were maintained throughout the study. Glipizide and alpha lipoic acid in combination produced control over the blood glucose at both dose levels (Table-2). The levels of HbA1c declined in groups treated with the combination of glipizide and alpha lipoic acid compared with the positive control ($p<0.05$, Fig 1).

Effect of combination of glipizide and alpha lipoic acid on cholesterol and triglyceride levels in diabetic rats

The combination of glipizide and α-lipoic acid significantly reduced the level of plasma cholesterol and triglycerides. Treatment with glipizide and alpha lipoic acid for 4 weeks was found to significantly lower the cholesterol and triglyceride levels to 123.65±7.82 mg/dl, 66.93±1.13 mg/dl which is statistically significant compared to positive control ($p<0.05$) (Fig. 2, Table 3)

Effect of combination of glipizide and alpha lipoic acid on liver marker enzymes.

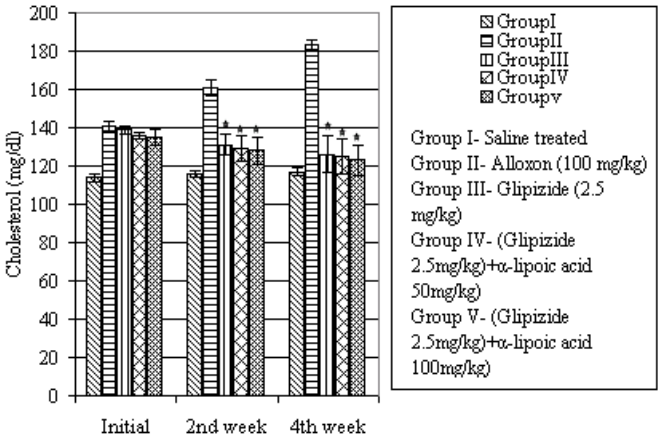

Figure 2: Effect of combination of glipizide and α-lipoic acid on cholesterol levels in diabetes rats

Table 3: Effect of combination of glipizide and α-lipoic acid on triglyceride levels in diabetes rats

S.NO	Group	Triglyceride (mg/dl)		
		Initial	2nd week	4th week
1	Group I (Negative control)	60.65±1.00	60.23±1.17	59.15±2.25
2	Group II (positive control)	74.84±0.83	80.93±1.18	102.31±2.86
3	GroupIII (standard glipizide 2.5mg/kg)	72.46±1.42	70.57±0.57*	68.27±1.02*
4	GroupIV (Glipizide 2.5mg/kg+α-lipoic acid 50mg/kg)	71.44±1.00	69.01±1.17*	67.09±1.50*
5	Group V (Glipizide 2.5mg/kg+α-lipoic acid 100mg/kg)	70.63±0.90	68.53±1.39*	66.93±1.13*

Values are mean ± SEM, n=6, *p<0.05 compared with positive control

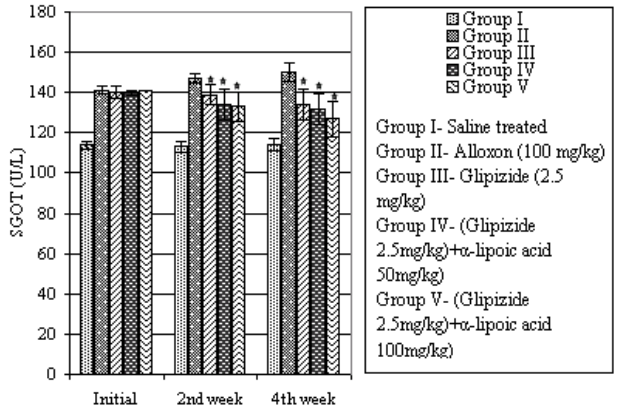

Figure 3: : Effect of combination of glipizide and α-lipoic acid on SGOT levels in diabetes rats

Diabetic state induced by alloxan produced a rise in the level of liver marker enzymes. Glipizide and alpha lipoic acid in combination exerted a lowering in these parameters implying that they are capable of minimising hepatocellular injury compared to positive control (p<0.05). Creatinine levels were elevated following exposure to alloxan. A decline in creatinine level was observed with the combination compared with the positive control (p<0.05) (Fig. 3-4, Table. 4).

DISCUSSION

The present study investigated the impact of combination of α-lipoic acid and glipizide on reduction of diabetes, control of hypercholesteremia and effect on liver marker enzymes. Alloxan has been widely employed to induce experimental diabetes in animals due to its ability to induce massive destruction of β-cells of islets Langerhans (Tedong, 2006). In the redox cyclic process, alloxan is converted to dialuric acid which is associated with the generation of reactive oxygen species (ROS). Oxidative stress which ensuies due to over production of ROS can accelerate the destruction of the β-cells creating paucity in insulin secretion. Persistant hyperglycemia can induce the mitochondria to overtly produce ROS which partakes in damage and destruction of β-cells (Lukacinova, 2008)

Glipizide, a second generation sulfonylurea has been extensively advocated in the management of diabetes mellitus. It binds to the sulfonylurea receptors located on the β-cell membrane leading to inhibition of stabilization of potassium efflux, thus causes depolarization of ATP sensitive K channels and accumulation of cal-

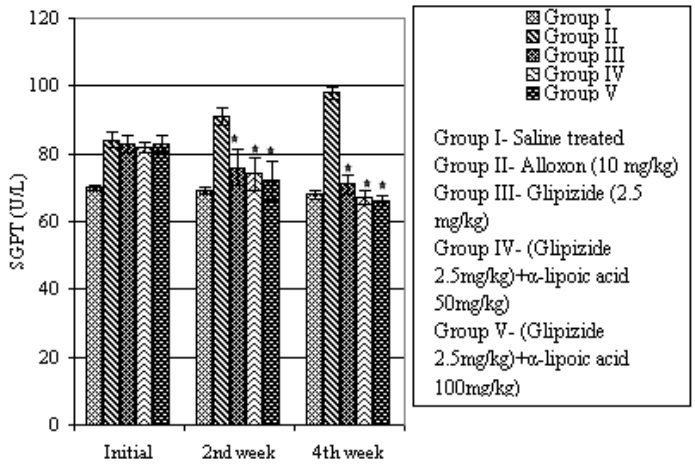

Figure 4: : Effect of combination of Glipizide and α-lipoic acid on SGPT levels in diabetes rats

Table 4: Effect ofcombination of glipizide and α-lipoic acid on bilirubin, creatinine and alkaline phosphatase in diabetic rats

S.NO	Group	Total Bilirubin (mg/dl)		Direct Bilirubin(mg/dl)		Creatinine (mg/dl)		Alkaline Phospha-tase (U/L)	
		2nd week	4th week	2nd week	4th week	2nd week	4th week	2nd week	4th week
1	Group I (Negative control)	0.45± 0.018	0.47± 0.006	0.342± 0.017	0.35± 0.026	0.316± 0.011	0.314± 0.010	85.77± 1.98	85.106± 2.41
2	Group II (positive control)	0.85± 0.010	0.88± 0.008	0.694± 0.007	0.724± 0.012	0.502± 0.028	0.528± 0.052	280.29± 3.31	294.98± 2.01
3	GroupIII (standard glipizide 2.5mg/kg)	0.72± 0.041*	0.66± 0.068*	0.67± 0.006*	0.65± 0.021*	0.46± 0.006	0.438± 0.045*	196.3± 6.12	167.38± 2.94
4	GroupIV (Glipizide 2.5mg/kg+α-lipoic acid 50 mg/kg)	0.68± 0.048*	0.64± 0.069*	0.59± 0.045*	0.55± 0.052*	0.42± 0.039*	0.40± 0.043*	173.13± 5.01*	169.01± 4.08*
5	Group V (Glipizide 2.5mg/kg+α-lipoic acid 100 mg/kg)	0.65± 0.056*	0.60± 0.078*	0.55± 0.056*	0.47± 0.085*	0.41± 0.034*	0.39± 0.022*	171.52± 5.26*	165.61± 2.87*

Values are mean ± SEM, n=6, *p<0.05 compared with positive control

cium intracellularly leading to the secretion of insulin from the β-cells. Glipizide stimulate insulin secretion and alters insulin sensitivity. We observed that glipizide significantly restores elevated blood glucose levels induced by alloxan to normoglycemic level with in 1 week of treatment with a consistant maintainance thereafter.

Supplementing current therapies with anti-oxidants could be capable of preventing the progression of the disease. α-lipoic acid is a powerful anti-oxidant capable of scavenging free radicals in a hydrophilic and lipophilic environment. α-lipoic acid is readily reduced in the body to dihydrolipoic acid. It is a popular therapy for the treatment of diabetic poly neuropathy by enhancing nerve conduction velocity. α-lipoic acid enhances glucose uptake in muscle, elevates glucose utilization by recruiting glucose transporter-4 to the cell membrane and glucogenesis. Treatment with the combination of glipizide and alpha lipoic acid exerted a better glycemic control compared to monotherapy with glipizide. This might be because α-lipoic acid exhibits an anti-diabetic effect in addition to its an antioxidant action. It exerts a multifaceted role by scavenging free radicals, chelating metal ions, enhances generation of vitamin E, potentates the action of vitamin C and facilitates its regeneration from dehydroascorbate there by bolstering the ability of β-cells to combat oxidative damage (El-Hossary, 2010)

Treatment with glipizide and α-lipoic acid considerably reduced weight gain induced by the diabetic state. The maintenance of body weight could be attributed to the action of α-lipoic acid on suppressing the central regulation of food intake and energy expenditure which is the hypothalamic AMP- activated protein kinase. Decrease in cholesterol and triglycerides might be due to better activation of the enzyme lipoprotein lipase by the combination compared to monotherapy (Harrihar, 2005).

Evaluation of liver and kidney marker enzymes with the combination showed a marked decline in their levels. The hepatocellular damage induced by the diabetic state is controlled by the combination of glipizide and lipoic acid possibly by its ability to scavenge free radicals

CONCLUSION

supplementing glipizide with α-lipoic acid could benefit individuals with diabetes as the dose of glipizide could be reduced.

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