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Biochemical evaluation of Antidyslipidemic properties of a new zinc-diosmin complex studied in high fat diet fed-low dose streptozotocin induced experimental type 2 diabetes in rats

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ABSTRACT

The substantial increase in caloric intake and reduction in physical activity throughout the world have resulted in a pandemic of obesity that in turn has led to an enormous increase in the incidence of T2DM which accounts for more than 90% of the diabetic population. Diabetes can results in severe morbidity and increased mortality as a result of secondary complications which affect multiple body systems via micro and macrovascular complications. Insulin resistance in T2DM leads to lipid accumulation in the muscle and liver aggravating the metabolic disturbances. Evidence continues to accumulate that glycemic and lipid control influences atherosclerotic disease progression. Reducing the burden of atherogenic lipoproteins in serum is unequivocally associated with reductions in the risk of cardiovascular events and may also ameliorate microvascular damage. Recently, we have synthesized a new zinc-diosmin complex, characterized by various spectral studies and evaluated its antidiabetic as well as antioxidant properties in HFD-low dose STZ induced experimental type 2 diabetes in rats. In this study, an effort has been made to explore the hypolipidemic potential of the zinc-diosmin complex in experimental diabetes. Diabetic rats were treated with zinc-diosmin complex (20 mg/kg b.w.) orally for 30 days. The levels of fasting blood glucose, glycosylated hemoglobin and plasma insulin were measured. In addition, the levels of lipids and lipoproteins were analyzed. Increased levels of fasting blood glucose, HbA1c and decreased plasma insulin level in diabetic rats were brought back to near normalcy upon oral administration of zinc-diosmin complex. Diabetic rats showed elevated levels total cholesterol, free fatty acids, triglyceride, and phospholipids in the serum, hepatic and kidney tissues, which was restored to normalcy in diabetic rats treated with zinc-diosmin complex. In addition, the diminished level of high-density lipoprotein cholesterol and elevated low-density and very low-density lipoprotein cholesterol levels in the serum of diabetic rats were normalized upon oral administration of zinc-diosmin complex. The data obtained clearly exemplify the hypolipidemic properties of the zinc-diosmin complex.

Key words: Insulin resistance; type 2 diabetes; lipoproteins; hypolipidemic; zinc-diosmin complex

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a polygenic disorder, accounts for 90–95 % of total diabetic population in which pancreatic insulin secretion does not meet the demands of insulin sensitivity [1]. More than 415 million people are affected by diabetes globally and this number will be suspected to increase 642 million forecasted in the year 2040 [2]. The etiology of type 2 diabetes is influenced by multifactorial interplay between genetic and environmental factors such as obesity, sedentary life style, population growth, ageing and urbanization [3]. T2DM leads to develop many secondary complications such as atherosclerosis, microangiopathy, nephropathy, cardiac abnormality and

retinopathy. Atherosclerosis is chiefly responsible for major cardiovascular events in diabetes and it is a heterogeneous condition, resulted from dyslipidemia coupled with peripheral insulin resistance due to persistent hyperglycemia in T2DM [4].

Dyslipidemia associated with diabetic condition includes reduced level of high-density lipoproteins (HDL), increased triglycerides and postprandial lipemia [5]. The association with T2DM, dyslipidemia as co-morbidity for cardiovascular disease eventually leads to a high rate of mortality has been a growing anxiety. However, the concentration of low-density lipoproteins (LDL) in T2DM is not significantly different from non-diabetic individuals. Nevertheless, modified LDL can promote atherogenesis, a condition in which abnormal fat formation occurs in arterial walls.

Non-enzymatic glycation can cause LDL to be rapidly internalized by macrophages, thus accelerating the process of atherosclerosis. Elevated glucose levels may also favor the production of oxidized LDL, the first step in the process of atherosclerosis [6]. In T2DM, the risk of coronary heart disease, cerebrovascular stroke, peripheral vascular disease was increased up to 2 to 4 folds and the mortality from cardiovascular complications remains as high as 75%. Diabetic dyslipidemia is a complex phenomenon, in which insulin inhibits lipolysis by suppressing hormone sensitive lipase activity in adipocytes [7]. Due to peripheral insulin resistance in T2DM, there is suppression of hormone sensitive lipase activity which increases intra-cellular hydrolysis of TG in the adipose tissue, consequently releasing free fatty acids (FFA) into the circulation. These FFA stimulate the assembly and oozing of VLDL from the hepatocytes, resulting in excess TG level in blood [8]. The elevated level of non-esterified fatty acids (NEFAs) in plasma in association with hyperglycemia leads to reduced insulin synthesis and impaired responsiveness to glucose in the β cells of pancreas, a condition termed as glucolipotoxicity, which cause β-cell dysfunction by chronic oxidative stress mediated by excessive generation of reactive oxygen species (ROS) [9].

Chronic oxidative stress is the key mechanism, which plays a cardinal role in the onset and progression of diabetes and its secondary complications resulting from defective insulin gene expression, insulin secretion and increased apoptosis of pancreatic β-cell that ultimately leads to interminable β-cell deterioration [10]. Hence, regulating β-cell homeostasis and its protection against glucolipotoxicity are vital which requires an efficient pharmacological therapy for the successful management of diabetes and its secondary complications.

Plenty of hypoglycemic drugs associated with hypolipidemic action are available for treating T2DM, that decrease peripheral insulin resistance but none of these have expected efficacy. The use of statins allied with diverse side effects even with their positive effects on lipid profile. Statins disrupts insulin-signaling pathway by affecting insulin sensitivity, pancreatic β-cell function and adipokine secretion [11]. During the development of synthetic agents for the treatment of diabetes, various metal ions and their synthetic complexes having hypoglycemic as well as hypolipidemic properties were studied. Metal ions play an important role in more than one third of proteins found in living systems, which involved mainly in a variety of key biological processes like gene regulation, DNA repair and replication, antioxidant defense, respiration, photosynthesis, neurotransmission, nitrogen and carbon cycling and biosynthesis of antibiotics [12]. Among these metal ions, zinc is one of the most important transition metal ion found almost all tissues of the body. Zinc plays a crucial role in the biosynthesis of insulin and maturation of insulin secretory granules [13]. Zinc complexes with insulin combining up to 11.6 ions in a hexamer and this feature means that zinc, both prolongs the action duration and facilitate insulin storage [14]. Zinc is necessary for the first line defense in body and it plays an important role in cell growth, cell division, wound healing and carbohydrates breakdown. An important advance in the use of zinc compounds for the treatment of diabetes and its complications has been the development of zinc complexes with various ligands in order to reduce the zinc toxicity and also it showed improved stability, absorption, utilization and efficiency.

Diosmin used as an organic ligand to synthesis a novel zinc-diosmin complex. Recently, we have reported that the antidiabetic and antioxidant properties of zinc-diosmin complex in HFD-STZ-induced type 2 diabetes in experimental rats [15-17]. The aim of the present study was to evaluate the ameliorative potential of zinc-diosmin complex against hyperglycemia-mediated dyslipidemia in HFD- low dose STZ induced diabetic rats. Metformin, as oral hypoglycemic drug having hypolipidemic activity was used as a reference drug [18].

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ), Zinc sulphate [$ZnSO_4.7H_2O$] and Diosmin ($C_{28}H_{32}O_{15}$) were purchased from Sigma-aldrich, St.Louis, USA. Ultra-sensitive ELISA kit for rat insulin assay was purchased from Crystal Chem inc. Life Technologies, India. All the other reagents used in the present study were of analytical grade.

Experimental animals

Male albino rats of Wistar strains weighing about 160–180 g were procured from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai. The rats were housed randomly in spacious polypropylene cages lined with husk under controlled environment (12:12 ± 1 h light/dark cycle; temperature 22°C ± 3°C; relative humidity 55% ± 10%). Before the initiation of the experiments, all animals acclimatized to standard husbandry conditions for one week to eliminate the effect of stress. Animals were fed with commercial pellet rat chow (Hindustan Lever Ltd., Bangalore, India), and had free access to water *ad libitum*. The experiments were designed and conducted in strict accordance with the current ethical norms approved by Ministry of Social justices and Empowerment, Government of India and Institutional Animal Ethical Committee guidelines [02.01.2012].

Synthesis of zinc-diosmin complex

Molar ratio method was followed in the synthesis of zinc diosmin complex. The zinc diosmin complex was synthesized as previously reported for the synthesis zinc complexes with slight modifications [19-21]. Briefly, a DMSO solution (10 ml) containing zinc sulphate heptahydrate (0.287g, 1mM) were added gradually to the hot solution of DMSO (15 ml) of diosmin (1.217g, 2mM). The resulting solution was dried in a pressurized rotary evaporator till the solution gets concentrated to one fifth of the whole solution. The obtained solution was allowed to evaporate in room temperature. The precipitated compound was washed with diethyl ether and kept under vacuum over anhydrous calcium chloride.

Experimental design

The rats were divided into four groups each comprising of six animals. The rats were allocated into two dietary regimens by feeding either normal pellet diet (NPD) or high fat diet (HFD) for 2 weeks of dietary manipulation [22]. After 2 weeks of HFD, the Group II-Group IV rats were injected with a single dose of STZ (35 mg/kg b.w/rat), while the Group I rats fed with NPD were injected with 0.5 ml of freshly prepared cold citrate buffer (pH 4.5) in a same volume, intraperitoneally, respectively. After one week of STZ injection, rats with fasting blood glucose levels ≥ 250 mg/dl were chosen for the experiment. The animals were divided into four groups, comprising of six animals in each group as follows:

Group I: Normal control rats.
Group II: HFD-STZ (i.p. 35 mg/kg b.w.) induced diabetic rats.
Group III: HFD-STZ induced diabetic rats treated with zinc-diosmin complex (20 mg/kg b.w/ rat/day) for 30 days.
Group IV: HFD-STZ induced diabetic rats treated with metformin (200 mg/kg b.w/rat/day) for 30 days.

At the end of the 30 days treatment period, the rats were fasted overnight, anesthetized with ketamine (80 mg/kg b.w i.p), and sacrificed by cervical decapitation. The blood sample was collected with and without anticoagulants for plasma and serum separation, respectively.

Biochemical estimations

The levels of fasting blood glucose [23] and glycosylated hemoglobin [24] were estimated. Insulin level was measured in plasma using the sensitive rat insulin ELISA kit (Linco Research, Inc., St. Charles, MO).

Assay of lipid profile

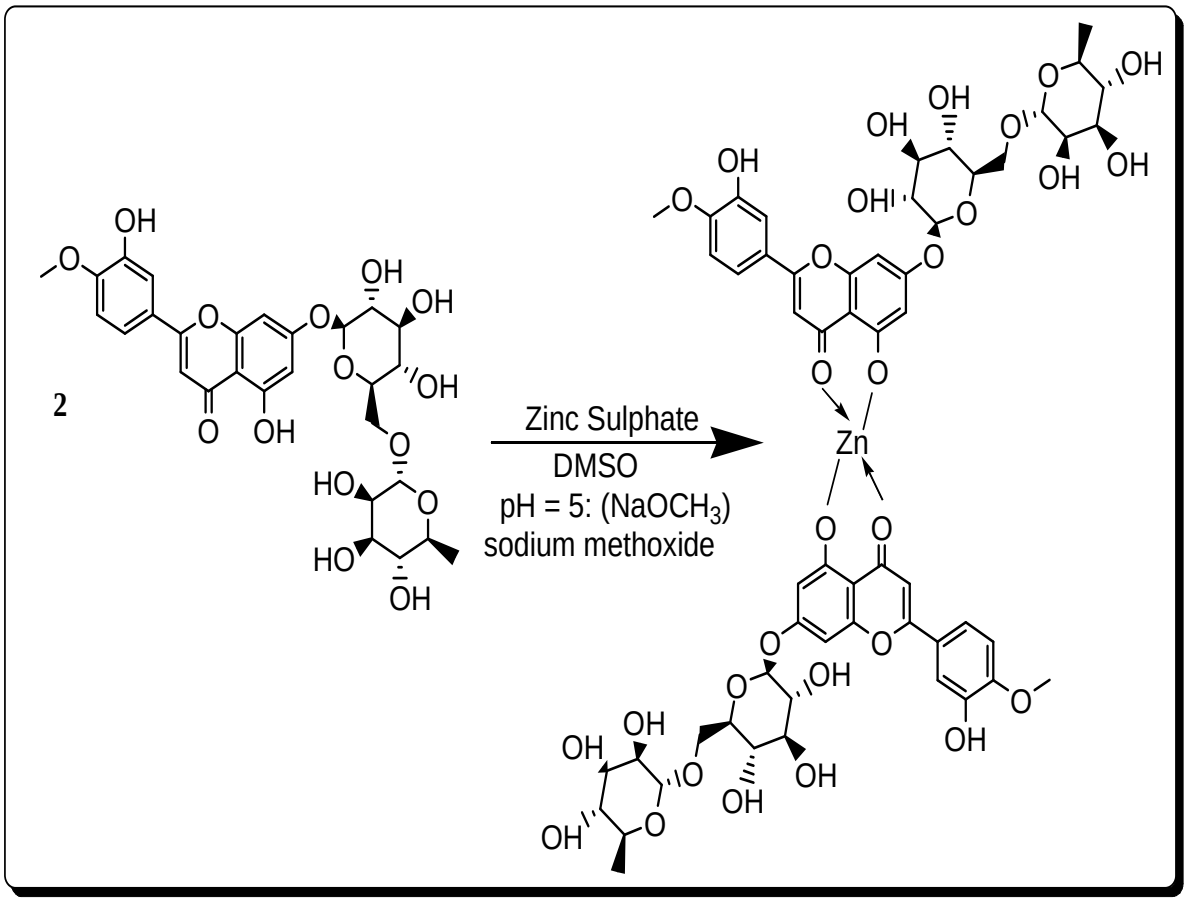
Cholesterol content [25], triglycerides [26], free fatty acids [27] and phospholipids [28] in serum, liver and kidney tissues were estimated. HDL and LDL were separated from the serum according to dual precipitation technique [29] and the cholesterol content of the lipoproteins was estimated.

Statistical analysis

SPSS 16.0 statistical package were used to perform statistical analysis was. The results were expressed as mean ± S.E.M. Analysis of variance (ANOVA) followed by post hoc test LSD was used to correlate the difference between the variables. Values were considered statistically significant if $P<0.05$.

RESULTS

The Zinc-diosmin complex ($C_{56}H_{62}O_{30}Zn$) was synthesized by molar ratio method and the yield was 76% (scheme 1)



Scheme 1: Structure of Zinc-diosmin complex

Table 1 shows the levels of fasting blood glucose, glycosylated hemoglobin and plasma insulin in the control and experimental groups of rats. The levels of fasting blood glucose, glycosylated hemoglobin are elevated with a concomitant decrease in the level of plasma insulin was observed in HFD- low dose STZ induced diabetic rats. Whereas, diabetic rats orally treated with zinc-diosmin complex showed significantly decreased levels of fasting blood glucose, glycosylated hemoglobin and an improvement in plasma insulin level. This action of zinc-diosmin complex is comparable with metformin.

Table 1: The levels of fasting blood glucose, plasma insulin and glycosylated hemoglobin (HbA1c) in control and experimental groups of rats

Groups	Blood glucose	Plasma Insulin	HbA1c
Control	85.39 ± 6.04	1.21± 0.07	5.01 ± 0.29
Diabetic control	316.16 ± 9.97 [a]	0.78 ± 0.03 [a]	13.26 ± 0.43 [a]
Diabetic + Zn-diosmin	110.69 ± 4.57 [b]	1.07 ± 0.05 [b]	7.14 ± 0.32 [b]
Diabetic + metformin	101.68 ± 5.40 [b]	1.12 ± 0.07 [b]	6.66 ± 0.97 [b]

Units are expressed as mg/dl for blood glucose, ng/ml for plasma insulin and % hemoglobin for HbA1c. Results are expressed as mean ± S.E.M [n=6]. One-way ANOVA followed by post hoc test LSD. Values are statistically significant at P<0.05. The results were compared with [a]Compared to control rats, [b]Compared to diabetic rats.

Figure 1 depicts the levels of total cholesterol, triglycerides, free fatty acids and phospholipids in the serum of control and experimental group of rats. HFD-STZ induced type 2 diabetic rats showed elevated lipid profile levels when compared to control rats. These elevated levels of lipid components were brought back to near normalcy in diabetic rats treated with zinc-diosmin complex as well as metformin treated diabetic rats.

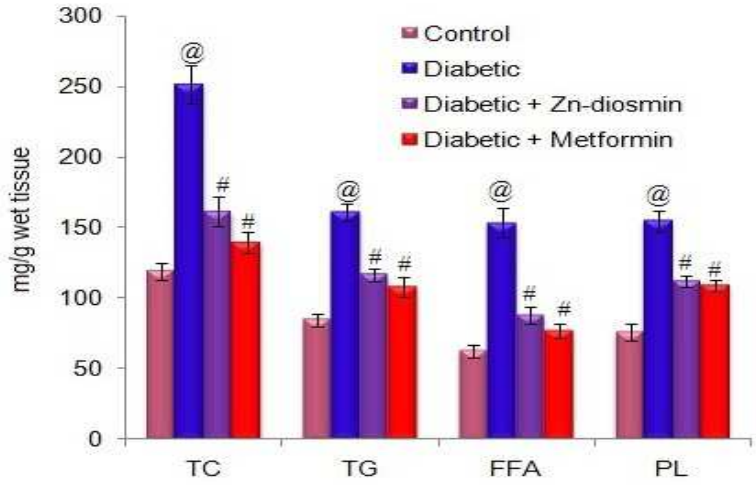


Figure 1: Levels of total cholesterol, triglycerides, free fatty acids and phospholipids in the serum of control and experimental group of rats. Results are expressed as mean ± S.E.M

[n=6]. One-way ANOVA followed by post hoc test LSD. The results were compared with [@]Control rats, [#]Diabetic rats. Values are statistically significant at P<0.05.

The levels of serum lipoproteins such as of HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol in control and experimental groups of rats were showed in table 2. The levels of LDL-c and VLDL-c were elevated significantly with a concomitant decline in the level of HDL-c in HFD-low dose STZ induced diabetic rats. Upon oral treatment with zinc-diosmin complex, the altered levels of plasma lipoproteins were significantly normalized.

Table 2: The levels of HDL-Cholesterol, LDL-Cholesterol and VLDL-Cholesterol in serum of control and experimental groups of rats

Group	HDL-C	LDL-C	VLDL-C
Control	33.68 ± 1.67	64.88 ± 4.88	16.10 ± 1.35
Diabetic control	11.86 ± 0.77[a]	224.19 ± 12.66[a]	33.20 ± 1.77[a]
Diabetic + Zn-diosmin	24.63 ± 1.99[b]	115.12 ± 6.23[b]	20.81 ± 1.00[b]
Diabetic + Metformin	28.19 ± 1.78[b]	106.45 ± 5.81[b]	21.94 ± 1.63[b]

Units are expressed as mg/dL. Values are expressed as mean ±SEM [n = 6].
[a]Statistical significance were compared with normal control rats.
[b]Statistical significance were compared with diabetic group of rats.
Values are statistically significant at P < 0.05.

The effect of zinc-diosmin complex on the levels of total cholesterol, triglycerides, free fatty acids and phospholipids in hepatic and kidney tissues of experimental group of rats were depicted in table 3 and 4 respectively. Diabetic rats showed significantly elevated levels of lipid components in hepatic and renal tissues. However, oral administration of zinc-diosmin complex as well as metformin to HFD-low dose STZ induced type 2 diabetic group of rats showed significant reduction in the levels to near normalcy.

Table 3: Effect of Zinc-diosmin complex on the levels of total cholesterol (TC), triglycerides (TG), free fatty acids (FFA) and phospholipids (PL) in the hepatic tissues of experimental group of rats

Group	TC	TG	FFA	PL
Control	7.52 ± 0.50	3.82 ± 0.27	6.69 ± 0.30	22.45 ± 1.22
Diabetic control	18.24 ± 0.85[a]	8.08 ± 0.44[a]	13.60 ± 0.65[a]	47.29 ± 2.26[a]
Diabetic + Zn-diosmin	11.99 ± 0.68[b]	5.57 ± 0.31[b]	9.89 ± 0.40[b]	25.24 ± 1.21[b]
Diabetic + Metformin	9.98 ± 0.76[b]	4.72 ± 0.31[b]	8.54 ± 0.47[b]	27.91 ± 1.51[b]

Units are expressed as mg/g wet tissue. Values are expressed as mean ±SD (n = 6).
Statistical significance was compared within the groups as follows: [a]compared with control rats, [b]compared with diabetic control. Values are statistically significant at p < 0.05.

Table 4: The levels of total cholesterol (TC), triglycerides (TG), free fatty acids (FFA) and phospholipids (PL) in the renal tissues of control and experimental group of rats

Group	TC	TG	FFA	PL
Control	5.61 ± 0.39	5.53 ± 0.43	4.31 ± 0.39	18.49 ± 0.86
Diabetic control	14.74 ± 0.69[a]	8.45 ± 0.51[a]	15.48 ± 0.81[a]	32.39 ± 1.91[a]
Diabetic + Zn-diosmin	9.53 ± 0.53[b]	6.79 ± 0.42[b]	7.66 ± 0.39[b]	21.17 ± 0.87[b]
Diabetic + Metformin	6.31 ± 0.35[b]	6.49 ± 0.36[b]	9.28 ± 0.48[b]	23.94 ± 1.48[b]

Units are expressed as mg/g wet tissue. Values are expressed as mean ±SD (n = 6).
Statistical significance was compared within the groups as follows: [a]compared with control rats, [b]compared with diabetic control. Values are statistically significant at p < 0.05.

DISCUSSION

The onset and progression of diabetes related micro and macrovascular complications is likely to involve a wide range of pathogenic conditions including lipoprotein glycation of both early and late stages. Glycated lipoproteins can directly cause damage such as related to toxic effects on vascular cells, foam cell formation and prothrombotic and proinflammation effect. The VLDL-c particles are easily taken up by scavenger receptors in macrophages leading to foam cell formation. The major abnormalities in patients with T2DM mainly include increased levels of triglycerides and low HDL-c levels. The decreased cholesterol level in diabetic individuals is mainly due to increased catabolism of HDL particles. This effect on HDL-c is related mainly due to insulin resistance. A normal individual is sensitive to insulin which regulates carbohydrate and fatty acid metabolism, lipogenesis, lipolysis and hence energy homeostasis. Insulin mediates the uptake of glucose into the cells wherein muscles and liver, it can be converted to and stored as glycogen. In insulin resistance, there is impaired signaling via the phosphoinositol 3-kinase pathway allowing the building of toxic lipid metabolites such as fatty acid acyl CoA, diacylglycerol and ceremide in numerous tissues including the liver, pancreatic beta cells and adipocytes [30]. It is the IR related lipid-mediated macrovascular complications, in large part related to atherogenic events that result in the high morbidity and mortality risk in T2DM.

The lipoprotein level in the blood depends on the balance of synthesis and degradation or turnover. Synthesis of the lipoprotein particle depends on cholesterol and fat absorption *de novo* cholesterol synthesis and *de novo* fatty acid synthesis. Cholesterol absorption depends on the availability of recirculated cholesterol via the enterohepatic circulation. *De novo* cholesterol synthesized in the intestine is also included in the cholesterol pool through the intestinal villi [31]. *De novo* synthesis of cholesterol occurs mainly in the liver, but virtually every cell in the body has the ability to synthesize cholesterol and the intestine is an important site of cholesterol synthesis. The larger lipoprotein particles consist of a triglyceride rich core and fatty acids which have been esterified to form phospholipid and cholesterol esters [31].

Most of the currently available drugs for the treatment for diabetes are unable to control the lipid metabolism and hence the lipid lowering drugs are often preferred as adjunct therapy to prevent the secondary complications due to hyperlipidemia. Many research groups are actively involved in search of drugs capable of controlling both the primary and secondary complications of diabetes mellitus. Metal ions play an important role in the biological system as they are essential for the maintenance of normal metabolic activities and their insufficiency leads to various diseases [32]. Among the various metal ions, zinc is one of the most important essential trace elements found to be present in almost all tissues and acts as a cofactor for more than 300 enzymes that participating in the synthesis and degradation of carbohydrates, proteins, lipids and nucleic acids as well as in the metabolism of other micronutrients. Zinc is a natural component of insulin and also possesses insulin mimetic activity [33]. Zinc is involved in the process of insulin synthesis, secretion and storage. In view of the unique credentials of zinc several zinc complexes

have been studied for their antidiabetic properties [34-36]. Recently, we have reported the antidiabetic and antioxidant properties of zinc-diosmin complex in HFD-low dose STZ induced experimental type 2 diabetes in rats [15-17].

Blood glucose management is a vital component in delaying or preventing primary as well as secondary diabetic complications [37]. However, insulin resistance is the initiating pathogenic factor in type 2 diabetes and pancreatic β-cell failure is accountable for insulin deficiency and impaired glucose tolerance to explicit type 2 diabetes [38]. Elevated levels of fasting blood glucose are directly related to increased glucose production in hepatic tissues. In the present study, an observed low level of plasma insulin in HFD-low dose STZ induced diabetic rats indicates perturbations in pancreatic β-cell function. Whereas, oral administration of zinc-diosmin complex to diabetic rats showed an increase in plasma insulin level and decreased the fasting blood glucose indicating that the complex is capable of stimulating insulin secretion from surviving β-cells in diabetic rats. More recently, we have reported the effect of zinc-diosmin complex on the regulation of carbohydrate metabolizing enzymes in the liver tissue of diabetic rats [17].

Under physiological conditions, circulatory hemoglobin exposures to elevated level of plasma glucose binds to it by non-enzymatic, irreversible covalent bond to form glycosylated hemoglobin (HbA1c). It is measured mainly to identify the average plasma glucose concentration maintained over a 2 – 3 month period of time [39]. The relationship between HbA1c and cardiovascular disease and total mortality was continues and apparent even among non diabetic individuals. The risk was lowest among persons with HbA1c level below 5% and increased there after throughout the range of non diabetic HbA1c levels up to 6.9%. Each one percent point increase in HbA1c above 5% was associated with a 20-25% increase in the relative risk for coronary heart diseases. Normal level of HbA1c reflects the maintenance of normal glucose concentration in blood whereas in diabetes, elevated glycosylated hemoglobin level, indicating poor control of blood glucose levels and increased glycation of hemoglobin due to elevated levels of blood glucose [40]. The increased level of HbA1c in HFD-low dose STZ induced diabetic rats returned to near normal after the oral administration of zinc diosmin complex for 30 days, may be due to the restoration of blood glucose levels, thereby reducing the intensity of hemoglobin glycosylation [15].

The levels of serum total cholesterol, triglycerides, LDL-c and HDL-c are the important risk factors for the overall lipid metabolic control in diabetes. Peripheral insulin resistance, diverse metabolic derangements and alterations in the regulatory mechanisms leads to increased accumulation of lipids [41]. Hyperlipidemia associated with persistent hyperglycemia is mainly characterized by elevated levels of total cholesterol, triglycerides, phospholipids, free fatty acids and alterations in the levels of plasma lipoproteins which contributes to major risk factor for cardio vascular diseases [42]. Insulinotropic property of FFA is an important protection against hypoinsulinemia on fasting, when the blood glucose is not available for the stimulation of insulin secretion in pancreas.

The availability of FFA derived from adipose tissue ensures sufficient insulin secretion to secure a negligible insulin level for preventing abandoned FFA outpour from adipose tissue [43, 44]. A large FFA inflow and TG accumulation in the pancreatic β-cells have a pessimistic effect on β-cell function resulted from increased pressure to secrete insulin results in β-cell deterioration up to apoptosis. Hyper-triglyceridemia and hypercholesterolemia are the most common findings in patients with diabetes mellitus. The elevated level of serum lipids in diabetics is essentially due to an increase in the mobilization of FFAs from the peripheral depots, since insulin inhibits the activity of hormone sensitive lipase [45]. However, insulin deficiency inactivates the lipoprotein lipase, which promotes conversion of free fatty acids into phospholipids and cholesterol, which resulted into elevated level of phospholipids in serum [46]. In the present study, oral administration with zinc-diosmin complex to HFD-low dose STZ induced diabetic rats showed decreased levels of FFA, TC and TG and phospholipids in serum, hepatic and kidney tissues, which were elevated in diabetic group of rats, may be attributed to the increased mobilization and transport of fatty acids due to decreased insulin resistance.

The dyslipidemia observed in insulin resistance and type 2 diabetes is associated with excess hepatic production of VLDL-c, LDL-c and low level of HDL-c [47]. Uncontrolled free fatty acid transport promotes the synthesis of lipoproteins rich in triglycerides. Increased level of FFAs and glucose, reduced activity of lipoprotein lipase and reduced LDL removal leads to an abnormality in the lipoprotein metabolism [48]. Elevated level of VLDL particles, increased triglycerides are mainly stored in peripheral tissues. In diabetic condition, increased activity of HMG CoA reductase leads to an increase in the synthesis of cholesterol, which is an important risk factor responsible for coronary heart diseases [49]. Hepatic and renal cholesterol levels are elevated due to an increase of cholesterol

biosynthesis as well as reduced reverse cholesterol transport [50]. The increased cholesterol levels in serum is due to reduced LDL clearance as a result of glycosylation of the lysyl residues of apoprotein B as well as from decreasing affinity for the LDL receptor [51]. Zinc-diosmin complex treatment to diabetic rats normalizes the altered levels of lipoproteins through the enhancement of clearance of lipoproteins cholesterol and triglycerides as well as enhanced lipoprotein lipase activity and diminished lipoprotein glycation.

CONCLUSION

As the prevalence of diabetes mellitus continues to increase, there will be an urgent need to develop therapies that may help to attenuate the cardiovascular risk in this high risk population. Oral administration of zinc-diosmin complex to HFD-low dose STZ induced type 2 diabetic rats exhibited significant hypolipidemic effect, which is evidenced from the restoration in the levels of lipids, lipoprotein cholesterol and fatty acids to near normal levels. The observed hypolipidemic properties of the complex might be due to the insulin stimulatory as well as insulin mimetic nature of the zinc-diosmin complex. Since, zinc-diosmin complex is non-toxic and possess significant hypoglycemic and hypolipidemic properties in addition to its antioxidant efficacy, it may be considered as a potential candidate for further detailed studies to develop as a successful drug.

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