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Evaluation of Anti-diabetic potential of Zinc-diosmin complex - an in-vivo study

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Abstract

Complex metabolic disorder, Diabetes mellitus affects most people in the globe. The present study was aimed to synthesis the Zinc diosmin complex and evaluating its antidiabetic potential in rat L6 myoblast cell lines. The effect of zinc-diosmin complex on the viability of L6 myotubes was evaluated by MTT assay at multiple concentrations of zinc diosmin complex. There was more than 90% of cell viability at concentrations of zinc-diosmin complex up to 200 µM, when compared with control survival. Dose dependant significant glucose uptake was observed in L6 myoblast at doses 50, 100 and 200 µM after 3 h incubation, when compared to control. The data obtained from this study revealed that Zinc-diosmin complex treatment may facilitate glucose uptake in skeletal muscles.

Keywords: Zinc-diosmin; Diabetes mellitus; L6 myoblast; MTT assay; skeletal muscles.

1. Introduction

Diabetes mellitus is a complex metabolic chronic illness associated with hyperglycemia, occurring from deficiencies in insulin secretion, action, or both. It affects more and more people and is the subject of many discussions [1]. The International Diabetes Federation published data according to which the prevalence of total diabetes was 15.8% in all adults. The prevalence of diagnosed and undiagnosed diabetes was 11.3% and 4.5%, respectively. Men had a higher prevalence of total and diagnosed diabetes (18.0% and 12.9%, respectively) compared with women (13.7% and 9.7%). Diabetes mellitus associated with chronic metabolic imbalance leads to puts patients at high risk for long term complications such as macro and microvascular complications which includes retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputations, autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular symptoms and sexual dysfunction [2]. Several pathogenic processes such as autoimmune destruction of the pancreatic β cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action are involved in diabetes development. According to an estimate, one person is detected with diabetes every 5ssomewhere in the world, while someone dies of it every 10s [3].

There are different ways to treat the diabetes. Several types of hypoglycemic drugs, which exert antidiabetic effects through various mechanisms, are currently used to treat diabetes mellitus [4]. Metformin, which is example of biguanides, is the first choice drug for type 2 diabetes. This drug is characterized by high effectiveness, low cost, and safety. Treatment of non-insulin-dependent diabetes mellitus (NIDDM) due to insulin dysfunction involves inhibiting or delaying intestinal carbohydrate digestion. Sugars are the main ingredient in our daily diet.

One possible therapeutic approach is inhibition of α-glucosidase activity (EC 3.2.1.20). An example of this antidiabetic medicine is acarbose and miglitol, widely used in some parts of Asia [5]. In turn, metformin increases the insulin sensitivity of tissues and reduces the production of glucose by the liver. However, with prolonged use of this

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medicine, there is a risk of lactic acidosis development [6]. Ever since, the role of zinc in diabetes was established by Coulston and Dandona in 1980 [7], several researchers attempted to elucidate the antidiabetic role of zinc ions. Very high dietary zinc supplementation (1000 mg Zn/kg diet) for 4 weeks in ob/ob mice attenuated fasting hyperglycemia and hyperinsulinemia in pancreatic islets [8]. Conversely, a Zn-deficient diet (3 mg Zn/kg diet versus a Zn adequate diet of 30 mg Zn/kg diet) for 6 weeks exacerbated fasting hyperglycemia in mice and this was associated with reduced circulating insulin. The combination of higher pancreatic zinc and lower circulating insulin concentrations in Zn-supplemented db/db mice versus control db/db mice suggested that zinc supplementation improved pancreatic ù-cell function and/or peripheral insulin sensitivity such that less circulating insulin was required for glucose uptake [9].

Phytochemicals are ecologically derived secondary metabolites produced by the plants to protect them against damage due to environmental stress such as UV radiation, high temperature, extreme cold, draught, flood and microbial invasion [10]. It is well-known that plants produce these phytochemicals to protect themselves, but also it protect human against various ailments. Diosmin (diosmetin-7-O-rutinoside) is one such naturally occurring bioflavone found profusely in the pericarp of citrus plants such as Meyer lemons and Buddha's finger fruits [11]. It was originally isolated from Scrophularia nodosa in and later it was readily obtained by the dehydrogenation of hesperidin. Diosmin is nontoxic and reported to exhibit a wide range of pharmacological properties including antioxidant, antiproliferative, anti-inflammatory and antidiabetic effects. Diosmin was also used in the treatment of venous disease, i.e., chronic venous insufficiency (CVI) and hemorrhoidal disease (HD), in acute or chronic hemorrhoids, in place of rubber-band ligation, in combination with fiber supplement or as an adjuvant therapy to hemorrhoidectomy, in order to reduce secondary bleeding. Having these beneficial as well as pharmacological aspects in view of the present study, an attempt has been made to synthesize a novel zinc complex using diosmin as an organic ligand and evaluating its antidiabetic potential in rat L6 myotubes.

2. Materials and Methods

2.1 Chemicals

The fine chemicals of analytical grade were used during the course of the present study and the same were purchased from Hi-Media Laboratories, Sigma Chemical Company, St. Louis, MO, USA, National Centre for Cell Science (NCCS), Pune, India (L6 myoblast).

Synthesis of zinc-diosmin complex

Molar ratio method was followed in the synthesis of zinc-diosmin complex as previously reported for the synthesis of various zinc complexes with slight modifications [12 & 13]. Because of the very low solubility of these compounds in water and other solvents, DMSO-d6 was used as a solvent. DMSO solution (10 ml) containing zinc sulphate heptahydrate (0.287g, 1mM) was gradually added to a hot solution of DMSO (15 ml) containing diosmin (1.217g, 2mM). The pH of the medium was adjusted to 7.5 with Tris-HCl buffer and the reaction mixture was constantly stirred, refluxed for 8 hours at 80oC over an oil bath. The resulting solution was dried in a pressurized rotary evaporator and the complex obtained was washed with diethyl ether and kept under vacuum over anhydrous calcium chloride.

Evaluation of role of zinc-diosmin complex on glucose uptake and insulin sensitivity in rat L6 skeletal muscle cells

2.2 Culture of rat L6 myoblast

The rat L6 myoblast cell line was procured from NCCS, Pune, India and were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4.5 g/L glucose, 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml) and amphotericin-B (250 ng/ml). Cultures were maintained at 37ºC with 5% CO2 in an incubator. After, the cells had reached 60–70% confluence; differentiation was induced by replacing the growth medium with DMEM supplemented with 2% fetal bovine serum instead of 10% fetal bovine serum [14].

Myotubes formation was achieved after 6-7 days with subsequent media change for every 48 h. All experiments were performed in differentiated L6 myotubes.

2.3 Cell viability assay

The effect of zinc-diosmin complex on the viability of the L6 myotubes was determined by colorimetric MTT assay [15]. After overnight fasting with serum free DMEM, the cells were treated with various concentrations (1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µM) of zinc-diosmin complex for 24 h at 37ºC with 5% CO2 in an incubator. After treatment, culture medium was removed from the wells, and 100 µl of MTT at a concentration of 5 mg/ml in D-PBS was added to each well. After 4 h incubation at 37ºC, MTT in D-PBS was removed and then the formazan crystals were solubilized in 100 µl of 2-propanol. The absorbance of dye was measured at a wavelength of 570 nm. The results were expressed as percentage of control cell viability

Determination of glucose uptake by cultured rat L6 myotubes

L6 myoblasts (5 X 104 cells/well) were seeded in 24-well tissue culture plates and for differentiation, cells were grown in DMEM with 2% fetal bovine serum for 6-7 days with subsequent change in media for every 48 h [14]. After differentiation, the cells were fasted overnight with serum-free DMEM containing low-glucose and then treated with insulin (100 nM) for 1 h as well as zinc-diosmin complex (1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µM) in fresh serum-free DMEM, for 3 h. After treatment, glucose concentration in the medium was determined by glucose oxidase method. The glucose level in the wells with cells was subtracted from the glucose in the blank wells to calculate the glucose uptake [16].

3. Results and Discussion

L6 myoblast cell line was obtained from National Centre for Cell Sciences (NCCS), Pune, India and maintained in DMEM with 10% fetal bovine serum (FBS) and supplemented with penicillin (120 units ml-1), streptomycin (75 Pg ml-1), getamycin (160 Pg ml-1) and amphotericin B (3 Pg ml-1) in 5% CO2 environment. For differentiation of myoblasts into myotubes, the myoblasts were transferred to DMEM with 2% FBS and the cells were seeded in a collagen-coated 96 well (4x103 cells/well) micro plate and cultured in DMEM for 3 days with 10% FBS till semi confluents. Subsequently, the cells were cultured in DMEM with 2% FBS for 5 days to differentiation to myotubes. Rat L6 myoblast cells morphologically differentiated in terms of alignment, elongation and fusion of mononucleated myoblasts into multinucleated myotubes. The effect of zinc-diosmin complex on the viability of L6 myotubes was evaluated by MTT assay at multiple concentrations of zinc diosmin complex (1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µM) [Figure 1].

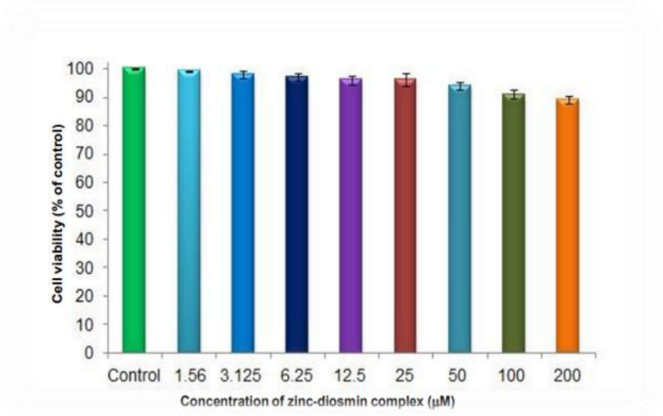

Figure 1. Effect of zinc-diosmin complex on L6 myotubes cell viability

After over night fasting with serum free DMEM, myotubes were treated with various concentrations of zinc-morin complex for 24 h at 37°C with 5% CO2 in an incubator. After treatment, culture medium was removed from the wells, and 100 µl of MTT at a concentration of 1mg/ml in D-PBS was added to each well. After 4 h incubation at 37° C, MTT reagent in D-PBS was removed and then the formazan crystals were solubilized in 100µl of 2-propanol. The absorbance of dye was measured at a wavelength of 570 nm. Cell survival was expressed as the percentage of control cells. Values are expressed as the mean ±S.E.M of triplicate experiments.

There was more than 90% of cell viability at concentrations of Zinc-diosmin complex up to 200 µM,

when compared with control survival Zinc-diosmin complex stimulates glucose uptake in muscle at dose and time dependent manner. Zinc-diosmin complex enhanced glucose uptake as percentage compared to control cells, 101.43%, 105.85%, 110.46%, 118.82%, 121.56%, 136.41%, 149.86% and 150.57% at concentrations, 1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µM respectively [Figure 2].

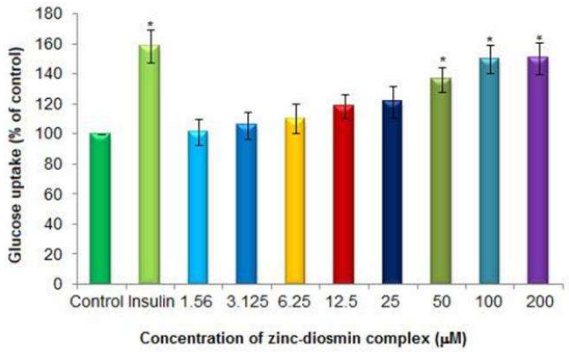

Figure 2. Effect of zinc-diosmin complex on glucose uptake in rat L6 myotubes

Myotubes were serum starved over night and then incubated with the indicated concentrations of zinc-diosmin for 3 h. Insulin (100 nM) was used as a positive control. After treatment, glucose concentration in medium was determined by glucose oxidase method. The glucose of the wells with cells was subtracted from the glucose of the blank wells to calculate the glucose uptake. Values are expressed as the mean ± S.E.M of triplicate experiments. *P < 0.05, compared with normal control culture cells.

Dose dependant significant glucose uptake was observed at doses 50, 100 and 200 µM after 3 h incubation, when compared to control group of rats. Though, zinc-diosmin complex at concentrations of 100 and 200 µM did not show any difference in activity of glucose uptake augmentation activity when compared to the effect of insulin (100 nM). The data obtained from this study revealed that zinc-diosmin complex treatment may facilitate glucose uptake in skeletal muscles. Insulin produces a variety of physiological effects on its target cells, including those on carbohydrate and lipid metabolism, protein synthesis and cell growth. The intrinsic tyrosine kinase activity of the insulin receptor plays a central role in mediating many of insulin's action [17]. A major breakthrough in insulin signaling came with the discovery of protein kinase B (PKB), as an enzyme, which becomes highly activated within the first minute of exposure of responsive cells to insulin (Kohn et al., 1996). Activated PKB also termed as Akt belongs to the family of ubiquitously expressed serine/threonine kinases and is a member of second messenger subfamily of protein kinases (Jones et al., 1991). PKB is implicated in glucose metabolism, transcriptional control and in the regulation of apoptosis in many different cell types. More significantly, the phosphorylation events were activated and regulated by PI3 Kinases [18].

4. Conclusion

Diabetes mellitus (DM) is a multisystemic, multifactorial disorder that arises due to absolute lack of insulin secretion (Type 1) or its action (Type 2). It is characterized by persistent elevation in both fasting and post prandial blood glucose levels. The prevalence of diabetes is increasing alarmingly worldwide. More than 95 percent of the diabetic individuals belong to type 2 diabetes. Most of the currently available drugs for the treatment of type 2 diabetes mellitus pose several problems such as undesirable side effects and resistance after prolonged use. For this reason, several Zinc compounds are being developed for pharmaceutical use to treat or prevent type 2 DM. Several zinc complexes have been proposed to be the new candidates in treating type 2 DM. Designing new zinc complexes on the other hand, requires attention to the stability and structural properties under physiological conditions. Zinc-diosmin complex significantly improved the uptake of glucose in rat L6 myotubes by increasing the translocation of GLUT4 to the plasma membrane. The data obtained through in vitro and in vivo studies clearly established the non-toxic nature of the Zinc-diosmin complex and its mechanism of action in ameliorating both the primary and secondary complications of type 2 diabetes. Thus, Zinc-diosmin complex may be considered as a potential new chemical

entity for further detailed studies to aid in the treatment of human type 2 diabetes.

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