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Design, synthesis and characterization of zinc–morin, a metal flavonol complex and evaluation of its antidiabetic potential in HFD–STZ induced type 2 diabetes in rats

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ABSTRACT

The present study deals with the synthesis, characterization of zinc–morin complex and evaluation of its antidiabetic efficacy in High Fat Diet (HFD)-fedStreptozotocin (STZ) induced diabetic rats. Oral administration of zinc–morin complex to diabetic rats (5 mg/kg body weight/day) for a period of 30 days resulted in the decreased levels of blood glucose and HbA1c. Oral administrations of the zinc–morin complex for 30 days significantly improved hyperglycemia, glucose intolerance, and insulin resistance. The elevated levels of lipid peroxides declined and the antioxidant competence was found to be improved in diabetic rats treated with the complex. The status of the lipid and lipoprotein profile in the serum was normalized upon treatment. Levels of TNFα decreased upon treatment with the complex. The altered levels of adipokines such as adiponectin and leptin were normalized upon treatment with the complex. In conclusion, the present study indicates that the zinc–morin complex possesses antidiabetic, antidyslipidemic and antioxidant potentials in HFD-fedSTZ induced diabetic rats.

1. Introduction

Diabetes mellitus (DM) is a multifactorial, multisystemic endocrine disorder often characterized by persistent elevation in both fasting as well postprandial glucose levels resulting in disturbances of carbohydrate, lipid and protein metabolism [1]. In DM, body does not produce (type 1) and/or properly respond (type 2) to insulin, a hormone essential for the entry of glucose from the plasma to cells for energy production [2]. Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are implicated in the development and progression of diabetes mellitus. These range from autoimmune destruction of insulin secreting β-cells of the pancreas with consequent insulin deficiency [3]. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

Currently available oral hypoglycemic drugs used for the treatment of diabetes such as sulphonylureas, biguanides, α-glycosidase inhibitors and thiazolidinediones are often associated with undesirable side effects or diminution in response after prolonged use. Hence, the search continues for novel drugs with effective antidiabetic activity at a low concentration without side effects.

There is accumulating evidence that the metabolism of several trace elements is altered in diabetes mellitus and that these elements might have specific roles in the pathogenesis and progress of this disease and its secondary complications [4]. Zinc is an essential trace element vital for the function of more than 300 enzymes and it is important for many of the cellular processes [5]. Hence, the concentration of zinc in the human body is regulated and disturbances of zinc homeostasis have been associated with several diseases including diabetes mellitus [6].

Zinc seems to exert insulin-like effects by supporting the signal transduction of insulin. Following the observations of *in vitro* insulin-mimetic activity of zinc(II) in 1980 and *in vivo* antidiabetic effect at high doses of zinc(II) ions after 1990, several anti-diabetic zinc complexes with different coordination structures have quite recently been disclosed, using experimental animal models [7].

Abbreviations: HFD, High Fat Diet; STZ, Streptozotocin; TC, total cholesterol; TGL, triglycerides; FFA, free fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase.

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For the development of clinically useful metallopharmaceutics, the research of zinc complexes on the long-term toxicity including side effects, clear-cut evidence of target molecule for the *in vivo* pharmacological action and good pharmacokinetic properties are essential in the current and future studies.

Flavonoids are plant derived secondary metabolites found rich in fruits and vegetables [8]. They are important constituents of the non-energetic part of the human diet and are thought to promote optimal health, partly via their antioxidant effects in protecting cellular components against reactive oxygen species. Flavanoids are classified as flavone, flavonol, flavanone, isoflavone, anthocyanidin and proanthocyanidins. Among these classes, flavonol is known to chelate metal ions in the presence of multiple hydroxyl groups and α-hydroxycarbonyl group [9].

High fat-fed STZ-induced diabetes is considered as a type 2 diabetic model since it arises from peripheral tissue insulin resistance followed by pancreatic β cell dysfunction and currently this model is widely used to study the *in vivo* effects of various natural products and synthetic complexes. Morin, a natural bioflavonol originally isolated from members of the *Moraceae* family plants exhibit several pharmacological properties including antioxidant, anti-inflammatory, nephroprotective, chemoprotective as well as insulin mimetic activity [10–12]. For the development of novel zinc complexes, in the present study, we have synthesized a zinc complex using morin and evaluated its antidiabetic efficacy in HFD–STZ induced type 2 diabetes in experimental rats.

2. Materials and methods

2.1. Chemicals

Zinc acetate [$Zn(CH_3COO)_2{\cdot}2H_2O$], morin ($C_{15}H_{10}O_7$) and Streptozotocin (STZ) were purchased from Sigma–Aldrich, St. Louis, USA. Ultra-sensitive ELISA kit for rat insulin was purchased from Crystal Chem Inc. Life Technologies, India. All the other reagents used in the present study were of analytical grade.

2.2. Analytical instruments

The IR spectral studies were carried out in the solid state as pressed KBr pellets using a Perkin-Elmer FT-IR spectrophotometer in the range of 400–4000 cm^{-1}. The mass spectrum of the complex was obtained using Jeol Gcmate. The ^{1}H NMR and ^{13}C NMR were obtained using Bruker AM-500 instrument at 500.13 and 125.758 MHz, respectively. The spectra were recorded without any correction for instrumental characteristics.

2.3. Synthesis of zinc–morin complex

Molar ratio method was followed in the synthesis of Zn–flavanol complex. Because of the very low solubility of these compounds in water, spectrophotometric graded ethanol was used. The zinc–flavonol complex was synthesized as previously reported with slight modifications [13–16]. Briefly, a ethanolic solution containing zinc acetate dehydrate (0.2195 g, 1 mM) was gradually added to an ethanolic solution of morin (0.6044 g, 2 mM). The pH of the medium was adjusted to 7.5 with Tris–HCl buffer and the reaction mixture was constantly stirred, refluxed for 8 h at 80 °C over an oil bath [17]. The resulting precipitate was filtered, washed with absolute ethanol, dried in vacuum and a pale yellow color solid (yield of 96%) was obtained. The solid product was characterized and used without further purification.

2.4. pH–potentiometric titrations

Potentiometric titrations were conducted for determining the formation constant of the synthesized Zn–morin complex using ELICO Li 120 pH meter fitted with glass and calomel electrodes. The electrode was initially calibrated as a hydrogen concentration probe by titrating known amounts of the standardized HCl with the standardized NaOH at 25 ± 10 °C and at least two independent titrations were performed. The program Origin 8.5 lab [18] was used to calculate the protonation and stability constants from the pH data. For all the samples prepared, the total volume was 20 mL, the ligand concentration was 1–10 mM. A total of six titrations for morin alone and fifteen titrations defined the zinc–morin equilibria. All the titrations were carried out over the range of pH 2–10 [19].

2.5. Experimental animals

Male albino rats of the Wistar strain weighing (160–180 g) were procured from the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai. The rats were housed in spacious polypropylene cages lined with husk. The experimental rats were maintained in a controlled environment (12:12 ±1-h light/dark cycle; temperature, 22 ± 3 °C; relative humidity 55%). Animals were acclimatized to standard husbandry conditions for one week to eliminate the effect of stress prior to initiation of the experiments. The rats were fed with commercial pelleted 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 the Ministry of Social justices & Empowerment, Government of India and Institutional Animal Ethical Committee guidelines [IAEC NO:03/10/12].

2.6. Experimental design

The rats were randomly divided into 2 groups, Group I ($n = 12$) was fed with normal pellet diet (NPD) and Group II ($n = 20$) fed with HFD (HFD) for 2 weeks. The composition of HFD is powdered NPD – 365 g/kg, lard – 310 g/kg, casein – 250 g/kg, cholesterol – 10 g/kg, vitamin and mineral mix – 60 g/kg, DL-methionine – 3 g/kg, Yeast powder – 1 g/kg, NaCl – 1 g/kg. Group II rats were injected with STZ (35 mg/kg body weight/rat), while the Group I rats were given vehicle citrate buffer (pH 4.5) in a same volume, intraperitoneally, respectively [20]. After one week of STZ injection, Group II rats with non-fasting blood glucose levels $\geqslant$300 mg/dL were randomly divided into 2 groups (Group 3 and 4) comprising 6 rats in each group. Group 3 and group 4 rats served as diabetic rats and diabetic rats treated with zinc–morin complex respectively. Then, Group I rats were divided into 2 groups (Group 1 and 2). Each group was comprised of a minimum of six rats and the rats were continued with their respective diets throughout the experimental period.

- Group 1: Normal control rats.
- Group 2: Normal rats treated with zinc–morin complex (5 mg/kg body weight/rat/day) for 30 days.
- Group 3: HFD–STZ induced diabetic rats.
- Group 4: HFD–STZ induced diabetic rats treated with zinc–morin complex (5 mg/kg body weight/rat/day) for 30 days.

Body weight was monitored on periodic intervals till the end of the experimental period.