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PHARMACOLOGICAL EVALUATION OF NOVEL MORIN-VANADIUM COMPLEX ON STREPTOZOTOCIN (STZ) INDUCED ALBINO FOSTER RATS IN DIABETIC NEPHROPATHY

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Abstract
Diabetic nephropathy, also known as Kimmelstiel–Wilson syndrome or intercapillary glomerulonephritis, is a progressive kidney disease caused by angiopathy of capillaries in the kidney glomeruli. The present study was undertaken to evaluate the effect of a novel Morin complex on post diabetic complications in in-vivo studies, using STZ induced rats as an animal model. Rats of either sex were divided into different groups such as normal control, diabetic control and test drug. The test drug exhibited anti-diabetic activity by significant decrease in blood sugar, blood cholesterol and increase in total protein when compared with the control group. Also, histopathological examination of kidney and pancreas showed improved cellular architecture in compare to normal control and diabetic control rats. From the above results, it was concluded that the Morin complex was found to be effective as an anti-diabetic agent in improving the post diabetic complication of nephropathy.

Key words
Nephropathy, Morin-vanadium complex.

INTRODUCTION

Diabetes mellitus is one of the world's oldest known Diseases. In 1997, diabetes prevalence was introduced as a "basic health indicator" for member states by the WHO. The ancient Chinese would test for diabetes by observing whether ants were attracted to a person's urine. Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia. The metabolic disturbance involves the disturbance in the metabolism of fats, proteins and carbohydrates, reflecting a state of insulin deprivation and possibly abnormally high amounts of glucagon and other counter regulating hormones such as glucagon hormone, sympathomimetic amines and corticosteroids. This occurs due to deficient insulin secretion and also to factors opposing the tissue effects of insulin or both. Diabetes mellitus is usually irreversible while it allows the patient to have reasonably normal life style, its complications results in a considerably reduced life expectancy [1]. In the present study our aim was to investigate the effect of vanadium Morin complex (both low and high dose) on the glucose homeostasis and the antioxidant system in streptozotocin induced diabetic rats. To the best of our knowledge this is the first study investigating the effects of eNOS in the pancreatic tissue in diabetic rat and tried to correlate the possible mechanistic approach of chemo preventive nature of vanadium in the alteration of eNOS expression in pancreatic challenged tissue.

MATERIALS AND METHODS

Materials

Morin monohydrate, vanadium oxide sulphate monohydrate, and STZ were purchased from Sigma Aldrich. (StLouis, MO). The Morin-vanadium (II) complex is prepared in our lab.

Animals

Animals were purchased from IICB Kolkata, India. Male & Female Albino Foster Rats (60–80g) were kept in a temperature- controlled environment on a 12:12h light/dark cycle with free access to food and water to acclimatized with the environmental conditions.

Preparation of Morin-Vanadium (II) Complex

The complex was prepared by dissolving 2.00 gm. of Morin into the water containing NaOH pallets. Into the solution a saturated solution of $VOSO_4$, H_20 in the VO: ligand 1:2 molar ratio was added while stirring continuously. The pH of the resulting solution was maintained by 1 (M) H_2SO_4 up to 6. After a few days a blackish solid crystal precipitated which was washed several times with water and dried in desiccators over $CaCl_2$.

Study design

Streptozotocin (STZ), 2-deoxy-2-(3-(methyl-3-nitrosoureado)-D-lucopyranose) is synthesized by "streptomycetes achromogenes" and is used to induce both IDDM and NIDDM. The range of STZ dose is not as narrow as in the case of alloxan. The frequently used single i.v dose in adult rats to induce IDDM is between 40 and 60 mg/kg b.w may be ineffective. Intracellular action of STZ results in changes of DNA in pancreatic β cells comprising its fragmentation. Recent experiments have proved that the main reason for the STZ induced β cells death is alkylation of DNA. The alkylating activity of STZ is related to its nitrosourea moiety, especially at the O6 position of guanine [2].

Histological assay

The Pancreas and kidney tissues from the specimens were fixed in 10% formaldehyde, passaged and embedded in paraffin. The paraffin blocks were then sectioned by3–5 mm thickness for H&E staining. For each case, five serial sections were used for hematoxylin and eosin (H &E) and immunohistochemical stains from each group. The photographs of histopathological sections of pancreas of control and experimental groups were shown in Figure 1, depicted the pancreatic tissue sections of normal control group showed well preserved normal cellular architecture of the pancreas. Langerhans cells (LC), Ductal cells (DC), Acinar cells (AC) and Centroacinar cells (CC) were clearly observed in the normal pancreatic tissue, where as in diabetic control group, showed pronounced degranulation and degeneration of cytoplasm was observed. Decrease the size of the cells and alteration of ductal cells structure were also found. The destruction of pancreatic tissue sections (Arrow heads) were more profound and cellular architecture was completely altered. In diabetic group treated with 10 mg/kg of vanadium-Morin, showed minimal improvement of the cellular architecture of the pancreatic tissue section and the cell destruction was quite observable (Arrow head). The number of Langerhans cells was increased but fails to regenerate its size. In vanadium-Morin (100 mg/kg) treated groups tissue destruction was quite minimal and size of the Langerhans cells were also increased. Vanadium-Morin low and high dose (Group-V & VI) control showed no observable distinct change in normal control.

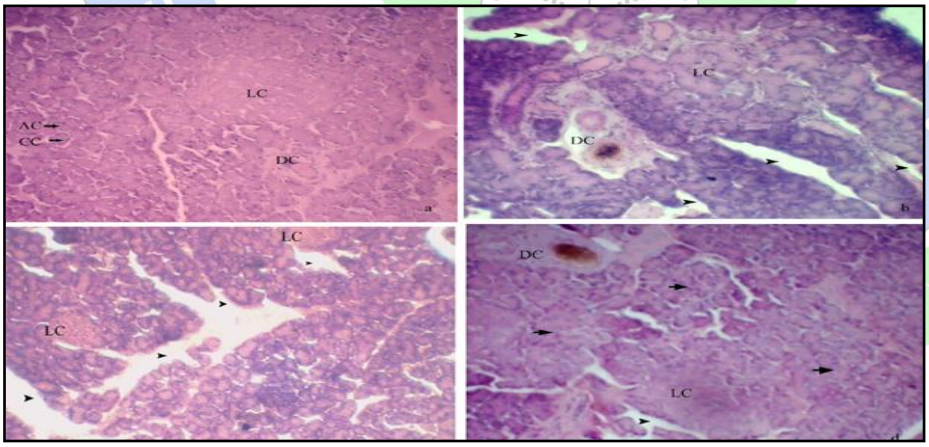
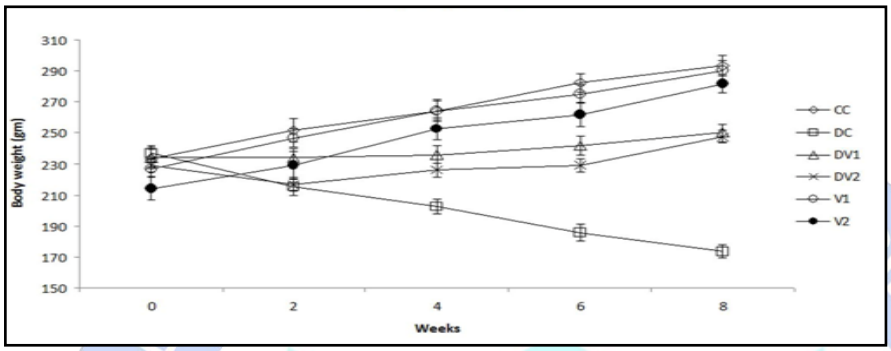


Fig.1 photomicrograph of histopathological staining of pancreas of control and experimental groups (H & E × 200)

The pancreatic tissue sections of normal control group showed well preserved cellular architecture and clearly observe the islets of Langerhans cells (LC), Acinar cells (AC), Centroacinar cells (CC) and Ductal cells (DC). Diabetic rats' showing pronounced degranulation and degeneration of cytoplasm was observed (arrow heads). In diabetic group treated with 10 mg/kg of vanadium-Morin showed minimal improvement of the cellular architecture of the pancreatic tissue. The much more profound effect of vanadium-Morin 100 mg/kg was clearly identified the tissue regeneration of the pancreatic cells (arrow).

RESULTS

The body weight changes between different groups of rats during the experimental period. The untreated diabetic rats gained weight at a much lower rate compared to control and Vanadium-Morin treated rats. Administration of low and high dose of Vanadium-Morin to diabetic rats results in an increase in body weight compared to diabetic rats. A gradual increase in body weight in the Vanadium-Morin treated groups was similar to that of control rats.



The effect of vanadium-Morine on the blood glucose levels of diabetic and non-diabetic rats is shown in Figure. A significant difference in the blood glucose levels of all groups was observed at the end of the 60 day experiment. The blood glucose levels were significantly increased in STZ- diabetic rats as compared to the control (P<0.01). Administration of low and high vanadium-Morine treatment to diabetic rats restored the blood glucose levels to near normal values (P<0.01). No significant difference was observed in rats treated with low and high dose of vanadium-Morine alone when compared to control groups.

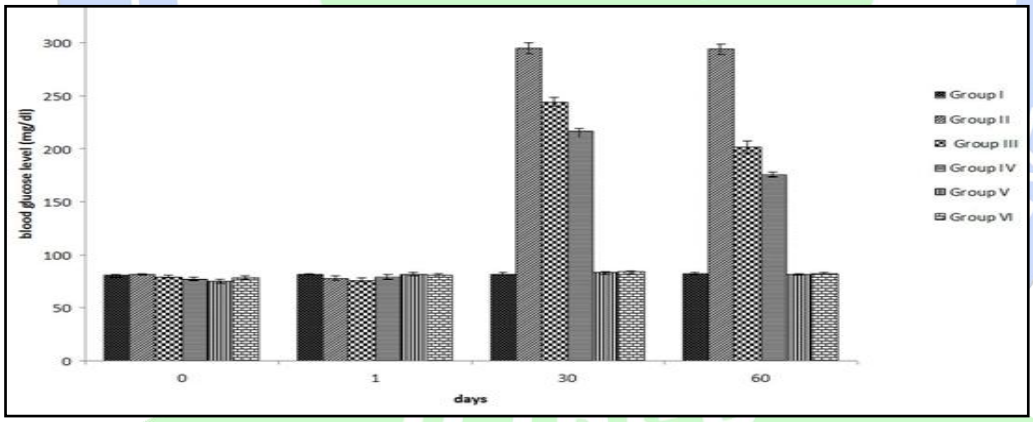


Fig.3: Variation in blood glucose level in normal challenged and complex treated group

Blood glucose levels of control, non-treated diabetic rats and treated diabetic rats. CC: control rats; DC: diabetic control rats; DV1: diabetic rats treated with 10mg/kg of vanadium-Morin; DV2: diabetic rats treated with 100mg/kg of vanadium-Morin; V1: healthy rats receiving 10mg/kg of vanadium-Morin and served as a vanadium control 1; V2: healthy rats receiving 100mg/kg of vanadium-Morin and served as vanadium control 2. Values are given as mean ± S.E.M. for groups of ten animals each; *Significantly different from non-treated diabetic rats (P < 0.01).

Serum parameters

The serum cholesterol levels of control and experimental rats are shown below. The serum cholesterol levels were significantly increased in diabetic group when compared to control group (P < 0.01). Administration of low and high dose of vanadium-Morin treated rats decrease the serum levels of cholesterol when compared to diabetic rats (P<0.01). No significant change in serum cholesterol

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was observed in rats treated with low and high dose of vanadium alone when compared to control rats.

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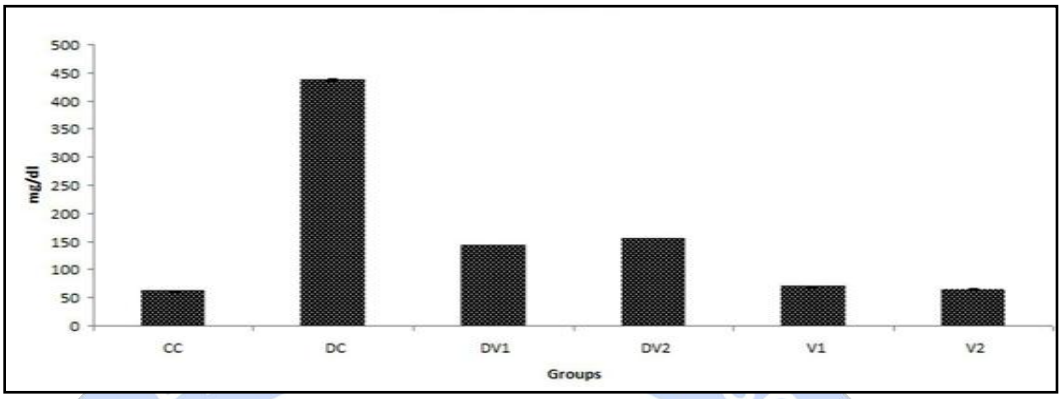

Fig.4: Variation in Blood Cholesterol level in normal challenged and complex treated group

Blood cholesterol levels of control, non-treated diabetic rats and treated diabetic rats.
CC: control rats; DC: diabetic control rats; DV1: diabetic rats treated with 10mg/kg of vanadium-Morin; DV2: diabetic rats treated with 100mg/kg of vanadium-Morin; V1: healthy rats receiving 10mg/kg of vanadium-Morin and served as a vanadium control 1; V2: healthy rats receiving 100mg/kg of vanadium-Morin and served as vanadium control 2. Values are given as mean ± S.E.M. for groups of ten animals each; *Significantly different from non-treated diabetic rats ($P < 0.01$).

DISCUSSION

There is several insulin like or insulin mimetic effects of vanadium-Morin complex confirmed by *in vitro* studies and *in vivo* experiments on animal models. There are also reported effects of vanadium-Morin that have not been compatible with the metabolic actions of insulin. In some tissues vanadate stimulated some of the actions of insulin while failing to mimic others. Both biologically active forms of vanadium, vananadyl, or vanadium-Morin have been demonstrated to possess insulin-like effects [3] Vanadium-Morin stimulated glucose

uptake [4], glucose transport and oxidation [5] decreased lipolysis [6] and increased lipid synthesis [7]. Vanadium-Morin also activated glycogen synthase in adipose tissue, liver, and muscles [8]; enhanced potassium uptake in cardiac muscle cells, Inhibited Ca/Mg ATPase [9], elevated intracellular pH [10], and suppressed secretion of apoliprotein B from rat hepatocytes [11]. In the livers of streptozotocin (STZ)-diabetic rats, vanadate restored levels of mRNA for glycolytic enzymes, glucokinase, L-type pyruvate kinase, Increased levels glycogen synthase, glycogen phosphorylase and restored liver glycogen [12], while decreasing levels of phosphoenolpyruvate carboxykinase (PEPCK.) and glucose transporter in liver (GLUT2) and muscles (GLUT4) [13]. Glucokiase and phosphotyrosine carboxykinase gane activation have also been attributed to vanadium-Morin [14]. Despite similarities between vanadium-Morin and insulin biological actions, it becomes obvious that vanadium-Morin may also produce actions different from insulin. Furthermore, there are many inconsistencies in experimental results by different researchers evaluating the insulin-mimetic action [15] of vanadium-Morin. Mechanism of insulin, which results in suppression of PEPCK mRNA levels [16-18], Additionally, vanadate seems to have a different central nervous system (CNS) mechanism than the insulin does [19-22].

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