Cognitive Dysfunction in Diabetic Rats. Effect of Minocycline

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Summary

Diabetes produced cognitive dysfunction in rats. We hypothesized that minocycline induced MMP-2 and MMP-9 inhibition. Diabetes was induced in female Wistar rats by streptozotocin (55 mg/kg i.p.). mg/kg i.p.). After diabetes induction test group rats were treated with minocycline (50 mg/kg, oral.) and standard group animals with glibenclamide (2.5 mg/kg, oral). At the end of 3 weeks and 4 weeks, cognitive dysfunction in diabetic rats was checked for the effect of test drug Minocycline with the help of water morris maze model and elevated plus maze model. Treatment with Minocycline significantly ameliorated dysfunction of cognition in diabetic rats. Inhibition of MMP-2 and MMP-9 could be the possible mechanism of action of Minocycline. Further studies are required to understand the mechanism.

Introduction

Diabetes Mellitus is a complex metabolic disease that can have devastating effects on multiple organs in the body. Cognitive dysfunction is not well recognized complication of diabetes. Patients with type 1 and type 2 diabetes mellitus have been found to have cognitive deficits that can be attributed to their disease. Both hypoglycaemia and hyperglycaemia have been implicated as causes of cognitive dysfunction. The pathophysiology underlying this complication is not well understood, and the most appropriate methods to diagnose, treat, and prevent cognitive dysfunction in diabetes have not yet been defined. In this, we will review the nature of cognitive dysfunction in type 1 diabetes mellitus, the pathophysiology of cognitive dysfunction secondary to diabetes, methodologies used to assess cognitive deficits [1]. Matrix metalloproteinase (MMPs) are a family of zinc dependent endopeptidase that mediate the degradation or remodeling of the extracellular matrix (ECM) [2]. The ECM is a multifunctional complex of proteins and proteoglycans assembled in a highly organized manner that contributes to the structural integrity of cells and tissue within an organ system [3]. Two groups of extracellular proteinases that have been shown to play a role in the retinal revascularization seen in the later stages of diabetic retinopathy, are the matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9).

Materials and Methods

Drugs and Chemicals: Streptozotocin was purchased from Sigma Aldrich Ltd. USA. Carboxy methyl cellulose (CMC) was supplied by Signet Chem., India. Minocycline tablet was purchased from Ranbaxy, India. Glucose oxidase–peroxidase (GOD/POD) glucose kit was purchased from Erba, India. Minocycline was suspended in aqueous solution of 0.5% carboxy methyl cellulose. STZ was freshly dissolved in ice cold citrate buffer (pH 4.5) solution.

Animals: Female Wistar rats (160-220g) were purchased from the Haffkine Institue, Mumbai, India and were housed at a temperature of 25 ± 10C and relative humidity of 45 to 55% in a clean environment under 12:12h light and dark cycle.

Healthy Female Wistar Rats (170-220 gm) were used in the experiment. The supplied animals used in this study are approved by in house institutional animal ethics committee of School of Pharmacy and Technolgy Management, NMIMS University. The animals had free access to food pellets and filtered water was made available *ad libitum*. The research protocol was approved by Institutional Ethical Committee (IEC) of School of Pharmacy and Technology Management, NMIMS University Mumbai, constituted under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Induction of Diabetes: A single dose (55 mg/kg, *i.p.*) of Streptozotocin (STZ) was used for induction of diabetes in rats [4]. Diabetes was confirmed after 72 h of STZ injection and again on weekly basis during the experiment. Plasma glucose levels were estimated using GOD/POD kit and rats with plasma glucose level > 300 mg/dl were considered for further studies.

Experimental Design: Animals judged to be suitable for testing would be selected. The animals were randomized base on their average weight (170-220 gm) was selected and were divided into 4 groups i.e Normal Group, Control Group, Test Group and Standard Group. All animals were feed with normal chow diet during study. Blood collection was performed on (by retro orbital puncturing), 4, 11, 18 and 25 day for estimation of blood serum glucose level. The blood samples were collected from the animals by retro-orbital vein puncturing. The serum was separated from the blood by centrifuging them at 6000- 7000 rpm and was analyzed for Blood Serum Glucose Level using an Erba instrument (ERBA®). Since the third day after the confirmation of diabetes induction, the animals of the Test Group were given Minocycline (50 mg/kg, oral) for a duration of 4 weeks (28 days) [5]. Minocycline was dissolved in o.5 % Carboxy Methyl Cellulose and then was given as oral dosage form to test group animals. After the confirmation of diabetes induction, the animals of the Standard Group were treated with Glibenclamide (2.5 mg/kg, oral) in 0.5 % Carboxy Methyl Cellulose. Water Morris Maze model and Elevated Plus Maze model were used to evaluate the cognitive dysfunction in diabetic rats.

Water Morris Maze Model: Several types of behavioral experiments, testing the learning and memory of the developing animals, can be conducted with the help of this model. One procedure, which is often used, is the Morris water maze, developed by Morris (1984). It is a behavioral experiment designed to test spatial memory. In this experiment, a rat is placed into a circular pool divided in quadrants which contains a platform, hidden a few millimeters below the water surface. The rat is places in the tank and must learn the location of the submerged platform through a series of trials. The time (latency) and distance (path) taken to reach the platform are indicators for the learning and memory abilities of the rat [6, 7].

Elevated Plus Maze Model: Animals are placed in the center of the elevated plus maze and activity is observed for five minutes. Total amount of time spent in the closed and in the open sections of the maze is *recorded by observing; an animal is considered to be completely within a section of the maze* when all parts of the body excepting the tail have crossed the threshold into that section. The number of times the animal crosses through the center of the maze is also recorded, with a crossing considered to occur when all parts of the animal excepting the tail have passed completely into and then completely out of the area of the center of the maze.

Statistics: Results were expressed as mean ± standard error of the mean (SEM).The results obtained for the different groups were analyzed by using statistical calculations.

Results

Animal Body Weight:

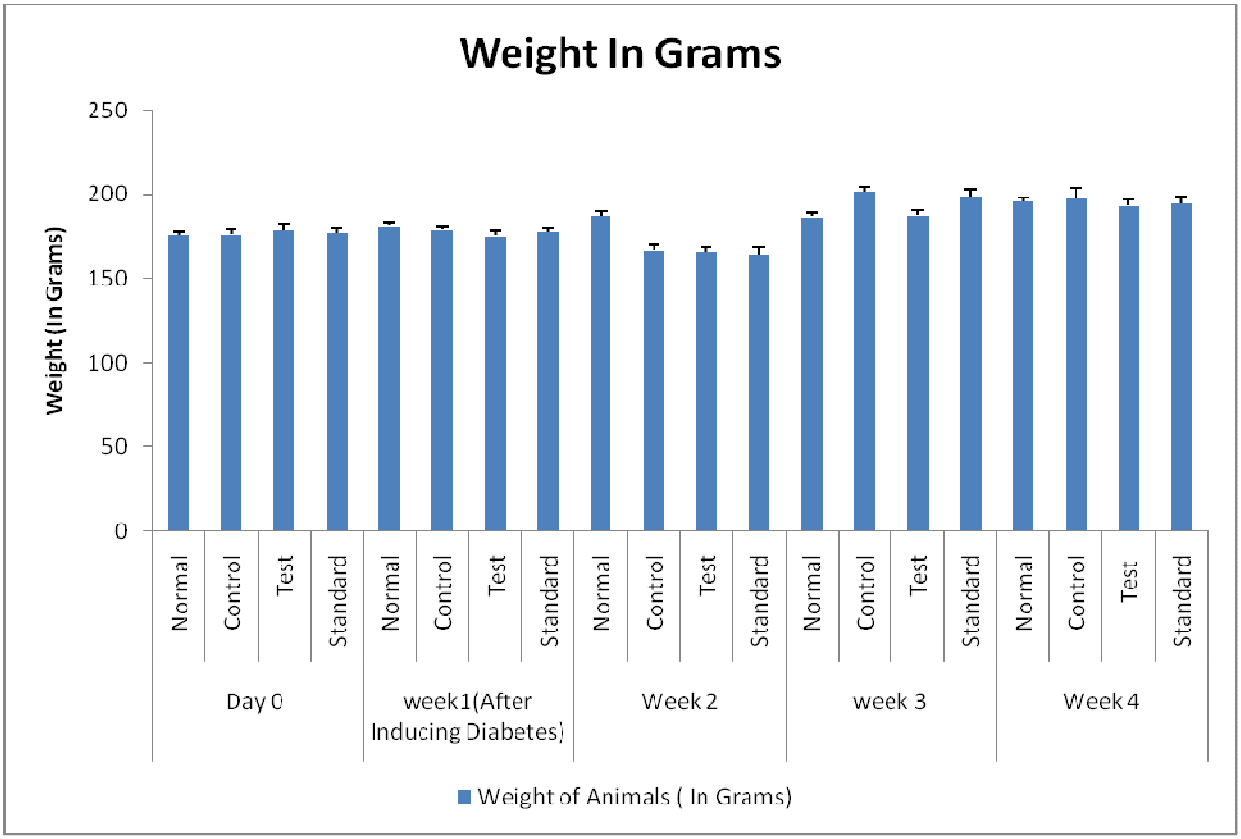

Figure No 1 : Comparison of weight of all groups of animals for 4 weeks

Before the inducing diabetes, the weight of all animals of all groups was around to 170-180 grams. After week-1 duration, no change was observed in the average weight of all the animals of all groups. In the week-2 duration, it was observed that there was slight decrease in the average weight of all groups animals which indirectly can confirm that diabetes has been induced successfully in all groups. Then the weight was recovered by the animals in next 3rd and 4th week.

Blood Plasma Glucose Levels:

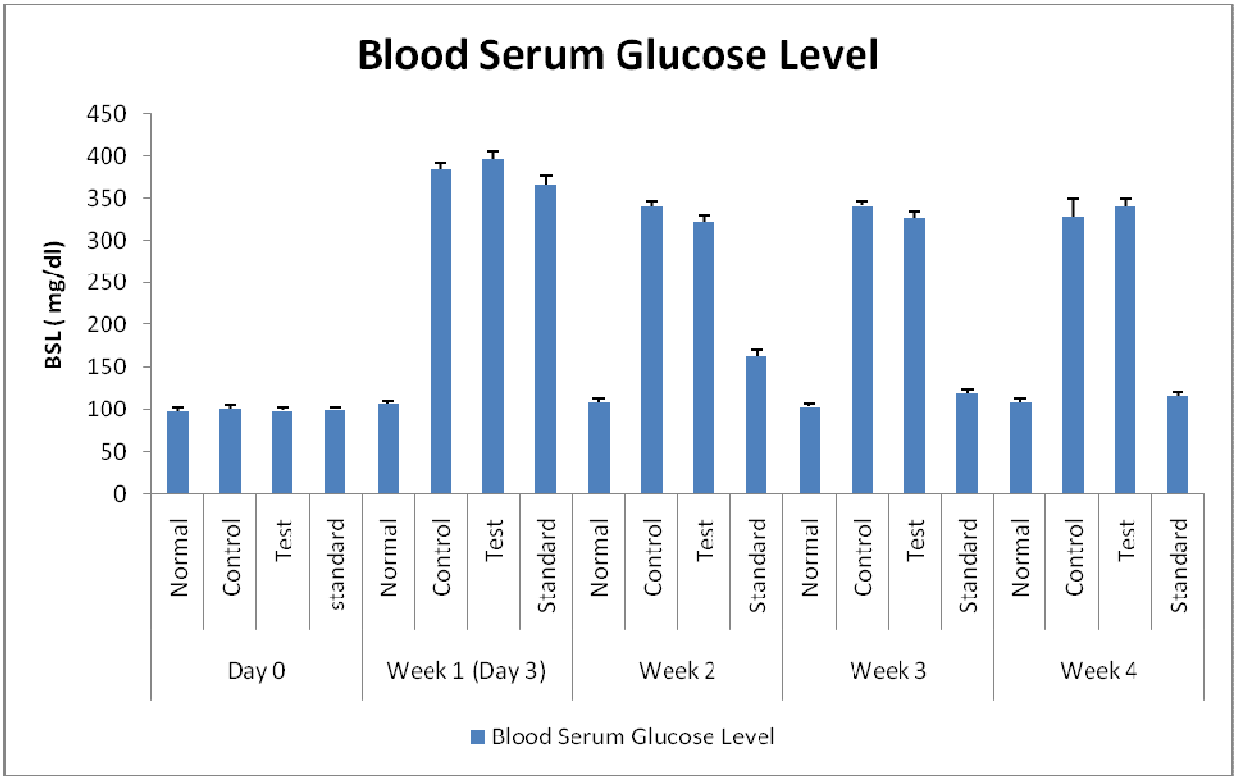

Figure No 2: Blood Serum Glucose Level for all animals for 4 weeks

STZ-induced diabetic rats showed approximately three-fold increase in the blood glucose levels after STZ administration, which was consistent throughout the study period. On the day 0 of induction of diabetes, the Blood Serum Glucose Level was found to be between range 95 – 105 mg/dl indicating the normal value of BSL in all animals of all groups. In week -1(Day 3) of induction of diabetes, it was found that the BSL of all animals of normal group remained in the range 95 – 110 mg/dl, which indicated the normal value of BSL in the animals. In other 3 groups i.e. Control, Test and Standard Group animals showed sudden increase in BSL value in range 360 – 400 mg/dl indicating that diabetes has been induced in all animals. In week – 2 after induction of diabetes, it was found no change in the BSL value of Control and Test group of week – 1. Due to treatment of Glibenclemide in standard group animals, it was found that BSL value was reduced to 165 mg/dl. The BSL value normal group animals remained in the same range. In week – 3 after induction of diabetes, the BSL value of control and standard group animals remained in range of 300 – 350 mg/dl, but a reduction was found in BSL value of standard group animals which came to around 120 mg/dl. In week – 4 after induction of diabetes, the BSL value of all group animals remained about same as that of week – 3. Diabetic rats showed slight decrease in body weight as compared to control rats. Treatment with Minocycline did not produce any change in plasma glucose levels.

Water Morris Maze Model: The average time taken by the normal group animals on day 0 was 11 seconds, and it reduced to 10.66 seconds on day 28. The average time taken by the control group animals on day 0 was 15.33 seconds, and it reduced to 14.6 seconds on day 28. The average time taken by the test group animals on day 0 was 13.33 seconds, and it reduced to 11.2 seconds on day 28. The average time taken by the standard group animals on day 0 was 11.83 seconds, and it reduced to 11.4 seconds on day 28.

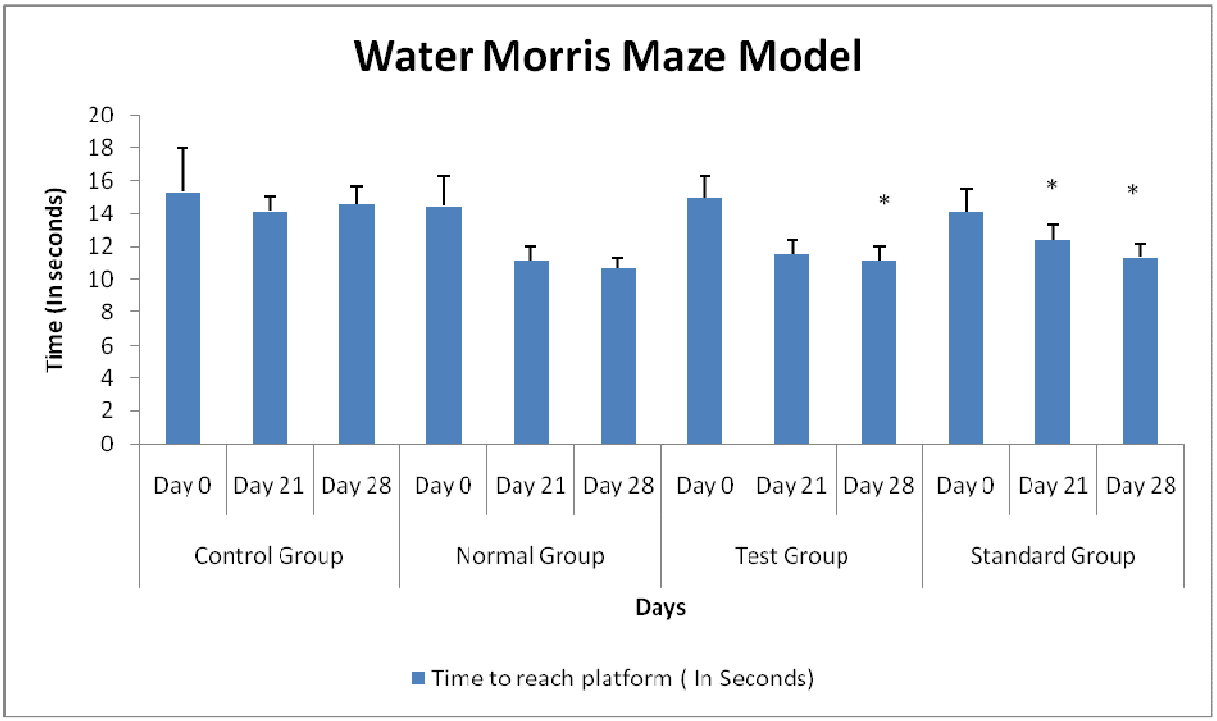

Figure No 3: Time taken by animals to reach the platform on different days in Water Morris Maze Model. ∗ p<0.05 compared to diabetic control.

Elevated Plus Maze Model: The average time taken by animals to reside in open arm is about 14.83 seconds on Day 0 and 16.16 seconds on Day 28. The average time taken by animals to reside in closed arm is about 179 seconds on Day 0 and 195 seconds on Day 28. : The average time taken by control group animals to reside in open arm is about 20.83 seconds on Day 0 and 43.4 seconds on Day 28. The average time taken by animals to reside in closed arm is about 153 seconds on Day 0 and 163 seconds on Day 28. The average time taken by control group animals to reside in open arm is about 20.83 seconds on Day 0 and 43.4 seconds on Day 28. The average time taken by animals to reside in closed arm is about 153 seconds on Day 0 and 163 seconds on Day 28. The average time taken by standard group animals to reside in open arm is about 14.5 seconds on Day 0 and 32.6 seconds on Day 28. The average time taken by animals to reside in closed arm is about 175 seconds on Day 0 and 175 seconds on Day 28.

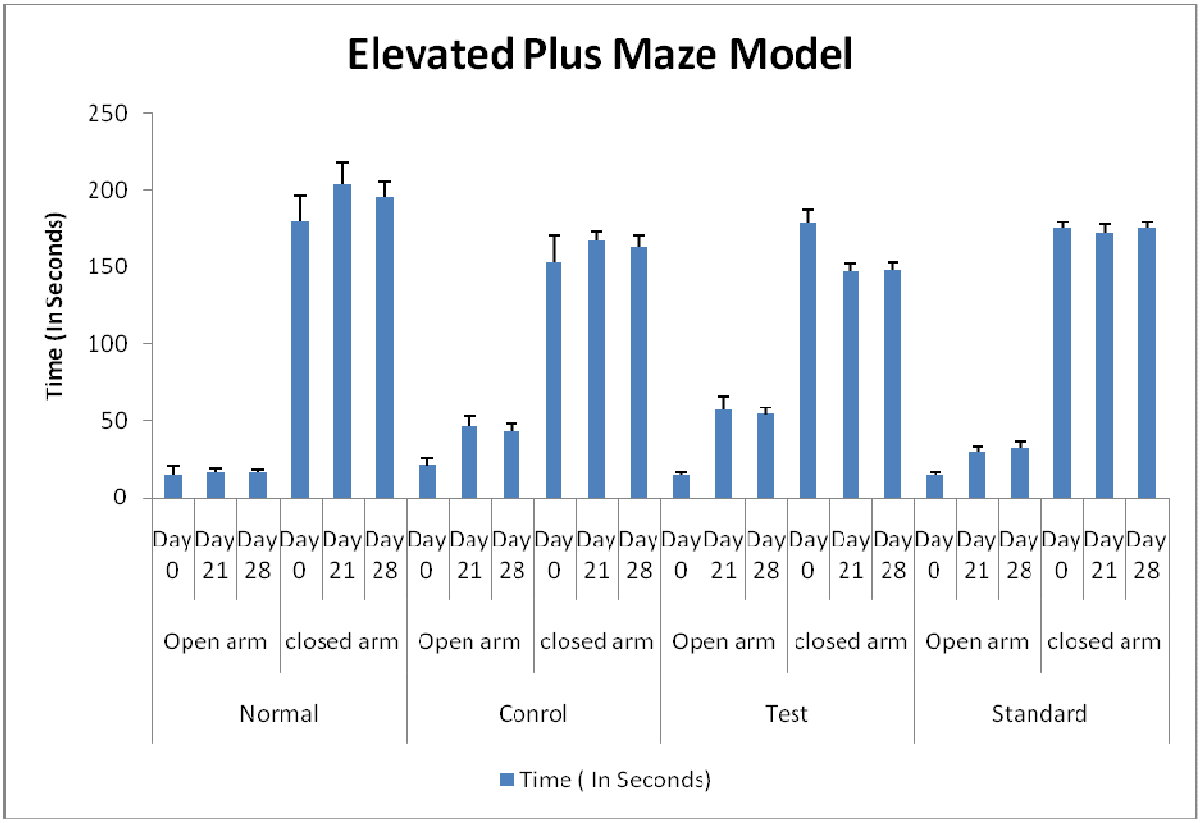

Figure No 4: Comparison of time in Open and Closed arm of all days of all animals for Elevated Plus Maze Model

Conclusion

Diabetes produced cognitive dysfunction in rats. Treatement with minocycline significantly amleorated dysfunction of cognition in diabetic rats. Since BSL is not changed by minocycline, it may be working through other glucose lowering mechanism. Inhibition of MMP-2 & MMP-9 could be possible mechanism. Further studies are required to understand the mechanisms.

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