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Study of the Behavioral Pattern in Type-2 Diabetic Mice Prepared by Introducing 10% and 15% Fructose Solution

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Abstract

An altered behavioral pattern is a deliberate fact in diabetic conditions that could be induced due to the consumption of foods rich in fructose content. The objective of this research was to develop a fructose-induced diabetic mice model to determine the behavioral changes. *Swiss albino* mice were involved in this study as the animal model which was subdivided into three groups (control, test groups 1 and 2). Diabetes was induced in test groups 1 and 2 by introducing 10% and 15% fructose solution orally for 8 weeks. After 8 weeks, behavioral tests including hole board test (HBT), tail suspension test (TST), elevated plus maze test (EMT), and forced swim test (FST) were performed to evaluate the behavioral pattern. Findings indicated that test group 1 showed a considerable increase in body weight but only a minor increase in fasting blood glucose (FBG) level than the control group. Neither the behavioral studies for test group 1 stretch any substantial alteration. Whereas test group 2 showed a considerable increase in FBG level and body weight as well as in the behavioral tests. (HBT: 47.87% lower head dipping than the control group; TST: 90.85% higher immobility time than the control group; EMT: 58% lower exploration to open arm than the control group; FST: 38.64% less mobility time than the control group). The findings of the study illustrate that consumption of high concentrations of fructose for long term could have a positive correlation with the induction of type 2 diabetes along with the altered behavioral pattern.

Key words: Diabetes, CNS behavior, fructose solution, behavioral test.

Introduction

Diabetes is a chronic metabolic disease highlighted through hyperglycemia, altered protein, lipid and carbohydrate metabolism that being induced due to defective insulin action or secretion or both which ultimately leads to different vascular complications (Jung *et al.*, 2022). About 90% of the diabetic cases involve the etiology of type 2 diabetes which is characterized by a lack of insulin sensitivity of the target organ due to the obliteration of a large number of beta cells which in turn induce insufficient insulin production that being unable to maintain glucose homeostasis induing a state of insulin resistance (Vandamme, 2014). Blood glucose homeostasis is maintained with the help of the opposing effect of glucagon and insulin along with amylin, glucose-dependent insulinotropic polypeptide (GIP) and glucose-like peptide (GLP) (King, 2012). The pathogenesis of type 2 diabetes is a combination of several mechanisms. Primarily it starts with insulin resistance at target sites like muscle, liver and adipose tissues (Biswas *et al*., 2016). To compensate for this resistance, beta cells increase insulin production. But this hyper insulinemic condition is a temporary one- and over-time beta cells continue to destroy which leads to diminished insulin production (Forouhi *et al*., 2014). These combined effects of insulin resistance and beta cell dysfunction cause increased hepatic glucose production and reduced glucose utilization and uptake by muscle and adipose tissue (Dekker *et al.*, 2010). Other factors have increased the concentration of free fatty acid (FFA),

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tumor necrosis factor-alpha (TNF-α) and hormone resistin. FFAs produce insulin resistance as they inhibit glucose uptake and glycolysis by skeletal muscle and also increase hepatic gluconeogenesis. TNF-α impairs insulin action and resistin antagonizes the effect of insulin. Thus, impaired insulin secretion and increased glucagon production ultimately caused the diseased condition (Zemdegs *et al.,* 2016).

Furthermore, diabetes mellitus brings about a variety of complications including diabetes retinopathy, ketoacidosis, nephropathy, neuropathy, dyslipidemia, adipocytosis and altered CNS behavior (Sartorius, 2012). Among the altered CNS behavior, depression is one of the most observed complications (Zemdegs *et al.,* 2016). Different studies have been conducted to evaluate the pathophysiology of this comorbidity between diabetes and depression. These studies suggested that hyperglycemia is associated with diabetes, being the primary metabolic cause of depression. This hyperglycemia causes dysregulated hypothalamic-pituitary-adrenal axis and neurotransmitter system which is another causative factor that promotes the development of depression during diabetes. Besides this, diabetes being a metabolic disorder induces oxidative stress and neuroinflammation which cause death of the cells present in the hippocampus and prefrontal cortex, the important brain areas that mediate and modulate emotional behavior (Badecsu *et al.,* 2016).

A number of risk factors including modifiable and non-modifiable factors potentiate the development of diabetes. Individuals of all generations tend to prefer canned foods that are rich in fructose, which has a substantial impact on the development of diabetes (DiNicolantonio *et al.,* 2015). High-concentration fructose solution has emerged as one of the major causes of non-alcoholic fatty liver disease as it induces fatty liver and insulin resistance which ultimately persuade diabetes among individuals (Fradkin, 2014). Given the fact that fructose is mainly metabolized within the liver, it escapes the rate-limiting step phosphorylation and promotes lipogenesis and reduces the rate of beta-oxidation which results in visceral fat accumulation This accumulated fat and their metabolite activate protein kinase C which inhibits the phosphorylation of insulin receptor substrate and impaired the insulin signaling that induces insulin resistance. Thus, due to the metabolism process of high-concentration fructose, it promotes the development of metabolic disorders including diabetes and fatty liver. (Stanhope *et al.,* 2008).

Diabetes has drawn the attention of researchers due to the surge in the number of patients with the disease and the repercussions accompanying it (Cho *et al.,* 2018). In reference to other research findings, we conducted research work to evaluate the impact of diabetes on CNS behavior in a fructose-induced type-2 diabetic mice model.

Materials and Methods

Animal model: Swiss albino mice (male and 6-7 weeks old weighing about 25-30 gm) were used as the animal model in the study. The mice were kept in polypropylene cages in an air-conditioned room (25degree Celsius ± 2) under a 12h light and dark cycle. The animals were given normal pellet food and water (Vandamme, 2014).

Induction of diabetes mellitus in mice: Diabetes was developed in *Swiss albino* mice by giving 10% and 15% fructose solution with normal drinking water for 8 weeks (Raghav, 2015).

Experimental design: The mice were divided into three groups containing control group (n=12), test group 1 (n=24), and test group 2 (n=24). The control group was given water and a normal pellet whereas the test group 1 and 2 were given 10% and 15% fructose solution respectively.

Measurement of body weight: Bodyweight of all groups of mice was taken twice each week and this process continued till the end of the study.

Fasting blood glucose (FBG) measurement: Blood was taken from the vein of the tail of mice. This process was done with a sharp needle and blood sugar level was measured with the help of a glucometer (GlucoLeader). Fasting blood glucose level was measured after the overnight fasting of

mice (Raghav, 2015). Fasting blood glucose was measured every two weeks.

Method for behavioral tests: Behavioral tests like hole board test (HBT), tail suspension test (TST), elevated plus maze test (EMT) and forced swim test (FST) were conducted to evaluate the alteration in behavioral pattern. To perform HBT, each mouse was placed on the hole board and the head dipping of them was observed for five minutes (Takeda *et al.*, 1998). For TST, the mice were brought into the testing room and were hanged by their tails with the handle of the board and immobility of the mouse was observed for five minutes (Cryan *et al.*, 2005). Elevated plus maze was used to perform EMT. EMT involved the observation of exploration to open and closed end of each mouse for 5 minutes (Carobrez, 2005). Eventually, for FST a bottle was filled with water up to 15 cm. The mice were placed in a bottle with water and the mobility time (response of the mice against the threat of drowning) of the mice was observed for 5 minutes (Yan *et al.*, 2015).

Measurement of triglycerides: The reagent and sample were mixed in test tubes. The sample and reagent were incubated at 37 °C for 5 minutes. The reading was zeroed out. The absorbance of the calibrator and sample was assessed in relation to a control at 505 nm (Huang *et al.*, 2004).

Measurement of total cholesterol: In test tubes, the sample and reagent were combined. At 37 °C, the sample and the reagent were incubated for 5 minutes. The gauge was set to zero. At 505 nm, the absorbance of the calibrator and sample were measured in comparison to a blank (Huang *et al.*, 2004).

Measurement of HDL cholesterol: The sample and precipitating reagent were combined and centrifuged for 10 minutes at 4000 rpm prior to 10 min of standing period. The supernatant was then collected and combined with the reagent and incubated at 37 °C for five min. The absorbance of the sample was observed at 505 nm (Huang *et al.*, 2004).

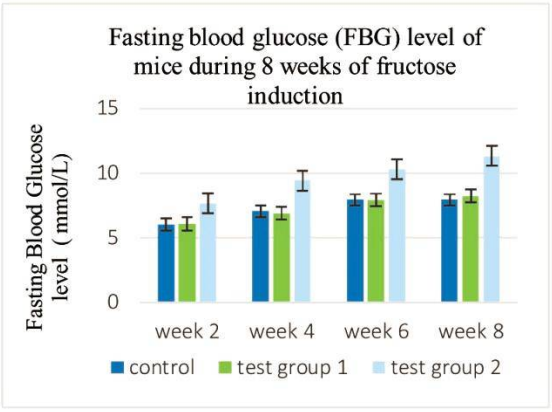
Data analysis: All data were plotted and calculated by using Microsoft Excel. The graphs and the percentage calculations were also calculated by using Microsoft Excel.

Results and Discussion

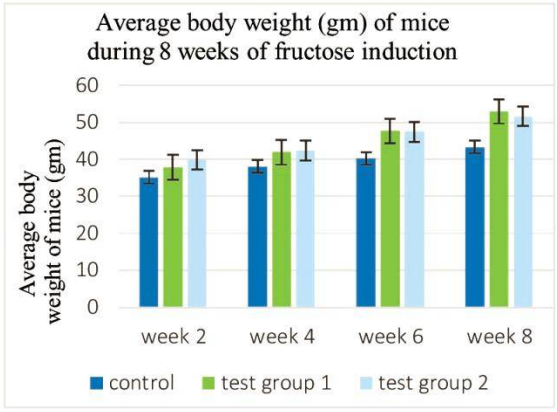
Graphs 1 and 2 contain the graphical representation of FBG level and body weight of the control group, test groups 1 and 2. Graph 1 illustrates the impact of fructose induction on FBG level and Graph 2 represents the impact on body weight among the research animals (*Swiss albino*). Findings indicated that although the FBG level among test group 2 was considerably increased (42.87%) only a 4% increase was observed among mice of test group 1. While, group 1 showed only a small increase in FBG level after 8 weeks of fructose induction but the body weight was markedly increased (22.25%) in comparison with that of the control group. The lipogenic nature of fructose could be a factor in increasing body weight among the test mice. On the contrary, test group 2 showed noticeably higher FBG level (42.87%) as well as body weight (19.24%) than the control group at the 8^{th} week of 15% fructose induction. These results of the FBG test and body weight suggest that consumption of high-concentration fructose solution may contribute to the development of diabetes mellitus, depending on the time period and concentration of fructose intake.

Different studies have shown that depression level increases with higher blood glucose level (Bădescu *et al.*, 2016). Graphs 3-6, represent the findings of behavioral tests of our study. Our study indicated that among test group 2 marked depressive behavior was observed (HBT: 47.87% lower head dipping than the control group; TST: 90.85% higher immobility time than the control group; EMT: 58% lower exploration to open arm than the control group; FST: 38.64% less mobility time than the control group) though promising change in behavioral pattern was unaccountable for the test group 1 (HBT: 2.17% lower head dipping than the control group; TST: 9.37% higher immobility time than the control group; EMT: 16.66% lower exploration to open arm than the control group; FST: 7.14% less mobility time than the control group). These variations

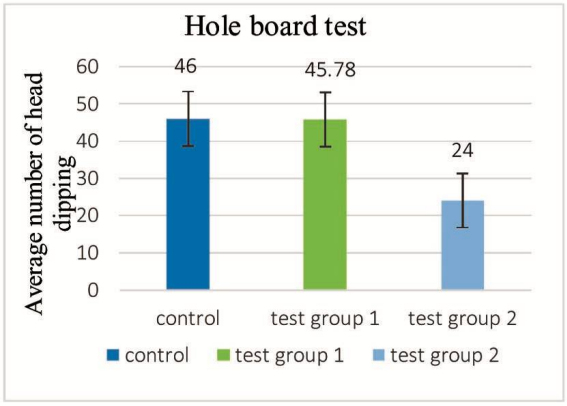
between the two test groups could be due to the fact that although test group 2 showed a considerable increase in FBG level but test group 1 had only minor increase in FBG level with the average FBG level of 7.2 mmol/L which can be considered as prediabetic state or minor diabetes has been induced. From these outcomes, we can interpret a positive correlation between the comorbidity of diabetes and depression.



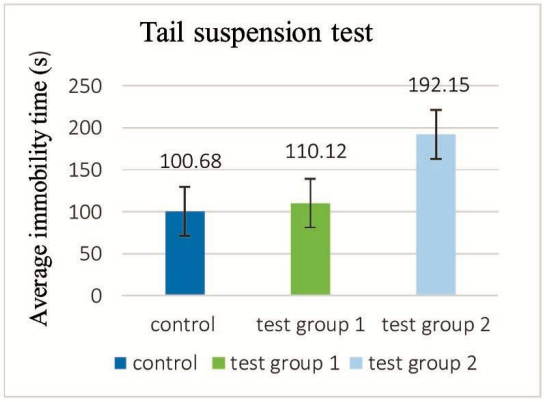
Graph 1. FBG level of mice



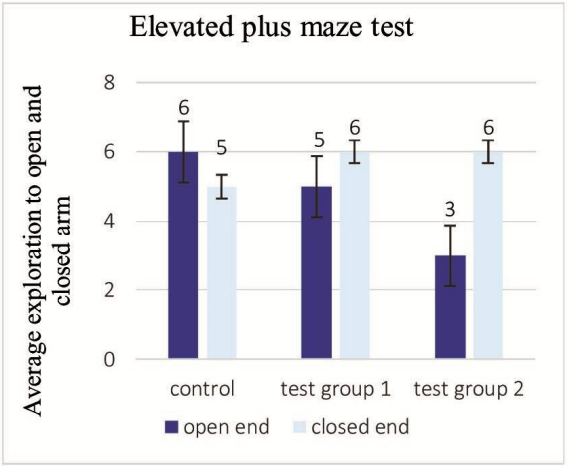
Graph 2. Average body weight of mice



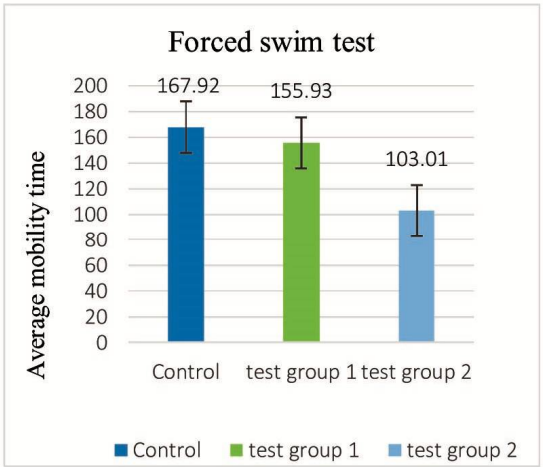
Graph 3. Hole board test



Graph 4. Tail Suspension test



Graph 5. Elevated plus maze test



Graph 6. Forced swim test

Along with these altered behavioral patterns, test group 2 also showed noticeable alteration in their lipid profile and visceral adiposity was also markedly increased in this group of mice.

The diabetic mice showed higher plasma total cholesterol l (23.4%), triglyceride (60.27%) and low-density lipoprotein (67.28%) whereas the level of plasma high-density lipoprotein (31.74%) was lower than the control group of mice.

Conclusions

One major factor contributing to the current rise in diabetes cases across all age groups is the preference of canned food that is highly concentrated in fructose solution. Because fructose is a lipogenic molecule, it fosters the development of metabolic syndromes, such as diabetes, which in turn causes several complications, including depression. The likelihood of developing depression is two to three times higher in those with diabetes than in the general population. These investigations imply that our finding corroborate with those of previous studies carried out by various scientists. Fructose-induced Swiss albino mice could be employed as a model for diabetes studies. These models can be used to understand the etiology of diabetes, its consequences, and the mechanism of action of new anti-diabetic drugs. Considering that the fructose-induced diabetic mice model is cost-effective model as they don't need costly equipment, special diets, or much maintenance effort they could be a popular choice for further research works to evaluate this paradigm, in which mice will be treated with pharmacological agents to evaluate unrevealed potentials.

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