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Phytomelatonin (*Zea mays*) Supplementation Restores the Damage Caused by Induced-Diabetes in the Golden Hamster *Mesocricetus auratus*

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Abstract

Diabetes is a lifestyle disorder with multiple etiologies, one of them being damage induced by free radicals. Melatonin, a neurohormone secreted by the pineal gland, is a well-known antioxidant or free radical scavenger. The melatonin found in plants is known as phytomelatonin. Phytomelatonin is potent in regulating stress management, apoptosis, seasonality and circadian rhythms in animals as melatonin. The supplementation of phytomelatonin is known to potentiate the antioxidant capacity. Therefore, in the present study, we proposed that the supplementation of corn seed (*Zea mays*) with regular diet may modulate the activity of antioxidative enzymes in diabetic hamsters. The supplementation of diet with phytomelatonin-rich corn did not reduce serum glucose level significantly. No significant elevation was noted in serum insulin level of animals after feeding corn. Glycogen level of both liver and muscle were also not significantly affected. However, phytomelatonin supplementation improved lipid profile by significantly reducing the cholesterol (TC) and LDL Cholesterol (LDL-C) and enhancing HDL Cholesterol (HDL-C). Significant reduction was noted in LPO level in pancreas. The supplemental diet led to significant increase in the level of Super Oxide Dismutase (SOD) and catalase (CAT) in pancreas. Diabetes produced a deleterious effect on oxidative stress markers, lipid profile, glucose, glycogen and insulin. Supplementation of corn in the diet for 40 days modified the biochemical parameters to various degrees. The phytomelatonin treatment improved most of the antioxidant parameters under investigation. The study has produced some positive outcomes, especially a strategy which may be relevant in prevention, development and/or slowing down of the progression of diabetes.

Keywords: Diabetes, Hamster, Melatonin, Phytomelatonin, *Zea mays*

1. Introduction

Diabetes is a disease with multiple etiologies, one of them being damage induced by free radicals[1]. Melatonin, a neurohormone secreted from the pineal gland, is a well-known antioxidant or free radical scavenger[2]. Melatonin has been detected in a number of edible plants and it has been proved that this classical indole is actually synthesized in plants and hence known as phytomelatonin[1]. The phytomelatonin was first identified in coffee beans in 1970[3], and later, different plant species like cereals and medicinal herbs have also been reported to contain high concentration of melatonin[4,5].

Phytomelatonin is equally potent in regulating stress management, apoptosis, seasonality and circadian rhythms in animals as melatonin does. The supplementation of phytomelatonin potentiates the antioxidant capacity of the serum and immunity in ruminants[6-8]. However, there is no report suggesting effect of melatonin supplementation from plant source on diabetes management.

Therefore, in the present study, we proposed that the supplementation of corn seed (*Zea mays*) with regular

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diet may modulate antioxidative enzymes activity in diabetic hamsters. Corn (*Z. mays*) seed, a common cereal that contains quite high levels of melatonin (1366 pg melatonin/g of corn seeds[4-5]), were selected for the supplementation with regular diet as source of exogenous melatonin to the hamsters.

2. Materials and Methods

2.1 Ethical Consideration

The experiments on the animals were conducted in accordance with prescriptions of the Institutional Animal Ethics Committee under the framework of CPCSEA (Committee for Purpose of Control and Supervision of Experiments on Animals), Government of India (2001).

2.2 Animals

Mesocricetus auratus (Golden hamster) were procured from CDRI, Lucknow. During the experiment the hamsters were kept in polypropylene cages in a well-ventilated room with ambient conditions (27±2°C, with gentle ventilation). The animals were provided with commercial feed and water *ad libitum*. Animals weighing 100±10 g were selected for the study.

2.3 Induction of Diabetes

Hamsters weighing 100±10 g were kept fasting for overnight and the next day a single dose of streptozotocin (STZ) at 50 mg/kg bw, dissolved in citrate buffer (pH=4), was injected intraperitoneally (*ip*)[2]. In order to avoid STZ-induced initial mortality the animals were given 20% glucose solution for twenty-four hours. After 72 hours of STZ injection fasting blood glucose (FBG) of the animals was checked (AccuChek, USA). The animals having FBG greater than 200 mg/dL were considered diabetic.

2.4 Experimental Design

The hamsters were divided into three groups of six animals each.

Group I: Control (normal, untreated)

Gropu II: Diabetic control (diabetes, induced through STZ)

Group III: Phytomelatonin supplemented (STZ induced diabetic hamsters receiving phytomelatonin)

Corn (*Zea mays*) was fed (supplemented) with regular diet of the diabetic hamsters at the dose of 100 mg /kg body weight for 40 days[8].

2.5 Sample Collection

The corn supplementation was continued for 40 days, after which the hamsters were fasted overnight. The next day, the hamsters were weighed and sacrificed by total body anaesthesia. Blood was collected directly from the heart, and the serum separated, was frozen at -80°C until ELISA for insulin determination (DIAMETRA, Lot no. DKO076), and biochemical estimations of serum glucose, total cholesterol (TC), HDL-Cholesterol (HDL-C), and LDL-Cholesterol (LDL-C). Pancreas, liver and muscle were dissected out on ice, blotted free of blood, and cleared of extra tissue. Pancreas was used for biochemical estimation of Lipid Per-Oxidation (LPO) assay, Super-Oxide Dismutase (SOD) and CATalase (CAT). Liver and muscles were used for biochemical estimation of glycogen.

2.6 Biochemical Estimations

Glycogen level in liver and muscle, serum glucose, Total Cholesterol (TC), HDL-C, and LDL-C, were determined according to manufacturer's protocol (BioLab Diagnostics, India). Serum insulin level was measured following the details in the kit (Diametra, DKO076). For finding the antioxidant status in pancreas SOD activity was determined using the method of Das *et al.*[9], CAT activity was determined following the protocol described by Sinha[10], and LPO was conducted using technique described by Okhawa *et al.*[11].

3. Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 8 (USA). The data were analyzed using students' *t*-test (For two groups). One-way analysis of variance (ANOVA) followed by Tukey's multiple-range test for multiple comparisons were conducted. All the data were expressed as the mean + Standard Error of Mean (SEM). Values of $p < 0.05$ were considered as statistically significant.

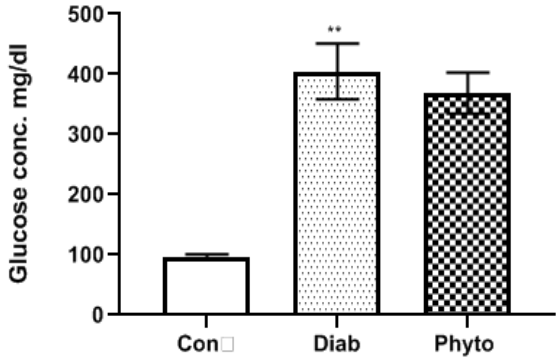

Figure 1. Bar graphs depicting no significant change in serum glucose concentration following *Z. mays* supplementation.

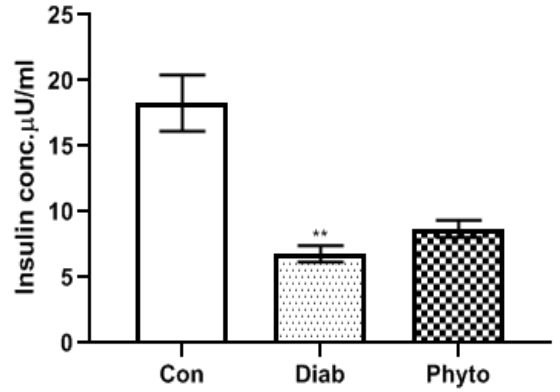

Figure 2. Bar graphs depicting no significant increment in serum insulin concentration following *Z. mays* supplementation.

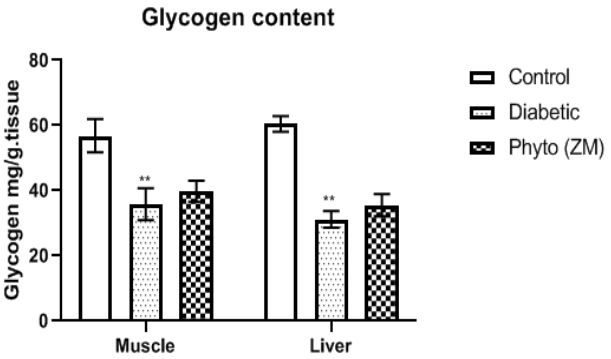

Figure 3. Bar graphs depicting no significant improvement in muscle and liver glycogen following *Z. mays* supplementation.

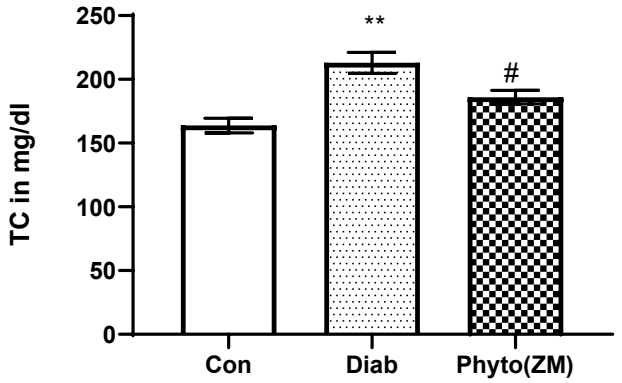

Figure 4. Bar graphs depicting significant reduction in total cholesterol (TC) following *Z. mays* supplementation.

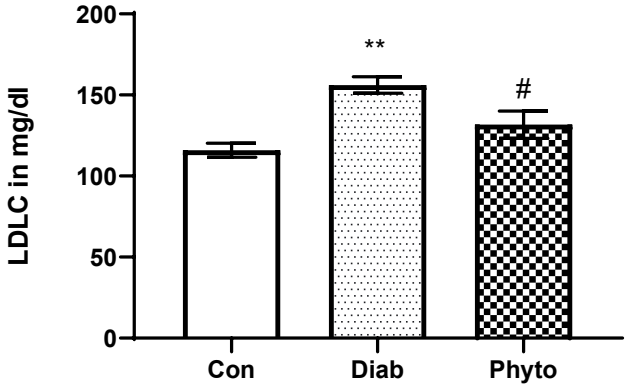

Figure 5. Bar graphs depicting significant reduction in LDL cholesterol (LDL-C) following *Z. mays* supplementation.

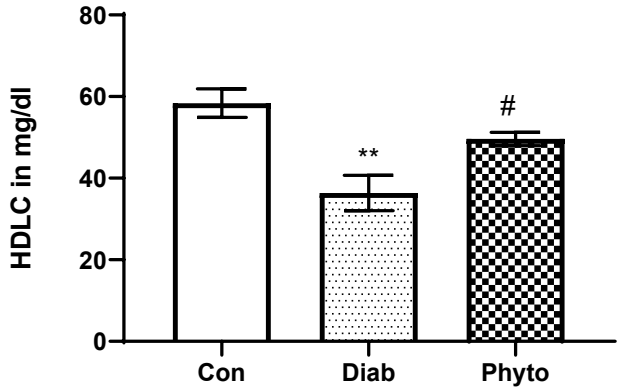

Figure 6. Bar graphs depicting significant increase in HDL cholesterol (HDL-C) following *Z. mays* supplementation.

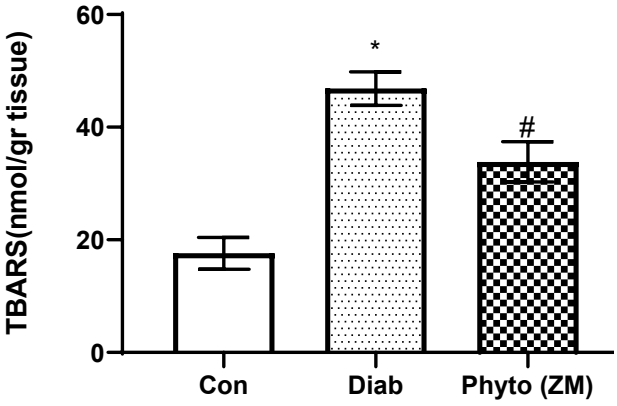

Figure 7. Bar graphs depicting significant reduction in Lipid Per-Oxidase (LPO) level in pancreas following *Z. mays* supplementation.

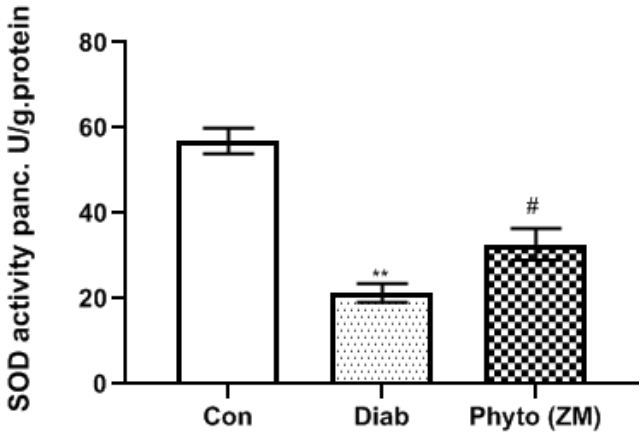

Figure 8. Bar graphs depicting significant increase in Super-Oxide Dismutase (SOD) level in pancreas following *Z. mays* supplementation.

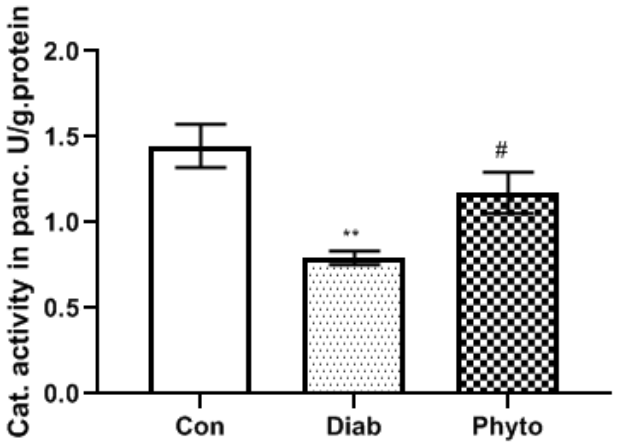

Figure 9. Bar graphs depicting significant increase in catalase (CAT) level in pancreas following *Z. mays* supplementation.

4. Results

There was no significant reduction in serum glucose level after giving phytomelatonin-supplemented diet (Figure 1). No significant increase in serum insulin was noted after feeding corn (Figure 2). Glycogen level of both liver and muscle were also not significantly affected (Figure 3). Phytomelatonin supplementation improved lipid profile by reducing the cholesterol (TC) (Figure 4) and LDL-C (Figure 5) and increasing HDL-C (Figure 6). Significant decrease was noted in LPO level in pancreas (Figure 7). The supplemental diet led to significant increase in the level of SOD (Figure 8) and CAT (Figure 9) in pancreas.

5. Discussion

Our study is the first of its kind to find the effect of plant indole amine (Melatonin) on diabetes. Though the effect of this phyto-indole melatonin is not directly as the prescribed drug it is definitely useful in reducing the blood sugar level indirectly *via* the management of Reactive Oxygen Species (ROS).

Diabetes is a chronic disease characterized by elevated blood sugar levels. In diabetic patients, oxidative stress induced by the release of excessive ROS and Reactive Nitrogen Species (RNS) is widely associated with chronic inflammation which would result in potential tissue damage. Thus, complications due to diabetes mellitus (such as retinopathy, nephropathy, neuropathy, ischemic heart disease, and peripheral vasculopathy) would entail challenging health problems[1]. Unsaturated phospholipids, glycolipids, and cholesterol in cell membranes and other organized systems are prominent targets of oxidant attack. This can result in lipid peroxidation wherein increased oxidative stress associated with lipid peroxidation in endothelial cells may be one of the major causes of hyperglycemia-associated diabetic complications.

Usually, the production and neutralization of ROS involves defence mechanisms such as repair and/or preventive mechanisms and antioxidation. Enzymatic antioxidant defences include SOD, CAT, Glutathione Pero-Xidase (GPx), and other enzymatic defences[2]. The oxidative stress state causes damage to cellular macromolecules such as proteins, lipids, and nucleic acids[1-2]. One of the main challenges of research in recent years has been finding ways to attenuate oxidative stress in order to manage diabetes. The present study demonstrates the effect of dietary supplementation of corn seed in the diabetic hamster.

The indirect consequence of phytomelatonin is believed to occur *via* lipid metabolism. We observed a significant improvement in lipid profile and a significant reduction in LPO level of pancreas, which is a highly important parameter for improvement of a diabetic patient. Though no significant improvement was noted in serum insulin, and glycogen in muscle and liver, phytomelatonin treatment improved the functional status of pancreas by significantly improving SOD and CAT activities.

The nutritional importance of melatonin/phytomelatonin in foodstuffs is gaining importance. It has been reported that consumption of melatonin-/phytomelatonin-containing foods increases circulating melatonin levels and it is correlated with the total antioxidant potential in humans and animals[8,12]. One melatonin/phytomelatonin molecule potentially scavenges 10 molecules of free radicals via the cascade reaction, which contrasts with the classic antioxidants that typically detoxify one radical per molecule[12]. Thus, the main function of melatonin in organisms, in physiological conditions, is to serve as an antioxidant to scavenge a variety of ROS and RNS[12]. When given exogenously, melatonin/phytomelatonin leads to an increase in antioxidant enzyme activities, while decreasing hydrogen peroxide, superoxide and malondialdehyde concentrations. Thus, phytomelatonin from any good source of dietary supplements might play an important role in preventing oxidative damage by increasing anti-oxidative property and scavenging excessive ROS and RNS that are generated in hyperglycemic conditions of diabetes mellitus[12].

In the present study we found that there was a non-significant change in serum glucose, insulin, and liver and muscle glycogen levels after giving phytomelatonin-supplemented diet. However, phytomelatonin supplementation improved lipid profile by significantly reducing the cholesterol (TC) and LDL-C and enhancing HDL-C. Significant reduction was noted in LPO level in pancreas. The supplemental diet led to significant increase in the level of SOD and CAT in pancreas.

In conclusion, diabetes has a deleterious effect on oxidative stress markers, lipid profile, glucose, and glycogen and insulin concentrations. Supplementation of corn in diet for 40 days affected the biochemical parameters to variable degrees. The phytomelatonin treatment improved most of the antioxidant parameters under investigation. This strategy may prove relevant in prevention, development and/or retardation of the progression of diabetes. Further studies are needed to explore the use of phytomelatonin as a dietary supplement in the treatment of diabetes.

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