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Original Article

Evaluation of the Antidiabetic Activity of Hesperidin on Streptozotocin-Induced Diabetes Mellitus in Swiss Albino Mice

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ABSTRACT

Objectives: The study is planned to investigate the antidiabetic activity of hesperidin on Streptozotocin (STZ)-induced diabetes mellitus in Swiss Albino mice. **Materials and Methods:** The Swiss Albino mice were divided into six groups *viz*., normal control, diabetic control, insulin 0.75 IU/kg, hesperidin 100, 200 and 400 mg/kg, respectively (n = 6/group). All groups received treatments once daily for 21 consecutive days, except normal and diabetic control groups. The random blood glucose and body weight were determined on the pre-study day, 7, 14 and 21st day of the experiment. At the end of the study, blood samples were collected through the retro-orbital plexus puncture and used for the biochemical analysis. **Results:** Throughout the study, the diabetic control mice showed a significant increase in glucose level when compared with that of the control group, whereas the animals treated with insulin or hesperidin showed a significant reduction in the levels of glucose when compared with that of the diabetic control group. In biochemical analysis, the mice administered with the STZ showed a significant increase in the levels of AST, ALP and creatinine when compared with that of the control group. The mice administered with insulin or hesperidin showed a significant decrease in the AST and ALP when compared with that of the diabetic control group. **Conclusion:** Hesperidin showed significant antidiabetic activity on STZ-induced diabetes mellitus in mice.

Keywords: Antidiabetic activity, Hesperidin, Streptozotocin-induced diabetes mellitus, Glucose reduction.

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INTRODUCTION

According to World Health Organization (WHO), diabetes is defined as a chronic, metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which leads over time to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The WHO statistics revealed that 422 million people worldwide have diabetes in 2014, and it is also one of the leading causes of death in the world. Diabetes death rates increased by 3% between 2000 and 2019.[1] Diabetes Mellitus (DM) is a heterogeneous metabolic disorder resulting from defective insulin secretion, resistance to insulin action or both leading to prolonged hyperglycemia. Chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of different organs especially, the eyes, kidneys, nerves, heart, and blood vessels.[1]

Globally, the number of people with DM has increased in the past three decades, and DM is the ninth major cause of death. There are two types of DM. Those are Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM). About 1 in 11 adults worldwide now have DM, 90% of whom have T2DM.[2] T1DM, also known as autoimmune diabetes, is a chronic disease characterized by insulin deficiency due to pancreatic β-cell loss and leads to hyperglycaemia. T2DM is a more commonly occurring diabetes worldwide which leads to microvascular and macrovascular complications. Although sufficient knowledge and awareness were created, still the incidence and prevalence keep on rising globally.[3,4] T2DM develops a number of serious and life-threatening complications. Severe diabetic patients will develop conditions like ischemic heart disease, peripheral vascular disease and cerebrovascular disease at a macrovascular level which is a very high chance of morbidity and mortality. At a microvascular level, complications like vision impairment (retinopathy), kidney disease (nephropathy) and neuronal damage. These complications will lead to irreversible blindness, chronic kidney diseases and non-traumatic lower limb amputations. Briefly, it can be said that these complications will compromise the patient's overall quality of health.[5]

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One of the therapies for DM is Tight Glycemic Control (TGC) which helps to reduce the risk for a few vascular events. Antidiabetic drugs include biguanides, sulfonylureas, meglitinide, thiazolidinedione, Dipeptidyl peptidase 4 (DPP-4) inhibitors, Sodium-Glucose Cotransporter (SGLT2) inhibitors, and α-glucosidase inhibitors are used in the management the DM.[6] Herbs, herbal formulations and plant bioflavonoids are also commonly used in the Asian continent as a Traditional Medicine for diabetes. Hesperidin is one of the safest and most important bioflavonoids which possesses a wide range of pharmacological properties including antiallergic, antimicrobial, antioxidant, anti-inflammatory and antihypertensive activities.[7] Hesperidin is rich in citrus fruits such as lemon, sweet orange and grapefruits.[8] In an *in silico* analysis, hesperidin exhibited antidiabetic activity by targeting Glucose Transporter Type 4 (GLUT4), which plays a major role in the pathophysiology of T2DM.[9] The antidiabetic effect of hesperidin is not clear. Hence, the present study is planned to investigate the antidiabetic activity of hesperidin on Streptozotocin (STZ)-induced diabetes mellitus in Swiss albino mice.

MATERIALS AND METHODS

Animals

Healthy, male Swiss albino mice weighing 20-25 g was used for the antidiabetic studies. All the animals were obtained from Central animal house, AIMST University, Malaysia. All the mice were maintained under standard laboratory conditions (temperature 24-28°C, relative humidity 60%-70%, and 12 h of dark light cycles) and the mice were fed with rodent-pelleted food and water *ad libitum*. Prior to the experiments, approval was obtained from the AIMST University Human and Animals Ethics Committee [AUAEC/FOP/2021/05] and the study was conducted according to the Animal Research Review Panel guidelines.

Compounds

Hesperidin and STZ were purchased from Sigma Aldrich.

Methods

Overnight fasted mice were administered with a single intraperitoneal injection of 55 mg/kg of STZ (freshly dissolved in 0.1 M cold citrate buffer [pH = 4.5]) to induce DM.[10] 30 min after the injection, the mice were allowed free access to food and water. After STZ injection, mice were given a 5% dextrose solution for the next 24 hr. The development of diabetes was confirmed after 48 h of the STZ injection and mice with fasting blood glucose levels >11 mmol/L were considered as diabetic and used for the experiments.[10] The animals were divided into control and treatment groups. The diabetic animals were randomly assigned to groups II – VI. The animals were divided (n = 6) as follows:

Group I: Normal control

Group II: Diabetic control

Group III: Insulin 0.75 IU/kg

Group IV: Hesperidin 100 mg/kg

Group V: Hesperidin 200 mg/kg

Group VI: Hesperidin 400 mg/kg

The animal dose of insulin and hesperidin were selected based on the literature.[8,11] All groups received treatments once daily for 21 consecutive days. During the experimental period changes in body weight and blood glucose levels were measured at regular intervals. At the end of the study, blood samples were collected through the retro-orbital plexus puncture and used for the biochemical analysis.

Body weight analysis

Changes in the body weight of experimental animals were recorded at regular intervals.

Biochemical analysis

On 7, 14 and 21 days of the study, a few microliters of the blood sample were collected in the tail vein for estimation of glucose levels using a glucometer (Sannuo GA-3 Blood Glucose Monitor, Changsha Sinocare Inc., China).[12] At the end of the experiment, a few Millilitres (mL) of the blood sample were collected from retro-orbital plexus puncture in the plain glass tube.[12] The serum was separated from the blood sample by centrifuging at 3000 RPM for 20 min. The serum samples were used for estimation of biochemical parameters such as Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), creatinine and urea using Reflotron Plus biochemical analyzer (Roche Diagnostics, Germany) with the help of commercially available Reflotron strips.

Statistical analysis

The mean ± Standard Error of the Mean (SEM) values were calculated for each group. Statistical differences among the groups were determined using One-way ANOVA. $P< 0.05$ was considered to be significant.

RESULTS

The effects of insulin and hesperidin on the body weights of diabetic mice were summarized in Figure 1. The diabetic mice administered with insulin 0.75 IU/kg and hesperidin 100, 200 and 400 mg/kg did not show any significant changes in body weight when compared with that of the diabetic control group. Decreases in body weight were observed in the diabetic control group when compared with that of the normal control group from the first week onward, but the results were not significant.

The effects of insulin and hesperidin on the blood glucose of diabetic mice were summarized in Figure 2. The mice administered with the STZ showed a significant increase in

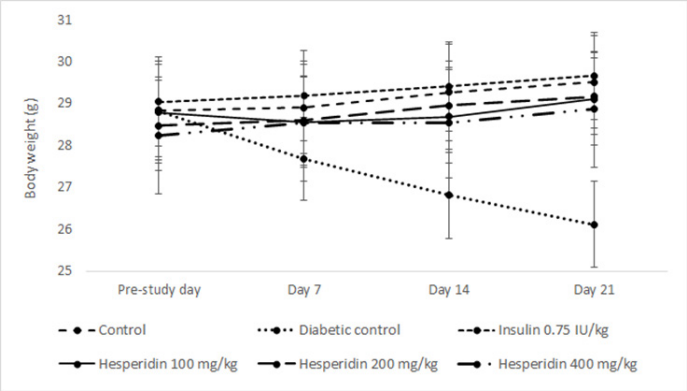

Figure 1: Effect of hesperidin on body weight (g) of diabetic mice. All the values are expressed as mean ± SEM ($n = 6$).

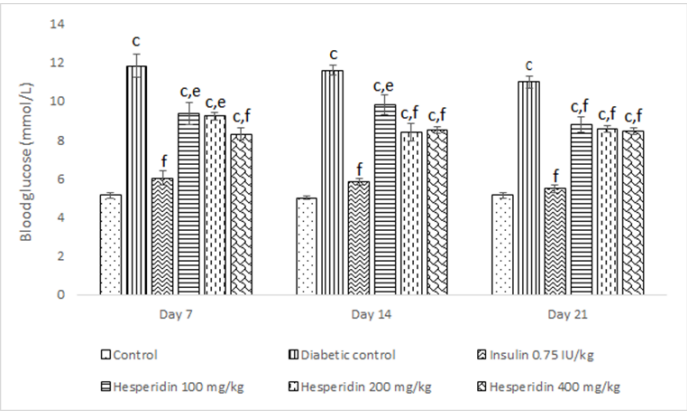

Figure 2: Effect of hesperidin on blood glucose (mmol/L) of diabetic mice. All the values are mean ± SEM ($n = 6$). $^{c}p<0.001$ compare with that of the control. $^{e}p<0.01$ and $^{f}p<0.001$ compare with that of the diabetic control (one-way ANOVA followed by Turkey's *post-hoc* test).

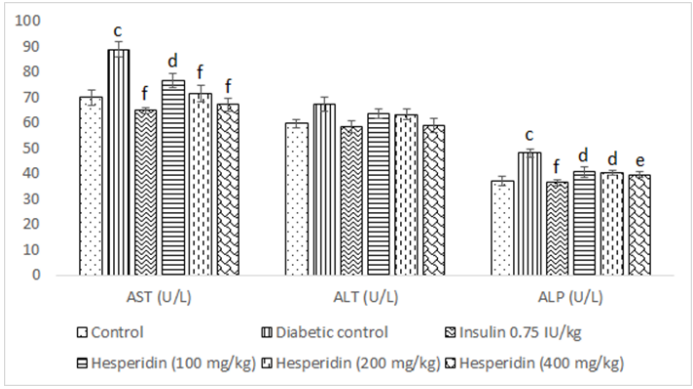

Figure 3a: Effect of hesperidin on biochemical parameters of diabetic mice. All the values are mean ± SEM ($n = 6$). $^{c}p<0.001$ compare with that of the control. $^{d}p<0.05$, $^{e}p<0.01$ and $^{f}p<0.001$ compare with that of the diabetic control (one-way ANOVA followed by Turkey's *post-hoc* test).

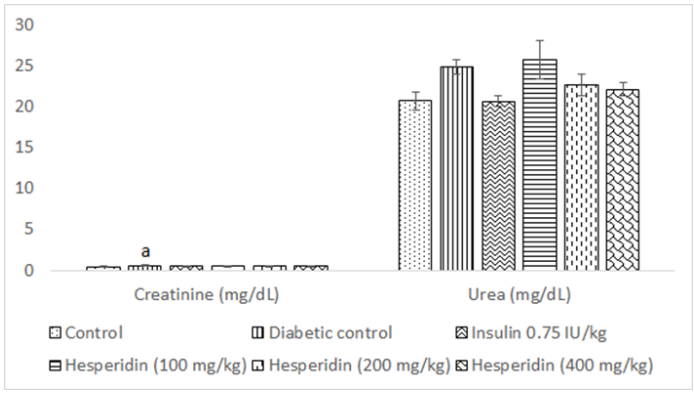

Figure 3b: Effect of hesperidin on biochemical parameters of diabetic mice. All the values are mean ± SEM ($n = 6$).

the levels of blood glucose level when compared with that of the control. The diabetic mice administered with insulin and hesperidin (100, 200 and 400 mg/kg) showed a significant decrease in the glucose level starting from Week 1 onwards when compared with that of the diabetic control.

Effects of insulin and hesperidin on the biochemical parameters of diabetic mice were summarized in Figures 3a and 3b. The mice administered with the STZ showed a significant increase in the levels of AST, ALP and creatinine when compared with that of the control. The diabetic mice administered with insulin or hesperidin showed a significant decrease in the levels of AST and ALP when compared with that of diabetic control. There were no significant changes in the levels of ALT and urea when compare with that of the control.

DISCUSSION

In this study, the antidiabetic effect of hesperidin was studied against STZ-induced diabetes mellitus in mice. STZ is known as the glucosamine-nitrourea and is obtained from the gram-positive bacterium, *Streptomyces achromogene*. STZ is used in the treatment of pancreatic beta cell carcinoma and is used to induce diabetes mellitus in rodents.[13] STZ induce diabetes mellitus by destroying the pancreatic beta-cells.[14] As per the study considered, the gender of the mice has a great influence on the development of diabetes mellitus. Healthy, male Swiss albino mice were used for the experiment because female mice are less sensitive to STZ than males. Moreover, female mice were associated with a diminished survival rate due to severe induction of diabetes by STZ.[15] Due to different sensitivities to the STZ will be displayed by animals of different strains, the Swiss albino species were chosen as they were reliably sensitive to STZ.

STZ-induced diabetes is characterised by hyperglycemia and decreased body weight. In the present study, diabetic mice showed hyperglycemia and a reduction in body weight. The diabetic mice administered with insulin or hesperidin prevented the STZ-induced changes in the body weight and blood glucose levels. Li *et al.*, reported the protective impact of hesperidin in STZ-induced body weight reduction in rats and the study results revealed that hesperidin reduced the negative effects of STZ on body weight.[16] Hesperidin also

improves insulin sensitivity by preventing the development of insulin resistance and diabetes in alloxan and High Fat Diet (HFD)-induced insulin resistance rats. Hesperidin also regulates glycolysis and gluconeogenesis by enhancing the activity of glucokinase, inducing the phosphorylation of Insulin Receptor (IR) and Phosphoinositide-Dependent Kinase 1 (PDK1), and decreasing the activity of Glucose-6-phosphatase (G6Pase) and Phosphoenolpyruvate carboxykinase (PEPCK) in the liver.[17]

In the present study, STZ-administered animals showed a significant increase in the levels of AST, ALP and creatinine when compared with that of the control. The elevated levels of AST and ALP in diabetic mice are considered significant markers of liver dysfunctions and elevated creatinine level is considered significant markers of renal dysfunctions.[18] In the present study, hesperidin attenuated the STZ effect on liver and renal markers, which indicates that hesperidin also exhibits hepatoprotective and nephroprotective effects. Hesperidin is known for its hepatoprotective and nephroprotective effects.[19,20]

CONCLUSION

Hesperidin showed significant antidiabetic activity on streptozotocin-induced diabetes mellitus in mice. The antidiabetic potential of hesperidin is comparable with that of insulin, which is evidenced by the restoration of blood glucose levels to normal levels.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ALP: Alkaline Phosphatase; **ALT:** Alanine Aminotransferase; **AST:** Aspartate Aminotransferase; **DM:** Diabetes Mellitus; **DPP-4:** Dipeptidyl peptidase 4; **G6Pase:** Glucose-6-phosphatase; **GLUT4:** Glucose Transporter Type 4; **HFD:** High Fat Diet; **IR:** Insulin Receptor; **mL:** Millilitres; **PDK1:** Phosphoinositide-Dependent Kinase 1; **PEPCK:** Phosphoenolpyruvate carboxykinase; **SEM:** Standard Error of the Mean; **SGLT2:** Sodium-Glucose Cotransporter; **STZ:** Streptozotocin; **T1DM:** Type 1 Diabetes Mellitus; **T2DM:** Type 2 Diabetes Mellitus; **TGC:** Tight Glycemic Control; **WHO:** World Health Organization.

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