MODULATORY EFFECT OF BLACKBERRY ON BIOCHEMICAL PARAMETERS AND INSULIN SIGNALING IN TYPE 2 DIABETIC ANIMAL MODEL

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1. INTRODUCTION

Diabetes Mellitus is a major health problem characterized by hyperglycemia and disturbances in metabolism, among which type 2 diabetes mellitus (T2DM) accounts for approximately 90%–95% of patients. The occurrence is usually the outcome of both genetic and environmental factors and their interactions (NING *et al.*, 2022).

Chronic hyperglycemia is the main root of ROS production and consequently oxidative stress induction and interruption in lipid and glucose metabolism. Most complications in diabetes come from dysregulation of glucose, metabolism of lipid and in insulin signaling pathways (POURFARJAM *et al.*, 2017).

The phosphatidylinositol 3-kinase (PI3K) and serine/threonine kinase protein B (AKT) pathway function as a key downstream component of insulin signaling. This PI3K/AKT cascade is critical for regulating cell growth, differentiation, and glucose metabolism, and its dysregulation is strongly associated with insulin resistance and the development of T2DM (MANNING; TOKER, 2017). Studies have shown that compounds capable of activating the PI3K/AKT pathway can markedly improve insulin sensitivity and contribute to the effective management of T2DM (HU *et al*., 2014).

Numerous studies indicate that natural compounds extracted from medicinal plants can be effective in managing metabolic disorders like T2DM (YEDJOU *et al.,* 2023). In this context, blackberry (*Rubus sp.*) is a rich source of anthocyanins and other essential phenolic compounds that play significant roles in the prevention and management of various health conditions, including diabetes, obesity, cancer and coronary heart disease (BADER UL AIN *et al.*, 2022). Multiple studies have highlighted the link between blackberry consumption and a range of health benefits (TONY *et al.*, 2023; GIL-MARTÍNEZ *et al.*, 2023). For this reason, the current study aims to explore its modulatory effect on biochemical parameters and insulin signaling pathway in type 2 diabetic experimental model.

2. METHODOLOGY

T2DM model was induced in male Wistar rats by using a high fat diet (HFD) for 3 weeks and a single i.p. dose of streptozotocin (STZ – 35 mg/kg). The animals were divided into 4 groups: Control, T2DM, T2DM plus *Rubus sp.* (200 mg/kg by gavage) and T2DM plus Metformin (250 mg/kg by gavage). At the end of the

experimental protocol, the animals were euthanized for sample collection to analyze biochemical parameters and insulin signaling pathway.

Serum was used for biochemical parameters analysis. Glucose, total cholesterol, fractions (VLDL, LDL and HDL), and triglyceride (TG) levels were determined using a colorimetric enzymatic method (Labtest, MG, Brazil).

For oxidative stress parameters and insulin signaling pathway the cerebral cortex was homogenized. RS levels were measured following the method described by ALI *et al*. (1992) by the oxidation of 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Results are expressed as µmol DCF/mg of protein. Superoxide dismutase (SOD) activity was determined according to MISRA; FRIDOVICH (1972). Catalase (CAT) activity assay was measured according to HAMZA; HADWAN (2020). Enzyme activities were reported as units/mg of protein.

For insulin signaling pathway, mRNA expression of NRF2, GSK3β, PI3K, IRS1 and FOXO3a were analyzed in the cerebral cortex by using RT-PCR. Statistical analyses were performed by GraphPad Prism software (version 8.0).

3. RESULTS AND DISCUSSION

HFD+STZ increased the blood glucose, cholesterol, TG, LDL levels in all groups when compared with CT ($p<0.05$). The treatment with BFE and Met did not reduce the blood glucose levels compared to T2DM group ($p>0.05$, Figure 1A). Both treatments (BFE and Met) ($p<0.001$) were able to reduce the cholesterol total levels compared to T2DM group (Figure 1B). Similar results were observed for TG levels (Figure 1C). LDL level was also prevented by both treatments ($p>0.05$, Figure 1D). A reduction of HDL was observed in T2DM group and BFE was able to protect against this alteration ($p<0.05$, figure 1E).

As regards redox status, HDF+STZ increased the level of RS and decreased the activity of SOD and CAT. However, treatment with Met and BFE significantly decreased the level of RS (Met: $p<0.001$, BFE: $p<0.001$) and increased the activity of SOD ($p<0.001$, figure 2B) and CAT ($p<0.01$, figure 2C).

Figure 3 illustrates the mRNA expression of PI3K, IRS-1, GSK3β, NRF2, and FOXO3a. First, PI3K ($p<0.001$, figure 3A), IRS-1 ($p<0.001$, figure 3B) and FOXO3a ($p<0.001$, figure 3E) were increased in the group treated with BFE in relation to control and T2DM groups. Also, GSK3β ($p<0.001$, figure 3C) and NRF2 ($p<0.001$, figure 3D) expression was reduced in the HFD+STZ group compared to control group and the treatment with BFE was able to protect against GSK3β ($p<0.001$) expression changes.

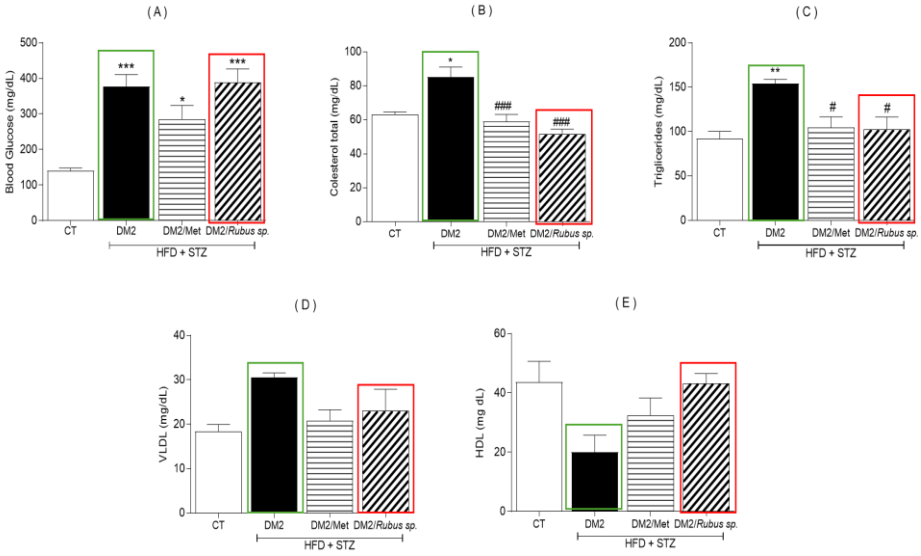

Figure 1. Effect of BFE and Met treatment on serum level of glucose (A), cholesterol (B), triglycerides (C), VLDL (D), HDL (E) in an animal model of T2DM.

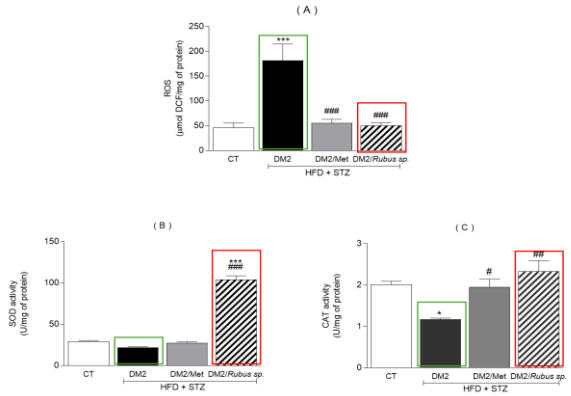

Figure 2. Effect of BFE and Met treatment on ROS, SOD and CAT activity in cerebral cortex from an animal model of T2DM.

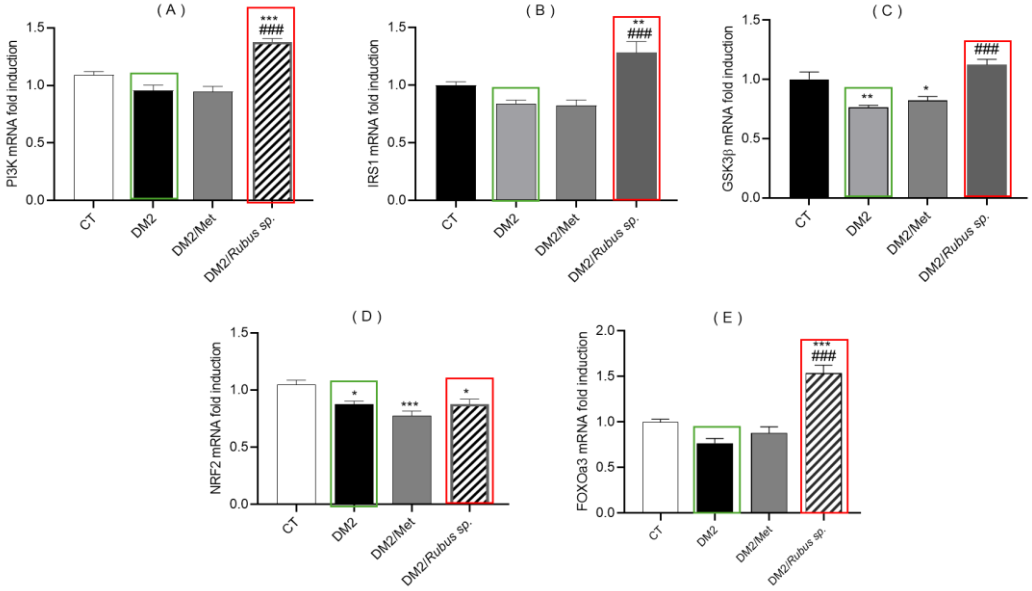

Figure 3. Evaluation of mRNA expression of PI3K, IRS1, GSK3β, NRF2 and FOXO3a in cerebral cortex from an animal model of T2DM.

4. CONCLUSION

In conclusion, our study showed that the *Rubus sp.* extract effectively modulated biochemical and oxidative stress markers, including enhanced activities of SOD and CAT, while also upregulating the downstream insulin signaling pathway. These findings suggest that *Rubus sp.* extract holds strong potential as a therapeutic agent for the treatment of T2DM.

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