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# Hepatoprotective effect of morin via regulating the oxidative stress and carbohydrate metabolism in STZ induced diabetic rats

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**ABSTRACT**

**Background:** Diabetes mellitus is widely recognized as one of the leading causes of death and disability worldwide. Hyperglycaemia-mediated oxidative stress plays a significant role in the development and progression of diabetes - induced liver damage.

**Objective:** The main aim of the study was to explore the modulatory effect of the flavonoid morin (3,5,7,2',4' - pentahydroxyflavone) on oxidative stress and carbohydrate metabolism in the liver of streptozotocin (STZ) induced diabetic rats.

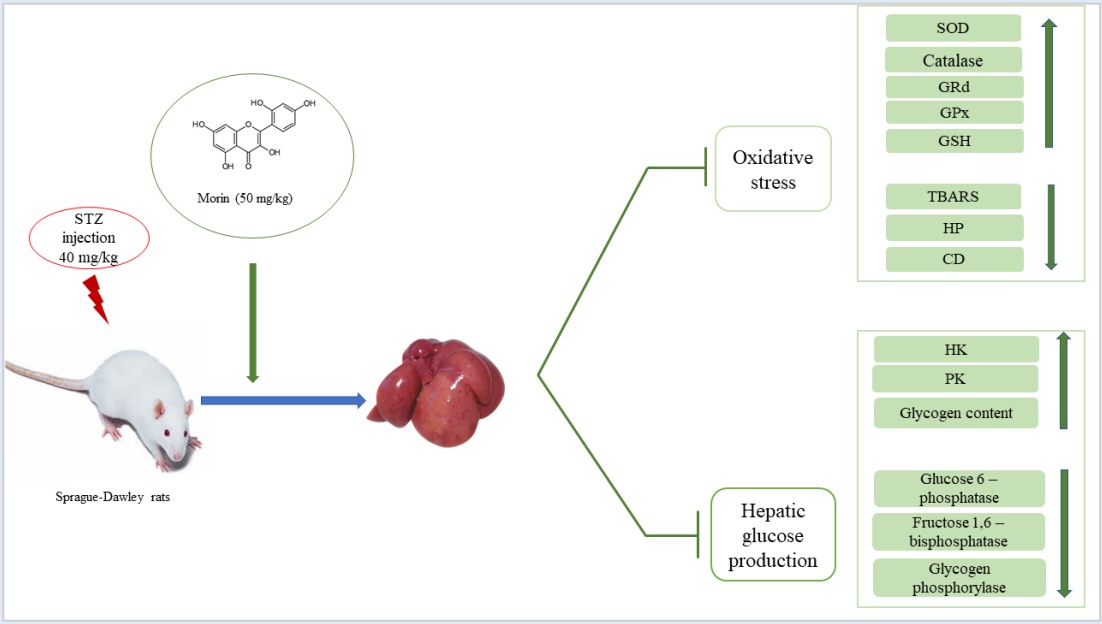
**Methods:** Diabetes was induced in male albino rats by intraperitoneal injection of streptozotocin (40 mg/kg body weight) and subsequently, the animals were given morin intragastrically at a dose of 50 mg/kg body weight for 60 consecutive days. At the end of the treatment period, the animals were sacrificed by an intraperitoneal injection of thiopentone sodium. Blood and liver tissue were collected for further biochemical evaluation and the effects were compared with diabetic rats administered metformin, a standard antidiabetic drug.

**Results:** Elevated blood glucose and HbA1c levels in diabetic rats were significantly decreased by morin administration. Morin effectively modulated the alternations in the concentration of lipid peroxidation products, activities of antioxidant

enzymes and carbohydrate metabolizing enzymes in the liver of diabetic rats. The overall effects were comparable with diabetic rats administered with metformin.

**Conclusion:** The results of our study proved that the morin administration exerts hepatoprotective activity by decreasing oxidative stress and regulating the altered activities of carbohydrate metabolizing enzymes in diabetes.

**Keywords:** diabetes, morin, liver, oxidative stress, carbohydrate metabolism






## INTRODUCTION

Diabetes mellitus is a group of chronic metabolic disorders characterized by hyperglycemia and is caused by inadequate production of insulin by pancreatic beta cells or by the action of insulin on peripheral tissues [1]. Complications of diabetes include diabetic nephropathy, neuropathy, retinopathy, cardiomyopathy, and macrovascular complications [2]. Diabetes is expected to affect 578 million people by 2030 and 700 million by 2045, which is 10.9% of the global adult population [3], making it a major health problem around the globe.

The term oxidative stress refers to an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses. Moreover, oxidative stress plays a key role in the development of diabetes and its complications [4]. All diabetes types are characterized by hyperglycemia, and persistent hyperglycemia produces ROS through various mechanisms, including the hexosamine pathway, polyol pathway, advanced glycation end-product formation and protein kinase C activation [5].

Liver is the master organ involved in oxidative reactions, detoxification reactions and glucose homeostasis [6-7]. Liver maintains glucose homeostasis by producing glucose during the postabsorptive phase and storing glucose as glycogen during the fed state [8]. About 30% to 60% of absorbed glucose is processed in the liver for storage and metabolism [9]. Several liver-related abnormalities have been associated with diabetes mellitus [10]. Hyperglycemia produced by

insulin resistance in diabetes affects carbohydrate, fat, and protein metabolisms, resulting in steatohepatitis, non-alcoholic fatty liver disease, cirrhosis and hepatocellular carcinoma. Combined effects of the elevated oxidative stress and inflammatory response is the main reason behind the development of these diabetes-induced liver damages [8, 10].

Currently several different classes of antidiabetic agents available and their usage depends on factors such as the type of diabetes, the situation of the patient, and the age of the person [11]. Anti-diabetic drugs are very effective; however, their side effects limit their use. The use of medicinal plants and phytochemicals as complementary and alternative therapies for the treatment of diabetes has grown dramatically over the past decade [12-13]. Morin (3,5,7,2',4'-pentahydroxyflavone) is a flavonoid present in a variety of fruits and vegetables exhibiting antioxidant [14], anticancer [15], anti-inflammatory [16], and neuroprotective [17] activities. Figure 1 represents the chemical structure of morin. In the present study, we evaluated the regulatory effect of morin on oxidative stress and the activities of carbohydrate metabolizing enzymes in the liver of STZ induced diabetic rats and compared with rats supplemented with metformin, an antidiabetic drug.

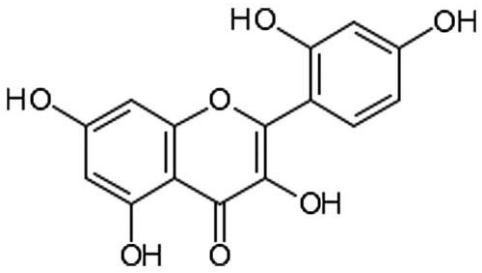



**Figure 1**. Chemical structure of morin

## MATERIALS AND METHODS

**Chemicals:** Chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), SRL Pvt. Ltd. (Mumbai, India), Merck Millipore (Molsheim, France) and Agappe Diagnostics Ltd., India.

**Experimental animals:** Male albino Sprague-Dawley rats (150-250 g) were used in the current study. The animals were housed in polypropylene cages and maintained under standard conditions [12 hours light and 12 hours dark cycles, (25 ± 10°C)]. They were fed with standard laboratory animal feed (VRK Nutritional Solutions laboratory animal feed with protein-14%, fiber-4%, fat-5%, carbohydrate-60%, minerals-1.5% and moisture-10%) and water *ad libitum*. All the animal care was taken as per the guidelines of The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the experimental protocol was approved by Institutional Animal Ethics Committee [IAEC-KU-07/2018-19-BCH-SM (41)].

**Induction of diabetes and experimental design:** Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin in 0.1M citrate buffer (pH 4.5) at a dose of 40 mg/kg body weight [18]. After three days of STZ injection, blood glucose was estimated and rats with blood glucose level > 250 mg/dL were considered as diabetic.

The study used 30 rats randomly divided into five groups with six rats in each group. N: Normal control, N+ Mo: Normal rats received 50 mg/kg body weight morin, D: Diabetic control, D + Mo: Diabetic rats received 50 mg/kg body weight morin, D + Met: Diabetic rats received 100 mg/kg body weight metformin. Commercially

available morin (Sigma-Aldrich, CAS Number: 654055-01-3) and metformin (Okamet-500, Cipla Ltd) were dissolved in distilled water and administered orally at a dose of 50 mg/kg body weight [19] and 100 mg/kg body weight [20], respectively. After the 60 days of treatment period, the animals were sacrificed, blood and liver tissue were collected for biochemical analysis.

**Biochemical analysis:** Blood glucose and glycated hemoglobin (HbA1c) levels were measured using kits purchased from Agappe Diagnostics, Pvt. Ltd. Insulin was estimated by ELISA method (Rat/mouse insulin ELISA kit; Merck Millipore).

The activities of antioxidant enzymes in the liver were assayed; catalase by the method of Maehly and Chance [21], superoxide dismutase (SOD) by the method of Kakkar et al. [22], Glutathione reductase (GRd) by the method of David and Richard [23], glutathione peroxidase (GPx) by the method of Agergaard and Jensen [24] and reduced glutathione content (GSH) by the method of Patterson and Lazarow [25]. A method of Ohkawa et al. [26] was used to estimate the concentration of lipid peroxidation products such as thiobarbituric acid reactive substances (TBARS), hydroperoxides (HPs) and conjugated dienes (CDs) in the liver.

Activities of glycolytic enzymes in the liver hexokinase and pyruvate kinase were determined by the methods of Crane and Sols [27] and Bucher and Pfleiderer [28], respectively. We assessed the activities of gluconeogenic enzymes, glucose 6 phosphatase using the method of Nordlie et al. [29] and fructose 1,6 bis phosphatase by Pontremoli [30]. The liver glycogen content was estimated by the method of Carroll et al. [31] and glycogen phosphorylase activity by Sutherland and Wosilait [32].

**Statistical analysis:** The values were expressed as mean ± SD. The statistical analysis was carried out by one-way analysis of variance using SPSS (version 22) statistical analysis program. Duncan's post hoc multiple comparison tests were used to determine significant differences among groups: $P < 0.05$ was considered significant.

## RESULTS

**Serum glucose and HbA1c level:** Serum glucose (Figure 2A) and HbA1c (Figure 2B) levels were significantly ($p<0.05$) elevated in diabetic control rats as compared to normal rats and normal rats treated with morin. Administration of morin and metformin significantly ($p<0.05$) decreased the fasting blood glucose and HbA1c levels in diabetic rats. There was no statistically significant difference in serum glucose and HbA1c levels in morin and metformin treated diabetic groups.

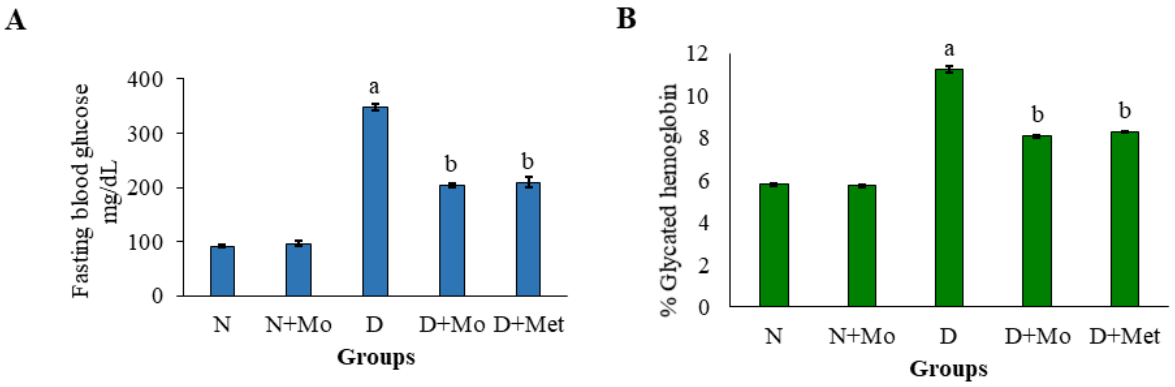



**Figure 2**. Effect of morin on blood glucose (A) and HbA1c (B). N has been compared with N + Mo and D ('a' indicates values were significantly different from N), and D is compared with D + Mo and D + Met ('b' indicates values were significantly different from D). Significance accepted at $p < 0.05$.

**Serum insulin level:** Figure 3 represents the serum insulin levels. Serum insulin level was significantly ($p<0.05$) decreased in diabetic rats than normal rats and normal rats treated with morin. Treatment of diabetic rats with morin and metformin significantly ($p<0.05$) increased serum insulin levels. The prominent effect was shown by morin treatment than metformin in diabetic rats.

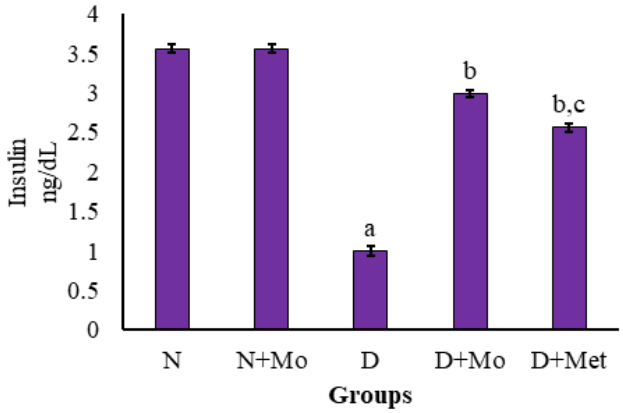



**Figure 3**. Effect of morin on serum insulin levels. N has been compared with N + Mo and D ('a' indicates values were significantly different from N), and D is compared with D + Mo and D + Met ('b' indicates values were significantly different from D) and D + Mo is compared with D + Met ('c' indicates values were significantly different from D + Mo). Significance accepted at $p < 0.05$.

**Activities of antioxidant enzymes:** Activities of enzymatic antioxidants such as SOD, catalase, GPx and GRd (Table 1) and the concentration of non-enzymatic antioxidant GSH (Figure 4) were significantly ($p<0.05$) decreased in the liver of diabetic rats. Whereas treatment of diabetic rats with morin and metformin significantly ($p<0.05$) improved the activities of these antioxidant enzymes.

**Table 1.** Activities of antioxidant enzymes in liver.

| Groups | SOD (U/mg protein) | Catalase ($\times10^{-3}$U/mg protein) | GPx (U/mg protein) | GRd (U/mg protein) |
|---|---|---|---|---|
| N | 1.82 ± 0.05 | 6.44 ± 0.34 | 50.65 ± 3.23 | 129.26 ± 3.89 |
| N + Mo | 1.90 ± 0.06 | 6.80 ± 0.21 | 51.72 ± 2.64 | 130.67 ± 4.53 |
| D | 0.42 ± 0.04[a] | 2.07 ± 0.18[a] | 20.47 ± 1.57[a] | 79.86 ± 4.71[a] |
| D + Mo | 1.02 ± 0.07[b] | 4.50 ± 0.15[b] | 34.94 ± 3.07[b] | 109.41 ± 4.99[b] |
| D + Met | 0.83 ± 0.04 [b, c] | 3.70 ± 0.15 [b, c] | 32.31 ± 3.17[b] | 107.00 ± 2.97[b] |

Values are expressed as mean ± SD (n=6). N has been compared with N + Mo and D ('a' indicates values were significantly different from N), and D is compared with D + Mo and D + Met ('b' indicates values were significantly different from D) and D + Mo is compared with D + Met ('c' indicates values were significantly different from D + Mo). Significance accepted at $p < 0.05$

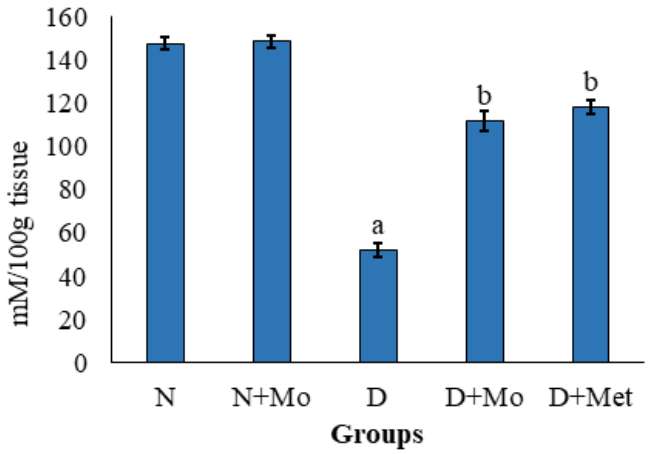



**Figure 4.** Effect of morin on the concentration of GSH in liver. Values are expressed as mean ± SEM (n=6). N has been compared with N + Mo and D ('a' indicates values were significantly different from N), and D is compared with D + Mo and D + Met ('b' indicates values were significantly different from D). Significance accepted at $p < 0.05$.

**Concentration of lipid peroxidation products:** The concentration of lipid peroxidation products such as TBARS (Figure 5A), CD and HP (Figure 5B) were significantly ($p<0.05$) elevated in diabetic rats. Supplementation of morin and metformin to diabetic rats significantly ($p<0.05$) decreased the concentration of TBARS, CD and HP. Effects were comparable in normal and normal rats treated with morin.

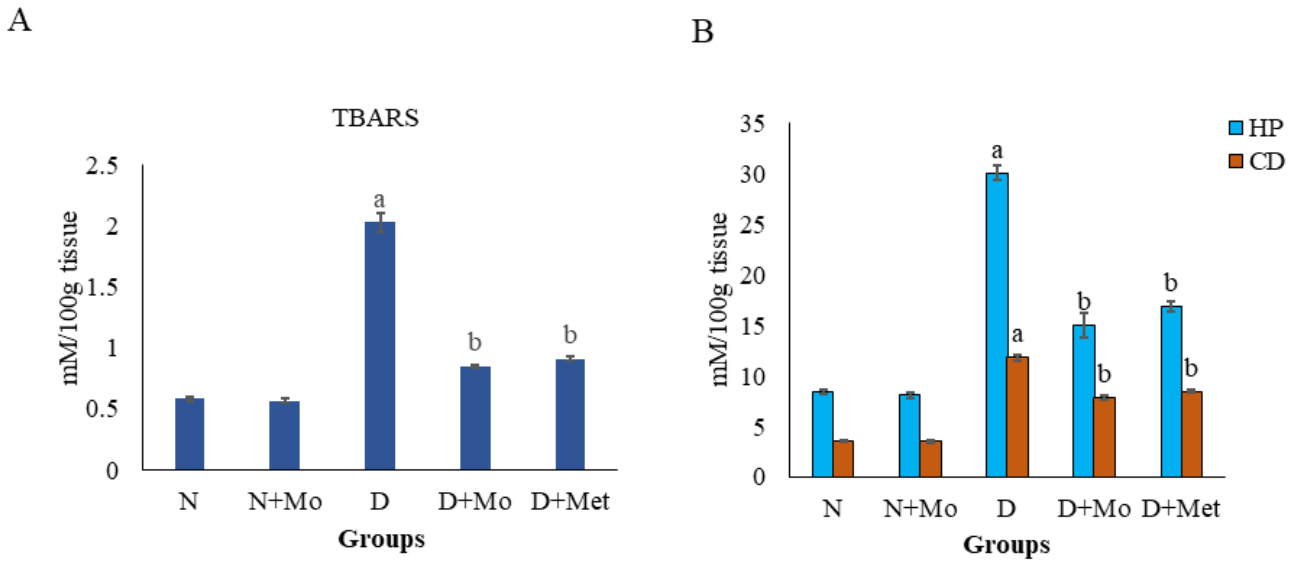



**Figure 5**. Effect of morin on the concentration of TBARS (**A**), HP and CD (**B**). Values are expressed as mean ± SEM (n=6). N has been compared with N + Mo and D ('a' indicates values were significantly different from N), and D is compared with D + Mo and D + Met ('b' indicates values were significantly different from D). Significance accepted at $p < 0.05$.

**Activities of glycolytic enzymes:** Activities of glycolytic enzymes hexokinase (HK) and pyruvate kinase (PK) were decreased significantly ($p<0.05$) in the liver of diabetic rats as compared with normal rats and normal rats treated with morin. Diabetic rats treated with both morin and metformin showed a significantly ($p<0.05$) improved activities of HK and PK. There was no significant difference in the activities of these enzymes in normal rats and normal rats treated with morin (Figure 6).

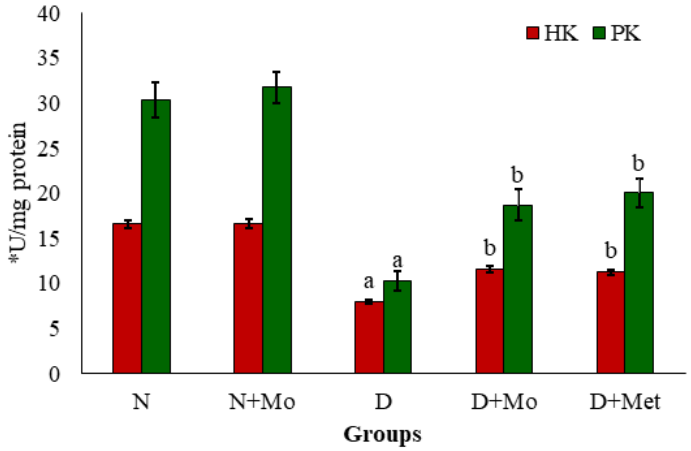



**Figure 6**. Effect of morin on the activities of glycolytic enzymes HK and PK in liver. Values are expressed as mean ± SEM (n=6). N has been compared with N + Mo and D ('a' indicates values were significantly different from N), and D is compared with D + Mo and D + Met ('b' indicates values were significantly different from D). Significance accepted at $p < 0.05$. *Units – mg glucose phosphorylated/min/mg protein.

**Activities of gluconeogenic enzymes:** Activities of gluconeogenic enzymes glucose 6 – phosphatase and fructose 1,6 -bisphosphatase were significantly ($p<0.05$) increased in diabetic rats as compared with normal rats and normal rats treated with morin. Whereas treatment of diabetic rats with morin and metformin significantly ($p<0.05$) decreased the activities of these enzymes in the liver than diabetic control rats. Results were comparable in normal and normal rats treated with morin (Table 2).

**Table 2.** Activities of gluconeogenic enzymes in liver.

| Groups | Glucose 6 – phosphatase (*U/mg protein) | Fructose 1,6 bisphosphatase (*U/mg protein) |
|---|---|---|
| N | 27.62 ± 1.38 | 72.09 ± 4.49 |
| N + Mo | 26.86 ± 1.34 | 71.77 ± 4.52 |
| D | 70.30 ± 3.51[a] | 130.83 ± 6.43[a] |
| D + Mo | 45.75 ± 2.28[b] | 100.65 ± 5.51[b] |
| D + Met | 42.15 ± 2.10[b] | 92.82 ± 2.49[b] |

Values are expressed as mean ± SD (n=6). N has been compared with N + Mo and D ('a' indicates values were significantly different from N), and D is compared with D + Mo and D + Met ('b' indicates values were significantly different from D). Significance accepted at $p < 0.05$. *Units - µmol of Pi liberated/h/mg protein.

**Activity of glycogen phosphorylase and glycogen content:** Diabetic rats showed significantly ($p<0.05$) higher activity of glycogen phosphorylase enzyme (Figure 7A) and a decreased concentration of glycogen (Figure 7B) in the liver as compared with normal and normal rats treated with morin. Diabetic rats supplemented with morin and metformin significantly ($p<0.05$) decreased the activity of glycogen phosphorylase and improved the liver glycogen content than untreated diabetic rats.

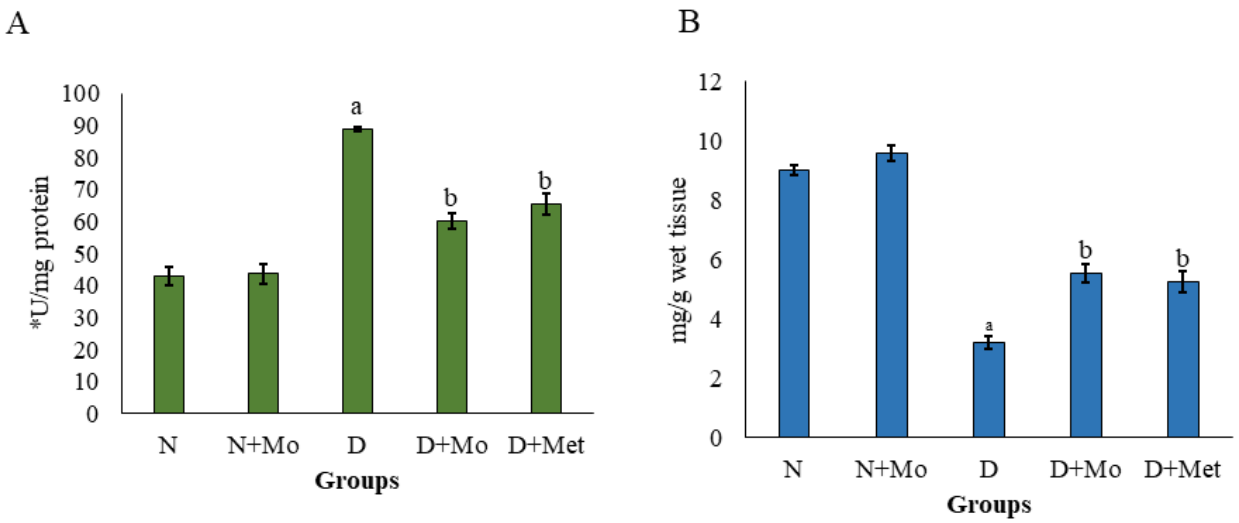



**Figure 7.** Effect of morin on the activity of glycogen phosphorylase (A) and glycogen content (B) in the liver. Values are expressed as mean ± SEM (n=6). N has been compared with N + Mo and D ('a' indicates values were significantly different from N), and D is compared with D + Mo and D + Met ('b' indicates values were significantly different from D). Significance accepted at $p < 0.05$.

## DISCUSSION

Liver plays a significant role in the body including vascular, metabolic, secretory, immunological, and excretory functions [33]. Type 2 Diabetes mellitus (T2D) is associated with alterations of liver functions [34]. Hyperglycemia in T2D results from insulin resistance and deficiency. Deficiency in insulin results in decreased insulin-dependent glucose uptake in muscle, increased glucose production from liver, and increased fat mobilization from adipose tissue [35]. Insulin resistance promotes adipocyte lipolysis, and the released fatty acids are eventually accumulated in the liver. Adipocytes also release TNF-α and leptin, resulting in further hepatocyte damage [10]. Oxidative stress plays an important role in the pathogenesis of diabetes, [36] whereas oxidative stress and hyperglycemia together induce inflammation and hepatocellular necrosis [10]. Moreover, the liver plays a unique role in maintaining glucose homeostasis by producing endogenous glucose during fasting and storing it postprandially. Hepatic glucose production is the outcome of glucose fluxes through glycolysis, gluconeogenesis, glycogenesis, glycogenolysis and other pathways. Under T2D conditions, these mechanisms are dysregulated, contributing to hyperglycemia in both fasting and postprandial states [37]. In the present study, we compared the effects of morin on oxidative stress and carbohydrate metabolism in the liver under experimental diabetes with those of metformin, the standard antidiabetic drug.

The characteristic glycemic markers of diabetes are blood glucose, HbA1c and insulin [38]. Elevated levels of serum glucose, HbA1c and decreased insulin were observed in STZ induced diabetic rats [39]. Like these reports, the present study showed that the diabetic rats exhibited decreased serum insulin and elevated levels of blood glucose and HbA1c. The oral administration of morin has previously been shown to effectively lower blood glucose levels and improve insulin levels in diabetic rats [19]. In our study also, the treatment of diabetic rats with morin positively regulated the glycemic control and increased insulin levels.

Defense against ROS produced under aerobic metabolism is performed by a system of antioxidant enzymes and compounds, which are capable of

neutralizing free radicals and preventing the excess production of ROS. Most important antioxidant enzymes are SOD, catalase, GRd, GPx and GSH. SODs are metalloenzymes that catalyze dismutation of superoxide anion ($O^{2-}$) into hydrogen peroxide ($H_2O_2$) and molecular oxygen [40]. Catalase converts $H_2O_2$ to molecular oxygen and water. GPx is another enzyme involved in the conversion of $H_2O_2$ to water [41]. The most abundant low-molecular weight thiol in cells, GSH plays a crucial role in protecting cells against ROS, xenobiotics, and other toxins [42]. The main function of GRd is maintaining the supply of reduced glutathione in cells [43]. Damage to the antioxidant system plays a vital role in the pathogenesis of various diseases and long-term complications of diabetes are partially attributed to elevated oxidative stress [44]. Increased rate of protein glycation, lipid peroxidation and low activities of antioxidant enzymes are the main reasons for the elevated oxidative stress in diabetes. Lowered activities of antioxidant enzymes were observed in the liver of diabetic animals [45-46]. Like these reports, reduced activities of SOD, catalase, GRd, GPx and reduced concentration of GSH were observed in the diabetic control rats. The activities of both enzymatic and non-enzymatic antioxidants were significantly improved in the liver of diabetic rats after morin administration as well. Antioxidative properties of flavonoids are mediated through direct elimination of ROS, activation of antioxidant enzymes, chelation of metals, inhibition of oxidases, reduction of α tocopherol radical and inhibition of oxidative stress-induced by nitric oxide [47]. The presence of a double bond between C2-C3 positions and hydroxyl groups at C3 and C4 positions contributes to the antioxidant properties of morin [48].

In lipid peroxidation, ROS attack the C-C double bonds of lipids, mainly polyunsaturated fatty acids (PUFA) [49]. ROS initiate lipid peroxidation by abstracting $H^+$ from PUFA and the remaining lipid carbon radical (L.) undergoes rearrangements to form conjugated dienes (CD). CDs reacts with molecular oxygen to forms lipid peroxyl radical (LOO.), which abstract H+ from other lipids species to form lipid hydroperoxides (LOOHs). LOOH are the primary products of lipid peroxidation and malondialdehyde is the aldehyde product of lipid peroxidation [50]. An elevated concentration of lipid peroxidation products was observed in STZ-induced experimental diabetes [51]. Likewise, lipid peroxidation products such as TBARS, HP and CD were increased in the liver of diabetic control rats. Supplementation with morin reduced the concentration of all these lipid peroxidation products and this result indicate the anti-lipid peroxidative ability of morin. Hydroxyl groups at 2’ and 4’ positions of morin are mainly responsible for the reduction in lipid peroxidation [48].

HK and PK catalyze the irreversible steps in glycolysis, and thus serves as the rate-limiting enzymes of glycolysis. HK is involved in the conversion of glucose to glucose 6 - phosphate, while PK is involved in the conversion of phosphoenolpyruvate to pyruvate. In previous studies, reduced activities of these enzymes were observed in diabetic rats; they are insulin-dependent enzymes, and the activities were increased by insulin treatment [52]. Like these reports, diabetic control rats displayed decreased HK and PK activity in the liver. There are several plant-derived compounds that have been shown to improve glycolytic enzyme activities under diabetic conditions [52-53]. The present study found that morin supplementation significantly improved the activities of HK and PK enzymes in the liver of diabetic rats.

Gluconeogenesis is important for maintaining glucose homeostasis under physiological conditions and crucial for the survival of animals by synthesizing glucose from non-carbohydrate substrates during fasting and starvation. Elevated gluconeogenesis is observed during insulin resistance and T2D mainly due to non – availability of insulin to suppress hepatic gluconeogenesis [54]. Rate

limiting enzymes of gluconeogenesis such as glucose 6-phosphatase [55] and fructose 1,6 bisphosphatase [56] were elevated during diabetic conditions. Similarly, diabetic control rats showed elevated levels of these enzymes, and morin and metformin reduced the activities of these enzymes in diabetic rats. Metformin reduces blood glucose levels by inhibiting gluconeogenesis [57].

Glycogen is the primary storage form of glucose in animals. Studies have shown that T2D patients have a reduced glycogen synthesis after consuming food [58]. Furthermore, the main cause of elevated hepatic glucose production under T2D is the breakdown of glycogen. Glycogen phosphorylase is the rate-limiting enzyme of glycogenolysis, catalyzes the breakdown of glycogen into glucose 1-phosphate. In our study, diabetic control rats displayed increased glycogen phosphorylase activities and decreased liver glycogen levels. In diabetic rats, morin treatment increased glycogen levels and decreased glycogen phosphorylase activity.

## CONCLUSION

Our study demonstrated that the flavonoid morin was effective in protecting diabetic rats against oxidative stress under STZ induction. The morin treatment improved the glycemic control in diabetic rats by improving liver carbohydrate metabolism. In conclusion, these findings suggest that morin may have therapeutic potential in diabetes induced liver damage.

**List of Abbreviations:** ROS: Reactive oxygen species, HbA1c: Glycated hemoglobin, SOD: Superoxide dismutase, GRd: Glutathione reductase, GPx: Glutathione peroxidase, GSH: Reduced glutathione, TBARS: Thiobarbituric acid reactive substances, HP: Hydroperoxides, CD: Conjugated dienes, STZ: Streptozotocin, T2D: Type 2 Diabetes mellitus, PUFA: Polyunsaturated fatty acids, LOOH: lipid hydroperoxides, HK: Hexokinase, PK: Pyruvate kinase

**Competing Interests:** The authors declare that there is no conflict of interest

**Authors' Contributions:** The original idea was conceived by K Sivan Shali and S Mini. This was discussed with N P Soumya and Sukanta Mondal. The focus and ideas of the paper were finally agreed upon by all authors. The experiments were conducted and analyzed by K Sivan Shali. S Mini conceptualized the main ideas behind the experiments. The main text of the paper was written by K Sivan Shali and S Mini. The manuscript was revised and edited by S Mini and N P Soumya, with Sukanta Mondal contributing to the editing and writing parts. All authors contributed to the writing and editing of the final draft.

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