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Hibiscus rosa sinensis L. anthocyanins prevent lipid peroxidation and improve antioxidant status in the liver of streptozotocin-induced diabetic rats

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ABSTRACT:

Background: Hyperglycemia and oxidative stress are hallmarks of diabetes mellitus (DM). Excessive oxidative stress is implicated in diabetic pathogenesis when endogenous antioxidants are defective.

Objective: The present study evaluates the effects of anthocyanins present in the petals of *Hibiscus rosa- sinensis* on oxidative stress and antioxidant status in streptozotocin-induced diabetic rats.

Materials and methods: Diabetes was induced in male Sprague-Dawley rats by a single intraperitoneal injection (30mg/kg) of streptozotocin. *Hibiscus rosa sinensis* anthocyanins (HA) extract (50 mg/kg body weight) orally administered to diabetic rats for 30 days. Results compared with diabetic rats provided with the standard drug metformin (150 mg/kg body weight).

Results: Altered levels of glucose, glycated hemoglobin, toxicity markers and lipid profile in serum were significantly modulated upon the administration of HA in diabetic rats. A supplementation of HA to diabetic rats reduced

Table 2: Hepatic antioxidant enzyme activities:

GROUPS	CAT (10^{-3}U/mg protein)	GPx (U/mg protein)	GRd (U/mg protein)	SOD (U/mg protein)
I	6.04 ± 0.57	30.9 ± 2.96	21.4 ± 2.05	4.48 ± 0.43
II	7.70 ± 0.73[a]	31.7 ± 3.04	25.1 ± 2.41[a]	6.01± 0.57[a]
III	3.11 ± 0.30 [a]	18.6 ± 1.78[a]	13.6 ± 1.30[a]	3.15 ± 0.30[a]
IV	4.97± 0.47[b]	27.1 ± 2.60[b]	20.1± 1.90[b]	4.19 ± 0.40[b]
V	3.90 ± 0.37 [b, c]	23.1 ± 2.20 [b,c]	17.2± 1.65[b,c]	3.70 ± 0.36[b]

Values are expressed as mean ± SD (n = 6). Group I: Normal control(N); Group II: Normal rats given 50 mg Hibiscus anthocyanin (HA) /kg body weight of rats (N+HA); Group III: Diabetic control (D); Group IV: Diabetic rats given 50 mg Hibiscus anthocyanin (HA)/kg body weight of rats (D+HA); V: Diabetic rats given 150 mg metformin/kg body weight of rats (D+M). 'a' indicates values significantly differ from normal control groups. 'b' indicates values significantly different from diabetic groups. 'c' indicates values are significantly different from HA treated groups. Significance accepted at $p<0.05$.

Reduced Glutathione content (GSH) in liver: The concentration of hepatic non-enzymatic antioxidant-reduced glutathione (GSH) has shown in figure 6. A significant decline was observed in the concentration of GSH in diabetic control rats compared to the normal control rats. In contrast, GSH concentration was significantly enhanced after treatment with HA or metformin in the liver of diabetic rats.

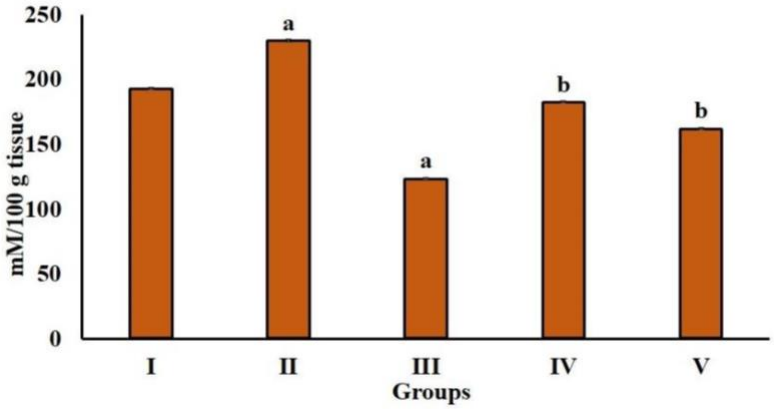

Figure 6. Reduced Glutathione content (GSH) in liver

Values are presented as mean ± SD (n = 6). Group I: Normal control(N); Group II: Normal rats given 50 mg Hibiscus anthocyanin (HA) /kg body weight of rats (N+HA); Group III: Diabetic control (D); Group IV: Diabetic rats given 50 mg Hibiscus anthocyanin (HA)/kg body weight of rats (D+HA); V: Diabetic rats given 150 mg metformin/kg body weight of rats (D+M). 'a' indicates values significantly different from normal control groups. 'b' indicates values significantly different from diabetic groups. Significance accepted at $p<0.05$.

Lipid peroxidation products in the liver: The levels of lipid peroxidation products, Thiobarbituric acid-reactive substances (TBARS), hydroperoxides (HP), and conjugated dienes (CD) in the liver of normal and experimental animals were shown in table 3. A significant elevation was observed in the levels of TBARS, HP, and CD in the liver of diabetic control rats as compared to normal control rats. Diabetic rats treated with HA or metformin showed a significant reduction in the levels of lipid peroxidation products compared to diabetic control groups (Table 3).

Table 3: Lipid peroxidation products in the liver

GROUPS	TBARS (mM/100 g tissue)	CD (mM/100 g tissue)	HP (mM/100 g tissue)
I	1.57 ± 0.149	27.8 ± 2.66	53.48 ± 5.12
II	1.44 ± 0.134	23.1± 2.22[a]	41.50 ± 3.97[a]
III	2.75 ± 0.260[a]	45.0 ± 4.35[a]	90.86 ± 8.7 [a]
IV	1.82 ± 0.175[b]	31.6 ± 3.04[b]	58.50 ± 5.61[b]
V	1.87 ± 0.80[b]	35.3 ± 3.41[b]	63.36 ± 6.09[b]

Values are expressed as mean ± SD (n = 6). Group I: Normal control(N); Group II: Normal rats given 50 mg Hibiscus anthocyanin (HA) /kg body weight of rats (N+HA); Group III: Diabetic control (D); Group IV: Diabetic rats given 50 mg Hibiscus anthocyanin (HA)/kg body weight of rats (D+HA); V: Diabetic rats given 150 mg metformin/kg body weight of rats (D+M). 'a' indicates values significantly different from normal control groups. 'b' indicates values significantly different from diabetic groups. Significance accepted at $p<0.05$

DISCUSSION

Hyperglycemia triggers oxidative stress and reduces the antioxidant defense mechanism by generating advanced glycation end products. (AGEs). As oxidative stress perturbs the antioxidant defense system, it plays a critical role in diabetes complications [29]. In the current study, we identified that the anthocyanins present in the *Hibiscus rosa sinensis* flower petals could attenuate hyperglycemia-induced hepatic injury by mitigating oxidative stress. The reduction in body weight associated with diabetes is caused by dehydration, carbohydrate loss, and the excessive breakdown of fats and proteins in tissues [30]. The results of our study corroborate this; body weights were significantly diminished in STZ-induced diabetic rats. The oral administration of HA or metformin to diabetic rats for 30 days significantly improved the body weight, which indicated the efficacy of Hibiscus anthocyanins in maintaining better glycemic control in diabetic rats.

Streptozotocin is one of the diabetogenic substances used to induce diabetes mellitus in experimental animals. The intraperitoneal injection of STZ partially damages the insulin-secreting pancreatic beta cells by breaking the DNA strand, resulting in decreased insulin biosynthesis and secretion [31-32]. The impairment in the synthesis of

insulin leads to persistent hyperglycemia [33-34]. In the present study, the blood glucose level was significantly elevated in diabetic control rats. However, treatment with HA or metformin significantly reduced the blood glucose level in diabetic rats, which suggests that *Hibiscus* anthocyanins protect the pancreatic β-cells against hyperglycemia-induced oxidative stress and could also preserve their functionality and insulin release. This result agrees with the previous studies that showed the protective role of flavonoid-rich ethyl acetate fraction of *Hibiscus rosa sinensis* petals in insulin secretion in β-cells under high glucose conditions in RIN-m5F cells [35].

Glycosylated hemoglobin (HbA1c) is a useful marker to assess the risk of diabetes mellitus. HbA1c is formed from the non-enzymatic and irreversible covalent bonding of glucose with Hb in circulation. The increase in HbA1c in diabetic patients is directly proportional to fasting blood glucose [36]. Similar to previous reports, we found that diabetic control rats had a significantly higher level of HbA1c. In contrast, rats that were treated with HA or metformin showed significant declines in HbA1c. The reduction in the level of HbA1c in HA-treated diabetic rats indicated the anti-hyperglycemic effect of *Hibiscus* anthocyanins by ameliorating glycemic control.

Hepatic marker enzymes (Alanine aminotransferase and Aspartate aminotransferase) elevated in the bloodstream indicate hepatocellular damage as these enzymes appear to have leaked from the liver into the bloodstream. [37]. Consistent with the previous reports, our results showed the activities of AST and ALT were significantly higher in diabetic control rats. However, the oral administration of HA or metformin significantly reduced the activities of AST and ALT in diabetic rats. The activities of toxicity markers were not altered in normal rats administered with HA, indicating the non-toxic nature of HA in normal conditions. These findings revealed the hepatoprotective nature of Hibiscus anthocyanins extract. Earlier studies have reported that anthocyanin-rich black rice extract protects against alcohol-induced liver damage in rats [38].

Hyperlipidemia is one of the major risk factors for cardiovascular disease in type 2 diabetes. The characteristic features of diabetic dyslipidemia include elevated plasma concentrations of triglycerides and apo B–containing lipoproteins, low HDL cholesterol, and increased concentrations of small, dense LDL-cholesterol particles [39]. Consistent with the previous reports, in the present study, diabetic rats exhibited a significant elevation of TC, TG, and LDL-C while decreasing HDL-C. Hibiscus anthocyanins or metformin administration lowered TC, TG, and LDL-C levels with an elevation of HDL-C level. A decline in serum lipid profiles observed in hibiscus anthocyanin administered diabetic rats suggests that its potential to regulate hyperlipidemia in the diabetic condition is mediated by the elevation of insulin level.

The increased production of ROS in hyperglycemia causes lipid peroxidation and tissue damage. The essential enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRd). Reduced glutathione (GSH) is a non-enzymatic antioxidant employed in free radical defense systems to remove radicals and protect biological sites [40]. SOD reduces superoxide radicals to hydrogen peroxide (H_2O_2), and catalase reduces hydrogen peroxide to water and protects tissues from reactive hydroxyl radicals [41]. The activities of the

enzymatic and non-enzymatic antioxidant systems are depleted during oxidative stress [42]. In the current study, there was a decline in the activities of antioxidants enzymes SOD, CAT, GPx and, GRd in the liver of diabetic control rats. Treatment with HA and Metformin in diabetic rats enhanced antioxidant enzymes SOD, CAT, GPx and, GRd in the liver of diabetic rats. These results suggest that Hibiscus anthocyanins extract could improve the activities of enzymatic antioxidants, reduce free radicals, and alleviate liver damage caused by oxidative stress in diabetic rats. Our results are consistent with other studies showing that an anthocyanin-rich ethanolic extract of *Vaccinium Arctostaphylos* fruit increases antioxidant markers in alloxan-induced diabetic rats. [43].

Glutathione (GSH) is a tripeptide (γ - glutamyl cysteinyl glycine) and a non-enzymatic antioxidant molecule. It is critical for cellular protection, such as detoxification of ROS, conjugation, excretion of toxic molecules, and controlling the release of inflammatory cytokines [44]. In previous studies, GSH concentration was found to decline in diabetic rats [45]. In the present study, supplementation of HA and Metformin significantly increased GSH levels in diabetic rats. We found that Hibiscus anthocyanins extract protected the liver from oxidative damage by stimulating GSH synthesis.

Oxidative processes, glucose homeostasis, and detoxification of major metabolites produced by excessive reactive oxygen species are all vital functions of the liver [46]. Lipid peroxidation is an important biomarker of free radical-mediated oxidative stress. During this process, free radicals interact with polyunsaturated fatty acids (PUFAs), leading to the formation of malondialdehyde (MDA) and 4-hydroxynonenal, which cause adverse effects such as cell necrosis and inflammation [47]. Increased lipid peroxidation induces disturbance of membrane organization, functional loss, modification of the protein, and DNA bases [48]. Diabetic rats have been shown to have elevated levels of lipid peroxidative markers (TBARS, HP, and CD) in the liver [49]. TBARS, HP, and CD were significantly elevated in the livers of diabetic control rats in the present study. Metformin and HA supplementation reduced TBARS, HP, and CD in the liver of diabetic rats. Hibiscus anthocyanins extract shows hepatoprotective properties against STZ induced hepatic injury by suppressing lipid peroxidation and restoring endogenous antioxidants. Results of the current study are consistent with previous studies showing that anthocyanin-rich mulberry extract inhibits oxidative stress induced by hyperglycemia by regulating the AMPK/ACC/mTOR pathway [50].

CONCLUSION

In conclusion, the present study showed that Hibiscus anthocyanins (50mg/kg body weight) extract might provide effective protection against oxidative stress damage in the liver of STZ induced diabetic rats. Hibiscus anthocyanins may reduce lipid peroxidation and free radical levels in hepatic tissues as well as increase enzymatic and non-enzymatic antioxidant defense. Results from this study suggest that anthocyanin fraction of *Hibiscus rosa sinensis* flower petals may be effective against hepatic complications associated with DM. This study confirmed that supplementation of anthocyanins present in the *Hibiscus rosa sinensis* flower petals could significantly modulate the complications associated with hyperglycemia by modulating oxidative stress and

regulating antioxidant enzymes and afford protection against hepatic damage.

List of abbreviations: CAT: Catalase, CD: Conjugated dienes, DM: Diabetes Mellitus, GPx: Glutathione Peroxidase, GRd: Glutathione Reductase, GSH: Reduced Glutathione, HA: *Hibiscus rosa sinensis* flower anthocyanin, HbA1c: Glycosylated Hemoglobin, HDL-C: High-density Lipoprotein cholesterol, HP: Hydroperoxides, LDL-C: Low-density Lipoprotein cholesterol, ROS: Reactive oxygen species, SGOT: Serum Glutamate Oxaloacetate Transaminase, SGPT: Serum Glutamate Pyruvate Transaminase, SOD: Superoxide dismutase, STZ: Streptozotocin, TBARS: Thiobarbituric acid reactive substances

Authors' contribution: The original idea was conceived by V S Kalpana, Jincy Mary, and S Mini. This was discussed with N P Soumya and Sukanta Mondal. The main focus and ideas of the paper were finally agreed upon by all authors. The experiments were conducted and analyzed by V S Kalpana and Jincy Mary. S Mini conceptualized the main ideas behind the experiments. The main text of the paper was written by V S Kalpana 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.

Conflicts of Interest: We wish to declare that there are no conflicts of interest associated with this study.

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