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Cardioprotective effects of Ferulic acid in Streptozotocin-induced diabetic rats

Sudha Anjali[1], Neelakanta Pillai Padmakumari Soumya[1], Sukanta Mondal[2], and Saraswathy Mini[1*]

[1]Department of Biochemistry, University of Kerala, Kariavattom Campus, Thiruvananthapuram, India- 695581; [2]ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India-560030

***Corresponding author:** Dr. Mini S, Professor, Department of Biochemistry, University of Kerala, Kariavattom Campus, Salem - Kochi - Kanyakumari Hwy, Karyavattom, Thiruvananthapuram, India- 695581

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ABSTRACT

Background: Diabetes mellitus causes changes in the structural or functional anatomy of the heart. A high blood glucose level and oxidative stress are key factors in diabetic cardiac damage. Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is a biologically active compound in many functional foods like fruits, vegetables, and medicinal herbs. It belongs to the group of cinnamic acid derivatives.

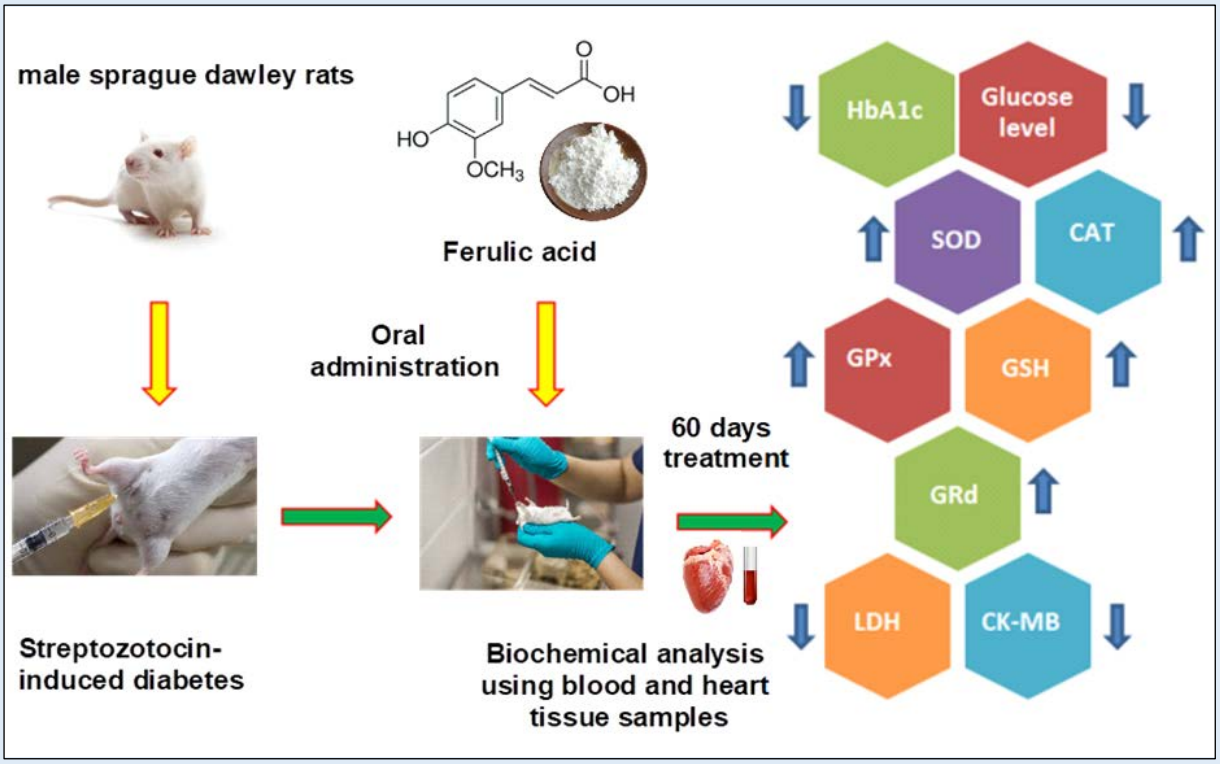
Objective: In the present study, we investigated the effects of Ferulic acid (FA) on Streptozotocin-induced diabetic cardiac damage in male Sprague Dawley rats.

Materials and methods: A total of 30 male Sprague-Dawley rats were divided into five groups of six each. Diabetes was induced by a single intraperitoneal injection of Streptozotocin (STZ) (40 mg/kg body weight). Group I consisted of normal rats (N); group II consisted of normal rats treated with FA (N+FA); group III consisted of STZ-induced diabetic rats (D), and groups IV and V consisted of STZ-induced diabetic rats treated with FA at a dose of 50 mg/kg body weight and glibenclamide at a dose of 5 mg/kg body weight respectively (N+FA and N+G) for 60 days. Rats were sacrificed after the treatment period, and blood and heart tissue were collected for analysis.

Results: STZ injection significantly increased blood glucose, HbA1c, cardiac marker enzymes LDH, CK-MB, and oxidative stress in heart tissue. The oral administration of FA to diabetic rats for 60 days significantly improved diabetic markers, oxidative stress, and cardiac markers.

Conclusion: The present study indicated that FA affords cardioprotective effect in diabetic rats, and this protection may be due in part to the attenuation of oxidative stress.

Keywords: ferulic acid, streptozotocin, diabetes, cardiac damage, oxidative stress



INTRODUCTION

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances in carbohydrate, protein, and lipid metabolism resulting from defects in insulin secretion or action [1]. The number of people living with diabetes worldwide in 2019 is approximately 463 million. By 2030, it will reach 10.2% (578 million), and by 2045, it will reach 10.9% (700 million) [2]. Diabetic patients are significantly more likely to suffer from micro and macrovascular disease and cardiovascular diseases, such as ischemic heart disease, stroke, and heart failure, than non-diabetic patients [3]. The majority of diabetes-related morbidity and mortality is caused by cardiovascular complications.

Diabetic-related cardiac dysfunctions are directly connected with redox imbalance and oxidative stress. Chronic elevations of blood glucose, the most defining clinical sign of diabetes, lead to an increase in reactive oxygen species (ROS) derived

from glucose autoxidation and glycosylation. This causes long-term and acute structural and functional changes in macromolecules that may impair cellular functioning, cause cell death, or damage organs [4]. Studies have shown that plants possess antidiabetic and antioxidant properties that can reduce the symptoms of diabetics and the effects of oxidative stress [5]. In this regard, antioxidant therapies based on plant bioactive constituents are getting more attention now against the progression of diabetic heart complications.

Bioactive compounds are substances found in foods with health benefits, including antioxidants, anti-inflammatory properties, antifungal properties, and various preventative properties [6]. Ferulic acid (FA) or 4-hydroxy-3-methoxycinnamic acid (Figure 1) is a bioactive compound found in many functional foods that have been linked to a wide range of health benefits. According to the Functional Food Centre, functional foods are defined as "Natural or processed foods that contain known or unknown biologically active compounds; which is effective, and non-toxic dose, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic diseases [7]. FA is rich in cereal seeds, wheat, rye, oats, barley, whole grains, spinach, parsley, grapes, rhubarb, etc [8]. FA has low toxicity and a wide range of physiological effects (anti-inflammatory, antioxidant, antimicrobial activity, anticancer, and antidiabetic activity) and is widely used in the pharmaceutical, food, and cosmetics industries [9]. FA is a free radical scavenger but also an inhibitor of enzymes that catalyse free radical generation and an enhancer of scavenger enzyme activity [10]. According to literature, FA has cardioprotective effect on diabetic hearts [11]. Based on preliminarily reported antioxidant and hypoglycemic properties of this molecule in STZ-induced diabetic rats [12–14], we designed the present study to evaluate its potential therapeutic role in ameliorating STZ-induced and oxidative stress mediated cardiac complications in Sprague Dawley rats.

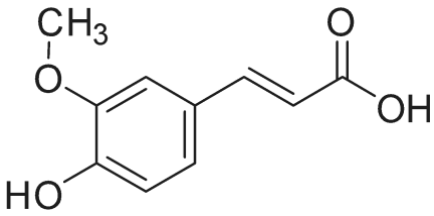


Figure 1. Structure of Ferulic acid

MATERIALS AND METHODS

***Chemicals*:** All the chemicals used in this study were of analytical grade and purchased from Sigma–Aldrich (St. Louis, MO), Hi-Media (Mumbai, India) and Sisco Research Laboratories (Mumbai, India).

Induction of experimental diabetes in rats: 30 Male Sprague Dawley rats (170-180 g) were bred in the Animal House, Department of Biochemistry, University of Kerala. Intraperitoneal injection of STZ in pH 4.5 citrate buffer at a dose of 40 mg/kg was used to induce diabetes in rats. To combat the drug-induced hypoglycemia, rats injected with STZ were given an overnight glucose solution of 20%. Rats with fasting plasma glucose levels greater than 250 mg/dl were considered diabetic and included in the study. All rats were housed in clean, sterile, polypropylene cages under standard vivarium conditions (12 h

light/dark cycles) with free access to a standard laboratory pellet diet (Hindustan Lever Ltd., Bangalore, India) and water. This study was approved by the Institutional Animal Ethics Committee (Ethical sanction number: (IAEC-3-KU-03/2018-19-BCH-SM (43)).

Experimental design: Rats were divided into five groups, with six rats in each group. Group I was normal rats (N); group II consisted of normal rats treated with FA (N+FA); group III with STZ-induced diabetic control rats (D), groups IV and V consisted of STZ-induced diabetic rats treated with FA at doses 50 mg/kg body weight [15] and glibenclamide at a dose of 5 mg/kg body weight [16] respectively (N+FA and N+G). The daily intragastric treatments using the fresh suspension of FA and glibenclamide were continued for 60 days. After the treatment period, the rats were fasted overnight, sacrificed, blood and heart tissue was collected in ice-cold containers for various estimations.

Biochemical studies: Blood glucose was estimated by the glucose oxidase method [17]. Glycated hemoglobin by HbA1c kit (Beacon Diagnostics Pvt Ltd) and Plasma insulin by ELISA kit (DRG Diagnostics, Marburg, Germany). The cardiac hypertrophic index is represented as the heart weight/body weight ratio. At the end of the study, each animal's heart weight (mg)/body weight (gm) weight ratio was calculated. The antioxidant status in heart tissue was determined from superoxide dismutase (SOD) [18], catalase (CAT) [19], glutathione peroxidase (GPx) and glutathione reductase (GRd) activity [20]. Estimation of serum lactate dehydrogenase (LDH) and creatine kinase myocardial band (CK-MB) was done as per the manufacturer instructions in protocol of kit from AGAPPE diagnostic Pvt.Ltd.

Statistical Analysis: Statistical analysis was done using the statistical package SPSS/PC+, Version 17 (SPSS Inc. Chicago, IL, USA), and GraphPad Prism 7.0. The data analyses for the single group were done by one-way analysis and grouped data by two-way analysis of variance (ANOVA). All the results were expressed as mean value ± SD (n = 6). 'P' values of 0.05 or less were considered significant.

RESULTS

Blood glucose and HbA1c: Figure 2A show that experimentally induced diabetic rats showed severe hyperglycemia with increased glucose levels estimated (371.20 ± 3.58 mg/dl). The supplementation of FA to the diabetic rats considerably improved the condition. Oral administration of FA at a dose of 50 mg/kg body weight for 60 days resulted in a significant ($P \leq 0.05$) fall in glucose levels (178.97 ± 5.1 mg/dl) at the end of the treatment period. Glycated hemoglobin level was significantly increased (13.93 ± 1.45 %) in diabetic control rats compared to normal groups. The administration of FA reduced the levels of glycated hemoglobin in diabetic control rats to 7.65 ± 0.70 %. The result was shown in figure 2B. The effect of FA was comparable with the standard drug glibenclamide.

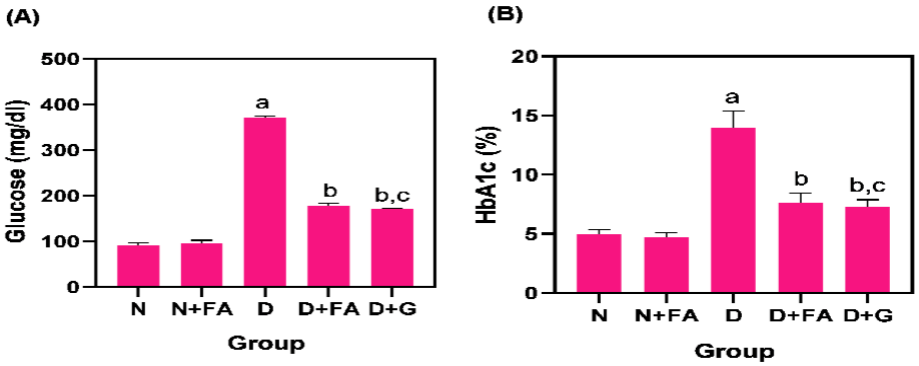


Figure 2. (A) Blood glucose (B) Glycated hemoglobin: Values are expressed as mean ± SD (n=6). 'a' indicates values were significantly different from N, D is compared with D+FA and DG ('b' indicates values were significantly different from D) and D+FA is compared with D+G ('c' indicates values were significantly different from D+G). Significance accepted at p ≤ 0.05. Abbreviations: N-Normal rats, N+FA- Normal rats treated with FA, D-Diabetic control rats, D+FA-Diabetic rats treated with FA, D+G- Diabetic rats treated with glibenclamide.

Cardiac hypertrophic index: Figure 3 represents the cardiac hypertrophic index in experimental animals calculated using the heart weight and body weight ratio of rats at the end of the study. There was a significant increase (P ≤ 0.05) in the cardiac hypertrophic index in the diabetic group (11.08 ± 1.02 mg/g) as compared to normal group (4.84 ± 0.84 mg/g), which was significantly decreased ((P ≤ 0.05) by glibenclamide and FA respectively treated groups (6.94 ±.60 and 7.00±0.84 mg/g) as compared with diabetic group.

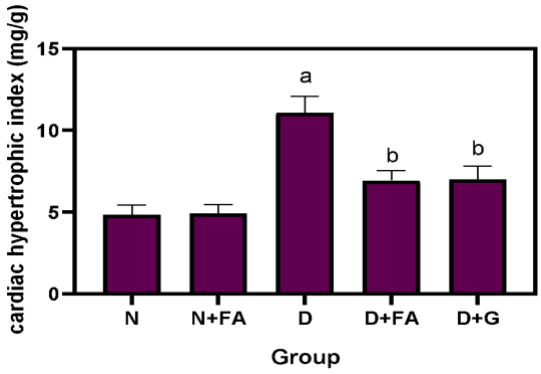


Figure 3. cardiac hypertrophic index: Values are expressed as mean ± SD (n=6). 'a' indicates values were significantly different from N, D is compared with D+FA and DG ('b' indicates values were significantly different from D). Significance accepted at p ≤ 0.05. Abbreviations: N-Normal rats, N+FA- Normal rats treated with FA, D-Diabetic control rats, D+FA-Diabetic rats treated with FA, D+G- Diabetic rats treated with glibenclamide.

LDH and CK-MB activity: Table 1 represents that in the diabetic group, there was a significant increase (P ≤ 0.05) in serum LDH levels as compared to normal group. Glibenclamide and FA treated groups showed significant decrease (P ≤ 0.05) respectively in serum LDH levels as compared with the diabetic group. A significant increase (P ≤ 0.05) in serum CK-MB levels were found in diabetic group as compared to the normal group. D+FA groups showed a significant decrease in serum CK-MB level compared to the diabetic group.

Table 1. Cardiac marker enzymes

GROUPS	LDH (U/L)	CK-MB (U/L)
N	56.86 ± 0.87	132.93 ± 3.21
N+FA	56.51 ± 1.49	131.66 ± 2.26
D	111.78 ± 1.81[a]	219.21 ± 1.54[a]
D+FA	76.49 ± 1.89[b]	163.29 ± 1.95[b]
D+G	71.83 ± 1.40[b,c]	154.47 ± 1.65[b,c]

Values are expressed as mean ± SD (n=6). 'a' indicates values were significantly different from N, D is compared with D+FA and DG ('b' indicates values were significantly different from D) and D+FA is compared with D+G ('c' indicates values were significantly different from D+G). Significance accepted at p ≤ 0.05. Abbreviations: N-Normal rats, N+FA- Normal rats treated with FA, D-Diabetic control rats, D+FA-Diabetic rats treated with FA, D+G- Diabetic rats treated with glibenclamide.

Antioxidant enzyme activities in heart tissue: Catalase and SOD activity was significantly (P ≤ 0.05) reduced in the heart tissue of diabetic rats compared to normal rats. Significant enhancement (P ≤ 0.05) in SOD activity and catalase activity was observed in FA (50 mg/kg body weight) treated animals. The activity of GPx and GRd in heart tissue decreased significantly (P ≤ 0.05) in diabetic rats compared to non-diabetic rats. Significant enhancement (P ≤ 0.05) in GPx and GRd activity was found in diabetic rats treated with FA. The effect of FA treatment was compared with the standard drug glibenclamide. The results are represented in table 2.

Table 2. Antioxidant enzyme activities in heart tissue

GROUPS	CAT (10^{-3}U/mg protein)	GPx (U/mg protein)	GRd (U/mg protein)	SOD (U/mg protein)
N	6.79 ± 0.29	26.68 ± 0.52	20.4 ± 2.04	2.07 ± 0.49
N+FA	6.90 ± 0.19	27.12 ± 0.51	23.1 ± 2.01	2.14 ± 0.39
D	2.31 ± 0.17 [a]	12.20 ± 1.10[a]	13.4 ± 1.20[a]	0.43 ± 0.10[a]
D+FA	4.27 ± 0.87[b]	16.23 ± 0.51[b]	17.82± 1.40[b]	0.83 ± 0.03[b]
D+G	4.41 ± 0.13 [b, c]	15.92 ± 0.90 [b]	15.82± 1.25[b,c]	0.87 ± 0.04[b,c]

Values are expressed as mean ± SD (n=6). 'a' indicates values were significantly different from N, D is compared with D+FA and DG ('b' indicates values were significantly different from D) and D+FA is compared with D+G ('c' indicates values were significantly different from D+G). Significance accepted at p ≤ 0.05. Abbreviations: N-Normal rats, N+FA- Normal rats treated with FA, D-Diabetic control rats, D+FA-Diabetic rats treated with FA, D+G- Diabetic rats treated with glibenclamide.

Reduced Glutathione content* (GSH*) in heart tissue: The concentration of non-enzymatic antioxidant-GSH in heart tissue is shown in figure 4. A significant decline ($P \leq 0.05$) was observed in the concentration of GSH in diabetic control rats (35.55 ± 1.13 Mm/100g of tissue) compared to the normal control rats (75.92 ± 1.42 Mm/100g of tissue). In contrast, GSH concentration was significantly enhanced after treatment with FA (51.85 ± 1.68 Mm/100g of tissue) or glibenclamide (51.71 ± 1.31 Mm/100g of tissue) in the heart tissue of diabetic rats.

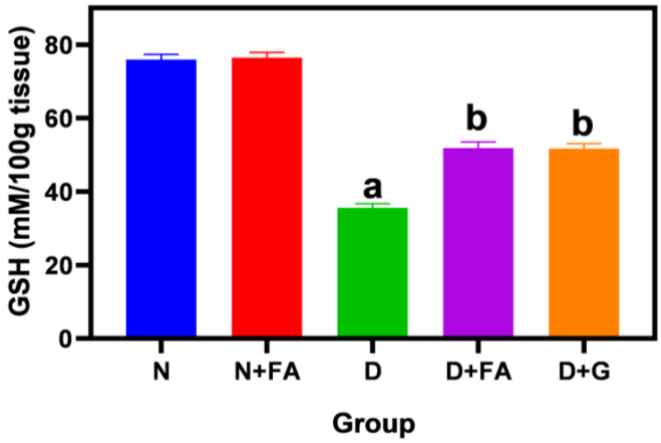


Figure 4. Reduced Glutathione content (GSH) in heart tissue: Values are expressed as mean ± SD (n=6). 'a' indicates values were significantly different from N, D is compared with D+FA and DG ('b' indicates values were significantly different from D). Significance accepted at $p \leq 0.05$. Abbreviations: N-Normal rats, N+FA- Normal rats treated with FA, D-Diabetic control rats, D+FA- Diabetic rats treated with FA, D+G- Diabetic rats treated with glibenclamide.

DISCUSSION

Cardiovascular problem is one of the most common complications of diabetes. Diabetes-induced oxidative stress has been implicated in the progression of the pathogenesis of cardiovascular complications [3]. There is a growing interest in antidiabetic remedies from plants sources because of their fewer side effects and low cost. In the present study, the protective effects of the phenolic phytochemical compound FA in the heart tissue of STZ-induced diabetic rats were determined. Tocompare the results of the study, glibenclamide was used as a standard antidiabetic drug.

STZ is one of the most common substances used to cause diabetes mellitus in experimental animals [21]. The destruction of beta cells by STZ injection and reduction of insulin production create diabetes. Here, male Sprague Dawley rats were induced diabetes using a 40 mg/kg dose of STZ injection, resulting in hyperglycemia with increased glucose and HbA1c levels in rats. These observations generally agree with other investigations on STZ-induced experimental diabetes [22–24]. A 60-day FA supplementation protected diabetic rats from this condition by regulating glucose levels. HbA1c is a reliable indicator of glycemic control in diabetes mellitus and is considered an indicator of diabetes prognosis. The administration of FA and glibenclamide reduced the levels of HbA1c in diabetic rats, showing its hypoglycemic potential. Several studies showed the hypoglycemic efficacy of FA in diabetic rats in

agreement with our results [25–27].

The heart-to-body ratio of rats during the experimental period represented the cardiac hypertrophic mass index here. The ratio may be greater in patients with hypertension and diabetes mellitus than those without diabetes mellitus [28]. In our study not a significant heart weight change was observed in diabetic animals while cardiac hypertrophic index was raised significantly in diabetic rats. 60 days of treatment with FA and glibenclamide showed a significant decrease in the cardiac hypertrophic index, showing its protection over diabetes-induced heart damage.

Myocardium contains high concentrations of CK-MB, one of the isoforms of creatine kinase. The amount of lactate dehydrogenase (LDH) and CK-MB level is an index for identifying cell injury and membrane integrity in cardiac tissue. In the process of cell membrane destruction, these enzymes leak out of cells. Thus, the level indicates the degree of injury and cell necrosis[29]. It has been previously reported that serum LDH and CK-MB activity increase with cardiomyopathy conditions [30]. In diabetic patients, CK-MB and LDH levels have been found to increase, possibly serving as markers for cardiovascular risk and cardiac muscular damage. In the present study, STZ-induced diabetes resulted in an increased serum LDH and CK-MB activity may be due to cardiac injury, whereas FA treatment decreased serum levels of LDH and CK-MB in diabetic rats. This suggests the effective cardioprotection activity of FA against diabetes-induced toxicity, which is in agreement with the previous findings [31].

Diabetes-associated heart damage and eventually cardiomyopathy are caused by increased production of ROS in the diabetic heart [3]. Increased ROS in cardiac tissue amplifies hyperglycemia and increases the formation of glucose-derived advanced glycation end products, which may contribute to developing cardiac complications in diabetes. Increased ROS generation may activate apoptotic signaling pathways, resulting in cell death, promoting abnormal cardiac remodeling, and contributing to the characteristic morphological and functional abnormalities [32]. The strategies that reduce ROS or enhance myocardial antioxidant defence mechanisms by increasing the activity of antioxidant enzymes such as SOD, catalase, GPx or GRd might be therapeutically effective in improving myocardial function in diabetes mellitus. Treatment with FA and glibenclamide increased the levels of heart tissue antioxidant enzyme levels in diabetic rats. FA is widely reported to have strong antioxidant properties. A complex antioxidant mechanism underlies the action of FA primarily through the inhibition of ROS and nitrogen oxidation and the neutralization of free radicals.

Additionally, FA can chelate protonated metal ions, such as Cu(II) or Fe(II), inhibit enzymes that generate free radicals, and enhances free radical scavenger enzyme activity, making it an excellent scavenger of free radicals [33, 34]. In this way, the antioxidant nature of FA can effectively prevent diabetes-induced oxidative damage to the heart. In our study, an increase in antioxidant enzymes level and reduced glutathione content in the heart was observed after treatment with FA in diabetic animals, which can effectively reverse the STZ-induced diabetic complications in heart.

CONCLUSION

This study demonstrated that Ferulic acid (50 mg/kg body weight) decreased glucose and glycated hemoglobin levels and effectively protected the heart tissues of STZ induced diabetic rats from oxidative

stress damage by increasing both enzymatic and non-enzymatic antioxidant defence. Together, these findings suggest that dietary Ferulic acid may have health benefits in the relief of oxidative stress and attenuation of the hyperglycemic response associated with diabetes-induced cardiac problems.

List of Abbreviations: ROS: Reactive oxygen species, HbA1c: Glycated hemoglobin, SOD: Superoxide dismutase, GRd: Glutathione reductase, GPx: Glutathione peroxidase, GSH: Reduced glutathione, STZ: Streptozotocin, FA: Ferulic acid, LDH: lactate dehydrogenase, CK-MB: creatine kinase-MB.

Authors' Contributions: Sudha Anjali and S Mini formulated the original idea and discussed it with N P Soumya and Sukanta Mondal. All authors finally agreed upon the main focus and ideas of the paper. Sudha Anjali conducted and analysed the experiments, and S Mini conceptualized the main ideas behind the experiments. Sudha Anjali and S Mini wrote the main text of the paper. 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.

Declaration of Interest: The authors report no declarations of interest

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