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Syringic acid affords antioxidant protection in the pancreas of type 2 diabetic rats

Sahari Shimsa[1], Neelakanta Pillai Padmakumari Soumya[2], Sukanta Mondal[3], and Saraswathy Mini[1*]

[1]Department of Biochemistry, University of Kerala, Kariavattom Campus, Thiruvananthapuram, India- 695581; [2]Department of Animal Husbandry, Government of Kerala, Kerala, India-695033; [3]ICAR- National Institute of Animal Nutrition and Physiology, Bengaluru, 560030, India

***Corresponding author:** Saraswathy Mini, PhD, Professor, Department of Biochemistry, University of Kerala, Kariavattom Campus, Thiruvananthapuram, 695581, India

Submission Date: January 17th, 2023; **Acceptance Date:** February 18th, 2023; **Publication Date:** February 27th, 2023

Please cite this article as: Shimsa S., Soumya N. P. P., Mondal S., Mini S. Syringic acid affords antioxidant protection in the pancreas of type 2 diabetic rats. *Bioactive Compounds in Health and Disease* 2023; 6(2):13-25. DOI: https://www.doi.org/10.31989/bchd.v6i2.1061

ABSTRACT

Background: Diabetes mellitus, is a multifactorial disease brought on by a complex interplay of metabolic, genetic, and lifestyle variables. Prolonged and chronic hyperglycemia is a complication of diabetes and might increase the risk of major health issues.

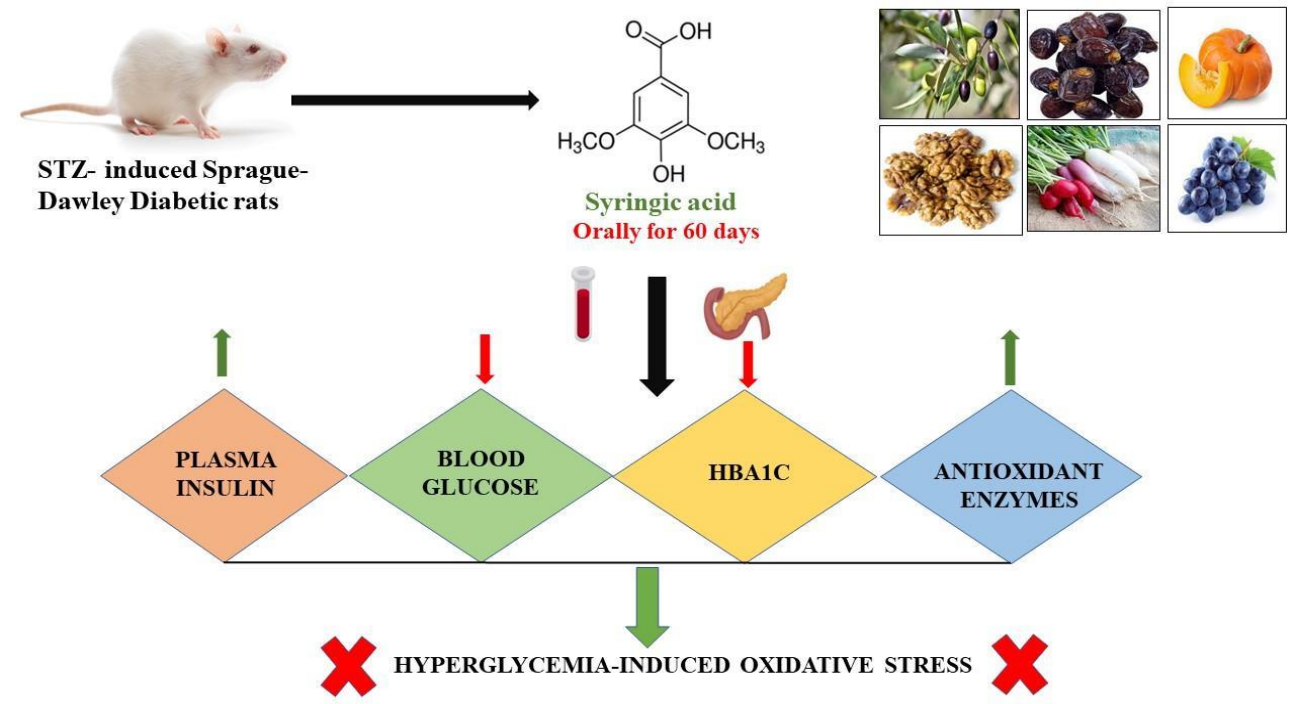
Objective: This investigation aims to determine whether the phenolic phytochemical syringic acid (SA) has any protective role on the pancreas of diabetic rats.

Methodology: Streptozotocin was injected intraperitoneally (40 mg/kg) into male Sprague-Dawley rats to induce diabetes. At a dosage of 50 mg per kg body weight, syringic acid (SA) was administered using an oral tube, once a day for 60 days. Our study examined plasma insulin, glucose, glycated hemoglobin, toxicity markers and antioxidant enzymes. The results were compared with those of diabetic rats receiving glimepiride (0.1 mg/kg) as the standard drug.

Results: Treatment with syringic acid significantly lowered hyperglycemia, improved insulin levels, reduced toxicity markers in diabetic rats. Further, Syringic acid also promoted activity of enzymes such as catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase in the pancreas.

Conclusion: These results imply that syringic acid, owing to its ability to control hyperglycemia, and reduce oxidative stress, affords antioxidant protection in the pancreas of diabetic rats.

Keywords: Diabetes mellitus, Syringic acid, Antioxidant protection, Glimepiride.



INTRODUCTION

Hyperglycemia is a defining trait of diabetes mellitus, which is caused by irregular insulin secretion, or by ineffective insulin action. Diabetes is on the rise, globally and in emerging nations like India, primarily due to unhealthy lifestyles [1]. Diabetes complications are primarily caused by chronic hyperglycemia, impaired metabolism, reactive oxygen species, and a reduced antioxidative system of defence [2]. The chronic hyperglycemia of diabetes increases the risk of serious health complications affecting vital parts like heart, liver, kidneys, nerves and blood vessels[3]. Due to the increases production of reactive oxygen species induced by hyperglycemia, microvascular and macrovascular complications arise. Even though the lifespan of patients with diabetes has improved with insulin therapy and newer hypoglycemic agents, chronic complications of the disease show a rising trend among diabetics.

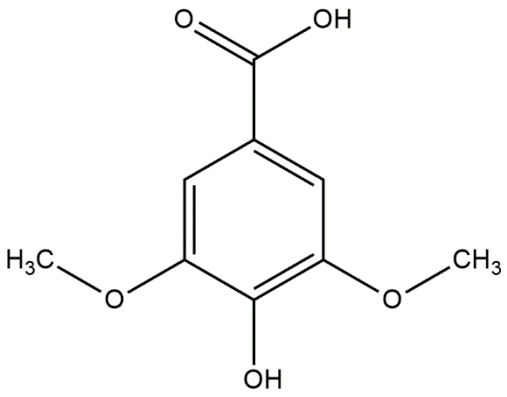
Untreated diabetes mellitus can lead to various diseases and long-term complications, and this is the major cause of morbidity among diabetic subjects. Free radical formation brought on by hyperglycemia causes oxidative stress, which can harm thestructure and functionality of vital organs.Superoxide dismutase, peroxidases, catalase, and the non-enzymatic antioxidant glutathione reduce ROS levels. In hyperglycemic conditions, however, antioxidant mechanisms malfunction and ROS-dependent signalling

pathways become active [4]. Impairment in antioxidant defense system makes it difficult to neutralize the reactive oxygen species (ROS). This imbalance between ROS and cellular defense system is deleterious to health [5].By altering membrane permeability and releasing cytochrome C into the cytosol, ROS can eventually trigger apoptosis[6].

Management of diabetic complications mainly centers on intense glycemic control through diet, oral hypoglycemicagents and by insulin therapy. These conventional therapies have several limitations and thus lose their potency in a significant percentage of patients [7]. Here comes the relevance of Natural products, with reduced risk of side effects, widespread availability and lower cost. A functional food is a food that has been enriched with nutrients or compounds that have a beneficial effect on health [8]. Function foods may contain high concentrations of biologically active ingredients that can have positive health effects when consumed properly [9].

The phytochemicals found in plants provide macro and micronutrients as well as health benefits [10]. Phytochemicals that impart health benefits may include alkaloids, flavonoids, tannins, or polyphenols.

Plant derived phenolic compounds are known for their therapeutic applications. Syringic acid is one such bioactive phenolic compound which is chemically 4-hydroxy-3,5-dimethoxy benzoic acid found mainly in swiss chard, olives, grapes, walnuts, dates, spices and cereals. Structure of syringic acid is shown in Figure 1. Syringic acid (SA) exhibits multipharmacologic properties such as antioxidant, antisteatosis, anti-inflammatory and antibacterial properties [11-14]. There are reports that syringic acid could afford antiglycation effects and is also known for its neuroprotective and hepatoprotective effects [12]. Syringic acid is also reported to modulate oxidative stress and mitochondrial mass in diabetic rats [13]. It is also known to reduce thromboembolism in mice [14]. This study investigates the beneficial impact of syringic acid on free radical induced oxidative damage in diabetic rats.



4-hydroxy-3,5-dimethoxybenzoic acid

Figure 1. Structure of syringic acid

MATERIALS AND METHODS: This study used analytical-grade chemicals from Sigma–Aldrich (St. Louis, Missouri, United States), and Sisco Research Laboratories Pvt. Ltd (Maharashtra, India).

Design of experiment: A total of thirty Sprague Dawley male rats (Body weight around 150-190 g) from the animal house, Department of Biochemistry was used for the study. The animal study design was approved by the Institutional Animal Ethics Committee [IAEC 3-KU-02/2018-19-BCH-SM (42)]. Rats were grouped into 5 groups of six rats each. The groups were Group I: normal (N); Group II: normal rats supplemented with SA, 50mg/kg body weight (N+SA); Group III: Diabetic control (DC); Group IV: diabetic rats administered with SA, 50mg/kg body weight (D+SA); Group V: diabetic rats provided with the standard drug glimepiride, 0.1mg/kg body weight(D+GM). Diabetes was induced in groups III, IV, and V with40 mg/kg of streptozotocin administered intraperitoneally in 0.1M citrate buffer (pH 4.5). Hypoglycemia caused by the drug was managed by giving the animals 5% glucose in their drinking water overnight. On the third day after receiving STZ injection, rats with blood sugar levels greater than 250 mg/dL were regarded as diabetic. Rats from groups II and IV were supplemented with syringic acid 50 mg/kg body weight intragastrically for two months [15]. Group V was supplemented with the standard antidiabetic drug glimepiride, 0.1mg/kg body weight [16].

Biochemical studies: Huggett and Nixon's glucose oxidase technique was used to determine blood glucose levels [17]. Utilizing an ELISA kit from DRG Diagnostics, Marburg, Germany, plasma insulin and glycated haemoglobin by using a kit from Beacon Diagnostics Pvt Ltd were measured. The kit from Agappe Diagnostics Pvt.Ltd was used to measure serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), and acid phosphatase (ACP)[18].

Tissue antioxidants: Activities of antioxidant enzymes in pancreatic tissue was evaluated by the following methods- Catalase was determined by the protocol previously explained [19]. Briefly at 4°C and 5000 rpm, the tissue was homogenised in 2 mL of phosphate buffer. Enzyme assay required supernatant. Following the drop in absorbance at 230 nm, the estimation was carried out spectro-photometrically. 0.1 mL of the enzyme solution and 3 mL of the H2O2-phosphate buffer were pipetted into the cuvette. Against a control cuvette containing phosphate buffer, absorbance was recorded every 10 seconds for 2 minutes.

Catalase activity (U/mg) =

$$\frac{2.303/60 \times \log OD \text{ at } 0'/OD \text{at } 60'}{\text{mg of protein}}$$

Superoxide dismutase (SOD)was estimated by the method of Kakkar et al., [20]. The tissue was homogenised with sucrose buffer at 4^0 C, centrifuged at 5000 rpm and collected the supernatant. This supernatant was used for the estimation of SOD, GPx and GRD. To 0.2 ml of supernatant, added 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of phenazine methosulphate, 0.3 mL of nitroblue tetrazolium, followed by 0.2 mL of NADH. The reaction was halted by adding 1 mL of glacial acetic acid after 90 seconds of incubation at 30 °C. 4 mL of n-butanol was added to the mixture and mixed well. Centrifuged the mixture and collected the butanol layer and colour estimated at 560 nm.

SOD activity (U/mg) =

$$\frac{(\text{Control OD} - \text{Test OD}) \times \text{Total volume}}{\text{mg protein}}$$

Glutathione peroxidase was estimated by the method by Agergaard and Jensen [21] was used for the estimation of glutathione peroxidase. To 0.2 ml of supernatant, 2mL of phosphate buffer, 0.3 mL of sodium

azide, 0.2 mL of EDTA, 0.1 mL of reduced glutathione, 0.1 mL of NADPH, and 0.5 mL of water was added and kept at room temperature for 5 minutes. The reaction was started by adding 0.2 mL of the H_2O_2 solution. For the following five minutes, the oxidation of NADPH was monitored at room temperature with a spectrophotometer at 340 nm. Distilled water was used as the blank.

Glutathione reductase (GRd) was estimated using David and Richard's methodology. To 0.2 ml of supernatant oxidized glutathione, EDTA, and 1.0 mL phosphate buffer (pH 7.2) was added followed by the addition of 0.05 mL NADPH.The absorbance was measured at 340 nm at intervals of 15 seconds for 1 minute. Micromoles of NADPH oxidized/min/mg protein is the unit of measurement for enzyme activity.

Statistical Analysis: SPSS/PC+ (Version 17.0 of SPSS Statistics for Windows. SPSS Inc., Chicago) were used for statistical examination. For the single group of data, an analysis of variance (ANOVA) was used. Results are presented as mean ± SD standard deviation (n = 6). Significant results were those with a p value of less than 0.05.

RESULTS

Blood glucose, Glycated hemoglobin and plasma insulin: In comparison to normal control groups, diabetic control (DC) rats had substantially higher blood glucose (Figure 2) and HbA1c levels (Figure 3). In comparison to untreated diabetic rats, SA and glimepiride treatment lowered glucose and HbA1c levels. When compared to normal rats, level of plasma insulin was considerably lower in the diabetes group. Comparative to diabetic control animals, diabetic rats treated with SA and glimepiride considerably raised the amount of plasmainsulin.

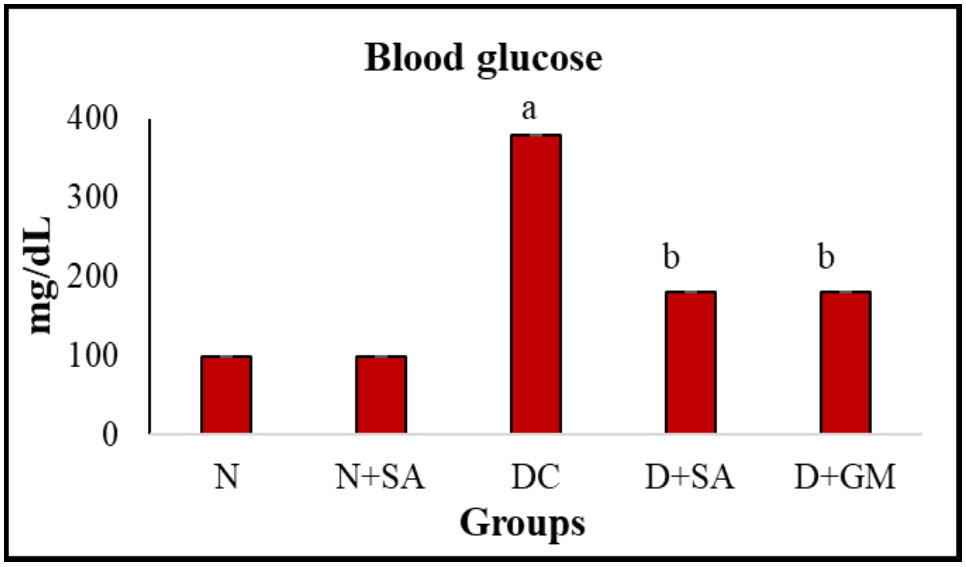


Figure 2. Blood glucose levels. DC is compared with D+SA and D+GM, and "a" denotes values that differed considerably from N whereas "b" denotes values that varied significantly from DC. P values less than P 0.05 is considered significant.

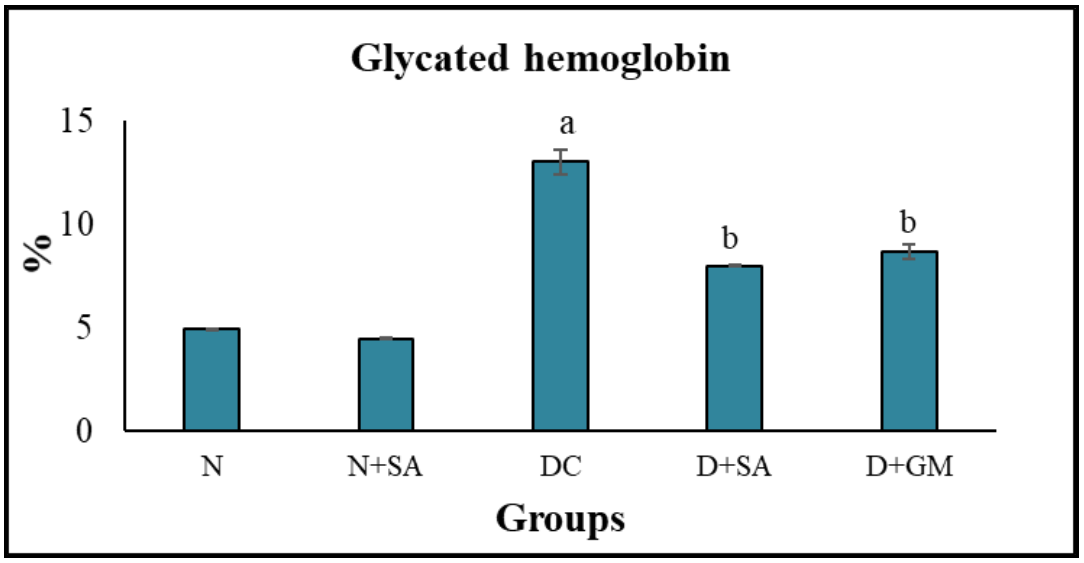


Figure 3. Glycated hemoglobin. DC is compared with D+SA and D+GM, and "a" denotes values that differed considerably from N whereas "b" denotes values that varied significantly from DC. P values less than P 0.05 is considered significant.

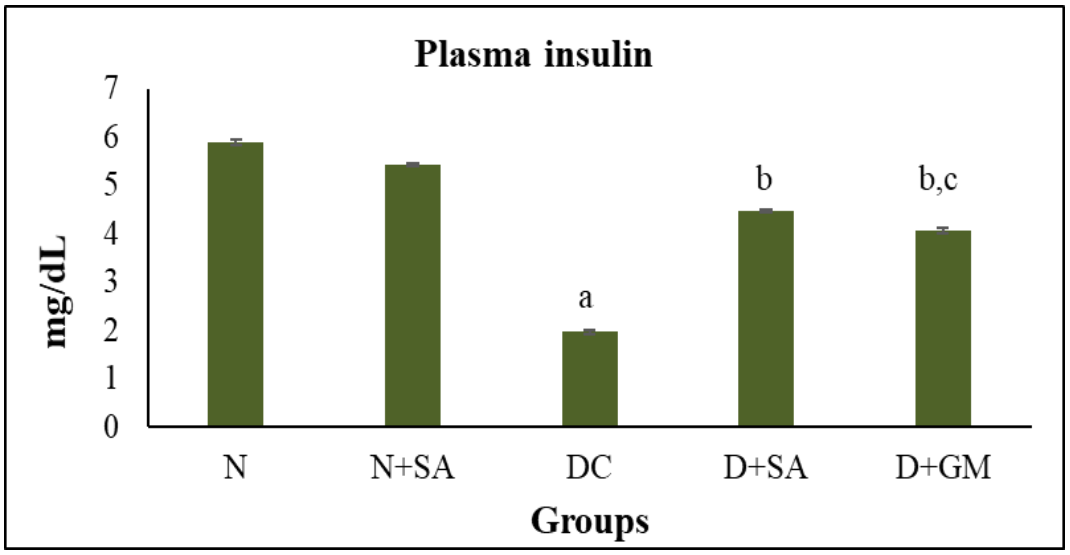


Figure 4. Plasma insulin levels. DC is compared with D+SA and D+GM, and "a" denotes values that differed considerably from N whereas "b" denotes values that varied significantly from DC."c" denotes values that varied significantly from D+SA. P values less than P 0.05 is considered significant.

Toxicity markers: Hepatic injury was assessed through the measurement of liver toxicity markers. Diabetic control rats showed increased SGPT, SGOT, ALP, and ACP activities. SA and Glimepiride treatment significantly reduced toxicity indicators in diabetic ratscontrary to diabetic control (table 1). Rats with diabetes showed noticeably elevated levels of urea, creatinine, and uric acid than normal rats. SA and glimepiride treatment in diabetic rats resulted in significantly lower levels of renal toxicity markers urea, creatinine, and uric acid, when compared to diabetic rats without treatment (Figure.5).

Table 1. Hepatic marker enzymes. Mean values with standard deviation are considered (n=6).

Groups	SGPT(U/L)	SGOT(U/L)	ALP(U/L)	ACP(U/L)
N	44.67±1.87	56.71±0.30	8.25±2.75	105.75±3.27
N+SA	45.72±1.26	56.98±0.25	9.17±1.59	106.17±3.25
DC	75.40±0.91[a]	103.90±0.98[a]	19.25±2.75[a]	207.25±3.12[a]
D+SA	52.58±2.74[b]	66.31±1.49[b]	13.67±2.63[b]	129.25±2.29[b]
D+GM	54.86±11.69[b]	65.84±2.42[b]	14.67±0.92[b]	144.08±11.64[b,c]

DC is compared with D+SA and D+GM, and "a" denotes values that differed considerably from N whereas "b" denotes values that differed significantly from DC. D+SA is contrasted with D+GM, and “c” denotes values that differed considerably from D+SA). P values less than 0.05 is considered significant.

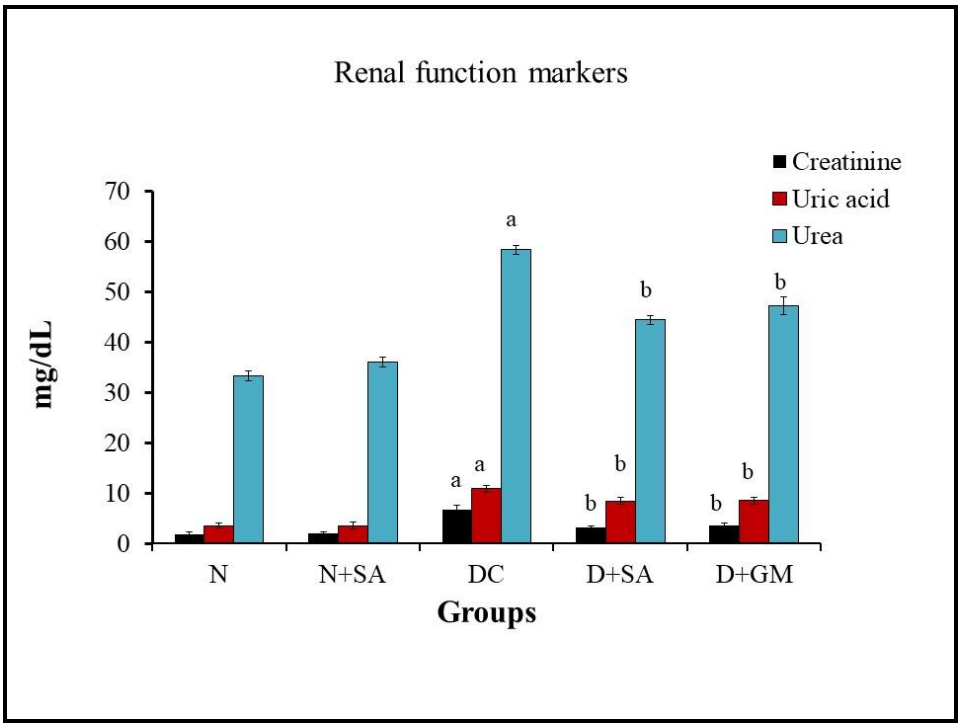


Figure 5. Renal function markers. Mean values with standard deviation are considered(n=6). DC is compared with D+SA and D+GM, and "a" denotes values that differed considerably from N whereas "b" denotes values that differed significantly from DC. D+SA is contrasted with D+GM and “c” denotes values that differed considerably from D+SA). P values lesser than 0.05 is considered significant.

Effect on Antioxidant enzymes in pancreas: The oxidation of glucose causes generation of free radicals which in turn leads to oxidative damage of tissues in diabetic rats [18]. Comparatively to the normal group, the activity of SOD, Catalase, GPx, and GRd were markedly ($p < 0.05$) reduced in the pancreas of diabetic rats. Treatment of SA and glimepiride considerably ($p < 0.05$) boosted the activity of pancreatic enzymatic antioxidants. Results are shown in Figure6 and Figure7.

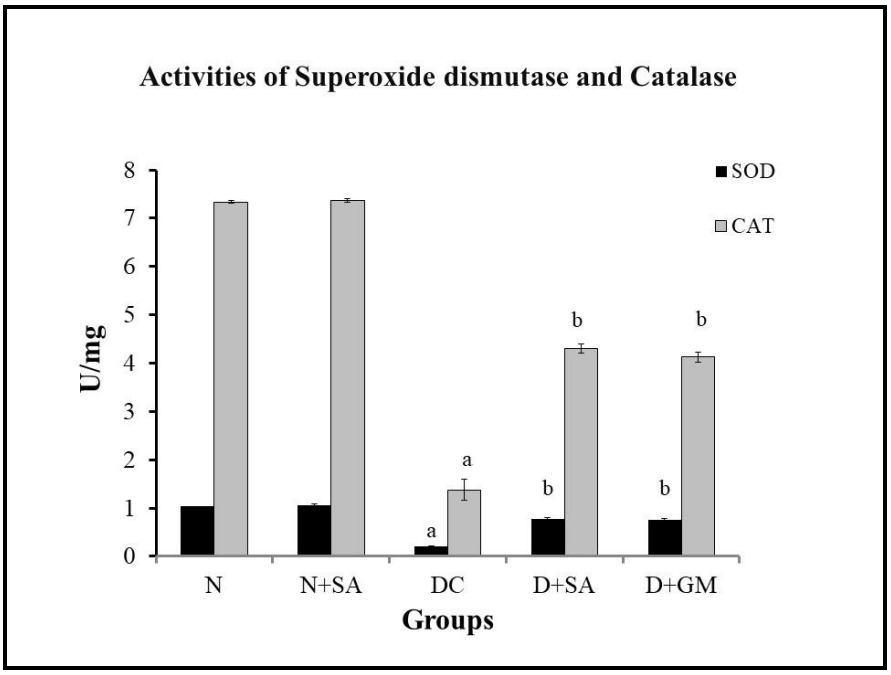


Figure 6. Activities of Superoxide dismutase and catalase in pancreas. In each group mean values with standard deviation are considered (n=6). DC is compared with D+SA and D+GM, and "a" denotes values that differed considerably from N whereas "b" denotes values that differed significantly from DC. P values lesser than 0.05 is taken as significant.

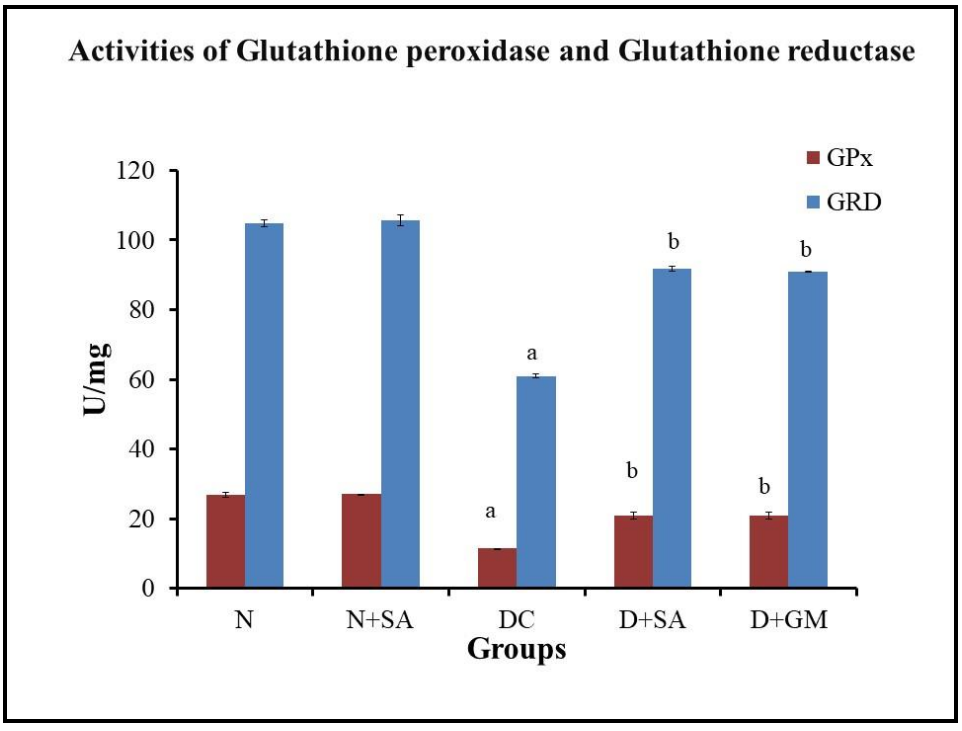


Figure7. Activities of Glutathione peroxidase and glutathione reductase in pancreas. In each group mean values with standard deviation are considered (n=6). DC is compared with D+SA and D+GM, and "a" denotes values that differed considerably from N whereas "b" denotes values that differed significantly from DC. P values lesser than 0.05 is taken as significant.

DISCUSSION

Diabetes is one among the most ubiquitous chronic illnesses around the world. India will have the highest rate of diabetes by 2025 making it, the “Diabetes capital of the world”. Hyperglycemia caused by abnormalities in production of insulin, insulin action, or a combination of both is a hallmark of diabetes mellitus (DM), that results from dynamic interaction between several genetic, environmental, and lifestyle components. Due to the significant harm, malfunction, and damage of numerous organs that arise when the ailment worsens, long-term diabetes is linked to a numberof comorbidities, including neuropathy, hepatopathy, blindness, delayed wound healing, kidney failure, heart disease, etc. Careful blood glucose monitoring, use of oral hypoglycemic agents and timely insulin injection help to manage diabetes, but even with perfect compliance to this therapy, patients may experience a variety of side effects. Management of diabetes with the usage of oral hypoglycemic agents and other conventional therapies have several adverse effects and are comparatively expensive. To combat these problems, phytotherapy may be a promising, inexpensive, eco-friendly way of managing diabetic complications. Dietary approaches based on functional foods have also been studied for the management of diabetes mellitus.Antioxidants found in functional foods and bioactive compounds can help to prevent and control diabetes [22].

Diets and functional foods have emerged as effective methods to prevent and treat a variety of illnesses in recent years. Functional foods are described as natural or processed foods that include known or unknown biologically active chemicals, which are effective, and at non-toxic dosage, give a clinically proven health benefit for the prevention, management, or treatment of chronic diseases [23]. Food bioactive compounds are primary and secondary metabolites of nutritive and non-nutritive natural components that promote health by preventing or controlling chronic disease or its symptoms [24]. Bioactive phytochemicals are being studied as a type of functional food that promotes good health. It has been demonstrated that food proteins provide health benefits in addition to satisfying the body's nutritional requirements [25]. Since life style diseases like diabetes often occurs in conjunction with obesity, the development and design of functional foods for lowering the risk of chronic diseases are crucial for reaching a global sustainable health [26].The majority of fruits and vegetables contain bioactive compounds, including phenolics, tannins, alkaloids, and carotenoids. These bioactive molecules are effective against free radicals and thus impart antioxidant protection to the body [27]. It has been reported that functional foods containing bioactive molecules can reduce cardiovascular disease and other chronic diseases, such as diabetes [28]. In view of the beneficial effects of polyphenols and their derivatives, aim of our research work was to analyze the protective impact of a bioactive polyphenolic compound, syringic acid in diabetic rats.

Chronic hyperglycemia resulting from inadequate insulin secretion combined with or without concomitant insulin action impairment is a crucial step in the pathophysiology of diabetes mellitus [29]. Hyperglycemia leads to glycation of proteins including hemoglobin and thus HbA1c is a standard biochemical marker for the assessment of diabetes. The extent of hemoglobin glycation is proportional to the amount of glucose in the blood [30]. In addition, imparing glucose oxidation and decreasing insulin biosynthesis and secretion is observed in diabetic rats [31]. In diabetic rats, ferulic acid has been shown to lower HbA1c levels, indicating its potential for controlling blood sugar levels [32]. Our findings support this, comparative to normal, diabetes control groups had substantially elevated

blood glucose and HbA1c levels and lower plasma insulin levels. SA and glimepiride supplementation lowered blood sugar and glycated haemoglobin levels (Figure 2 and Figure 3). In addition, they could also enhance plasma insulin levels (Figure .4). HbA1c and blood sugar levels in the N and N+SA treated groups did not vary significantly. Both SA and glimepiride showed comparable effects except for plasma insulin where SA showed superior effect. These findings are consistent with earlier research from Muthukumaran et al. (2013) [33].

Altered levels of hepatic and renal toxicity markers are found to be associated with diabetes. Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), and acid phosphatase (ACP) are the first and most significant indicators in liver injury [34]. The main contributing reasons to the hepatic and renal impairment associated with diabetes are the increased generation of free radicals as a consequence of the oxidation of glucose, non-enzymatic protein glycation, and impaired antioxidant system [18]. Chronic hyperglycemia leads to pathophysiological changes in liver and kidney and because of this, diabetic conditions result in higher levels of hepatic and renal toxicity indicators. When compared to the normal groups in our study, the activities of SGPT, SGOT, ACP, and ALP were substantially elevated in the diabetic control group. Supplementation of both SA and glimepiride could reduce their activities, (Table.1). N and N+SA treated groups does not show any significant difference. Renal toxicity indicators such as urea, creatinine and uric acid were also reduced by the supplementation of syringic acid (Figure. 5). Similar results were also obtained in previous studies where administration of various phytochemicals to diabetic rats reduced the activities of these toxicity markers [35]. Similar findings were also found in a prior investigation on the beneficial role of syringic acid on hepatotoxicity in rats [36].

Under certain conditions, excess accumulation of ROS may damage the tissues. Oxidative stress promotes cardiomyocyte hypertrophy in hyperglycemic conditions [37]. Two vital enzymes for scavenging free radicals are superoxide dismutase and catalase. SOD converts superoxide radicals to H_20_2 and oxygen while catalase shield the tissues from hydroxyl radicals, which are extremely reactive [38]. Hydrogen peroxide reduction is catalysed by glutathione peroxidase and glutathione [39]. A crucial molecule in preventing oxidative stress is glutathione reductase, which catalyses the conversion of glutathione disulfide (GSSH) to sulfhydryl from glutathione (GSH)[40]. In the diabetes control group, comparative to the normal groups, our study found that the activities of antioxidant enzymes were dramatically decreased. Natural antioxidants are also effective in the scavenging of free radicals and thus are frequently used in the treatment of many disorders. There are reports that treatment of diabetic rats with hibiscus anthocyanins and metformin increased the levels of the antioxidant enzymes SOD, CAT, GPx, and GRd [41]. Our findings also showed that administering SA and glimepiride to diabetic rats markedly increased the activities of antioxidants such superoxide dismutase, glutathione reductase, Catalase, and Glutathione peroxidase. Both SA and glimepiride showed comparable effects (Figure.6 and Figure.7). These results are in agreement with previous study conducted by shali et al who revealed that morin could improve the activities of antioxidant enzymes in diabetic rats [42].

CONCLUSION:

This study revealed that SA could significantly reduce hyperglycemia and restore the activities of antioxidant enzymes such as superoxide dismutase, catalase,

glutathione peroxidase and glutathione reductase in the pancreas of diabetic rats. Syringic acid could afford antioxidant protection under diabetic condition leading to the abatement of oxidative stress in pancreatic tissue. Furthermore, our results indicate that syringic acid may have similar effects as the commercial antidiabetic drug glimepiride and may thus provide an alternative to synthetic drugs. Syringic acid is a natural bioactive molecule that does not cause side effects or other complications like those caused by synthetic antidiabetic drugs. It is therefore possible to use SA as a potent candidate for the management of diabetes, although clinical trials are required to prove its practical efficacy.

Abbreviations: ACP-Acid phosphatase, ALP- Alkaline phosphatase, CAT-Catalase, GM-Glimepiride, GPx-Glutathione peroxidase, GRd-Glutathione reductase, SA-Syringic acid, SGOT-Serum glutamate oxaloacetate transaminase, SGPT-Serum glutamate pyruvate transaminase, SOD-Superoxide dismutase, STZ-Streptozotocin.

Authors' contribution: The basic idea was formulated by S Shimsa and S Mini and discussed with N P Soumya and Sukanta Mondal. All authors ultimately agreed on the paper's basic theme and ideas. Shimsa carried out the experiments, with Mini conceptualizing the main concepts. S Shimsa and S Mini authored the full text of the manuscript. The writing and editing for the manuscript were done in part by Sukanta Mondal., which was revised by S Mini and N P Soumya. The final draft was written and edited by all authors.

Declaration of interest: Conflicts of interest are not reported by the authors.

Acknowledgments: CSIR-Junior Research Fellowship, provided by the CSIR, New Delhi, India, as well as the range of amenities offered by the University of Kerala are sincerely appreciated.

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