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Effect of Ginger on Cardiac Oxidative Stress and Carbohydrate Metabolic Profiles in STZ Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The purpose of this research was to investigate how ginger can protect oxidative stress (antioxidant status) and carbohydrate metabolism in rats with induced diabetes. In this study we observed in diabetic rats, activities of SOD, CAT, GSH content, total carbohydrates, and total proteins decreased, while glycogen content was increased. However, administering ginger to diabetic rats led to an improvement in antioxidant status, total carbohydrates, total proteins and a decrease in glycogen levels. These results indicate that ginger has antioxidant and other medicinal properties that can regulate carbohydrate metabolism in diabetic rats.

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1. INTRODUCTION

"Diabetes is a metabolic disease that is the consequence of a combination of hereditary and environmental factors. This disease causes hyperglycaemia and other classical symptoms, especially polyuria, polydipsia, and polyphagia" [1]. "The global prevalence of diabetes for all age groups was estimated to be 2.8 % in 2000 and is projected to rise to 4.4% in 2030" [2]. "Reactive oxygen species (ROS) are an important part of the defense mechanism against infection, but the excessive generation of free radicals in unsaturated fatty acids has been implicated in the pathogenesis of vascular disease" [3]. "Under diabetic conditions, persistent hyperglycaemia may cause high production of ROS via glucose auto-oxidation and/or protein glycation in various tissues. This over production of ROS causes an imbalance between radical production and protective antioxidant defence system. The free radical mediated peroxidation of membrane lipids increases membrane fluidity and permeability with loss of its integrity" [4]. "Carbohydrate, protein and lipid metabolic defects causes diabetes mellitus. To regulate these metabolic activities insulin is very essential. Due to defect in secretion or action of insulin, the blood glucose level has elevated that is specific sign of diabetes mellitus" [5]. "Liver plays a central role in carbohydrate metabolism which function can be affected in diabetes" [6]. "Prolonged high levels of blood sugar can result in the glycation of cellular proteins, leading to complications in various organs such as the eyes, nerves, kidneys, and arteries" [7]. "Insulin deficiency hampers the body's ability to effectively metabolize glucose, as well as other sources of energy like lipids and proteins" [8].

"Numerous antidiabetic drugs are commercially available, but these drugs give many adverse effects like gastrointestinal disturbances, diarrhoea, lactic acidosis, hepatotoxicity, weight gain and sever hypoglycaemia after prolonged treatment" [9]. To treat diabetic patients without any adverse effects is a challenging task in health care system. Therefore, many new drug development researchers are concentrating to develop antidiabetic drugs without any adverse effects from natural compound derived from medicinal plants.

"Traditionally, medicinal plants are extensively used in India due to their low cost, easy accessibility to everyone and perceived fewer side effects. In many respects, the mechanism of action of the herbal drugs differs from that of the synthetic drugs or pure compounds. Zingiber officinale (Roscoe), commonly known as ginger is one of the commonly used spice in India and around the world. Ginger has been used to treat a number of diseased conditions including headache, cold, arthritis, postoperative nausea and vomiting, motion sickness, and reduces symptoms in patients with nausea of pregnancy. Ginger has hypoglycemic, insulinotropic, hypolipidemic and antioxidant properties. Ginger is used to treat alcoholism, bronchitis, cancer, ulcer and dyspepsia" [10]. A preliminary study has reported that ginger contains a large number of bioactive compounds like polyphenols, alkaloids, saponins and tannins. Some of the chemical constituents isolated from ginger include (6) - gingerol, α-zingiberene, phenolic compounds, essential oils and oleoresins. In this study, we investigated the effects of ginger on the cardiac antioxidant enzymes activities (SOD, CAT, GSH content) and on biochemical parameters (carbohydrate, glycogen, and protein) in experimentally induced diabetic rats to determine if this herb has the potential to be used in the treatment of diabetes.

2. MATERIALS AND METHODS

2.1 Animals

Male albino rats of the Wistar strain, weighing 180±20g, were obtained from the Indian Institute of Science in Bangalore. These rats were housed in polypropylene cages, with six rats in each cage, and were kept in a temperature-controlled room at 27±2°C with a 12-hour light-dark cycle. They were provided with a standard rat pellet diet and had unrestricted access to water.

2.2 Chemicals

Streptozotocin (STZ) was bought from Sigma in the USA, while all other chemicals and reagents were of analytical grade. Glibenclamide was obtained from a local pharmacy in Hyderabad, India.

2.3 Induction of Diabetes

The animals were fasted overnight and diabetes was induced by administering a single intraperitoneal injection of a freshly prepared

solution containing streptozotocin (STZ) at a dose of 50 mg/kg body weight in 0.1 M cold citrate buffer with a pH of 4.5. The animals were allowed to consume a 5% glucose solution overnight to counteract any potential drug-induced hypoglycaemia. Diagnosis of diabetes was confirmed if the blood glucose levels of the animals exceeded 250 mg/dl on the third day post-STZ injection. Ginger treatment commenced on the eighth day after STZ administration, marking the beginning of the 30-day treatment period.

2.4 Plant Material and Extraction

The fresh rhizomes of Zingiber officinale was locally and identified and authenticated by botanist, Dr. Madhva Chetty in the department of Botany, S.V. University. Tirupati. Two kilograms of air-dried rhizomes of the herb was milled into fine powder mechanically and extracted in cold percolation with 95% ethanol for 24h. The extract was recovered and 95% ethanol was further added to the ginger powder and the extraction was continued. This process was repeated three times. The three extracts were pooled together, combined, filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resulting ethanolic extract was air-dried, finally give 80 grams of dark brown, gelatinous extract of ginger dried rhizomes. Without any further purification, the crude ethanolic extract was used for the experiments. Dose equivalent to 200 mg of the crude drug per kg body weight, was calculated and suspended in 2%, v/v Tween 80 solution for the experiment.

2.4 Experimental Design

The rats were divided into 5 groups, six rats in each group and treated as follows:

1. Group I- Normal control (NC). This group of rats received vehicle solution (2% of tween 80).
2. Group II -Diabetic control (DC). Streptozotocin (50 mg / kg body weight) is given intraperitonially for the induction of diabetes to this group.
3. Group III - Ginger treatment (DC+Gt): Diabetic rats received ginger ethanolic extract via orogastric tube for a period of 30 days at the dose of 200 mg/kg body weight.
4. Ginger treatment (Gt): This group of rats received ginger ethanolic extract via oragastric tube for a period of 30 days at the dose of 200 mg/kg body weight
5. Group V - (DC+Glb) diabetic animals were treated with 20 mg/kg/day of glibenclamide for 30 days.

After completing one month of treatment, the animals were euthanized by cervical dislocation, and their heart tissue was excised at 4°C. The tissue was rinsed with ice-cold saline, flash-frozen in liquid nitrogen, and immediately stored in a deep freezer at -80°C for further biochemical analysis. Superoxide dismutase (SOD) activity was assayed in the tissue homogenates by the method of Misra and Fridovich [11] at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer. Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 U per milligram of protein. Catalase (CAT) activity was determined at room temperature by using the method of Aebi [12] and absorbance of the sample was measured at 240 nm for 1 min in a spectrophotometer. The concentration of reduced glutathione (GSH) in brain homogenates was measured, as described by Akerboom and Sies [13]. The levels of selected carbohydrate metabolic profiles including total carbohydrates, glycogen, total proteins, were assessed using methods described by Carroll et al. [14], Kemp and Van Hejnigen [15], Lowry et al. [16], respectively.

2.5 Statistical Analysis

The data was analyzed using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel software to determine the significance of the main effects, factors, treatments, and their interactions. One-way ANOVA with Dunnett's multiple comparison test was utilized for comparison, with significant differences noted at $p < 0.001$.

3. RESULTS

After administering the ethanolic extract of ginger orally for a 30-day period, comparing diabetic control rats to normal control rats, a significant decrease in SOD, CAT, GSH, total carbohydrates, Total proteins, and along with an increase in glycogen levels was noted. However, when diabetic rats were treated with ginger there was a notable increase in antioxidant status, total carbohydrates, total proteins, and, with a decrease in glycogen levels indicating a restoration of antioxidant enzymes, carbohydrate metabolic profiles to near-normal values.

Table 1. Changes in Total Carbohydrates (TC), Total protein (TP) levels and Glycogen (Gly) in the heart tissue of Normal Control (NC), Diabetic Control (50 mg/kg body weight) (DC), Diabetic+ ginger treated (DC+Gt), ginger treated (Gt) (200 mg/body weight), Diabetic + Glibenclamide treatment (20mg/kg) (DC+Gli) rats

Parameter	Group I (NC)	Group II (DC)	Group II (DC+Gt)	Group IV (Gt)	Group V (DC+Glb)
ΨTotal Carbohydrates	1.304± 0.057	0.936 ±0.049*(-15.498)	1.251±0.078*(+1.162)	1.363±0.063*(+21.852)	1.089±0.016(-4.532)
ΨΨTotal proteins	10.865±0.321	7.320±1.021*(-21.452)	9.286±0.547*(+2.326)	10.894±1.156*(+2.362)	9.789±0.057*(+0.258)
ΨΨΨ Glycogen	1.786±0.045	2.89±0.023*(-14.563)	1.985±0.069*(+0.489)	1.759±0.052*(+3.201)	1.982±0.043*(+0.872)

All the values are mean, ± SD of six individual observations,
Ψ value are expressed mg of glucose/gram wet weight of the tissue.
ΨΨ expressed in mg of protein /gram wet weight of the tissue.
ΨΨΨ expressed as mg of glycogen/gram wet weight of the tissue.
**Significant at p< 0.001 with normal contro*

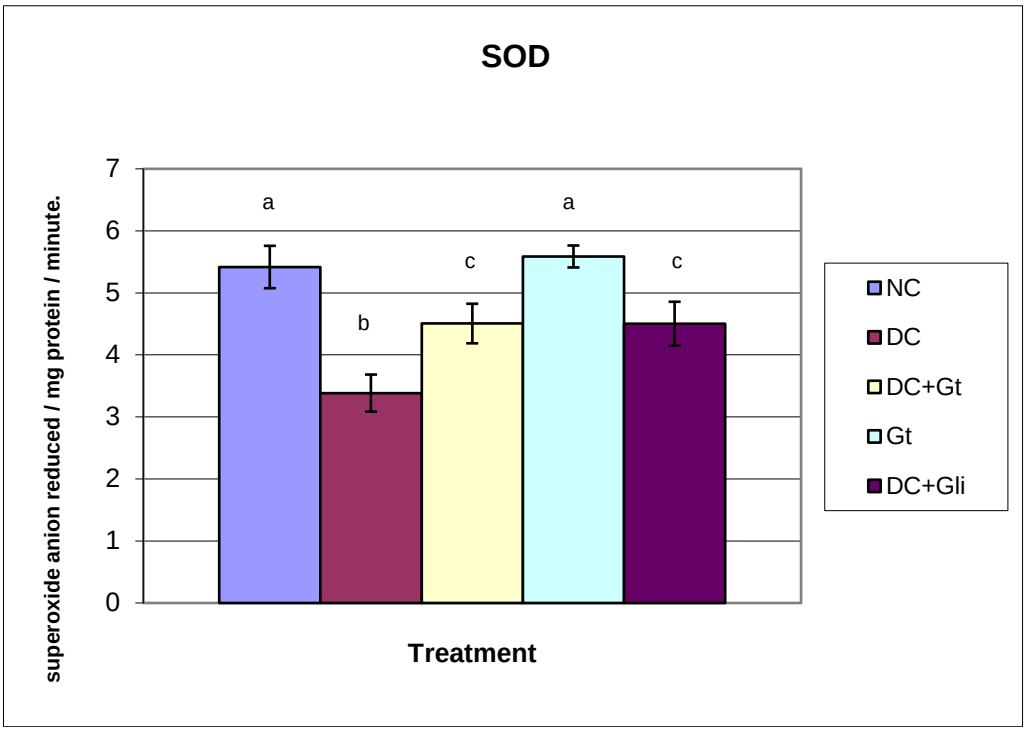

Fig. 1. Changes in SOD activity in the heart of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with ginger extract (DC+Gt), ginger extract (Gt), Diabetic rats treated with Glibenclamide. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.001

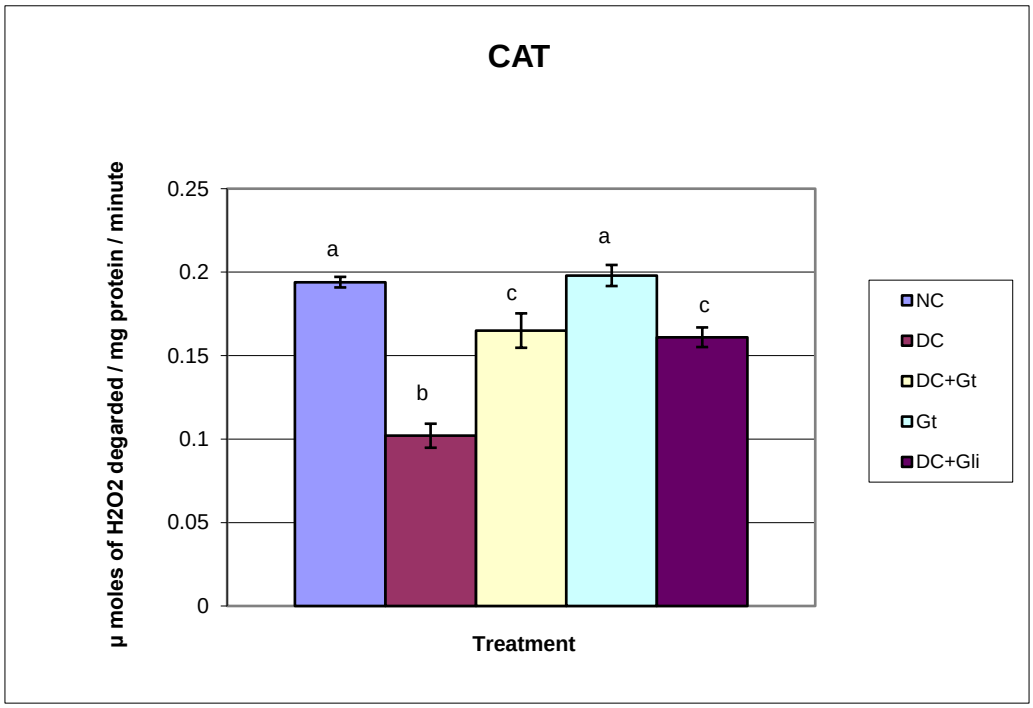

Fig. 2. Changes in CAT activity in the heart of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with ginger extract (DC+Gt), ginger extract (Gt), Diabetic rats treated with Glibenclamide. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.001

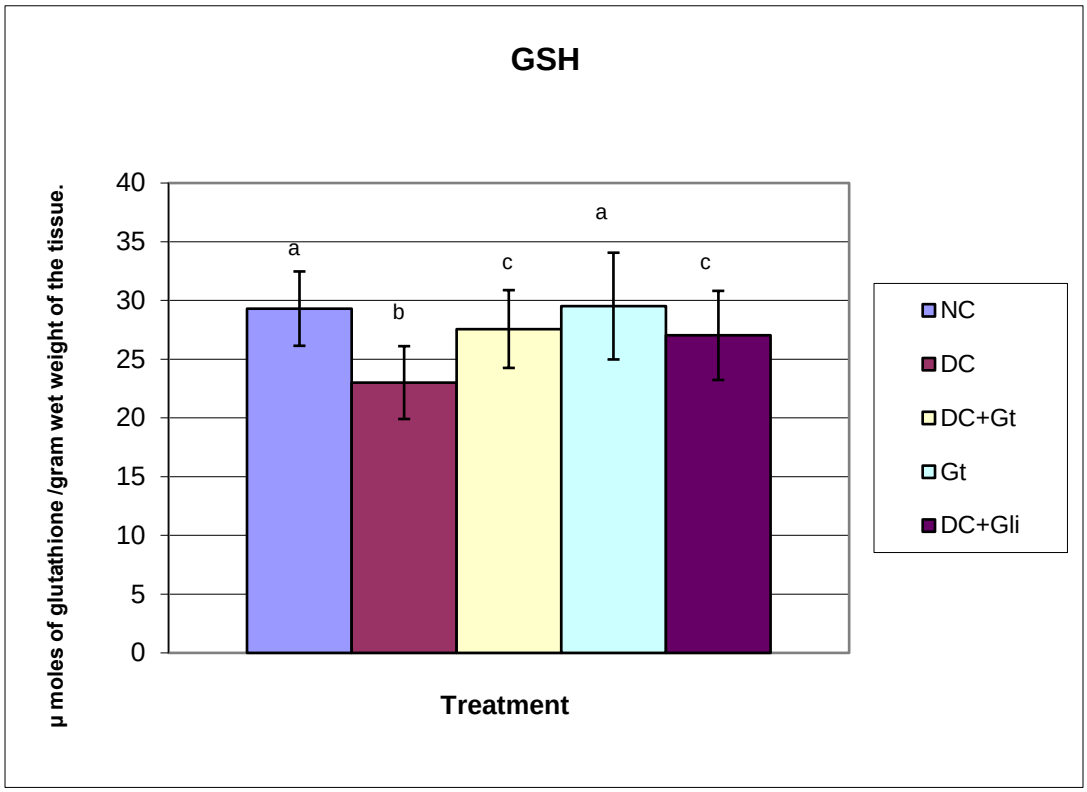

Fig. 3. Changes in GSH content in the heart of Normal Control (NC), Diabetic control (DC), Diabetic rats treated with ginger extract (DC+Gt), ginger extract (Gt), Diabetic rats treated with Glibenclamide. Each vertical bar represents the mean ± SD (n=6). Top of the vertical bars having the same letter do not differ significantly at p<0.001

4. DISCUSSION

In the current research, it was found that the activities of SOD and CAT were significantly reduced (P<0.01) in diabetic conditions compared to the normal control group. Oxidative stress is a key factor in the development of chronic complications associated with diabetes, likely due to increased efforts to combat free radicals. The decrease in SOD activity may be caused by inactivation from H_2O_2 or enzyme glycation, common occurrences in diabetes [17]. "The antioxidative defence system enzymes like SOD and CAT showed lower activities during diabetes and the results agree well with the earlier published data" [18]. The formation of α-hydroxyethyl radicals can lead to enzyme inactivation and the accumulation of harmful free radicals, resulting in detrimental effects on cell membrane integrity and function. The lowered CAT and SOD activities could be a response to the increased production of H_2O_2 and superoxide through glucose autoxidation and non-enzymatic glycation [19]. Interestingly, treatment with ginger extract and glibenclamide was able to restore the activities of SOD and CAT. This may be attributed to the presence of alkaloids, flavanols, flavones, and volatile oils in ginger, which possess antioxidant properties. These compounds could directly scavenge superoxide radicals.

Research conducted on diabetic rats has indicated a reduction in glutathione (GSH) levels, contributing to cellular damage from oxidative stress. The depletion of GSH in diabetic rats, as highlighted in a study by Rastogi et al. [20], suggests heightened utilization of GSH against reactive oxygen species. However, treatment with ginger in diabetic rats has been shown to normalize GSH levels, showcasing the antioxidant properties of ginger. Studies Ajith et al. [21], have documented ginger's ability to decrease lipid peroxidation, elevate GSH levels, and maintain the balance of antioxidant enzymes. Furthermore, researchers have pointed out the antioxidant activity of ginger's phenols, tannins, and terpenoids in various experimental models, as noted by Young et al. [22].

Carbohydrates serve dual purposes in cells, providing structure and serving as sources of stored energy in the form of polysaccharides, typically glycogen in the liver. They play a crucial role in metabolism by acting as a fuel source for oxidation and generating energy for various metabolic functions. This study investigates the impact of ginger extract on carbohydrate metabolic profiles in diabetic rats induced by STZ. Our results indicate that STZ induction caused significant changes in carbohydrate metabolic profiles. Intriguingly, supplementation of ginger to the rats showed promising results in alleviating the adverse effects of STZ, suggesting potential for cardiac protection.

“In a recent study, it was noted that diabetic rats displayed decreased levels of total carbohydrates, which are linked to disrupted glucose regulation and impaired carbohydrate metabolism primarily due to insufficient insulin production. These pathological changes play a crucial role in the onset and advancement of various complications associated with diabetes, such as neuropathy, nephropathy, cardiovascular issues, and cerebrovascular diseases” [23] “The significant drop in total carbohydrate levels in the brains of diabetic rats suggests a potential shift towards utilizing carbohydrates to meet energy demands during streptozotocin (STZ) toxicity. A similar pattern of altered carbohydrate levels has been observed in the liver and other tissues of rats in conditions of STZ-induced diabetes” [24,25]. However, treatment with ginger in diabetic rats led to an increase in total carbohydrate levels. This rise could be linked to the presence of pharmacological and antioxidant compounds in ginger which might aid in raising total carbohydrate levels in STZ-induced diabetic rats.

“Proteins play a vital role in cellular metabolism, with each tissue boasting its own distinct protein profile. In diabetes, several proteins undergo nonenzymatic glycation, potentially leading to long-term complications” [26]. “This study indicated a reduction in total protein content, possibly due to factors like diminished amino acid absorption, lower essential amino acid concentrations, heightened conversion of glycogenic amino acids to carbon dioxide and water, and reduced protein synthesis from limited mRNA availability” [27]. The decrease in protein levels has been linked to microproteinuria and increased protein breakdown [28]. However, administering an ethanolic extract of ginger to diabetic rats led to a remarkable elevation in total protein levels, surpassing normal levels.

“Glycogen, the main carbohydrate reserve found in muscle, liver, and kidney tissues, plays a crucial role in sustaining normal metabolic functions. In the present study we observed glycogen was reduced in the heart tissue. The levels of glycogen stored in these tissues can vary based on dietary intake and physiological circumstances” [29]. Derived mainly from glucose, glycogen stored in the liver, muscle, kidney, and other tissues serves as an immediate energy source. In animals, glycogen serves as the primary form of carbohydrate storage that is vital for various biological processes, and maintaining appropriate glycogen reserves is essential for ensuring proper metabolic function. Recent research has indicated an increase in glycogen levels in the kidney tissue of diabetic rats, while previous studies have highlighted elevated glycogen levels in the kidney tissue of diabetic rats [30]. Interestingly, treatment with ginger in diabetic rats was found to reduce glycogen content, returning it to normal levels. This suggests that ginger may have a regulatory impact on carbohydrate metabolism.

5. CONCLUSION

Based on the findings, it can be inferred that ginger demonstrates antioxidant and various pharmacological properties. This plant also influences on antioxidant status and carbohydrate metabolism, effectively restoring oxidative stress and carbohydrate profiles to normal levels in diabetes management.

ETHICAL APPROVAL

The experiments were carried out in accordance with guidelines and protocol approved by the Institutional Animal Ethics Committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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