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Hepato-Protective and Antioxidant Effect of Ginger on Hepatic Tissue in Experimental Diabetic Rats.

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ABSTRACT

Antioxidant defense system plays a key role in diabetic complications. The effects of ginger ethanolic extract on ruined antioxidant system in liver tissue remains unclear in diabetic rats. In this study, we investigated the impact of oral administration of ginger ethanolic extract on antioxidant parameters in experimental diabetic rats. Thirty rats were equally divided into five groups, including normal control (NC), diabetic control (DC), ginger treatment (Gt), diabetics treated with ginger (D+Gt) and diabetics treated with glibenclamide (D+Glb). The parameters like SOD, CAT, GPx, GR, GST activities and GSH levels were significantly decreased in liver of diabetic rats, which indicates impaired antioxidant status. The lipid peroxidation marker MDA levels were drastically increased in diabetic rats. However, we found that the administration of ginger to diabetic rats restored the altered antioxidant status that is almost similar to normal control group, simultaneously depleted MDA levels in liver of diabetic rats. Further, evidences from our histopathological studies also prove that ginger protects the liver from oxidative stress in diabetic rats.
Keywords: Diabetes, antioxidant enzymes, liver, rats.

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INTRODUCTION

Diabetes is one of the most pressing global health problems and it is estimated that the prevalence of diabetes will be more than 300 million in 2025 [1]. Increased oxidative stress and changes in antioxidant capacity was observed in both clinical and experimental diabetes and thought to be the etiology of diabetic complications [2]. Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with antihyperglycemic activity and fewer side effects. Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances and some may inhibit insulinase activity [3]. Some plants are involved in the stimulation of β-cells to produce more insulin and others may increase β-cells in the pancreas by activating regeneration of pancreatic cells.

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 [4,5]. 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 oilsand oleoresins6. We had recently reported the renal protective effect of ginger in alcoholic subjects [5].

So for there was no work on hepatoprotective effect of ginger in diabetic rats. Hence, the present study was carried out to know the impact of ginger on hepatic antioxidant enzymes against STZ induced diabetic rats.

MATERIALS AND METHODS

Animals

Male albino Wistar rats, body weight of 180±20 grams, were used in this study. The rats were housed in clean polypropylene cages having 6 rats per cage under hygienic conditions and maintained under temperature controlled room (27±2 C) with photoperiod of 12 hours light and 12 hours dark cycle. The rats were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, and approved by the Institutional Animal Ethical Committee No: 09(iv)/a/CPCSCA/IAEC/07-08/SVU/Zool/KSR-CHRK/ dated: 26/6/08. S.V.University, Tirupati, Andhra Pradesh. The rats were allowed standard rat pellet diet (Lipton India Ltd., Mumbai, India) and water *ad-libitum* for the duration of the experiment.

Chemicals

All the chemicals used in the present study were Analar Grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fischer (Pittrsburg, PA, USA), Merk (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Induction of Diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ) (50 mg/kg body weight) in 0.1 M cold citrate buffer (pH: 4.5). The animals were allowed to drink 15% glucose solution overnight to overcome the drug- induced hypoglycemia. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The animals are acclimatized one week in diabetic condition after one week ginger treatment was given to the diabetic rats for 30 days.

Preparation of Ginger extract

The fresh rhizomes of ginger was locally purchased in Tirupati in the month of July 2009. Two kilograms of air-dried rhizomes of the herb was milled into fine powder mechanically and extracted cold percolation with 95% ethanol for 24h. The extract was recovered and 95% ethanol was further added to the plant material and the extraction was continued. The process was repeated three times and the three extractions were pooled together, combined filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resulting ethanol extract was air-dried, finally giving 180g of brown, jelly ginger. Without any further purification the plant crude ethanol extract was used in the study. Dose equivalent to 200 mg/kg body weight of the crude extract was calculated and suspended in 2% v/v Tween 80 solution for the experiment.

Experimental design

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

Group I – Normal Control (NC): Six rats were received tween 80 solution via orogastric tube for a period of 30 days.
Group II – Diabetic Control (DC): Six rats were used as diabetic control by the induction of streptozotocin (STZ) intraperitonial injection of 50mg/kg body weight after fasting.
Group III – Ginger treatment (Gt): Six rats were received the ethanolic extract of ginger, 200mg/kg bodyweight via orogastric tube for a period of 30 days.
Group IV – Diabetic + Ginger treatment (D+Gt): Six diabetic rats were received the ethanolic extract of ginger, 200mg/kg bodyweight via orogastric tube for a period of 30 days.
Group V - Diabetic + Glibenclamide treatment (D+Glb): Diabetic rats treated with glibenclamide (600 µg/kg body weight) in aqueous solution for a period of 30 days.

The animals were sacrificed after 24 hours of the last treatment by cervical dislocation and the liver tissue was excised at 4°C. The tissues was washed with ice-cold saline, immediately immersed in liquid nitrogen and stored in the deep freezer at -80˚C for further biochemical analysis and enzymatic assays. Before assay, the tissues were homogenized under ice-cold conditions. Selected parameters were estimated by employing standard protocols.

Biochemical parameters estimation

Hepatic superoxide dismutase (SOD) activities were assayed in the tissue homogenates by the method of Misra & Fridovich [7], at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer and the activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 Unit per milligram of protein. Whereas Catalase (CAT) activity was estimated by using the method of Aebi [8]. The Activity of glutathione peroxidase (GPx) was determined by the method of Flohe & Gunzler [9], in the presence of NADPH, and absorbance was measured at 340 nm, using hydrogen peroxide. Glutathione reductase (GR) activity was determined according to the method of Carlberg & Mannervik [10].

All of the enzyme activities were expressed as per mg of protein and the tissue protein was estimated according to the method of Lowry *et al* [11], using bovine serum albumin (BSA) as a standard. The blood glucose levels were measured by using an Accucheck glucometer (Roche – Germany).

Statistical analysis

The data are expressed as Mean values with their SD. Readings of the five different groups were compared using one-way ANOVA analysis with Dennett's multiple comparision test. Statistical analysis was performed using SPSS (Version 13.5; SPSS Inc., Chicago, IL, USA). Using M.S. Office, Excel Software, the data has been analyzed for the significance of the main effects (factors), and treatments along with their interactions. Differences were considered significant at ($*p < 0.001$), ($\Psi p < 0.01$).

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RESULTS

Blood glucose and Body weight changes

Blood glucose levels were found to be significantly increased after STZ-induction. The administration of ginger, decreased the blood glucose levels significantly (Ψp<0.01) in diabetic rats than that of diabetic control group (Fig-1). These decreasing levels of blood glucose may be due to the regeneration of β - cells of the pancreas, which were destroyed by STZ.

A significant decrease in body weight in the diabetic control rats was observed than normal control rats. Whereas with ginger treatment, the bodyweight was significantly (Ψp<0.01) increased in diabetic rats (Fig-2).

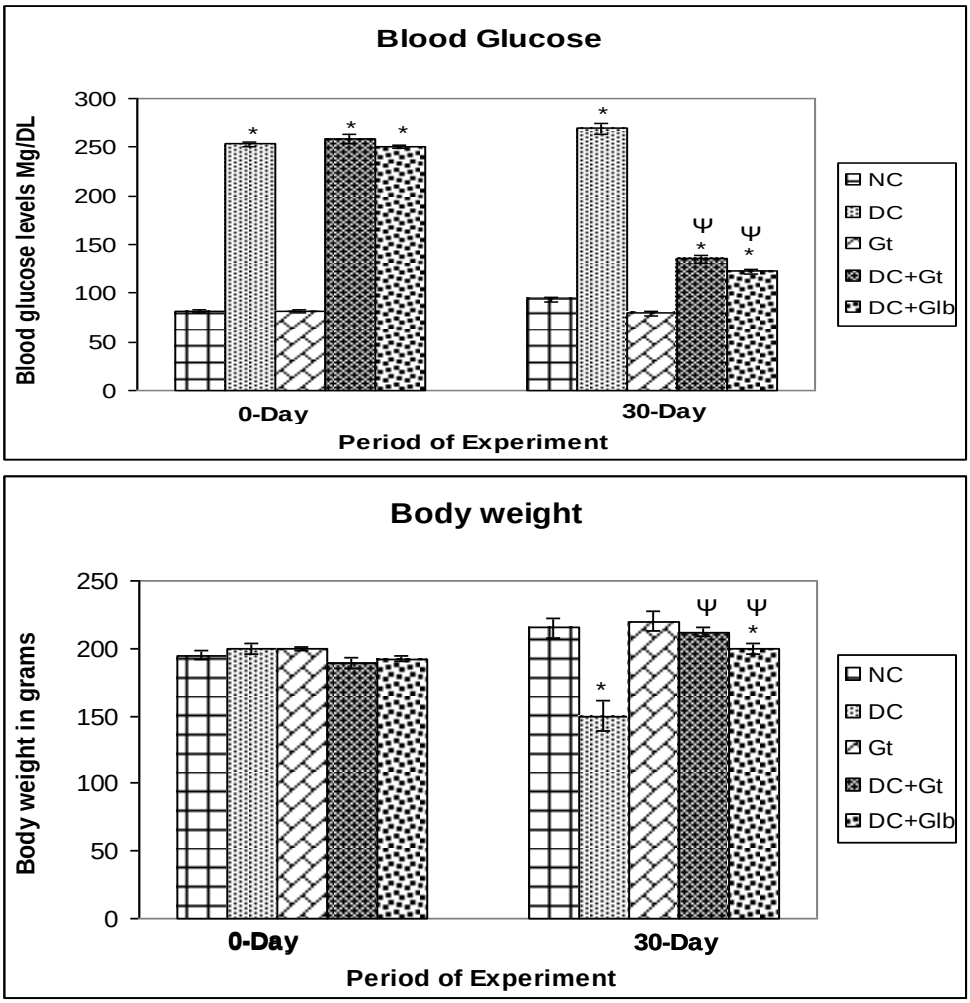

Figure 1 & 2: The effect of ginger on Blood glucose levels and Body weight changes in diabetic rats. The values are significant compared to the following: control ($^{*}p < 0.001$), diabetic ($^{\Psi}p< 0.01$)

Results of SOD, CAT, GPx, and GR in the Liver tissue of all Experimental groups

The ethanolic extract of ginger was selected for antioxidant activity in diabetic rats. We observed significant (*p < 0.001) decrease in the activities of SOD, CAT, GPx GR in liver tissue of diabetic rats, where as MDA level was increased in diabetic rats. Ginger treatment to diabetic rats significantly (Ψp<0.01) increased the SOD, CAT, GPx GR activities.

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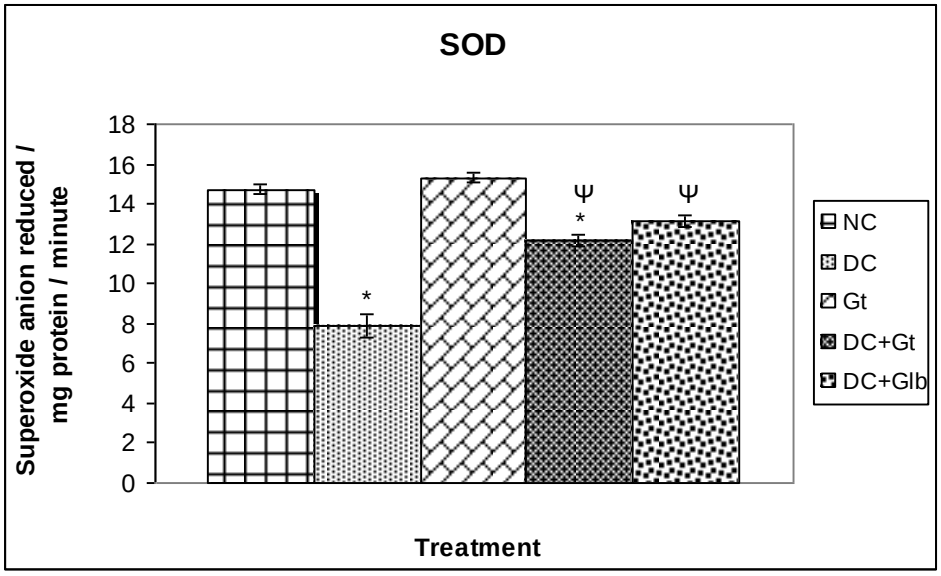

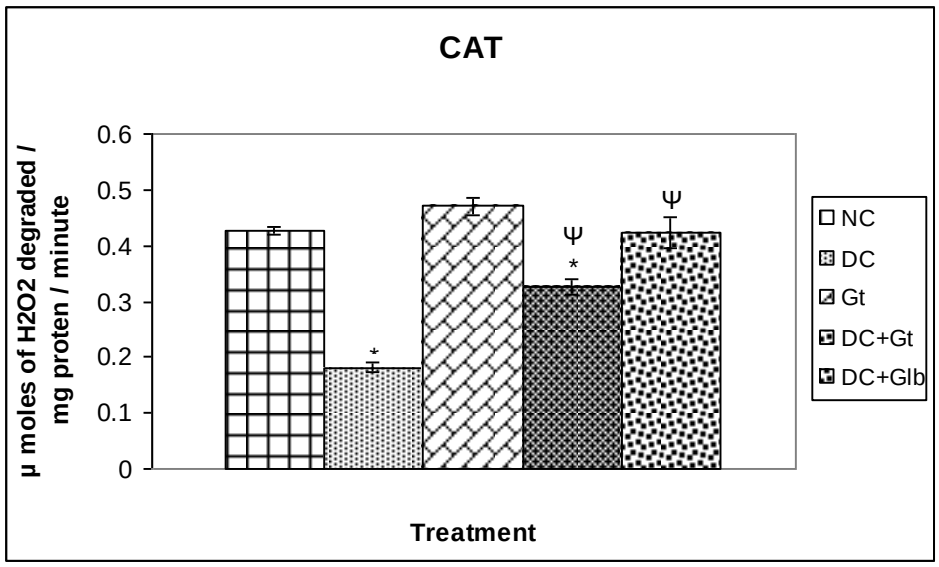

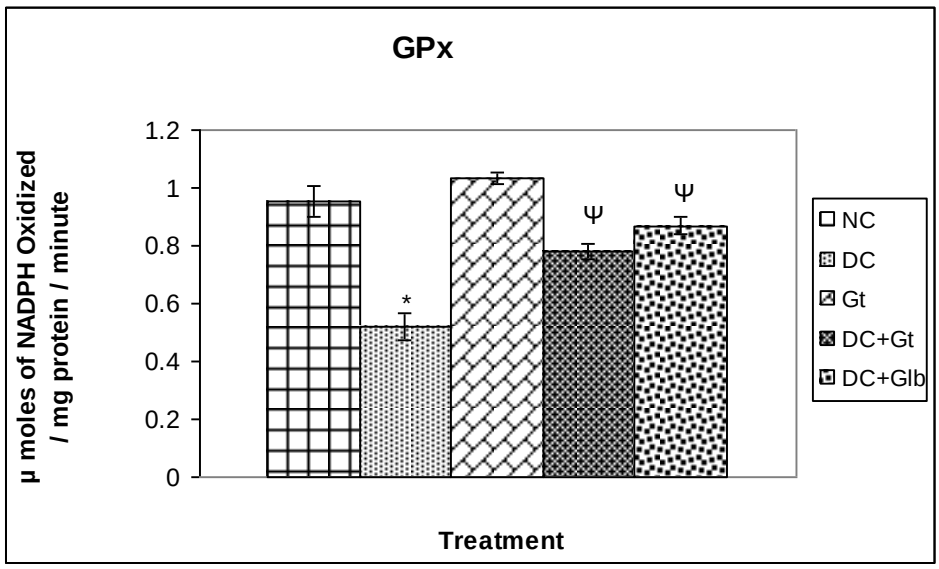

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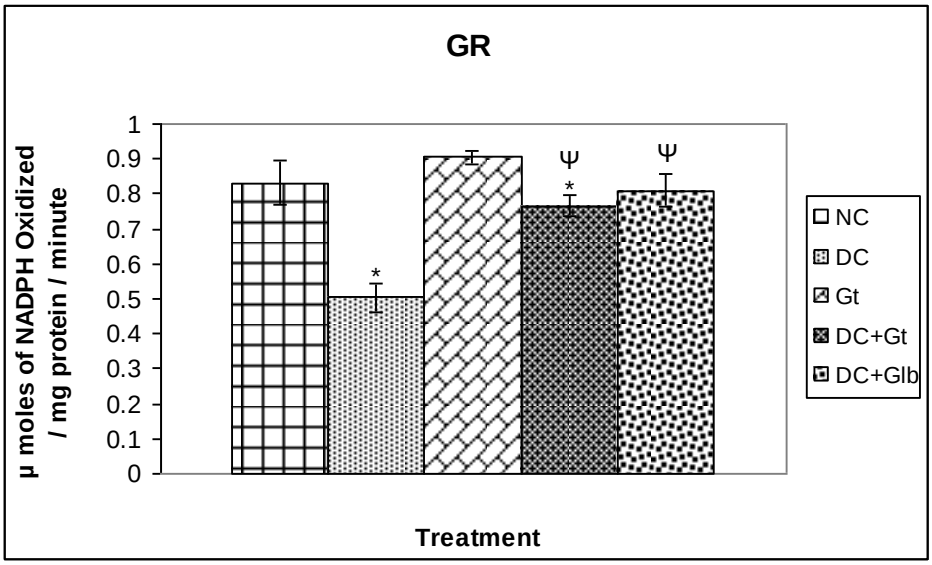

Figure 3: Status of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) activities, reduced glutathione (GSH) content in Liver. The values are significant compared to the following: control ($^{*}p < 0.001$), diabetic ($^{\Psi} < 0.01$) (Dunnett's multiple comparison test).

DISCUSSION

The present study investigates the effects of ginger antioxidant property in STZ- induced diabetic rats. Oxidative stress is suggested to be a potential contributor to the development of complications in diabetes. Increased free radical production or reduced antioxidant defense responses, both of which occur in the diabetic state may give rise to increased oxidative stress [12]. Consequences of oxidative stress are cell injury, i.e. damage lipids, antioxidant enzyme system, disruption in cellular homeostasis and accumulation of damaged molecules [13].

In Streptozotocin-induced diabetic rats, we observed increased blood glucose levels. The elevation of glucose in STZ-induced rats was due to an oxidative stress produced in the pancreas, due to a single strand break in pancreatic islets DNA [14]. Whereas with ginger treatment in diabetic rats, we observed that blood glucose levels are decreased this may be due to the free radical scavenging and hypoglycemic activity of ginger. Ginger inhibits lipid peroxidation and prevents streptozotocin-induced oxidative stress and protects β-cells from diabetic shock.

We have registered a decrease in body weight in STZ-induced diabetic rats. The characteristic loss of body weight associated with STZ-induced diabetes is due to increased muscle wasting in diabetes [15]. This indicates polyphagic condition and loss of weight due to excessive break-down of tissue proteins in diabetic rats [16]. Hakim *et al* [17] have stated that decreased body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by diabetic rats [18]. When ginger was administered to diabetic rats, the body weights seemed to be increased, as was the ability to reduce hyperglycaemia. However, it could not normalize the body weight completely. The treatment with ginger showed a significant increase in body weight in diabetic rats. The administration of ginger to STZ diabetic rats reduced blood glucose levels, in accordance with earlier reports [19].

In the present study, a reduced activity of SOD and CAT in liver tissue has been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. However, with ginger treatment in diabetic rats, we observed increased activity of SOD and CAT. Ginger treated rats showed decreased lipid peroxidation, which is associated with an increased activity of SOD and CAT in diabetic rats. This means that the ginger can reduce reactive oxygen free radicals and improve the activities of the hepatic antioxidant enzymes.

Glutathione peroxidase (GPx), an important antioxidant enzyme, and was significantly decreased in diabetic liver tissue, which indicates impaired scavenging of H_2O_2 and lipid hydroperoxides. This result is consistent with the studies of Friesen *et al* [20]. GPx catalyses the reaction of hydro peroxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydro peroxide [21]. The decreased GPx activity represents a compensatory mechanism to degrade H_2O_2. Treatment of the diabetic animals with ginger restored the altered GPx. This may be due to the presence of many antioxidant compounds, such as gingerols, shogaols, phenolic ketone derivatives, volatile oils and flavonoids of ginger. These antioxidant compounds may modulate the antioxidant enzymes in diabetic rats [5].

In the current study, GR activity was decreased in diabetic rats. GR serves to regenerate reduced GSH from oxidised GSSG by the activation of GPx. The decrease in GR activity may reflect the decline in the production and availability of GSH to overcome H_2O_2 [22]. In the combination treatment (D+Gt) group, the GR activity was increased significantly ($\Psi p<0.01$). It may be inferred that ginger provokes sustained higher levels of GR. Afshari *et al* [23] reported that plasma antioxidant capacity is increased by ginger treatment in diabetic rats.

In conclusion, our results demonstrated that STZ is capable of causing marked oxidative stress in addition to deplete the antioxidants and inhibiting the activities of antioxidant enzymes. Finally, the results of our work reveals that ginger would be a valuable source of anti-diabetic and antioxidant agents, with a potential use in pharmaceutical preparations. In all, we suggest that the ginger diets have health promoting effects especially in metabolic disorders. Moreover further studies of ginger extracts and *in-vivo* studies of inhibition are warranted.

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