

The Antidiabetic and Nephroprotective Effects of *Zingiber officinale* Rosc in Diabetic Nephropathy Rats

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ABSTRACT

Diabetes is a metabolic syndrome characterized by a high blood sugar level over a prolonged period of time. Blood vessels, eyes, kidney, heart and nerves are particularly damaged by diabetes. About one-third of diabetic patients develop diabetic nephropathy (DN) and progress to end-stage renal disease (ESRD). Ginger has a broad spectrum of pharmacological properties and it is used against various illness including diabetes mellitus. The study is specifically planned to see the potential therapeutic effects of Ginger (*Zingiber officinale* Rosc) and compare their effects with metformin in the treatment of diabetic nephropathy. A total of 36 adult male Albino Wistar rats weighing between 150 and 200g were used. All experimental animals were divided into four groups. Group-I was normal control rats. Alloxan (100mg/kg of body weight; IP) was administered to group II, III and IV animals to induce diabetes. Group-I and II rats were treated with normal saline, 500mg of ginger extract/kg of B.W and 100mg of standard drug metformin/kg of B.W were administered daily for 30 days using oral gavage to Group-III and IV rats respectively. According to our findings we confirmed that ginger restrained the alloxan induced oxidative stress by increasing the activity of CAT, SOD, GSH and decreasing MDA activity. Plasma urea and serum creatinine levels were also significantly reduced by ginger treatment. This study also demonstrated that reduced HbA1C concentration and increased serum nephrin concentration by ginger treatment. These results suggest that ginger ameliorated the diabetes induced complications and this provides a strong basis for ginger to be used clinically for diabetes treatment.

INTRODUCTION

About 6.7 million deaths are caused by diabetes mellitus - one every 5 seconds. The current data reports that 537 million people (20-79 years) are suffering from diabetes - 1 in 10. It is expected that this figure may rise to 643 million by 2030 and 783 million by 2045 (Ogurtsova *et al.*, 2022). Diabetes is common in low- and middle-income countries as over 3 in 4 adults are living with diabetes. It is affecting 26.7% adults and approximately 33 million cases of diabetes are found in Pakistan. This number is increasing every year and it is alarming. Diabetes is a metabolic syndrome characterized by a high blood sugar level over a prolonged period of time. Blood vessels, eyes, kidney, heart and nerves are particularly damaged by

diabetes and may lead to severe diabetic complications such as retinopathy neuropathy, nephropathy and cardiovascular complications (Forbes and Cooper, 2013).

Glucose control is not adequate, some pharmacological mediations such as oral antidiabetic drugs are also required along with lifestyle changes the patients need to make (Gaede *et al.*, 2003). The main objective of diabetes management would be to minimize the risks of microvascular and macrovascular complications, improve symptoms, and decrease mortality. A common approach would be to set targets for hyperglycemia, blood pressure, and blood cholesterol; to make suitable food choices and maintain a healthy and normal weight; perform a regular exercise and self-monitoring of blood glucose (American Diabetes Association, 2016). Herbal medicines are organic substances which are obtained from leaves, roots, seeds, or flowers of the plants and are used for therapeutic purposes. The herbal supplements are cost effective, accessible, have mitigated risk of side effects and natural healing.

Zingiber officinale Rosc is a perennial plant. Its rhizome is commonly used as a seasoning and herbal remedies (Ozkur *et al.*, 2022). It belongs to Zingiberaceae family. A large number of volatile and nonvolatile compounds are found in different concentrations. It contains more than 300 chemical constituents (Liu *et*

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Key words

Diabetic nephropathy, Ginger, Metformin, Antidiabetic, Nephroprotective, End stage renal disease

al., 2019). Major components of ginger include lipids (3–8%), carbohydrates (50–70%) phenolic chemicals and terpenes (*Grzanna et al., 2005*). While gingerol, paradols, and shogaol are phenolic compounds, zingiberene, β -bisabolene, α -farnesene, β -sesquiphellandrene, and α -curcumene are terpene elements (*Shukla and Singh, 2007*). The rhizomes of ginger have been used to cure cold, fever, infectious diseases, arthritis, hypertension, bronchospasm, cerebral infarction, type-I and type-II diabetes etc. It is also used as a medicine for treatment of digestive complaints like constipation, belching, bloating, indigestion, nausea, and vomiting etc. Ginger has a very long and well-documented history of use as a medicine in humans, and many human trials have demonstrated its efficacy in treating a variety of conditions, including gastrointestinal disorders (*Lete and Allue, 2016*). Many studies have shown the anti-inflammatory, antioxidant, antidiabetic and cardioprotective effects of ginger (*Lantz et al., 2007; El-Bassossy et al., 2016*). Researchers have found that ginger possess a potent antioxidant activity (*Abolaji et al., 2017*) and an aqueous extract of ginger demonstrated strong free radical scavenging activity (*Gabr et al., 2019*). *Zhu et al. (2015)* have reported that both 6-shogaol and 6-gingerol prevent the progression of diabetic complications, by inhibiting the production of AGEs by trapping methylglyoxal (MGO which is the precursor of AGEs). This exploratory work was specifically planned to see the potential therapeutic effects of ginger (*Zingiber officinale Rosc*) and compare their effects with metformin in the treatment of diabetic nephropathy.

MATERIALS AND METHODS

Drugs and chemicals

Alloxan with metformin were purchased from Sigma Aldrich Chemicals Pvt. Ltd. Moringa powder was purchased from the Earth's Afil Industries, Karachi, Pakistan. Fresh ginger roots were purchased from local market. Aqueous ginger extract was prepared from the protocol established by *Al-Amin et al. (2006)*.

Animals

A total of 36 adult male albino wistar rats weighing between 150 and 200g were purchased from the animal facilities of the International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi. Each animal was numbered and housed separately in cages (49 x 40 cm cages of polypropylene with a stainless-steel top grill) having saw-dust covered floor. 12 h light/12 h dark cycle, humidity and temperature-controlled room were maintained. All animals were acclimatized for one week. The bedding was changed after every 2 days. Experimental

animals had access to water and food. All these conditions were maintained throughout the study.

Induction of diabetes

The body weights and blood glucose concentrations of experimental rats were recorded before alloxan injection. Alloxan, dissolved in 0.9% NaCl, was administered intraperitoneally (I.P.) at a dosage of 100 mg per kg of body weight to induce diabetes mellitus (*Lee et al., 2008*). Following 72 h of Alloxan injection, the blood glucose levels were checked using a glucose assay kit. For the current research, diabetic rats whose blood glucose levels were above 126 mg/dL were considered diabetic and selected (*Shirwaikar et al., 2006*).

Animals grouping

All experimental animals were divided into four groups of 9 rats each (Group I, II, III and IV). Group-I was normal control rats. Alloxan (100mg/kg of body weight; IP) was administered to group II, III and IV animals to induce diabetes and blood samples were obtained for blood glucose levels estimation. Rats in group I received normal laboratory diet and 0.9% saline. Rats of group II were treated with normal saline daily for 4 weeks using oral gavage following one week of alloxan treatment (*Ola et al., 2015*). Group III animals were treated with 500mg of ginger extract/kg of body weight daily for 30 days using oral gavage (*Thomson et al., 2002*) following alloxan treatment. Rats of group IV received 100mg of standard drug metformin/kg of B.W daily for 30 days using gavage (*Erejuwa et al., 2011*).

Sample collection and preparation of tissue homogenates

The experimental animals were sacrificed by using chloroform and blood was drawn by cardiac puncture at the end of experimental schedule. Blood sample in heparinized glass tubes was centrifuged for 5 min at 3000rpm to obtain plasma and was stored at -80°C. Blood in clean glass tubes was allowed to clot at room temperature and left it for 30-60 min. Then sample was centrifuged for 5 min at 3000rpm to obtain clear serum which was then separated and stored at -80 °C. After dissection, liver, kidneys and pancreas were removed, cleaned with cold normal saline, weighed and stored at -80 °C.

The liver, kidney and pancreas tissues were homogenized in buffer solution and the homogenates were centrifuged at 5000xg at 4°C for 20min. The clear supernatant was obtained and called as post-mitochondrial supernatant. PMS was stored at -80 °C and used for estimation of tissue malondialdehyde (MDA) and antioxidants (catalase, superoxide dismutase and reduced glutathione) (*Noori et al., 2009; Erejuwa et al., 2010*).

Biochemical estimations

Blood glucose level was measured by using commercially available glucose kit (Accuchek). Plasma urea concentrations were measured by Kit method (Erba Diagnostics, Germany) (Talke and Schubert, 1965). Serum creatinine concentrations were estimated by method of Jaffe using kit (Biogene Diagnostics, USA) (Lamb et al., 2006).

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by using enzymatic kit (Randox, UK) (Bergmeyer and Horder, 1980). Serum alkaline phosphatase (ALP) was determined by using enzymatic kit of Erba Diagnostics, Germany (Tietz et al., 1983). Tissue malondialdehyde (MDA), catalase (CAT) and superoxide dismutase (SOD) and glutathione (GSH) were estimated according to Okhawa et al. (1979), Sinha (1972), Kono (1978) and Carlberg and Mannervik (1985), respectively.

Hemoglobin A1C and nephrin (NPHN) concentrations were determined by ELISA (Enzyme linked Immunosorbent Assay) using ELISA kit (BT LAB).

Histological examination

Tissues were fixed in 10% neutral buffered formalin and prepared for embedding in paraffin wax according to the routine histological examination protocol. 5 to 10 μm thick sections were cut and then stained with hematoxylin/eosin following (Palipoch and Punsawad, 2013).

Data analysis

The data is presented as Mean \pm SEM. One-way analysis of variance (ANOVA) was used to calculate the statistically significant differences between the experimental groups, with significance levels of $P < 0.05$, $P < 0.01$, and $P < 0.005$ acceptable. SPSS-16.0 and Microsoft Excel were used to perform statistical analysis and draw tables and graphs, respectively.

RESULTS

Effect of ginger extract administration on blood glucose

Glucose concentrations were estimated in animals at the start of the experiment. The average blood glucose concentration was about 80mg/dL in all experimental groups before induction. The diabetic animals showed glucose values of $\pm 140\text{mg/dL}$ ($p < 0.005$) after induction of diabetes. In the second, third and fourth week, glucose concentrations in the diabetic groups treated with ginger and metformin reduced in comparison to diabetic control rats. The control rats and diabetic rats treated with ginger and metformin did not exhibit difference throughout this period (Table I).

Effect of administration of ginger extract on MDA levels

There was a highly notable ($p=0.000$; $p < 0.005$) rise in liver, kidney and pancreas MDA concentration in GP-II in comparison to GP-I. The treatment of ginger extract highly significantly ($p=0.000$; $p < 0.005$) decreased liver, kidney MDA and non-significantly pancreas MDA levels compared to GP-II. Diabetic metformin treated rats (GP-IV) revealed a greatly significant ($p=0.000$; $p < 0.005$) reduction in tissues MDA concentration compared to GP-II but exhibited a remarkable ($p=0.000$; $p < 0.005$) rise in liver MDA concentration in comparison to diabetic ginger treated rats (GP-III). Nevertheless, no remarkable change was observed in kidney MDA levels in GP-III and diabetic metformin treated rats (GP-IV) and showed an insignificant reduction in pancreas MDA levels in diabetic metformin treated rats as compared to GP-III (Table I).

Effect of administration of ginger extract on antioxidant enzymes

The liver, kidney and pancreas CAT, SOD and GSH concentrations were remarkably ($p=0.000$; $p < 0.005$) reduced in GP-II in comparison to GP-I in the present study. The concentration of tissues CAT, SOD and GSH was highly significantly ($p=0.000$; $p < 0.005$) rise in GP-III and GP-IV in comparison to GP-II. GP-IV exhibited a remarkable ($p=0.000$; $p < 0.005$) reduction in liver and kidney CAT, SOD and GSH concentration compared to GP-III. GP-IV showed a non-significant reduction in pancreas CAT concentration and a non-significant rise in tissue SOD concentration in comparison to GP-III. GP-IV exhibited a remarkable ($p=0.000$; $p < 0.005$) decrease in tissue GSH concentration as compared to GP-III (Table I).

Effect of administration of ginger extract on liver function enzymes

Serum ALT concentration was markedly ($p=0.000$; $p < 0.005$) rise in GP-II compared GP-I in the ongoing study. However, GP-III exhibited a marked ($p=0.000$; $p < 0.005$) reduction in ALT concentration in comparison to GP-II. GP-IV showed a highly significant ($p=0.000$; $p < 0.005$) rise in ALT concentration as compared to GP-III and it was non-significant compared to GP-II (Table I).

In our study, a non-significant change was observed in serum AST concentration in GP-II and GP-I. AST concentration was markedly ($p=0.000$; $p < 0.005$) reduced in GP-III in comparison to GP-II. GP-IV exhibited a marked ($p=0.000$; $p < 0.005$) rise in AST concentration in comparison to GP-II and GP-III (Table I).

In the current study there was a marked ($p=0.000$; $p < 0.005$) increase in serum ALP concentration in GP-II in comparison to GP-I. However, GP-III exhibited a highly significant ($p=0.000$; $p < 0.005$) decrease in ALP

concentration in comparison to GP-II. A significant ($p=0.000$; $p<0.005$) rise was observed in ALP concentration in GP-IV compared to GP-III but it was non-significantly increased compared to GP-II (Table I).

Effect of administration of ginger extract on renal function parameters

There was a marked ($p=0.000$; $p<0.005$) increase in plasma urea concentration in GP-II in comparison to GP-I. Plasma urea concentrations was significantly reduced

($p=0.001$; $p<0.005$) in GP-III in comparison to GP-II. GP-IV did not show a significant change in plasma urea concentration compared to GP-II and GP-III (Table I).

The serum creatinine concentration was highly significantly ($p=0.000$; $p<0.005$) rise in GP-II compared to GP-I. A marked ($p=0.000$; $p<0.005$) reduction was observed in serum creatinine concentration in GP-III and GP-IV compared to GP-II. GP-IV showed a non-significant rise as compared to GP-III (Table I).

Table I. Effect of aqueous extract of *Zingiber officinale* on blood glucose level, tissue antioxidants in liver, kidney and pancreas liver function test, renal function test, HbA1C and nephrin of diabetic nephropathy (DN) rats.

| | Normal control (GP-I) (n=9) | DN control (GP-II) (n=9) | DN rats treated with | |
|-------------------------------------|--------------------------------|-----------------------------|---|---|
| | | | Ginger (500mg/kg body weight/day) (GP- III) (n=9) | Metformin (100mg/ kg body weight/day) (GP-IV) (n=9) |
| Antioxidants | | | | |
| MDA(nmol/g) | | | | |
| Liver | 34.79 ± 0.63 | 51.92 ± 0.95** | 26.29 ± 0.81** | 44.08 ± 0.70**/** |
| Kidney | 43.98 ± 0.70 | 55.78 ± 1.24** | 46.04 ± 0.59** | 44.52 ± 0.56**/NS |
| Pancreas | 42.3 ± 0.97 | 58.46 ± 1.60** | 53.79 ± 1.68NS | 50.12 ± 2.14**/NS |
| CAT (μmol/g) | | | | |
| Liver | 3.62 ± 0.1 | 2.66 ± 0.17** | 5.55 ± 0.11** | 3.71 ± 0.16**/** |
| Kidney | 5.07 ± 0.13 | 4.12 ± 0.16** | 7 ± 0.13** | 5.79 ± 0.10**/** |
| Pancreas | 6.93 ± 0.37 | 2.66 ± 0.18** | 5.81 ± 0.13** | 5.42 ± 0.11**/NS |
| SOD (U/mL) | | | | |
| Liver | 10.95 ± 0.18 | 7.55 ± 0.30** | 12.19 ± 0.12** | 9.04 ± 0.28**/** |
| Kidney | 14.76 ± 0.13 | 11.92 ± 0.31** | 18.09 ± 0.47** | 14.03 ± 0.37**/** |
| Pancreas | 11.1 ± 0.35** | 5.2 ± 0.24** | 8.11 ± 0.44** | 9.71 ± 0.17 **/NS |
| GSH (U/g) | | | | |
| Liver | 5.67 ± 0.10 | 4.27 ± 0.36** | 6.09 ± 0.17** | 5.11 ± 0.13** |
| Kidney | 5.4 ± 0.15 | 3.71 ± 0.16** | 7.7 ± 0.21** | 5.56 ± 0.08**/** |
| Pancreas | 5.86 ± 0.18 | 2.4 ± 0.17** | 6.79 ± 0.14** | 5.69 ± 0.10**/** |
| Blood glucose level (mg/dL) | | | | |
| Before induction | 80 ± 2.16 | 71 ± 2.14 | 77 ± 2.12 | 80 ± 2.16 |
| Day 7 after induction | 87.77 ± 0.87 | 140.67 ± 2.67** | 138.89 ± 0.37 | 139.78 ± 0.54 |
| Day 28 post treatment | 87.77 ± 0.87 | 140.67 ± 2.67** | 95.33** ± 1.65 | 95.33 ± 1.65 **/NS |
| Liver function tests (IU/L) | | | | |
| ALT | 22.32 ± 0.75 | 27.2 ± 0.5** | 19.53 ± 0.32** | 28.89 ± 0.76NS/** |
| AST | 41.69 ± 0.71 | 42.39 ± 0.65NS | 34.09 ± 1.17** | 49.05 ± 1.6**/** |
| ALP | 52.14 ± 1.59 | 67.82 ± 0.77 | 48.57 ± 1.03** | 71.49 ± 1.33NS/** |
| Renal function tests (mg/dL) | | | | |
| Plasma urea | 32.19 ± 0.79 | 52.54 ± 1.3** | 45.93 ± 0.82** | 48.86NS/NS ± 1.29NS/NS |
| Serum creatinine | 0.24 ± 0.006 | 0.6 ± 0.01** | 0.36 ± 0.008** | 0.41** ± 0.01NS |
| Blood urea nitrogen | 15.03 ± 0.37 | 24.54 ± 0.61** | 21.39 ± 0.36** | 22.82 ± 0.6NS/NS |
| HbA1C (ng/ml) | 130.14 ± 3.02 | 135.48 ± 4.91NS | 110.51 ± 5.33NS | 125.93 ± 2.24*0/NS |
| Nephrin | 23.26 ± 0.39 | 22.6 ± 0.36NS | 25.04 ± 0.34** | 24.38 ± 0.21*/NS |

NS, Non-significant; * P<0.05; **P<0.005

There was a marked ($p=0.000$; $p<0.005$) rise in blood urea nitrogen concentration in GP-II in comparison to GP-I. However, treatment of ginger extract ($p=0.001$; $p<0.005$) significantly decreased blood urea nitrogen concentration in comparison to GP-II. GP-IV showed insignificant decrease in blood urea nitrogen concentration in comparison to GP-II and non-significant rise compared to GP-III (Table I).

Effect of administration of ginger extract on serum HbA1C levels

There was a non-significant rise in HbA1C in GP-II in comparison to GP-I. HbA1C concentration was non-significantly reduced in GP-III in comparison to GP-II. Diabetic metformin treated rats ($p=0.001$; $p<0.005$) GP-IV exhibited a marked reduction in HbA1C in comparison to GP-II and it was non-significantly rise in GP-IV compared to GP-III (Table I).

Effect of administration of ginger extract on serum nephrin levels

The nephrin concentration was insignificantly reduced in GP-II as compared to GP-I. The level of nephrin was significantly rise in GP-III and GP-IV in comparison to GP-II. GP-IV did not show change compared to GP-III (Table I).

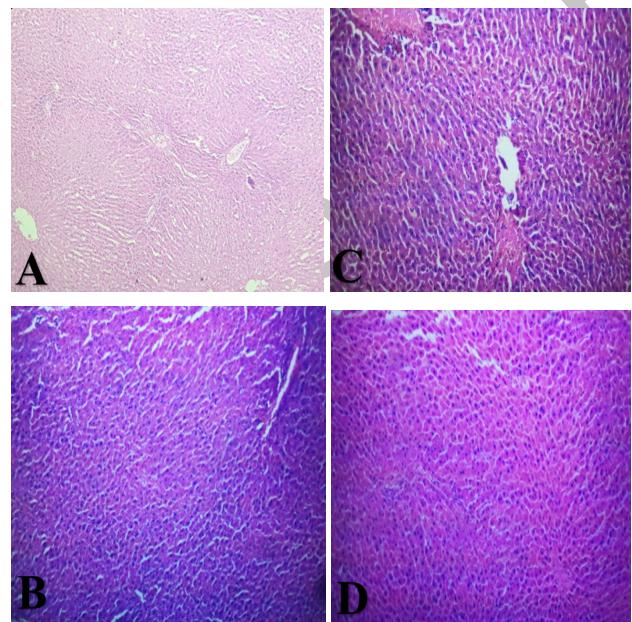


Fig. 1. Effect of ginger extract on histopathological changes in the liver after 4 weeks of treatment in experimental groups. A, Normal control rats; B, Diabetic control rats; C, DN ginger treated rats; D, DN metformin treated rats. Magnification: 20x; Stain: Hematoxylin and Eosin

Histopathological studies

Histopathological examination showed that there was no change in the size and color of the kidneys in every single experimental group of animals. No marked changes in tissue fat contents were noticed in normal control rats, diabetic control rats and other treated groups. Histology showed no identifiable periportal necrosis and bridging necrosis but showed identifiable focal necrosis in diabetic control rats and other treated groups. These focal necrosis changes were less in ginger and metformin treated groups compared to diabetic control rats. It also showed identifiable periportal and lobular inflammation in different experimental groups but these inflammatory changes were less in ginger and metformin treated groups compared to control groups. No significant changes were observed in fibrosis and bile duct proliferation (Fig. 1).

No morphological changes were observed in the size and color of the kidneys. Histological examination showed that the blood vessels and glomerulus were unremarkable. There was no change in interstitial inflammation, tubular atrophy and autolysis. It showed renal infarction and necrosis but the infarction and necrosis were less in ginger and metformin treated groups compared to control group (Fig. 2).

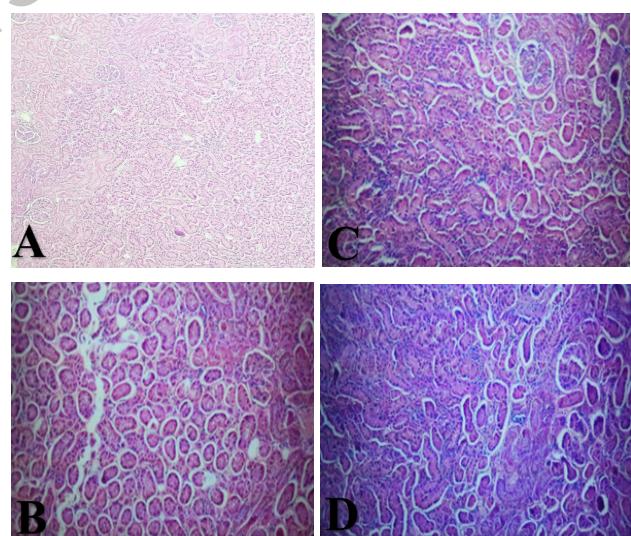


Fig. 2. Effect of ginger extract on histopathological changes in the kidney after 4 weeks of treatment in experimental groups. A, Normal control rats; B, Diabetic control rats; C, DN ginger treated rats; D, DN metformin treated rats. Magnification: 20x; Stain: Hematoxylin and Eosin

Histology of pancreas showed identifiable inflammation, lymphocytes infiltrate and atrophy of acinar cells in diabetic control rats and ginger and metformin treated groups. The changes in inflammation, lymphocytes

infiltrate and atrophy of acinar cells were less in ginger and metformin treated groups compared to control rats. No change has been observed in islets cells except in metformin treated rats that showed hyperplastic islets cells (Fig. 3).

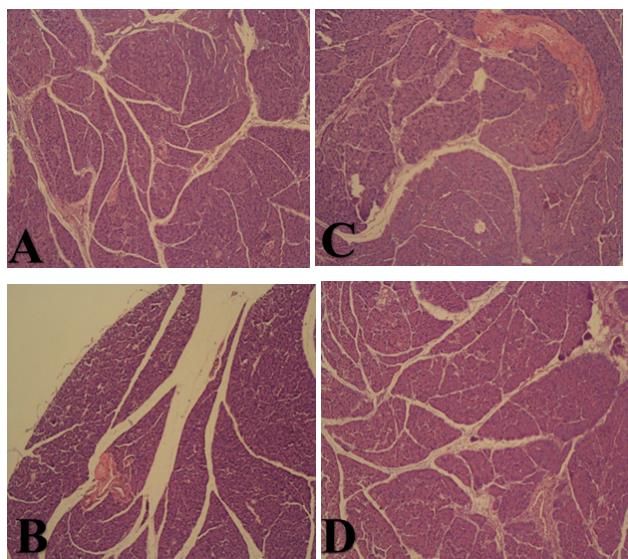


Fig. 3. Effect of ginger extract on histopathological changes in the pancreas after 4 weeks of treatment in experimental groups. A, Normal control rats; B, Diabetic control rats; C, DN ginger treated rats; D, DN metformin treated rats. Magnification: 20x; Stain: Hematoxylin and Eosin.

DISCUSSION

The present study demonstrates strong hypoglycemic effect of ginger in diabetic rats induced by Alloxan. We have observed that *Zingiber officinale* Rosc treatment provides protection against diabetes. The protective effect may be regulated by decreasing oxidative stress, reducing MDA levels, increasing antioxidant activity, decreasing lever enzymes, controlling HbA1C levels and transmembrane Nephron concentration.

According to Gvazava *et al.* (2018), streptozotocin and alloxan are conventional antidiabetic agents that cause diabetes. Federiuk *et al.* (2004) reported that administration route and drugs dosage may change and depends on the animal species. Alloxan was used as an antidiabetic agent in our study. It is a poisonous glucose substance that mainly accumulates in beta cells of pancreas via the GLUT 2 glucose transporter and selectively kills beta cells that produce insulin when given to rodents and many other animal species. Free radicals are produced in redox reactions that cause beta cell damage by alloxan. Because of its strong affinity for cellular molecules containing SH,

alloxan reduces GSH contents. Glucokinase activity is also inhibited by alloxan as glucokinase is essential for insulin secretion (Szkudelski, 2001).

Sangi *et al.* (2018) reported that ginger treatment exhibited a strong hypoglycemic effect in diabetic Wistar rats when it was administered for 30 days (Table I). This antidiabetic effect may be due to pancreatic cellular framework reconstruction. It resumes normal functioning of beta cells and following insulin release which might be due to strong antioxidant action of ginger (Ezez and Tefera, 2021). Increase production of reactive oxygen species leads to peroxidation of lipids (Juan *et al.*, 2021). Free radicals production weakens the antioxidant defense mechanisms. It damages cellular enzymes as well as organelles. MDA is a biomarker. MDA levels are also increased and is produced by membrane lipids oxidation and deterioration. The lipid peroxidation is assessed by MDA levels. In the ongoing study, administration of alloxan significantly increased MDA concentration in diabetic rats and it suggests increased free radicals generation which is in concurrence with Yasin *et al.* (2022) who reported elevated MDA levels in liver (Arabloe Sani *et al.*, 2022), kidney (Pei *et al.*, 2022) and pancreas (Giribabu *et al.*, 2014) in diabetic rats (Table I). The current study showed that ginger treatment highly significantly decreased MDA levels compared to diabetic control rats in liver, kidney and pancreas tissues and results are in agreement with (Cui *et al.*, 2018; Almatroodi *et al.*, 2021).

CAT, SOD and GSH are antioxidative enzymes and they play a crucial role in cell injury protection against free radical impairment. In our research study, CAT, SOD and GSH concentrations are markedly decreased in the PMS of liver, kidney and pancreas in the diabetic group in comparison to the normal control group (Table I). Sadri *et al.* (2017) reported that antioxidant enzyme activities are greatly reduced in diabetes which is caused by increase ROS production that leads to development and progression of DM. The effects caused by an overproduction of ROS and it contributes to liver, kidney and pancreas injury. In our study, we observed that diabetic ginger treated rats revealed a significant rise in tissue CAT, SOD and GSH levels in comparison to diabetic control rats and it is in accordance with research of Rostamkhani *et al.* (2022) (Table I).

ALT, AST and ALP are the liver enzymes and markers of liver functions. High AST, ALT and ALP levels indicate liver damage (Music *et al.*, 2015). Research studies have shown that serum AST, ALT and ALP concentrations are high in diabetic patients (Idris *et al.*, 2011). In our current study, increased serum ALT and ALP were noted in diabetic rats compared to control rats and it is in agreement with the findings reported by Arabloe Sani *et al.* (2022)

but no significant change was observed in AST. The ginger and metformin treatment showed a significant reduction in liver enzymes (Alshathly, 2019; Arabloe Sani *et al.*, 2022) (Table I).

One of the main causes of renal dysfunction in diabetes is free radical-mediated stress, which is characterized by elevated levels of plasma urea, serum creatinine, and BUN as a result of persistent hyperglycemia and changes in the renal tissues (Yang *et al.*, 2022). The elevated levels of plasma urea and serum creatinine can be used as indicators for excessive protein catabolism because urea and creatinine are products of the protein's metabolism. According to this study, plasma urea and serum creatinine levels were considerably higher in diabetic rats than those of the healthy control rats (Yang *et al.*, 2022). Administration of ginger extract significantly reduced the concentration of urea and creatinine levels compared with diabetic control rats. It suggests that ginger extract may exert a preventive and curative effect on kidney damage in diabetes conditions due to its antioxidant properties. These findings are consistent with previous findings reported by (Almatroodi *et al.*, 2021; Ghudhaib, 2018) (Table I).

The risks of microvascular and macrovascular complications can be evaluated and assessed by HbA1c. It is a highly sensitive marker and used to detect diabetes in high-risk individuals with normal fasting blood glucose levels (Perry *et al.*, 2001). It also assesses blood glucose control to evaluate the effectiveness of diabetes treatment (Wu, 1993). Many studies have shown that alloxan treatment also causes increase in HbA1C concentration. In the present study, Administration of ginger non-significantly decreased HbA1C concentration in GP-III in comparison to GP-II. The administration of Metformin (GP-IV) showed a highly significant reduction in HbA1C concentration in diabetic rats compared to GP-II and this is in agreement with previous findings (Pérez Gutiérrez *et al.*, 2021) (Table I).

Nephrin is a transmembrane protein which is found in glomerular podocytes. It is an integral part of podocytes and it forms the glomerular filtration barrier with endothelial cells and the basement membrane. In podocytes, nephrin also mediates significant signaling pathways. The expression of nephrin is frequently reduced in adult kidney disorders, such as diabetic nephropathy. In our study Serum Nephrin concentration was non-significantly reduced in diabetic control rats compared to normal control rats. Administration of ginger extract markedly increased the nephrin concentration compared with diabetic rats. Rafieian-Kopaei and Nasri (2014) reported that the renal nephrin expression was significantly raised after ginger treatment and it might be due to renoprotective effects of ginger (Table I).

Histopathological examination showed that the hepatic focal necrosis, inflammatory changes, renal infarction, renal necrosis and the pancreatic inflammatory changes, lymphocytes infiltrate and atrophy of acinar cells were less in ginger and metformin treated groups compared to diabetic control rats. These findings agree with the results published by Sakina *et al.* (2022).

CONCLUSION

There are numerous distinct types of diabetic medications, some of that act in similar ways, such as by enhancing insulin resistance, regulating the level of blood sugar, and lowering oxidative stress and inflammation. The vast majority of these anti-diabetic medications, however, have a low level of efficacy and a number of adverse side effects, including drug resistance, weight gain, dropsy, and high rates of secondary failure. As a result, further research is required to create low-toxicity, cost-effective anti-diabetic medicines and manage diabetes complications, particularly in long-term treatment. Due to their low toxicity, accessibility, affordability, and simplicity of use, plant-based medications have gained popularity worldwide in recent decades for the treatment of various ailments. In this regard, *Zingiber officinale* Rosc's hypoglycemic, antihyperlipidemic, antioxidant, anti-inflammatory, and numerous other benefits have been reported in many studies. The findings of the current study could increase the depth of understanding regarding ginger's beneficial effects in regulating blood glucose levels in diabetes and related complications including diabetic nephropathy, as well as in providing additional defense against any negative effects on the structure or function of the liver, kidneys, or pancreas. Consequently, it is essential to develop a strategy for using ginger as a safe adjuvant or supplemental medicine for the treatment of diabetes.

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IRB approval

Departmental Research Committee of Department Of Physiology, University Of Karachi with reference to letter DRC No. - 215/23 approved the study.

Ethical statement

Experimental setup for this study was designed in accordance with guidelines of internationally accepted principles for laboratory use and care in animal research (Health research extension ACT of 1985).

Statement of conflict of interest

The authors have declared no conflict of interest.

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