Efficacy of Ginger (*Zingiber officinale*) in Ameliorating Streptozotocin-Induced Diabetic Liver Injury in Rats: Histological and Biochemical Studies

Mona R. Alshathly[1,2]

[1]Department of Biological Sciences, Faculty of Science, Northern Border University, Arar, [2]Department of Biology, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

Ginger (*Zingiber officinale*) was reported to have an antioxidant, antidiabetic effect. This study was done to investigate its therapeutic effect against functional and structural alteration in liver of diabetic rat (intraperitonal streptozotocin (STZ) in a dose of 60 mg/kg/bw). Thirty adult male rats (three-months-old and 250 g weight) were sorted into five groups (*N*=6). G1 used as control, G2 was diabetic rats without any treatment, G3 was diabetic rats given oral ginger in a dose of 500 mg/kg/bw, G4 was diabetic rats treated with metformin (500 mg/kg/bw) while G5 received ginger orally. The experiment lasts for six weeks, animals were anesthetized by ether, body weight was recorded for all animals. Blood was collected for further analysis of lipid profile, liver enzymes and total antioxidant. Liver was dissected, weighted and samples were processed for histopathological study. The results showed significant decrease of glaucous level and liver enzymes in ginger treated rats. Total antioxidant was preserved. Ginger lowered blood glucose, level, regained body weight and liver index to near normal values. Diabetes induced degenerative changes and micro-vesicular lipid deposition in hepatocytes with moderate portal area fibrosis. Ultrastructure study confirmed such changes beside demonstrating increased lipid deposition in fat storing cells. Ginger was found to ameliorate those changes in treated animals. Results were matching metformin effects. In conclusion, Ginger as a natural safe Herbal medication can be used to support liver functions in diabetic status.

Keywords: Diabetes, ginger, histology, liver, streptozotocin, ultrastructure

INTRODUCTION

Diabetes mellitus (DM), a lifelong progressive disease, diagnosed upon the presence of hyperglycemic status characterized by high circulating blood glucose level as a result of body's inability to produce or use insulin deficiency or resistance to its full potential.[1] The global prevalence of the disease all over the world for all age groups was estimated to be 4.4%, and the number of diabetic adult persons was expected to reach 439–552 million by 2030.[2-5] In Saudi Arabia, several studies reported that the risk of developing DM would continue to rise.[6-9]

Diabetes occurs when the pancreas is no longer able to synthesize insulin, and it is classified into two major categories of diabetes which were clinically well known – Type 1 and Type 2. Type 2 DM (T2DM) is the most prevalent form. It accounts for 90%–95% of cases.[10] It is characterized by insulin resistance, progressive decrease of pancreatic β-cell mass and accumulation of human islet amyloid peptide deposits, and progressive loss of pancreatic β-cell mass beside the presence of insulin resistance[11] and associated with subsequent alteration metabolic disorder of lipid and carbohydrate metabolism.[12] Furthermore, low physical activity and high-energy intake are usually associated with it.[13]

When the cells become unable to utilize glucose and/or the liver and skeletal muscles cannot store glycogen, hyperglycemia occurs.[14] The increased glucose concentrations within

Address for correspondence: Dr. Mona R. Alshathly,
Department of Biological Sciences, Faculty of Science,
Northern Border University, Arar, Saudi Arabia.
E-mail: mabdulah@kau.edu.sa

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cellar vicinity result in oxidative stress due to subsequent to production of free radicles in addition to decrease in natural antioxidant defenses.[15] Oxidative stress plays a key role in the onset and development of diabetes complications, was mostly attributed to generation of free radicals resulting in tissue damage notably diabetic nephropathy.[16,17]

Given that, the liver was known to play a major role in the homeostasis of insulin and plasma glucose besides other functions such as glycogen synthesis and gluconeogenesis. Estimation of serum liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase can be used as liver markers to independently predict T2DM[18,19] or metabolic syndrome.[20,21] These markers have been considered indirect measurements of insulin resistance status.[22]

Glucophage (metformin hydrochloride), originated from the plant *Galega officinalis*, is the clinically used as an oral treatment of T2DM and was once considered a complementary and alternative medicine for diabetes.[23]

It was well known that the most synthetic drugs prescribed for treating obesity or diabetes have many unneeded side effects, and therefore, searching for an alternative safe natural agent from medicinal plants, herbs, and spices become mandatory.[24]

Ginger (*Zingiber officinale*) is well known and one of the most widely consumed as a spice worldwide[25] and one of the top five antioxidant foods. Ginger has several beneficial effects and traditional uses in controlling hyperglycemia as well as hyperlipidemia.[26-28] Antioxidant and hepatoprotective effects were also reported,[29] while antiobesity[30,31] and hypocholesterolemic effects were reported in human[32] and experimental animals.[26]

Gingerols were considered an important active ingredient in ginger root.[33-35] The compound 6-gingerol appears to be responsible for its characteristic taste. The analyzed chemical compositions of aqueous extracts of ginger root contain polyphenols, Vitamin C, B, β-carotene, flavonoids, and tannins.[36] Rao and Rao[37] and Butt and Sultan[38] mentioned that all major active ingredients of *Z. officinale* roots and leaves have antioxidant activity by virtue of its components (scavenge free radicals, hydroxyl, and superoxide radicals and that protect cell membrane membranous lipids from oxidative stress damaging effect oxidation). A recent study has revealed that ginger can reduce blood sugar and to an improvement of lipid profiles by ginger based on increasing the activity of antioxidant enzymes,[39] while Khandouzi *et al.*[40] confirmed the antidiabetic effect of ginger in both experimental diabetic rat and diabetic patients, respectively.

Literature regarding testing possible toxic effect of short- or long-term administration of ginger studies abroad safety of ginger usage for treating many clinical conditions as well as treating nausea during pregnancy at dose up to 2500 mg/day was reported.[41-43] The LD$_{50}$ of methanol and water extracts

of ginger for mice are calculated at 10.25 g/kg and 11.75 g/kg body weight (bw) by oral administration.[41,44]

Metformin is clinically used as an antihyperglycemic agent for T2DM because it lowers high blood level in diabetic independent of increasing insulin secretion,[45,46] does not significantly bind to plasma proteins, does not undergo hepatic metabolism, and reaches a steady state in 24–48 h. Besides its antihyperglycemic in T2DM patients, it helps to control body weight, improved lipid profiles and maintained endothelial function.[47] Due to these such characters, it was used in insulin-resistant states.[48,49]

Based on the previous data, in the present study, the role of fresh ginger juice in protecting rat liver tissue from streptozotocin (STZ)-induced diabetes was investigated using both biochemical and light and electron microscopic studies.

MATERIALS AND METHODS
Streptozotocin
STZ was purchased from Sigma-Aldrich Chemical Company (CO., St. Louis, MO, USA). STZ was freshly prepared before use within 15 min by dissolving in 0.01 M sodium citrate buffer, pH 4.5 (solution containing 150 mM NaCl), and used at a dose of 60 mg/kg body weight.[50-52]

Induction of diabetes
Previously prepared STZ was injected intraperitoneal (1 ml/rat) to fasted rats (12 h). After the administration of STZ, the animals were given 5% sucrose solution overnight to prevent hypoglycemia and enhance STZ entrance to β-cells through GLUT2 glucose transporter.[53-55] After 7 days of STZ injection, hyperglycemic status was estimated using Bayer's Contour meter (Japan) in samples taken from the rat tail vein. Blood glucose levels > 250 mg/dl were considered an indicator for developing Type 2 diabetic and selected for the study.[56,57] In the present study, 5% glucose solution in drinking bottle was allowed for injected rats to guard against STZ-induced hypoglycemia.

Metformin preparation
Metformin hydrochloride (Shanghai Shi Guibao Medicine Co., Ltd., China) dose for rat was calculated at 500 mg/kg/day and administrated orally to diabetic rats[58] through gastric tube after dissolving in 0.9% (W/V) sodium chloride for 6 weeks.[59,60]

Preparation aqueous extract of ginger
Ginger fresh roots were obtained from commercial sources in Jeddah, Saudi Arabia. Roots (50 g) were blended after mixing with 75 ml sterile, cold 0.9% NaCl solution for 2 min. The mixture was filtered, centrifuged at 2000 rpm for 10 min, the supernatant fraction was separated, and volume made up to 100 ml with cold normal saline. The concentration was considered 500 mg/ml pertained to the weight of the initial weight (50 g/100 ml). The prepared extract was stored in refrigerator at 4°C weekly. Ginger extract 500 mg/kg/bw/day was given orally to experimental rats for 6-week accord to previously mentioned methodology.[61-64]

Experimental animals and study design

Ethical approval was obtained from the Ethical Committee of King Abdulaziz University (Jeddah, Saudi Arabia) and in accordance with the OECD guidelines for the proper care and use of laboratory animals.

Male rats (*Rattus rattus*) Sprague-Dawley breed were purchased from Animal House, KAU. Thirty male rats, 3 months old, weighing 200–250 g at the age of 8–10 weeks, were kept in standardized rat cages, with light–dark cycle (12/12) at 22°C ± 2°C and left for 1 week for acclimatization. The standard diet and drinking water (provided in graduated polyethylene bottles placed in metal grids in the upper part of the cages) were available *ad libitum* throughout the study. All doses were given daily for 6 weeks through gastric gavage.[63,64]

Animals were then sorted into six groups (*n* = 6):
- G1: control (C) group, which received a vehicle citrate buffer and normal saline (Pierre *et al*., 2012)
- G2: Type 2 nontreated diabetic (D) group provided by standardized food pellets and water *ad libitum*
- G3: Diabetic Type 2 treated with ginger (D + G) group, given previously prepared ginger orally
- G4: Diabetic Type 2 treated with metformin (D + M) group, received 500 mg/kg/bw metformin for 6 weeks
- G5: normal ginger (G) group, received previously prepared ginger orally.

Body weights

Rats were individually weighted by means of a sensitive balance. Whole body weights were record to the nearest 1 mg to determine weekly changes. Initial body weight before starting the experiment as well as at the end was recorded for all animals.

Relative liver weights

Fresh livers from sacrificed rats were blotted dry and subsequently weighted. Absolute liver weights were recorded to the nearest 0.1 mg using an electric balance. To obtain a more precise measure of the change in organ weights, liver weights were recorded relative to body weight.

Blood sampling and biochemical analyses

After 6 weeks from the experiment, rats were fasted for 10–12 h. Blood (1.5 ml) was collected by special capillary tube from retro-orbital venous plexus.[65] Serum was separated by centrifugation at 3000 rpm for 15 min. Serum samples were directly frozen at −80°C for further biochemical analysis.[66] Enzymatic glucose kits (Human Gesellschaft für and Diagnostica mbH, Germany) was used for estimation of blood glucose. Serum level of liver enzymes including ALT, AST, and alkaline phosphatase (ALP) was estimated. Lipid profile including, triglyceride, total cholesterol, low-density lipoprotein, and high-density lipoprotein cholesterol aware also was determined.

Antioxidant analysis

Biodiagnostic kit method was used to estimate the total antioxidant capacity. Steps applied were carried out in accordance to Koracevic *et al*.[67]

Histological examinations

For histological processing of liver, animals of both control and experimental groups were fasted overnight. The rats were sacrificed by decapitation under deep ether anesthesia, the abdomen was opened and the liver was removed, washed in saline. For light microscopy, the largest lobe of the liver was sliced into 2 mm × 3 mm samples, fixed by immersion in 10% neutral-buffered formalin (phosphate buffer PH 7.4) for 24 h, and then processed routinely for paraffin embodying. Blocks were cut in 4 μm. Sections were dewaxed, hydrated in a series of alcohols, stained with hematoxylin and eosin, and further examined by the light microscope (Olympus, USA) connected to digital camera. Photographs from all groups were compared for histological changes.[62]

For electron microscope examinations, samples of liver were cut into 1 mm^3 thick slices and further fixed in 3% glutaraldehyde in phosphate buffer (pH 7.4) at 4°C. Routine processing for electron microscopy was carried out in highly specialized electron microscopy unit. Semi-thin sections (0.5–1 mm in thickness) were then stained with 1% toluidine blue for general orientation using the light microscope. Ultrathin sections, 60 nm thick, were prepared and stained by 2% uranyl acetate and lead citrate.[68] Photographing was done on (JEM-100 Cx11, JEOL, Egypt), at 80 kV, transmission electron microscope.

Statistical analysis

One-way analysis of variance was used for the present data using statistical processor system support (SPSS) for Windows software, version 16.0, SPSS Inc. (Chicago, IL, USA), to compare all the treated groups. Data were presented as mean ± standard deviation. LSD comparisons were performed to assess the significance of the difference among various treated groups, with the statistical significance level of $P < 0.05$.

RESULTS

Body weight investigations

Insignificant decrease in body weight was observed of diabetic rats compared to control [Table 1]. However, there was significant decreased in body weight in diabetic ginger-treated rats compared with both normal and nontreated diabetic.

Liver weight and liver index

In diabetic rats treated with both ginger and metformin, significant decrease was observed in liver weight compared to both nontreated and control animals. On the other hand, there was insignificant difference between all groups.

Liver index of both metformin and ginger treated showed significant different compared to control. Nevertheless, only liver index of metformin showed significant differences with nontreated diabetics. Ginger showed insignificant differences with control [Table 2].

Blood glucose levels

At the beginning of experiments, blood glucose level was

Table 1: Total body weight in the 2nd and 6th weeks comparison of different parameters versus control and diabetic control

Groups	2nd week body weight (g)	6th week body weight (g)	Body weight changes (%)
G1 (control)	161.36±8.47	298.17±22.41	45.77±2.71
G2 (diabetic control)	244.76±14.51	294.32±36.84	15.85±10.48
G3 (diabetic + ginger)	205.80±8.83 (P=0.0001)	254.62±51.71 (P=0.031)	20.14±15.18
G4 (diabetic + metformin)	242.58±14.07	320.57±16.74	24.21±4.98
G5 (normal ginger)	168.82±13.84	285.19±16.37	40.61±6.29

Data were expressed as mean±SD. P: Significance versus diabetic control (P≤0.05). SD: Standard deviation

Table 2: Comparison of liver weight (g) and liver weight index (%) in different weeks versus normal and diabetic control

Groups	Liver weight (g)	Liver weight index (%)
G1 (control)	11.34±1.43	3.91±0.50
G2 (diabetic control)	10.22±1.29	3.49±0.38
G3 (diabetic + ginger)	8.68±1.38 (P=0.035)	3.44±0.24
G4 (diabetic + metformin)	9.40±1.27	2.92±0.24 (P=0.008)
G5 (normal ginger)	10.18±0.78	3.58±0.31

Data were expressed as mean±SD. P: Significance versus diabetic control (P≤0.05). SD: Standard deviation

Figure 1: (a) Liver from control (central vein region) with normal hepatocytes radiating from the central vein. Cells have slightly basophilic cytoplasm and vesicular rounded central nuclei (black arrows). Blood sinusoids are lined by endothelial cells (white arrows). (b) Liver from control (portal vein region with normal content bile duct, hepatic artery, and portal vein) is not visible. There is minimal fibrous tissue and connective tissue cells (star). Nearby, hepatocytes have rounded vesicular active nuclei (H and E, ×400)

within normal level in all groups. After the 6th week of STZ injection, the level of blood glucose showed significantly increased values reached 454.83 ± 9 4.43 mg/dl as compared with normal control (110.33 ± 13.91 mg/dl) [Table 3]. Treatment with either ginger or metformin recorded decreased the level of blood glucose compared to diabetic and normal control group. Ginger significant decreased blood glucose level after 2 weeks compared to control.

Liver function test
Values of ALT and AST recorded increase in diabetic rats as compared with normal control, indicating liver damage [Table 4]. These values reversed insignificantly after the treatment of diabetic rats with ginger and metformin.

Histopathological studies
Light microscopy
The liver from control rat group showed normal architecture of hepatic lobules. Hepatocytes were arranged in the form of branching cords, separated by blood sinusoids, and radiated from the central vein. The cells appeared polyhedral in shape and containing basophilic granules and central rounded vesicular nuclei [Figure 1a]. Portal spaces were also normal, with minimal or no inflammatory infiltration cells and normal contents of connective tissue cells [Figure 1b]. In contrast, the liver parenchyma in diabetic rats showed mild dilation of blood sinusoids, prominence of Kupffer cells. Microstates (in the form of tiny vesicles) were observed in hepatocytes near the central vein. Hepatocyte nuclei were deformed and dark stained (sign of cell apoptosis) [Figure 2a]. Portal vessels are dilated and surrounded by increased fibrous tissue and cell infiltrates [Figure 2b].

Liver tissues from diabetic rats treated with ginger showed slight dilatation of blood sinusoids but still prominent Kupffer
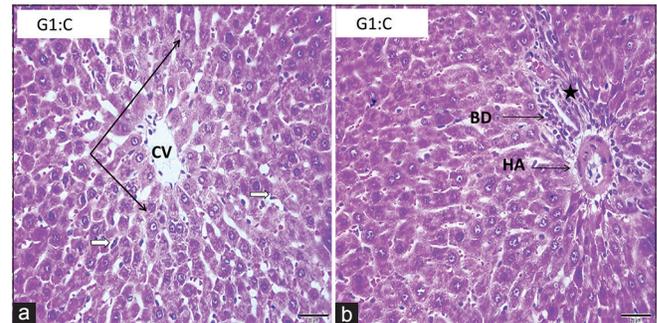
cells. Hepatocytes have homogeneous cytoplasm, with no lipid deposition; they have normal central vesicular nuclei [Figure 3a]. Portal area showed normal vessels and nearly normal hepatocytes [Figure 3b]. In the liver of diabetic rats treated with metformin, hepatocytes central vein and portal areas looked more or less similar to control. Binucleated cells were evidently increased [Figure 4a and b].

In control animals receiving oral ginger, liver parenchyma looked normal with histological features similar to control [Figure 5a and b].

Electron microscopy
Semi-thin sections prepared from the same samples showed more cleared features of liver parenchyma. In control animals, the polyhedral shape of hepatocytes and normal arrangement around central vein were observed. The nuclei of cells are vesicular with prominent one or two nuclei. The endothelial and von-Kupffer cells were also clearly observed [Figure 6a].

Ultrastructure micrographs from control liver [Figure 7a and b] showed that hepatocytes have normal population of mitochondria, rough endoplasmic reticulum, and glycogen granules with few fat globules. Blood sinusoids are lined by normal von-Kupffer cells. Fat-storing cells contain few fat globules.

In semi-thin section from diabetic liver, the central vein showed thickened wall with increased connective cells and fibers. Hepatocytes showed numerous fat globules near sinusoidal

Table 3: Comparison blood glucose levels (mg/dl) in different studied groups in different weeks versus control and diabetic control

Groups	0-week blood glucose (mg/dl)	6th week blood glucose (mg/dl)
G1 (control)	77.00±9.38	110.33±13.91
G2 (diabetic control)	84.67±9.20	454.83±94.43
G3 (diabetic + ginger)	81.83±16.42	320.33±135.94 (P^b=0.027)
G4 (diabetic + metformin)	87.50±8.91	282.83±188.15 (P^b=0.005)
G5 (normal ginger)	63.83±9.91 (P^a=0.031)	126.67±14.36

Data were expressed as mean±SD. P^a: Significance versus normal control ($P \leq 0.05$); P^b: Significance versus diabetic control ($P \leq 0.05$). SD: Standard deviation
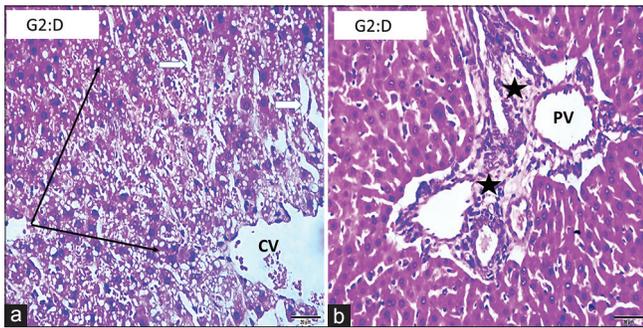

Figure 2: (a) Section of the liver (central vein region) from diabetic rat showing hepatocytes with small lipid droplets (microsteatosis). Their nuclei looked deformed and dark stained (black arrows). Some of the sinusoids are dilated and disorganized (white arrows). The central vein is dilated and showed degenerated endothelial lining. (b) Liver from diabetic group (portal area) showing dilated portal vessels, increased fibrous tissue, and cells infiltrate (stars) (H and E, ×400)
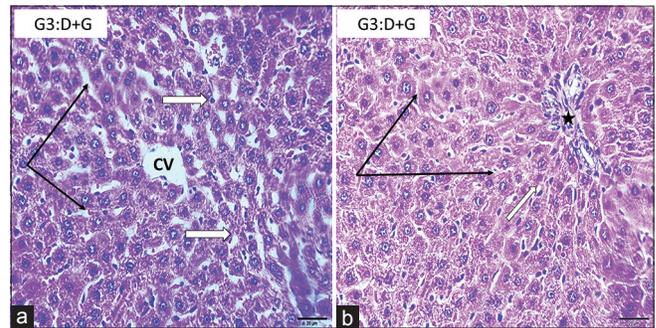

Figure 3: (a) Liver from diabetic and ginger group showing normal hepatocytes radiating from the central vein. Cells have homogeneous cytoplasm free from lipid deposition. They have vesicular rounded central nuclei (black arrows). Blood sinusoids in between are lined by endothelial cells (white arrows). (b) Liver from diabetic and ginger showing normal portal region content (star) and nearly normal liver architecture with normal hepatocytes (black arrows) and blood sinusoids (white arrow) (H and E, ×400)
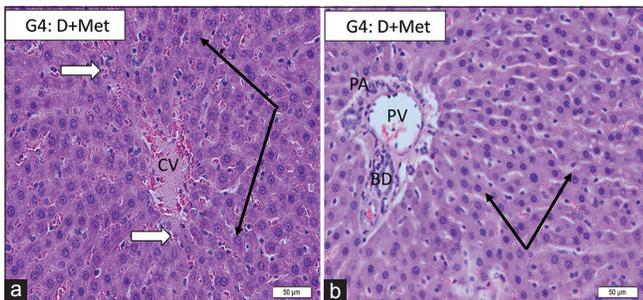

Figure 4: Section in rat liver of diabetic with metformin showing: (a) central vein region with normal hepatocyte cell cord (arrow) and slightly congested sinusoids (white arrows). (b) Portal area, with normal portal vein and bile ducts. Nearly, hepatocytes showed normal cytoplasm and nuclei (arrows)

Figure 5: (a) Liver from the ginger group with normal hepatocytes radiating from the central vein. Some cells have slightly basophilic cytoplasm (arrows), vesicular rounded central nuclei (white arrows). Blood sinusoids in slightly basophilic cytoplasm (arrows), vesicular rounded central nuclei (white arrows). Blood sinusoids in between are lined by endothelial cells (stars). (b) Liver from the ginger group with bile duct, hepatic artery that is not visible and portal vein. Hepatocytes have rounded vesicular active nuclei

wall which it has numerous Ito cells. Portal area showed increased collagen fibrous tissue, and hepatocytes showed also fat globules [Figure 6b].

In electron microscopic graphs, evidence prominence of Ito cells overloaded with fat globules was observed. The hepatocytes showed more lipid droplets and microbodies. Increased bundles collagen in space of Disse was observed [Figure 8a and b]. Some sample hepatocytes showed increased swollen mitochondria with loosen cristae, whereas Ito cells are hypertrophied with large amount of fat cells [Figure 8c and d].
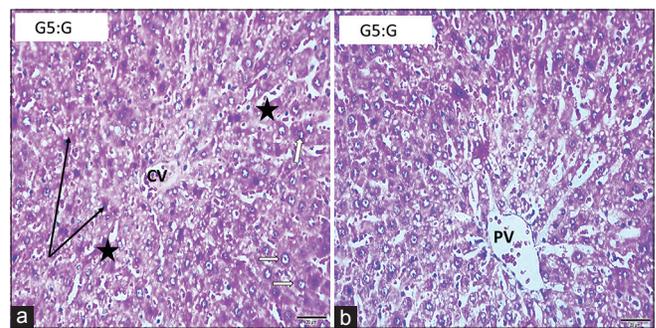
In samples treated with ginger, semi-thin section showed hepatocytes with few number of fat droplets. The nuclei are vesicular with prominent nucleolus. Blood sinusoids are normal [Figure 6c].

Electron micrograph showed hepatocytes with normal cell organelle population. Blood sinusoids are dilated with widening of space of Disse, and hypertrophy of von-Kupffer
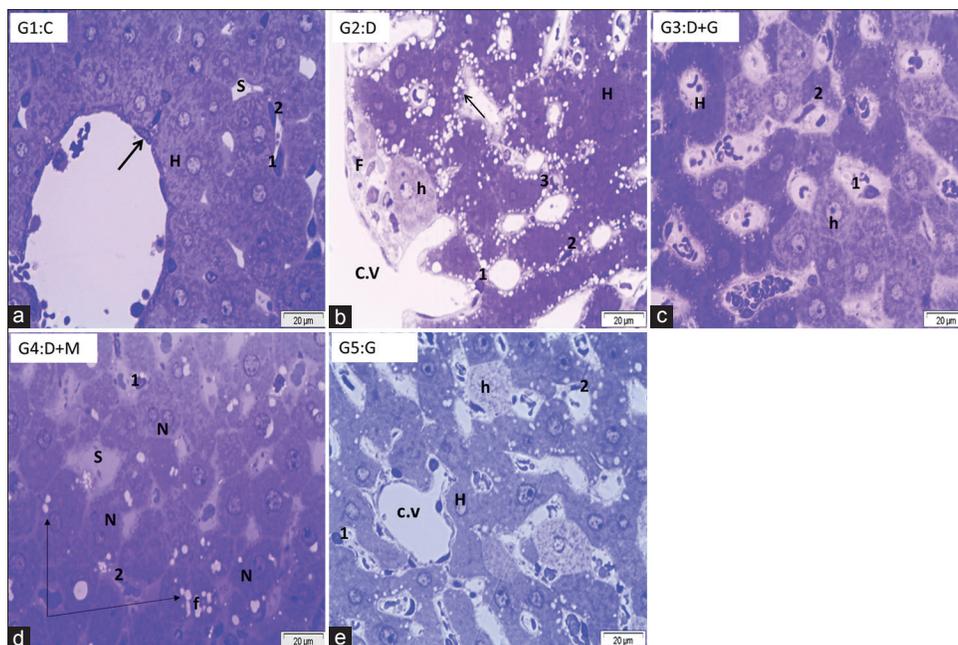
Figure 6: Semi-thin sections from rat liver stained by toluidine blue. (a) G1: Control with normal hepatocytes (H) having rounded vesicular nuclei. Thin wall blood sinusoids (s) lined by Kupffer cells (1) and endothelial cells (2). Central vein is lined by endothelial cells (arrow). (b) G2: diabetic liver with thickened wall of central vein, subintimal fibrosis (F). Hepatocytes (H) showed spherical vacuoles mostly fat globules related to sinusoidal wall (arrows). Sinusoids are lined by Kupffer cells (1), endothelial cells (2), and numerous Ito cells (3). A faintly stained hepatocyte (h) was seen related to central vein. (c) G3: diabetic + ginger showing nearly normal hepatocytes with faint (h) or dark (H)-stained cytoplasm. The nuclei are rounded and vesicular with prominent nuclei. The sinusoidal wall is lined by Kupffer cells (1) and endothelial cells (2). (d) G4: diabetes + metformin showing normal hepatocytes, most are binucleated (N) with prominent nucleoli. Some hepatocytes contain tiny fat globules (f; arrows). Hepatic sinusoids (s) showed moderate congestion and lined with Kupffer cells (1) and endothelial cells (2). (e) G5: ginger group showing hepatocytes (H) with rounded vesicular nuclei. Hepatic sinusoids are dilated and are lined with Kupffer cells (1) and endothelial cells (2)

Table 4: Comparison of liver function tests in different studied groups versus normal control and diabetic control

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
G1 (control)	57.60±13.87	111.80±24.36	154.20±35.24
G2 (diabetic control)	88.50±26.10	140.33±24.43	151.67±30.34
G3 (diabetic + ginger)	73.83±11.77	128.50±26.65	449.95±65.33 (P=0.0001)
G6 (diabetic + metformin)	62.67±14.79 (P=0.004)	119.33±25.17	125.83±35.88
G7 (normal ginger)	48.67±16.62	106.83±20.28	158.00±15.52

Data were expressed as mean±SD. P: Significance versus diabetic control ($P \leq 0.05$). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, SD: Standard deviation

cells with numerous electron-dense bodies, hepatocytes showed small fat globules [Figure 9a and b].

Semi-thin section from metformin-treated liver shows binucleated hepatocytes with normal feature. Blood sinusoids are normal [Figure 6d]. Electron micrograph showed that both hepatocytes and sinusoids have ultrastructure feature similar to control with some samples still showed lipid droplets [Figure 10a and b].

In liver from rats receiving ginger only, semi-thin section [Figure 6e], and electron micrograph [Figure 11a and b] showed features similar to control.

Antioxidant analysis

In STZ-induced diabetic rats, serum total antioxidants were significantly decreased compared to controls (199.1206 ± 12.7327 and 297.5683 ± 25.85752, respectively) [Table 5]. Meanwhile, administration of ginger to STZ-induced diabetic rats preserved total antioxidant level near normal values (292.098 ± 22.1368) and was found to match that of metformin administration (266.7733 ± 15.89884) [Table 5]. Ginger given to control normal rats was found to increase the level of serum antioxidant (339.9392 ± 32.20223) [Table 5].

DISCUSSION

In the present study, the effectiveness of fresh ginger juice on controlling hyperglycemia induced by STZ in rat and its impact on liver functions and structure was evaluated. Diabetes was found to reduce both animal and liver weight compared to nondiabetics. This loss of body weight was attributed to increased muscle loss and degradation of tissue proteins.[69] On the other hand, ginger-treated diabetic group showed significant body weight gain, which has been previously confirmed by other studies before.[63] This effect was suggested to be due to the improvement in insulin secretion and overwhelming muscle wasting and loss.[63] Diabetes is characterized by increased levels of plasma glucose, which in turn modifies blood plasma
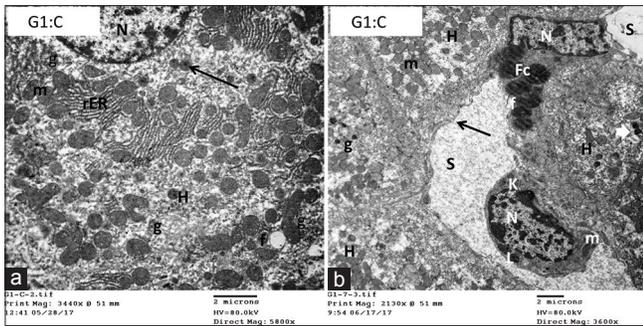
Figure 7: (a) T. E. micrograph of hepatic cell (H) belonging to control group showing the normal ultrastructure, Nucleus (N), rough endoplasmic reticulum, mitochondria (m), microbodies (arrows), glycogen granules (g), and small fat vacuole (f). (b) T. E. micrograph of hepatocyte of control group showing the wall of hepatic sinusoid (S) formed by Kupffer cell (k) having electron-dense lysosomes (L), mitochondria (m) and nucleus, flattened fenestrated endothelial cells (arrows) and fat-storing or Ito cell (Fc) having numerous fat globules (f) and nucleus (N). Notice numerous hepatic cells (H) present around the sinusoid of normal morphological structure containing mitochondria (m), microbodies (arrows head), and glycogen granules (g)

Table 5: Level of serum total antioxidant in all experimented groups

Group	Total antioxidant (mM/g tissue)
G1 (control)	297.5683±25.85752
G2 (diabetic control)	199.1206±12.7327
G3 (diabetic + ginger)	292.0980±22.1368 (P^a<0.00001)
G4 (diabetic + metformin)	266.7733±15.89884 (P^a<0.00001)
G5 (normal ginger)	339.9392±32.20223 (P^b=0.015375)

Data were expressed as mean±SD. ^aP: Significance versus diabetic control ($P\leq0.05$); ^bP: Significance versus normal control ($P\leq0.05$). SD: Standard deviation

proteins by a nonenzymatic reaction called glycation; the effect of ginger matches that of metformin which was known to work through direct inhibition of advanced glycation formation.[59] Metformin was found to reduce plasma glucose levels without the use of endogenous insulin.[70]

In the present study, ginger administration to diabetic rats showed remarkable and almost equal decrease glucose levels compared to the metformin-treated group. However, blood glucose did not decrease to normal level. Many studies have reported that ginger has antihyperglycemic effect by reducing glucose levels.[63,71] This was explained by the ability of the aqueous extract to increase glucose uptake and glycogen synthesis and to increase phosphorylation of the insulin receptor.[40] Furthermore, Li *et al*.[10] stated that ginger promoted glucose clearances in insulin-responsive peripheral tissues and augmented insulin release, which maintained blood glucose homeostasis. The same authors added that ginger extract and its gingerol active ingredient enhanced glucose uptake in cultured rat skeletal muscle cells. In addition, Ramakrishna *et al*.[72] reported that decreased blood glucose levels might be due to the free radical scavenging activity. STZ-induced oxidative stress was prevented by ginger
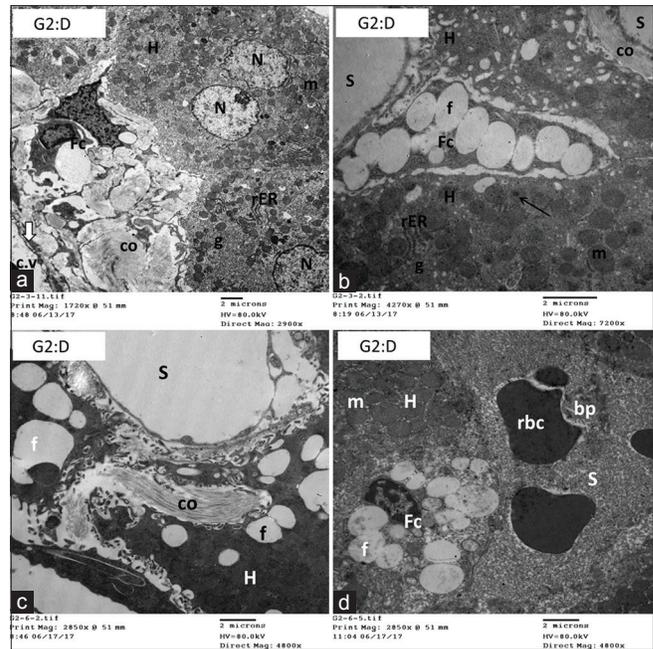

Figure 8: (a) T. E. micrograph of hepatocytes (H) with its cell organelles such as mitochondria (m), rough endoplasmic reticulum, glycogen granules (g), and deformed-shaped nucleus (N). Noticed fibrous deposition formed by bundle of collagen fiber (co), Ito cell (Fc), and connective tissue cells (white arrow) in the wall of the central vein. (b) T. E. micrograph of diabetic liver showing Ito cell or fat-storing cell (Fc) overloaded with numerous fat vacuoles (f). The hepatic cells (H) contain mitochondria (m), microbodies (arrows), rough endoplasmic reticulum and glycogen granules (g). Notice presence of bundle of collagen fiber (co) in space of Disse and the hepatic sinusoid (S) lined with flattened endothelial cell. (c) T. E. Micrograph of part of liver belonging to diabetic group showing hepatic sinusoid (S) with the presence of bundle of collagen fiber (Co) in space of Disse and presence of numerous fat vacuoles (f) in the hepatocytes (H). (d) T. E. micrograph of the liver belonging to diabetic group showing hepatic sinusoid (S) contains red blood cells and blood platelets. Notice the hepatic cell (H) showing increase populations of their mitochondria (m) with swelling and loss of their cristae. Furthermore, the fat-storing or Ito cell (Fc) is hypertrophied and containing large amount of fat vacuoles

administration based on its ability to inhibit lipid peroxidation and hence protect β-cells from diabetic free radicals damaging effect.[73]

The active compounds of ginger are 6-gingerol, tannins, polyphenolic compounds, flavonoids, and triterpenoids.[74] In particular, prostaglandin and leukotriene, the inflammatory mediators were reported to be inhibited by 6-gingerol.[75] Similar effect was reported on angiogenesis.[76] Lowering blood glucose (as confirmed by glucose tolerance test) due to the enhancement of insulin release was reported in an *in vivo* study carried by Ojewole.[77] Involvement of 5-HT3 receptor was thought to be the mechanism underlying this action of ginger through binding to a modulatory site receptor-ion channel complex.[78]

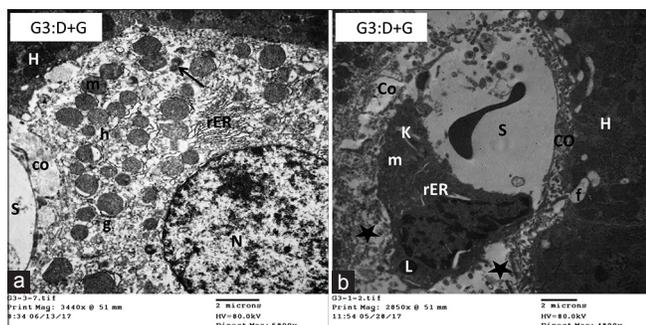The present results showed that ginger extract did not only exhibit hypoglycemic activity in diabetic rats group but

Figure 9: (a) T. E. micrograph of the liver diabetic + ginger group showing hepatic sinusoid (S), space of Disse contains cross sections of collagen bundle (Co). Nearby, hepatocyte (h) looked normal with electron-lucent cytoplasm showing the presence of rough endoplasmic reticulum, mitochondria (m) microbodies (arrow), glycogen granules (g). The nucleus (N) is large and euchromatic. Notice the presence of part of other hepatic cell with electron-dense cytoplasm (H). (b) T. E. micrograph of the hepatic tissue from diabetic + ginger group showing Disse space (stars), hypertrophy of the Kupffer cell (k) having numerous electron-dense lysosomes (L), rough endoplasmic reticulum and mitochondria (m). The hepatic cell (H) showing increases of electron density of the cytoplasm and contain small fat vacuole (f)

Figure 10: (a) T. E. micrograph of the liver from diabetic and metformin group showing the hepatocytes (H) contain large vesicular nucleus (N), numerous fat vacuoles (f), microbodies (arrows), and increased mitochondria (m) population. The hepatic sinusoid (S) contains RBCs (rbc) with widening of the Disse spaces (star). (b) T. E. micrograph of the liver from diabetic and metformin group showing blood platelets (bp) and its wall formed by fenestrated endothelial cells (yellow arrow), Kupffer cell (k) having electron-dense lysosomes (L) and fat-storing or Ito cell (Fc) containing numerous fat globules (f) compressing the cell nucleus (N), and presence of cross section of collagen bundle (Co). Notice the hepatocyte (H) containing indented nucleus (N), rough endoplasmic reticulum, mitochondria (m), microbodies (arrow), and glycogen granules (g)

also decreased glucose level in control nondiabetic rats as well. This action has been also observed by Akhani et al.[26] This pointed to the importance of taking ginger in healthy individuals as protective supplement against diabetes. Similar observation was reported by El-Sharaky et al.[79] who found that pretreatment of healthy rats protects them from toxic effect of bromobenzene-induced hepatotoxicity.

The pathophysiology of T2DM and its complication in different body tissues could be reversed by putative activities of ginger (anti-inflammatory, antioxidant, and antiobesity) and possible hepatoprotective effect. Ginger may not have a direct effect on diabetes but acts indirectly by suppressing factors that lead to impaired glucose control. This was supported by a study showing that ginger root powder (200 mg/kg bw) in a T2DM rat model reversed symptoms of metabolic syndrome, blood glucose, blood lipids, and decreased oxidative stress.[80] Literature proved that increased generation of ROS and reactive nitrogen species are implicated in various liver diseases.[81] Ginger extracts and gingerol are effective in scavenge, superoxide, hydroxyl, and nitric oxide *in vitro*, as they possess free radical scavenging effects.[82,83] These phytochemicals reported to be an effective free radical's scavenger like superoxide.[84,85] Phosphamidon-induced hepatotoxicity was found to be ameliorated by ginger extract.[86]

Analysis of liver function parameters showed elevated levels of ALT and AST in serum compared to control. Such elevation is a well-known effect of T2DM as observed from the previous studies.[87-89] On the other hand, the group treated with metformin showed significant decrease, although not to control levels, indicating liver recovery and homeostasis commencement [Table 4]. Furthermore, the ginger group also
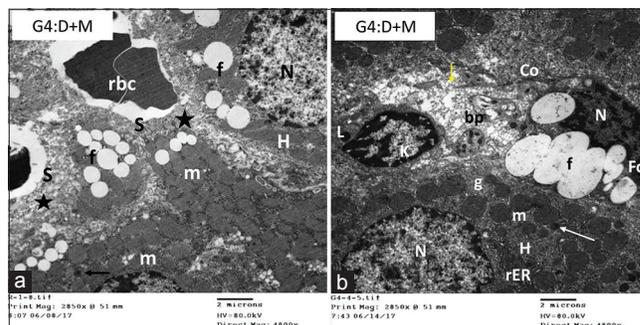
showed almost as decrease levels as the metformin, which demonstrated its protective and ameliorating effect.

Ginger in the present study was found to preserve histological structure of diabetic rat liver as proved by both light and electron microscopy. At biochemical level, ginger aqueous extract was found to significantly decrease serum liver enzymes (AST, ALT, and ALP) in obese diabetic rats.[90] The antioxidant activity of ginger seemed to be underlying potential mechanism promoting hepatoprotective effect of ginger.[29] The antioxidant activity of ginger is possibly preventing membranous lipid peroxidation that results in cell damage and necrosis. Antioxidant markers were assayed in the present study. Ginger was found to prevent the decrease in total antioxidant induced by STZ and hyperglycemia in rat liver tissue. Similar reports with a decrease in dismutase, catalase, glutathione peroxidase, and glutathione reductase were reported by Shanmugam et al.[91] in hepatic tissue of diabetic rat. Similar to the present study, Ramakrishna et al.[72] found that the administration of ginger to diabetic rats restored the altered antioxidant status.

CONCLUSION

The results of the current study can provide a further proof for the ameliorating effect of ginger in controlling blood glucose levels in case of diabetes and further protection against any effect on liver function or structure. Therefore, can provide a plan to utilize ginger as an adjuvant and/or complementary therapy, which is safe in treating T2DM or as a prophylactic in healthy individuals.
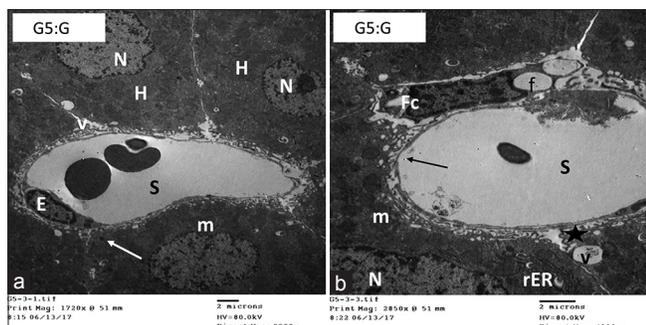
Acknowledgments

Figure 11: (a) T. E. micrograph of the liver from ginger group showing hepatocytes (H) with nuclei (N) having irregular outlines. The hepatocytes contain mitochondria (m), microbodies (arrow). The hepatic sinusoidal (S) looked dilated and is lined by fenestrated endothelial cells (E). (b) T.E. micrograph of the liver from the same showing the sinusoidal wall formed by fenestrated thin endothelial cell (arrow) and fat-storing or Ito cell (Fc) located between the endothelial cell and hepatic cells containing nucleus (N) and fat globules (f). Notice hepatocyte contains nucleus (N), mitochondria (m), rough endoplasmic reticulum, and few small fat vacuoles (v)

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Conflicts of interest
There are no conflicts of interest.

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