European Review for Medical and Pharmacological Sciences 2023; 27: 2428-2442

Potential therapeutic effect of medium chain triglyceride oil in ameliorating diabetic liver injury in a streptozotocin-induced diabetic murine model

K.M. MEHDAR[1], S.A.M. ALSAREII[2]

[1]Department of Anatomy, Faculty of Medicine, Najran University, Najran, Kingdom of Saudi Arabia
[2]Department of Surgery, Faculty of Medicine, Najran University, Najran, Kingdom of Saudi Arabia

Abstract. – OBJECTIVE: Diabetes mellitus is one of the most commonly arising endocrine conditions. The disorder gives rise to enduring damage to a number of body tissues and viscera as a result of related macrovascular and microvascular complications. In patients who are unable to maintain their nutritional status independently, medium-chain triglyceride (MCT) oil is frequently added as a supplement to parenteral nutrition. The aim of the present research is to establish whether MCT oil has a therapeutic influence on the hepatic damage occurring in male albino rats as a result of streptozotocin (STZ)-induced diabetes.

MATERIALS AND METHODS: 24 male albino rats were randomized into four cohorts, i.e., controls, STZ-diabetic, metformin-treated and MCT oil-treated. The rodents were fed a high-fat diet for 14 days; a low dose of intraperitoneal STZ was then administered in order to induce diabetes. The rats were subsequently treated for 4 weeks with metformin or MCT oil. Analysis included an appraisal of liver histology and biochemical indices, i.e., fasting blood glucose (FBG), hepatic enzymes and glutathione (GSH), the latter obtained from hepatic tissue homogenate.

RESULTS: A rise in FBG and hepatic enzymes was observed, but in the STZ-diabetic cohort, hepatic GSH levels were diminished. Treatment with either metformin or MCT oil led to a decline in FBG and hepatic enzyme titers whereas GSH concentrations increased. Liver histology findings were notable amongst rodents within control, STZ-diabetic and metformin-treated groups. The majority of histological changes were resolved following therapy with MCT oil.

CONCLUSIONS: The anti-diabetic and antioxidant characteristics of MCT oil have been substantiated by this work. MCT oil led to a reversal of the hepatic histological changes seen in STZ-induced diabetes in rats.

Key Words:
Hepatic damage, Diabetes mellitus, Histological changes, Antioxidant, medium chain triglyceride oil, Liver injury, streptozotocin.

Introduction

Diabetes mellitus (DM) is well-established as one of the most frequently arising chronic pathologies[1]. It is associated with grave complications which affect most physiological systems; these are likely to be correlated with the severe category of coronavirus-19 disease (COVID-19)[2]. The World Health Organization (WHO) has published contemporary statistics on the global casualty rate from the metabolic disorder. By 2030, the Worldwide prevalence of DM is anticipated to increase to between 10.2% and 10.9%, i.e., 578 and 500 million individuals, respectively[2]. Numerous studies[3,4] have observed the relationship between DM and the severe form of COVID-19, although, as yet it is not clear whether individuals suffering from DM exhibit greater susceptibility to coronavirus infection. However, serum glucose titers and the presence of DM are recognized as independent markers of morbidity and mortality in patients with severe acute respiratory syndrome[5].

DM can be broadly classified into type I (T1DM) and type II (T2DM). Elevated serum glucose levels can promote impaired cell function within the pancreas. This may induce tissue resistance to insulin, a situation typical of T2DM, a disorder which is characterized by insulin deficiency and which involves numerous viscera, such as the liver, musculature and adipose tissue[6].

Corresponding Author: Khlood M. Mehdar, MD; e-mail: khlood.mehdar02@gmail.com

DM is associated with a spectrum of hepatic disorders, e.g., abnormal glycogen deposition, non-alcoholic fatty liver disease (NAFLD), fibrosis, cirrhosis, hepatocellular carcinomas, abnormally elevated liver enzymes, acute hepatic disease and viral hepatitis[7,8]. Surplus adiposity within the liver can promote tissue resistance to insulin and marked metabolic abnormalities. Raised glucose levels may precipitate the death of liver cells and cause a condition referred to as fatty liver in individuals with DM, which is associated with elevated mortality and morbidity rates[7].

The most commonly arising hepatic pathology globally is non-alcoholic fatty liver disease (NAFLD). It occurs in 60% of individuals with hyperlipidaemia; in patients with hyperlipidaemia and elevated liver enzymes, NAFLD has a prevalence of 80%[9]. Insulin resistance is considered to be the common factor connecting NAFLD and metabolic conditions[10,11]; this factor also impacts other metabolic tissues, i.e., the musculature and adipose tissue[12].

There have been notable recent advances in pharmaceutical agents for the treatment of DM, insulin delivery system and glucose monitoring apparatus. Recent guidelines[6,13] suggest that the optimum management strategies should be patient-centric, designed on individual patient's distinctive features, requisites and context. Options for therapy encompass oral anti-diabetic agents, insulin or a combination; their use is guided by ongoing glucose surveillance. Metformin is a long-standing and well-established oral hypoglycemic agent. However, the use of metformin and insulin are associated with adverse effects, such as anorexia, cerebral atrophy (brain shrinking) and insulin resistance[14,15].

A challenge for individuals suffering from T2DM is their inability to control their serum glucose titers over prolonged periods. The appropriate utilization of anti-diabetic agents, together with suitable nutrition plans and physical activity schedules can contribute to the maintenance of normoglycemia. Nevertheless, adverse events have been documented with the use of pharmaceutical agents[16]. The newer anti-diabetic formulations and therapeutic methods are currently highly sought after, particularly nutraceuticals, which have a more benign adverse event profile and greater potential anti-diabetic potency. Advocates of drugs synthesized from naturally arising compounds propose that their use in DM may be more cost-effective. More traditional medicinal practitioners have claimed that substances derived from wild plants may have a therapeutic effect in DM[17,18,19,20].

A naturally arising substance which is derived from *Streptomyces achromogenes*, streptozotocin (STZ) is poisonous to mammalian pancreatic β-cells responsible for insulin release[21]. Clinically, STZ is utilized as chemotherapy for tumours of the islets of Langerhans. In clinical research, STZ can be administered in order to create an *in vivo* experimental model for DM[22,23]. The mechanisms underlying the use of STZ include the production of free radicals and damage to genetic material and its toxicity for pancreatic β-cells, as just one dose can lead to permanent β-cell necrosis and DM[24,25].

Medium-chain triglycerides (MCTs) arise naturally, and typically have between 6 and 12 carbon atom chains, e.g., capric and lauric acids, respectively. Examples of plant oils that have a high MCT content are coconut oil, palm oil, and Cuphea seed oil. Lipases within the stomach and pancreas are responsible for the digestion of MCTs in neonates and nursing animals; the MCTs form a rapid energy source for the metabolic processes that take place within the intestinal cells and the liver[26]. As MCTs undergo rapid absorption into the blood circulation, these substances are valuable for individuals with gastrointestinal disorders, e.g., diarrhea, steatorrhea, and coeliac disease, and following partial gastrectomy or enterectomy[27]. In premature neonates, who have significant energy needs and in whom the gastrointestinal tract is still immature, MCTs are used as a rapid energy source[28]. MCTs are also used as supplementation by sportspeople, as they can enhance athletic performance, diminish body fat and promote muscle gains[29].

An excessive fat percentage in the diet leads to conditions of overweight and obesity. Low-fat or fat-free dietary regimens may be unsatisfactory as the vitamin content is often insufficient and there is an elevated likelihood of cardiovascular disease. Another approach is to substitute some of the traditional long-chain triglycerides (TG) in the diet with MCT oil[30]. A ketogenic nutritional regime complemented by MCT, referred to as the MCT diet, has been commonly utilized therapeutically in individuals with neurodegenerative conditions; in pediatric patients with refractory epilepsy, there have been encouraging data published regarding seizure control[31].

Contemporary ketogenic diets can generally be classified into two types. The conventional form provides approximately 60-80% of energy from

the diet though long-chain lipids, which have 16-20 carbon atoms[32]. There is an extremely reduced carbohydrate component, making the diet especially strict; adherence can be challenging. A second MCT diet was therefore designed, in which lipids take the form of triglycerides which contain 8-carbon and 10-carbon fatty acids, i.e., ~60% octanoic and 40% decanoic acid, respectively. These shorter fatty acids are broken down swiftly within the body, thus leading to a more efficacious production of ketones[33].

In animal experiments and clinical trials, MCTs have demonstrated a hepatoprotective effect. For instance, Li et al[34] observed that a MCT diet diminished alcohol-induced hepatic lipid dyshomeostasis. MCTs also had a prophylactic effect against hepatic injury in a rodent model of NAFLD[35]. Additionally, in clinical trials involving individuals with liver cirrhosis, protection is conferred by MCTs with respect to liver damage caused by alcohol[36].

Therefore, the aim of the current study is to establish whether the administration of MCT oil has a therapeutic outcome in male albino rats with hepatic damage caused by STZ-induced DM.

Materials and Methods

Drugs and Chemicals

Nestle Health Science® MCT Oil, which comprised 53.02% caprylic acid (C8:0) and 46.20% capric acid (C10:0), was purchased from Nestle HealthCare Nutrition, Inc. (Bridgewater, NJ, USA).

Metformin HCl and STZ were obtained from Merck Sante (S.A.S., Lyon, France) and Sigma-Aldrich Chemical Co. (St. Louis, MO, US), respectively.

Siemens Healthcare (Issaquah, WA, US) formed the source of the Flex® reagent cartridge kits utilized for the measurement of glucose levels. Rat alkaline phosphatase (ALP), aspartate aminotransferase (AAT) and alanine aminotransferase (ALT) assays were performed using the respective ELISA Kits, procured from DiaSys Diagnostic Systems (Germany). An ELISA kit selective for rat reduced glutathione (Catalog Number: MBS724319) was employed in order to facilitate a comparison of reduced glutathione titers in various tissues.

Animals

24 male albino rats, weighing between 150 g and 250 g, were obtained from the King Fahad Research Centre. The rodents were kept in cohorts of six in plastic cages containing wood chips. The ambient conditions included a temperature of 23 ± 2 ˚C and a normal circadian cycle. A daily chow diet and a high-fat diet (HFD) were recommended by the research center. The former included 5%, 53% and 23% fat, carbohydrate and protein, respectively; the latter, 22%, 48% and 20% fat, carbohydrate and protein, respectively. The energy content for the daily chow was 25 kJ/kg, and for the HFD, 44.3 kJ/kg[37]. The criteria set by the King Fahd Medical Research Centre's Animal House, Department of Medicine, Faculty of Medicine, King Abdul-Aziz University, Jeddah, Saudi Arabia experimentation were met at all times. Ethical approval was obtained from the institution's Bioethics and Research Committee under the reference number (No.:444-42-39961-DS).

Induction of Diabetes

The HFD was fed to all the animals, regardless of the randomization, for 14 days; this was followed by a low dose (35 mg/kg) of SZT given intra-peritoneally (ip)[37,38]. The STZ was made up to solution in a *de novo* generated 0.01 M citrate buffer (pH 4.5). The first low glucose levels were resolved using oral glucose; 10% glucose was added to the drinking water for the following 24-hour period[39]. After being injected with SZT, fasting blood glucose (FBG) concentrations were assayed. Phlebotomy was performed using the rodents' tail veins; at this juncture, ether gas was utilized for anesthesia. FBG were repeated after a 14-day observation period, and the results for the four groups were compared[40].

An automated glucose analyzer (Glucometer, On Cal; San Diego, CA, USA) was used to assay FBG concentrations, using blood samples taken from the tail veins of fasted rodents. DM was defined in animals after 14 days as FBG titers ≥180 mg/dL[41]. Once the intervention was begun, i.e., 14 days after the induction of DM, the rodents from cohorts II, III and IV were returned to their typical daily chow nutrition. The duration of therapy with either metformin or MCT was 28 days.

Experimental Design

The rodents were randomly assigned to one of four cohorts, each containing six rats. The regimes for each experimental group are detailed below.

Group I (control): animals received routine daily chow as described above and distilled water only.

Group II (diabetic): a 14-day HFD and low-dose ip injection of STZ was utilised to induce DM; fol-

lowing this the rats were given a routine daily chow diet and distilled water for a period of 28 days.

Group III (metformin-treated): 14 days after the induction of DM, rats were commenced on oral daily metformin *via* gavage in a dose of 250 mg/kg bodyweight. This was given for 28 days, based on the recommendations of previous studies[42].

Group IV (MCT oil-treated): 14 days after the induction of DM, rats were administered oral daily MCT in a dose of 500 mL/kg bodyweight. This was continued for 28 days. In previous studies[43,44], the MCT oil contained 14 g gat and 115 calories per 15 mL, and so for the current study, the dose was modified to retain consistency. In order to promote intestinal tolerance, the maximum advised dose is 50-100 g per day, which equates to 60-100 mL. This includes 56-88 g lipid and between 460 and 805 calories[43]. Eight studies have investigated the acute toxicity profile of oral MCTs, i.e., caprylic and capric TG in murines, and concluded that a safe quantity was within the dose range 4.5-36 mL/kg. For mice and rats, the LD450 was established as 25 mL/kg and 36 mL/kg, respectively[44].

Biochemical Analysis: Serum Glucose Level and Liver Profile

At the study's conclusion, the rodents were anaesthetized with diethyl ether. Cardiac puncture was used to obtain blood specimens; these underwent centrifugation in order to separate the cellular component from the serum. The serum was then sampled in order to assay FBG and liver enzymes. The specimens were stored directly for later analysis at a temperature of -20°[45]. The glucose assay was performed as directed in the instructions from the kit vendor. ALP was used for qualitative enzyme analysis only; the vendor's guidelines were again followed.

Liver Homogenization for Evaluation of Oxidative Stress

In order to enable biochemical analysis of reduced GSH levels in the tissue homogenate, a sample of hepatic tissue was stored in an Eppendorf tube at a temperature of 80 ˚C for future analysis. A second specimen underwent preservation in 10% neutral formalin for 48 hours; this was reserved for histological evaluation. In order to assay the GSH levels in the tissue homogenate, a reduced glutathione-horseradish peroxidase admixture was utilized for the initiation of enzyme activity. The degree of coloration was immediately correlated with the quantity of reduced glutathione. In keeping with the vendor's guidelines, the procedures were performed with reference to the standard curve.

Histological Examination of the Liver Tissue

The hepatic samples fixed in 10% neutral formalin were rinsed assiduously and then dehydrated; the latter process was achieved with the use of gradually increasing titers of ethanol. The samples were then infiltrated and embedded in the center of paraffin wax blocks, and sectioned into 5 µm thick slices utilizing a microtome. The slices were immersed in distilled water and hematoxylin and eosin (Sigma, St. Louis, MO, USA) staining was applied[46].

Statistical Analysis

Mean ± standard error (SE) values were obtained from the data by utilizing one-way analysis of variance. The mean differences from each cohort were compared with the use of the Tukey post hoc test. Statistical significance was defined as a p-value < 0.05. Statistical analysis was facilitated by the use of GraphPad Prism (2020) version 9.0 (San Diego, CA, USA). Any relationships between the biochemical assays were determined by Pearson's correlation analysis.

Results

Effects on Fasting Blood Glucose

When compared to controls, FBG concentrations were elevated in the rats with STZ-induced DM ($p < 0.001$) but not in the cohorts treated with either metformin ($p = 0.05$) or MCT oil ($p = 0.8633$). Rodents receiving MCT oil showed a reduction in FBG compared to the STZ-induced diabetic cohort ($p < 0.0001$). A trend towards a lower FBG in the MCT oil-treated group was seen when compared with those given metformin but this failed to reach significance ($p = 0.4136$) (Table I; Figure 1).

Effects on Liver Enzyme Markers

ALT, AST and ALP were elevated in the rats with STZ-induced DM ($p < 0.0001$) compared to the control group (Table I; Figure 2 A-C). A trend towards a rise in hepatic enzymes was seen in the rats receiving either metformin or MCT oil, but this failed to reach significance ($p > 0.05$). When the rats with STZ-induced DM and those treated with MCT oil were compared, ALT, AST and LP

Table I. Serum fasting blood glucose and liver profile and oxidative stress parameters in liver homogenate in different groups.

Parameters	Groups (N=6)	Mean±S.E.
Serum Fasting blood glucose (FBG) (mg/dl -1)	**Control**	87.17±3.70
	STZ-Diabetic	238.33±16.39
	Metformin	129.83±11.20
	MCT oil	103.33±4.47
Serum Alanine aminotransferase (ALT) (IU/L)	**Control**	17.48±.53
	STZ-Diabetic	72.00±4.79
	Metformin	18.90±.48
	MCT oil	18.91±.66
Serum Aspartate aminotransferase (AST) (IU/L)	**Control**	20.96±1.82
	STZ-Diabetic	105.58±3.60
	Metformin	24.50±2.49
	MCT oil	20.18±1.53
Serum Alkaline phosphatase (ALP) (IU/L)	**Control**	51.00±2.80
	STZ-Diabetic	135.66±5.43
	Metformin	52.50±2.26
	MCT oil	48.33±2.13
Reduced glutathione (GSH) (ng/mg protein) in Liver Homogenate	**Control**	18.78±.99
	STZ-Diabetic	2.61±.41
	Metformin	18.03±.76
	MCT oil	19.35±.52

N = number of animals/groups; S.E. = standard errors.

were diminished in the latter cohort ($p < 0.0001$). No differences in liver function tests were observed between the groups receiving MCT oil or metformin ($p > 0.05$).

Effects on Antioxidant Levels

The antioxidant levels are documented in (Table I; Figure 2D). GSH was markedly diminished in the rat cohort with STZ-induced DM ($p < 0.0001$) compared to all the other experimental groups. When compared with the controls, no increase in GSH was observed in the rats administered either metformin or MCT oil ($p > 0.05$). However, in the rats treated with MCT oil, GSH levels were reduced ($p < 0.001$) in comparison to those seen in the STZ-induced diabetic animals, but equivalent to those seen in rats given metformin ($p > 0.05$).

Table II. Pearson's correlation to test the correlation among the biochemical values in different groups.

Correlations		FBG	ALT	AST	ALP	GSH
FBG	r	1	.860**	.886**	.888**	-.895**
	Sig. (2-tailed)		.000	.000	.000	.000
ALT	r	.860**	1	.982**	.968**	-.949**
	Sig. (2-tailed)	.000		.000	.000	.000
AST	r	.886**	.982**	1	.968**	-.965**
	Sig. (2-tailed)	.000	.000		.000	.000
ALP	r	.888**	.968**	.968**	1	-.963**
	Sig. (2-tailed)	.000	.000	.000		.000
GSH	r	-.895**	-.949**	-.965**	-.963**	1
	Sig. (2-tailed)	.000	.000	.000	.000	

**. Correlation is significant at the 0.01 level (2-tailed). r = Person's correlations; N = number of rats/groups. Fasting blood glucose (FBG), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), and reduced glutathione (GSH).

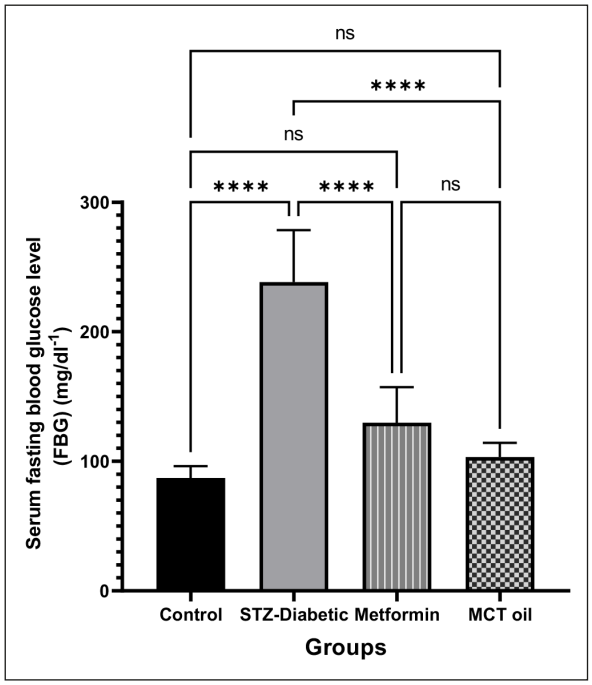

Figure 1. Mean± S.E of serum fasting blood glucose level (FBG) mg/dl-1 on control, STZ-diabetic, Metformin, and Medium-chain triglycerides (MCT) oil groups at the end of the experiment (n=6 rat/group). ****$p \leq 0.0001$, ns = non-significant to the corresponding group.

Biochemical Parameters: Pearson's Correlation Analysis

A positive relationship was observed between the FBG concentration and titers of ALT, AST and ALP (Figure 3A). Notably, negative associations were present between GSH, the marker for oxidative stress, and FBG levels and hepatic enzymes within all the experimental cohorts (Table II; Figure 3B).

Histological Analysis of Liver

Group 1 (control)

In this cohort, histological analysis demonstrated typical appearances of structurally normal liver lobules, with liver cells arranged as anastomosing branching plates emanating from central venous vasculature. The blood sinusoids, which lie between the liver cells, were lined with Kupffer cells and flat endothelial cells. The hepatic cells were characterized as acidophilic, and contained simply a central vesicular nucleus. The cells were densely arranged; a number had two nuclei (Figure 4).

Group II (diabetic)

In contrast to the histology seen in group I, group II rats exhibited notable degenerative alterations in liver cell morphology in the peri-central and peri-portal regions. Deposited lipid droplets were common within parenchymal hepatocytes, giving the appearance of macrovesicular and microvesicular steatosis. Intracellular nuclei demonstrated either a shift or were disrupted. The portal veins appeared distended and congested; the Kupffer cells lying amongst the liver cells demonstrated hypertrophy. These appearances were consistent with marked dilatation and congestion of the sinusoids (Figure 5).

Group III (metformin-treated)

In rats from the metformin-treated cohort, the histological changes recognized in the rodents from group II had partially resolved over the course of the study. The liver cells appeared less rarefied than in the group II animals, with occasional vacuolated cytoplasm. There was only modest sinusoidal congestion and dilatation of the portal vein tributaries, although marked congestion was noted in some areas. The frequency of Kupffer cell hypertrophy was notably less (Figure 6).

Group IV (MCT oil-treated)

In this cohort, the appearances of the liver cells around the central vein were essentially within normal limits. Replicating bile duct and *de novo* liver cells in the vicinity of the portal vein were noted to have dark oval to round nuclei. The morphology and characteristics of the blood sinusoids and Kupffer cells were similar to those seen in group I (Figure 7).

Discussion

The hepatic tissue is a key viscera for metabolic processes, including homeostasis, breakdown, manufacture, hormone synthesis and detoxification. Owing to the key functions performed by the liver in the metabolism of carbohydrates and control of glycemic levels, this organ has been the subject of considerable interest for a number of years in DM. A number of studies[47-49] have reported alterations in the liver in proportion individuals with DM. Biopsy samples have demonstrated lipid accrual within the liver cells, causing a rise in the weight of the hepatic tissue.

Typically, T2DM and NAFLD are comorbidities, with the latter being recognized as a manifestation of metabolic syndrome. The spectrums

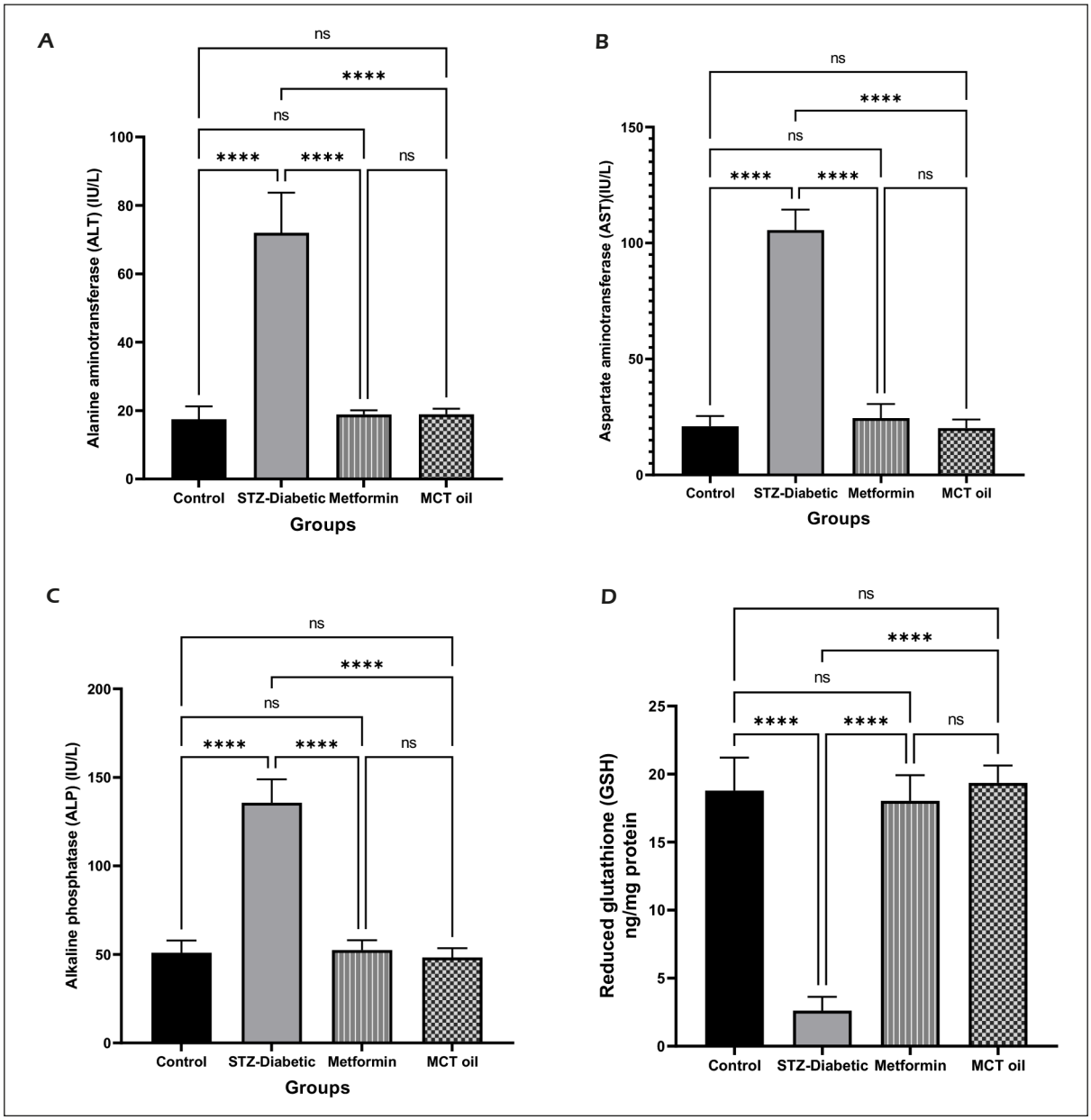

Figure 2. Mean± S.E of serum liver function enzymes: (**A**) Alanine aminotransferase (ALT), (**B**) Aspartate aminotransferase (AST), (**C**) Alkaline phosphatase (ALP) and (**D**) Glutathione (GSH). on control, STZ-diabetic, Metformin, and Medium-chain triglycerides (MCT) oil groups at the end of the experiment (n=6 rat/group). $^{****}p \leq 0.0001$, ns = non-significant to the corresponding group.

of presentations arising from NAFLD include straightforward steatoses (NAFL), non-alcoholic steatohepatitis (NASH) and cirrhosis. Approximately 70% patients with T2DM have NAFLD. This is notably linked with conditions of overweight and obesity, and tissue resistance to insulin[50-52]. Given that obesity is a major etiological factor underlying NAFLD, weight reduction comprises a first line approach to treatment. Contemporary trends have popularized conventional ketogenic nutritional regimens for the reversal of obesity, T2DM and NAFLD[53]. However, as a result of the strict exclusion of most carbohydrates, this diet is poorly tolerated and its preparation is challenging, features which led to poor compliance. Supplementing a diet with MCT is more palatable and gives rise to a greater ketogenic effect than the conventional ketogenic guidelines[54].

Therefore, the aim of the current research was to design an apposite and stable rodent model of

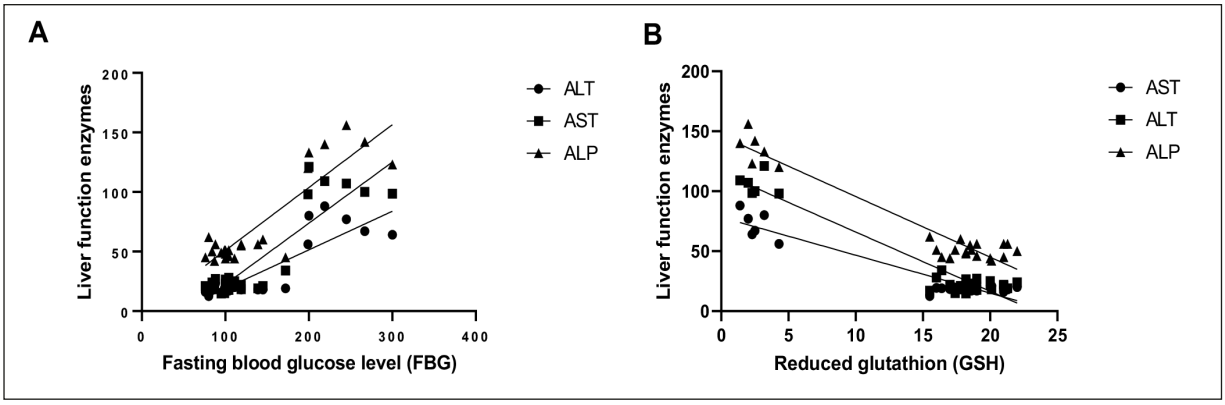

Figure 3. **A**, Correlations between liver function enzymes and fasting blood glucose level. **B**, Correlations between liver function enzymes and reduced glutathione (GSH). ALT= alanine aminotransferase; AST= aspartate aminotransferase; ALP= alkaline phosphatase.

human T2DM, induced following a HFD and an ip injection of STZ, and to establish the potential therapeutic influence of MCT oil on the arising hepatic damage.

A principal objective of the study was to generate a T2DM model in rodents that would simulate the normal progression and metabolic characteristics of the metabolic disorder in humans, and exhibit a response to treatment with pharmaceutical agents[55]. The animal model of T2DM in this research was generated following the use of a HFD and a single ip injection of STZ. In earlier studies[41,55-57], a variety of nutritional regimens and STZ doses have been employed in order to induce T2DM. Numerous researchers have demonstrated the rise in insulin resistance in peripheral tissues that occurs in rodents receiving HFD; this condition is re-

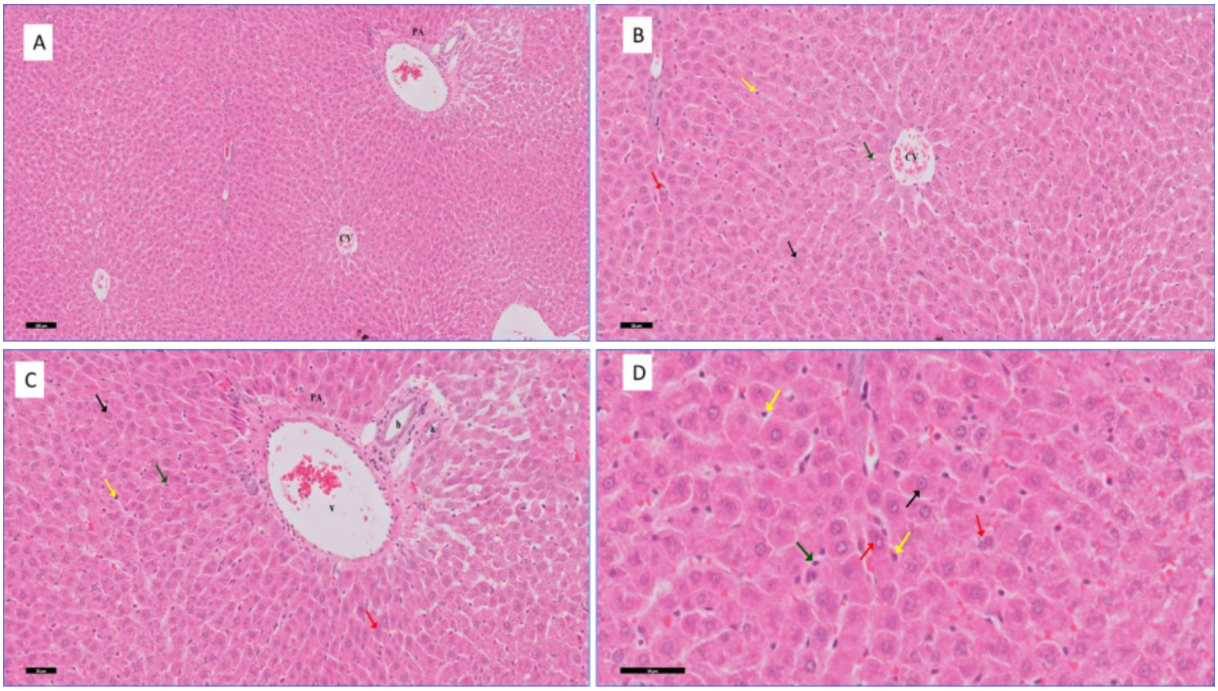

Figure 4. Group I (control) rat liver sections: (**A**) (X10) Hepatocytes distributed radially in cords radiating from the central vein (CV), (**B-C**) (X20) normal vesicular basophilic nuclei (↑) and granular cytoplasm are seen. **D**, (X40) at blood sinusoids Kupffer cells (yellow ↑) and the thin, flat endothelium (green ↑) are lined it. There are normal hepatocytes in the portal region (P.A.) around the portal vein (v), bile duct (b), and hepatic artery (a) with their vesicular nuclei. A few of them (red↑) are binucleatedm, H&E stain were used.

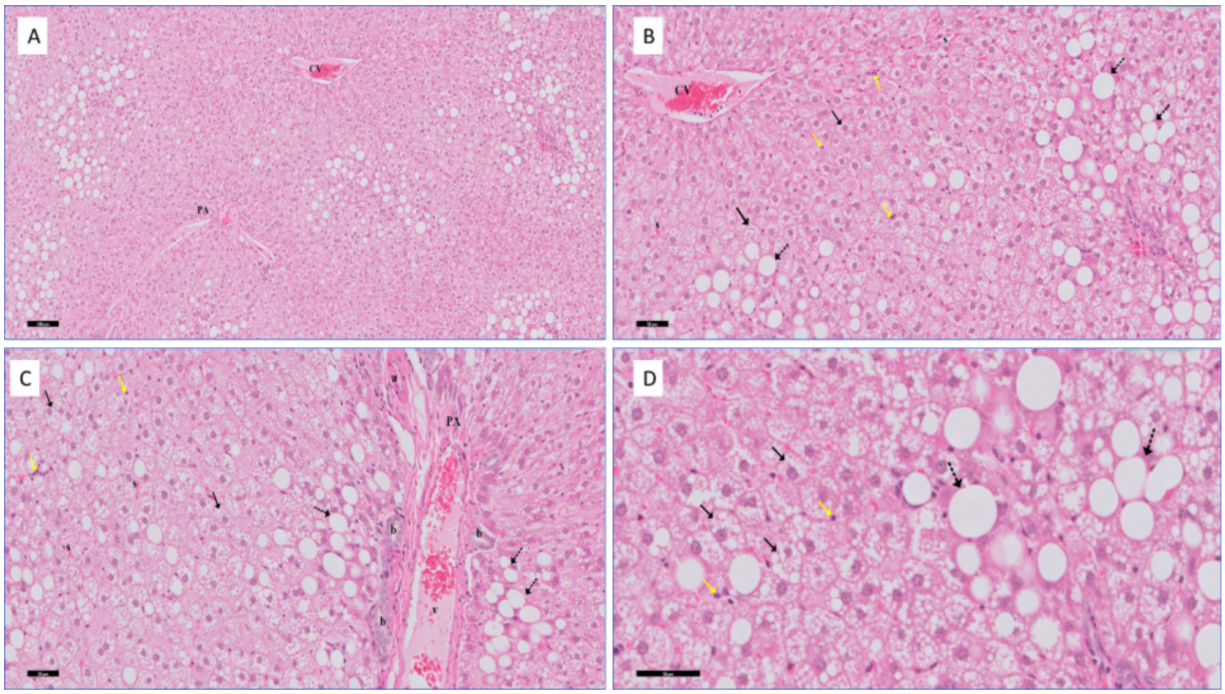

Figure 5. Group II (STZ- diabetic group) rat liver sections: (**A**) (X10) rat liver section, (**B-C**) (X20) rat liver section demonstrating macrovesicular (↑) and (**D**) (X40) rat liver section showing microvesicular (dot arrowhead) steatosis around a congested and dilated central vein (CV) and portal vein (v) in the portal region (P.A.) of degenerated liver cells. Hypertrophied Kupffer cells (yellow↑) and congested blood sinusoids(s) are seen, H&E stain were used.

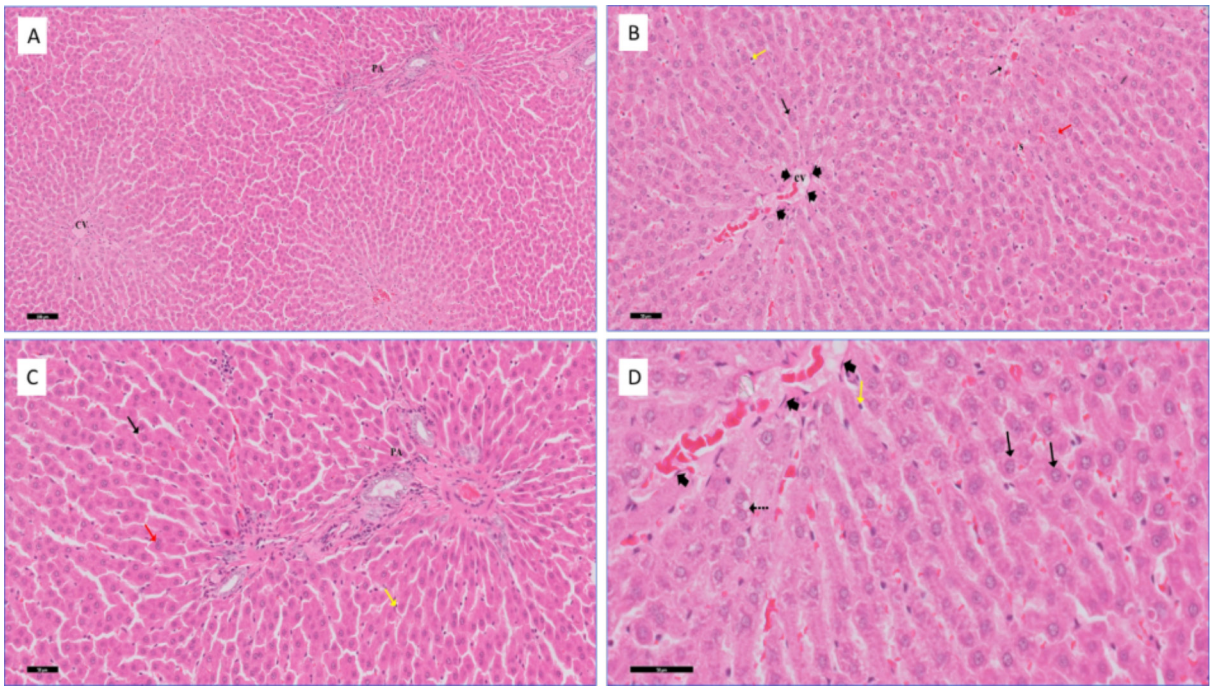

Figure 6. Group III (Metformin treated rats) rat liver sections: (**A**) (X10) hepatocytes are seen in surrounding the central vein (C.V.) and portal area (P.A.), (**B-C**) (X20) surrounding (C.V.) and (P.A.) hepatocytes are seen with typical central vesicular nuclei (↑) and binucleated cells (red ↑) and (**D**) (X40) some vacuolated hepatocytes (dot arrow) and hypertrophied Kupffer cells (yellow ↑). Observe the center vein's flat endothelial cells (arrowhead), H&E stain were used.

ferred to as lipotoxicity, and is characterized by the accrual of lipids[58-62].

The current regime, when judged against the HFD given in the study published by Srinivasan et al[41], had a reduced proportion of lipids, i.e., 22% of the total calories as opposed to 58%. Nevertheless, STZ in a low dose has a low suppressive effect on the release of insulin, which is equivalent to features seen in late-stage T2DM[41,63-65]. The fact that the objectives were accomplished was evidenced by the results, in that the histology and the biochemical analysis indicated that the HFD-STZ protocol had generated marked raised blood sugar levels in the animals within the experimental cohorts[45-66].

The MCT class predominantly comprises caprylic (C8) and capric (C10) fatty acids, but additionally encompasses caproic (C6) and lauric (C12) fatty acids. The range of applications of these lipids is rapidly expanding; they are used in meals, pharmaceutical agents and cosmetic products, as well as in parenteral feeds for patients who require them. The metabolic and pharmacokinetic profiles of MCTs are different to those for TG owing to the abridged carbon chains. In comparison to chylomicrons, MCT absorption occurs immediately into the hepatic portal vein, following which the lipids are rapidly broken down by intestinal and liver cells to generate energy[67]. Furthermore, the anti-inflammatory, antioxidative and anti-obesity characteristics of MCTs mean that they play a key role in energy metabolism[68-70]. *In vivo* experimental models of alcoholic liver disease and NAFLD have been used to demonstrate the hepatoprotective traits of MCT oil[34,35]. In the current work, the second objective was to investigate the latter properties of MCT oil on the injury to the liver caused by STZ-induced DM in rodents.

The principal goal of treatment in patients with DM is to restore normoglycemia. In the rats with STZ-induced DM in the current study, the administration of MCT oil notably diminished FBG titers. In the rats with STZ-induced DM, animals receiving nutrition from normal chow supplemented with MCT oil exhibited a superior glycaemic profile to those on normal chow alone. The beneficial impact of MCT on pancreatic β-cells was investigated in an experimental *in vivo* model by Pujol et al[71]. Raised glucose levels were improved and insulin synthesis was enhanced in elderly rodents; the authors concluded that MCT had the potential to act as both a therapeutic and prophylactic agent in T2DM.

Histological assessment of the rodents with STZ-induced DM in this study demonstrated that the liver had lost some of its typical architectural configuration. In keeping with the current observations, Gawad et al[45] noted that the pathogenesis of DM and its complications may be related to an imbalance between radical-producing and radical-scavenging processes, thus generating oxidative stress. They proposed that an abundance of radicals could arise from glucose protein glycation and autoxidation; these could then stimulate the peroxidation of lipids. Conversely, the synthesis of reactive oxygen species (ROS) within the mitochondria has been demonstrated in response to raised glucose levels; these may play a major role in the advancement of the complications seen in patients with DM[45]. A further explanation is that the low insulin levels and inhibition of fatty acid β-oxidation could be associated with the observed deterioration in the integrity of liver cells in rodent models of DM; this results in triglyceride deposits within the hepatocytes[72]. These earlier findings may underlie the relationships noted between GSH and FBG in the present research.

The identification of Kupffer cell hypertrophy – which seemed to be more prevalent in the rodents with STZ-induced DM, and which only arose in those receiving metformin – was a notable finding in the current study. The role that Kupffer cells play in hepatic injury is currently being readdressed. In one study[73], Kupffer cell hypertrophy and stimulation were the most marked adaptations associated with alterations in the appearances of the liver cells. In defined disease contexts, hepatic stem cells, referred to as oval cells in rodents, have the ability to replicate, whereas liver cell growth is suppressed in severe hepatic injury[74]. Replicating *de novo* liver cells were observed in association with the bile duct following therapy with MCT oil. Yuan et al[75] reported that oval cells may differentiate into either hepatocytes or epithelial cells within the biliary tract; these can precipitate regeneration following liver cell damage, an observation which is in keeping with the results from the current study. The proposal that oval cells are *de novo* generated liver cells may underpin this observation[74].

The control or regulation of apoptosis, described as programmed cell death which arises in order to maintain the tissue's equilibrium, may be lost leading to injurious disease processes, e.g., DM. Elevated FBG titers have been robustly associated with oxidative stress and apoptosis[67]. The rise in oxidative stress and consequent demise of

both hepatocytes and endothelial cells have been described. The present study also demonstrated this process, as there was clear evidence that programmed cell death following the STZ injection was exaggerated. The administration of MCT oil induced restorative changes within the hepatic tissue; the histological arrangement of the tissue was practically identical to that of the control sample. These findings are in keeping with the potential of MCT oil to suppress the activity of cytochrome P450 and/or is potentiation of glucuronidation[76,77].

The antioxidant enzymes form essential constituents of cellular defenses towards ROS and ultimately, towards oxidative stress. The balance between the synthesis of ROS and defense capabilities are therefore indicative of the level of oxidative stress. GSH is one of the antioxidant enzymes responsible for eradicating ROS[78]. From the perspective of pharmaceutical toxicity, the viscera most often targeted is the liver. The production of radical species, and especially ROS, has therefore been proposed as a marker of liver toxicity. GSH is the antioxidant that has the most functions within the cell and is typically assigned a defensive role in relation to the toxicity of xenobiotics[79]. In the present study, GSH levels were diminished in the diabetic rats; in contrast, GSH titers were noted to be elevated in those receiving MST oil. These data are consistent with the proposal by Wollin et al[80], who observed heightened antioxidant levels, e.g., GSH, in cardiac and hepatic tissue following MCT oil delivery. This implies that MCT oil maintains normal oxidation equilibrium in mammals and thus, notably suppresses peroxidative changes in lipids.

The analysis of the liver enzymes affirms the hepatic oxidative damage that has occurred in rats with STZ-induced DM. In the present work, elevated AST, ALT and ALP titers are indicators of necrosis or steatosis within the liver. Significant associations between the hepatic enzymes and FBG were observed. Nevertheless, the investigated liver enzyme levels were markedly diminished in the rats given MCT oil; this was reflected by the normal liver structure and the absence of hepatic steatosis seen on histology in these animals. The binucleated cells that were noted in this cohort suggested the regeneration of cells. Thus, it could be surmised that significant hepatoprotective effects against oxidative damage and ensuing liver steatosis were obtained from the MCT oil[81].

In line with the abnormal liver function tests observed in the rats with STZ-induced DM, the histological analysis in these animals indicated lipid droplet deposition within the hepatic parenchyma. Activation of the enzyme, cytochrome P450 can amplify hepatic enzyme titers *via* reversible pathological changes within the liver, e.g., steatosis, glycogen build-up, and centrolobulillar hypertrophy[38].

In one study, following delivery of a 14-day HFD with a 58% fat content and a 35 mg/kg STZ injection delivered ip in order to induce DM in a cohort of rodents, MCT oil was demonstrated to lead to the preservation and regeneration of liver tissue[38]. Sung et al[38] noted comparable results to those achieved in the current study, in that rodents with DM fed a HFD and who were administered MCT oil demonstrated changed histology in their hepatic tissue and altered glucose levels compared to rodents fed a HFD containing soybean oil. Hepatic disease was practically absent when the isocaloric fat content of MCT oil, which contained C8:0 and C10:0 medium chain fatty acids, was elevated; a dose-dependent decline in steatosis was demonstrated which was presumed to underlie the fall in liver enzyme titers, e.g., ALT[35]. These findings may explicate the positive histological findings in the rodents receiving MCT oil, and the encouraging prophylactic effect of MCT oil against hepatic damage.

Limitations

The limitation of this study is that an additional confirmation on whether MCTs play a synergistic role in other tissues in the animal such as adipose tissue was not conducted. Also, the glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide was not measured after the administration of MCTs as they are secreted in gut as response to nutrient to endorse glucose-dependent insulin. In addition, this study was conducted on male rats, as female may have different responses because of female hormones. Therefore, further investigation needed.

Conclusions

The current study endeavors to attract the interest of academics and scientists with respect to therapeutic options for DM, and focuses on the impact of MCT oil on serum glucose concentrations and liver function tests. The results substantiate the presence of anti-diabetic and antioxidant properties of MCT oil. In rodents with STZ-induced DM, it was demonstrated that MCT returned the deranged liver histology to a

near-normal appearance, and led to more optimal histological and biochemical indices than seen in animals administered metformin. Thus, MCT oil could potentially be recommended as an efficacious therapeutic agent in patients with DM in combination with metformin, an area which merits additional study. The results from the present study may be of advantage to academics in pharmacy, biochemistry, medical and nutritional spheres.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Data Availability
Presented data are available upon the request from corresponding author.

Acknowledgment
The authors are thankful to the Deanship of Scientific Research of Najran University for funding this work through grant research code NU/NRP/MRC/11/8.

ORCID ID
Mehdar KM, 0000-0001-6652-2002.
Alsareii SA, 0000-0003-3579-1386.
.

Ethics Approval
Ethical approval was obtained from the institution's Bioethics and Research Committee of Najran University (No.: 444-42-39961-DS).

Informed Consent
Not applicable.

References

1) Huang I, Lim MA, Pranata R. Diabetes mellitus is associated with increased mortality and severity of disease in COVID-19 pneumonia–a systematic review, meta-analysis, and meta-regression. Diabetes Metab Syndr Clin Res Rev 2020; 14: 395-403.
2) Kononenko I V, Smirnova OM, Mayorov AY, Shestakova M V. Classification of diabetes. World Health Organization 2019. What's new? Diabetes Mellit 2020; 23: 329-339.
3) Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The lancet 2020; 395: 1054-1062.
4) Guo W, Li M, Dong Y, Zhou H, Zhang Z, Tian C, Qin R, Wang H, Shen Y, Du K, Zhao L. Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes/metabolism research and reviews 2020; 36: e3319.
5) Yang JK, Feng Y, Yuan MY, Yuan SY, Fu HJ, Wu BY, Sun GZ, Yang GR, Zhang XL, Wang L, Xu X. Plasma glucose levels and diabetes are independent predictors for mortality and morbidity in patients with SARS. Diabet Med 2006; 23: 623-628.
6) Degli EL, Perrone V, Saragoni S, Blini V, Buda S, D'avella R, Gasperini G, Lena F, Fanelli F, Gazzi L, Giorgino F. Insulin Glargine U100 Utilization in Patients with Type 2 Diabetes in an Italian Real-World Setting: A Retrospective Study. J Diabetes Res 2019; 2019: 3174654.
7) Mohamed J, Nazratun Nafizah AH, Zariyantey AH, Budin SB. Mechanisms of Diabetes-Induced Liver Damage: The role of oxidative stress and inflammation. Sultan Qaboos Univ Med J 2016; 16: e132-e141.
8) Guven A, Yavuz O, Cam M, Ercan F, Bukan N, Comunoglu C, Gokce F. Effects of melatonin on streptozotocin-induced diabetic liver injury in rats. Acta histochemica 2006; 108: 85-93.
9) Mavrogiannaki AN, Migdalis IN. Nonalcoholic fatty liver disease, diabetes mellitus and cardiovascular disease: newer data. Int J Endocrinol 2013; 2013: 450639.
10) Utzschneider KM, Kahn SE. The role of insulin resistance in nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2006; 9: 4753-4761.
11) Fruci B, Giuliano S, Mazza A, Malaguarnera R, Belfiore A. Nonalcoholic fatty liver: a possible new target for type 2 diabetes prevention and treatment. Int J Mol Sci 2013;14: 22933-22966.
12) Hebbard L, George J. Animal models of nonalcoholic fatty liver disease. Nat Rev Gastroenterol Hepatol 2011; 8: 35-44.
13) Task Force on diabetes and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD) pre-diabetes. ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD–summary. Diabetes Vasc Dis Res 2014; 11: 133-117.
14) Bahrambeigi S, Yousefi B, Rahimi M, Shafiei-Irannejad V. Metformin; an old antidiabetic drug with new potentials in bone disorders. Biomed Pharmacother 2019; 109: 1593-1601.
15) Sekar V, Mani S, Malarvizhi R, Nithya P, Vasanthi HR. Antidiabetic effect of mangiferin in combination with oral hypoglycemic agents metformin and gliclazide. Phytomedicine 2019; 59: 152901.

16) Veerapur VP, Prabhakar KR, Kandadi MR, Srinivasan KK, Unnikrishnan MK. Antidiabetic effect of Dodonaea viscosa aerial parts in high fat diet and low dose streptozotocin-induced type 2 diabetic rats: A mechanistic approach. Pharm Biol 2010; 48: 1137-1148.

17) Ngala RA, Ampong I, Sakyi SA, Anto EO. Effect of dietary vegetable oil consumption on blood glucose levels, lipid profile and weight in diabetic mice: an experimental case—control study. BMC Nutr 2016; 2: 1-8.

18) de Lima FF, Traesel GK, Menegati SE, Dos Santos AC, Souza RI, de Oliveira VS, Sanjinez-Argandoña EJ, Cardoso CA, Oesterreich SA, do Carmo Vieira M. Acute and subacute oral toxicity assessment of the oil extracted from Attalea phalerata Mart ex Spreng. pulp fruit in rats. Food Res Int; 91: 11-17.

19) Baldissera MD, Souza CF, Grando TH, Cossetin LF, Sagrillo MR, Nascimento K, da Silva AS, Machado AK, da Cruz IB, Stefani LM, Klein B. Antihyperglycemic, antioxidant activities of tucumã oil (Astrocaryum vulgare) in alloxan-induced diabetic mice, and identification of fatty acid profile by gas chromatograph: New natural source to treat hyperglycemia. Chem Biol Interact; 270: 51-58.

20) da Silva PV, Ramiro MM, Iriguchi EK, Corrêa WA, Lowe J, Cardoso CA, Arena AC, Kassuya CA, Muzzi RM. Antidiabetic, cytotoxic and antioxidant activities of oil extracted from Acrocomia aculeata pulp. Natural Product Research 2019; 33: 2413- 2416.

21) Lenzen S. Alloxan and streptozotocin diabetes. Diabetologia 2007; 51: 216-226.

22) Eleazu CO, Eleazu KC, Chukwuma S, Essien UN. Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans. J diabetes Metab Disord 2013; 12: 1-7.

23) El Rabey HA, Al-Seeni MN, Bakhashwain AS. The antidiabetic activity of Nigella sativa and propolis on streptozotocin-induced diabetes and diabetic nephropathy in male rats. Evidence-Based Complement Altern Med 2017; 2017: 5439645.

24) Szkudelski T. Streptozotocin–nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med 2012; 237: 481-490.

25) Elsner M, Guldbakke B, Tiedge M, Munday R, Lenzen S. Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. Diabetologia 2000; 43: 1528-1533.

26) Zentek J, Buchheit-Renko S, Ferrara F, Vahjen W, Van Kessel AG, Pieper R. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Anim Heal Res Rev 2011; 12: 83-93.

27) Łoś-Rycharska E, Kieraszewicz Z, Czerwionka-Szaflarska M. Medium chain triglycerides (MCT) formulas in paediatric and allergological practice. Prz Gastroenterol 2016; 11: 226.

28) Telliez F, Bach V, Leke A, Chardon K, Libert J-P. Feeding behavior in neonates whose diet contained medium-chain triacylglycerols: short-term effects on thermoregulation and sleep. Am J Clin Nutr 2002; 76: 1091-1095.

29) Nosaka N, Suzuki Y, Nagatoishi A, Kasai M, Wu J, Taguchi M. Effect of ingestion of medium-chain triacylglycerols on moderate-and high-intensity exercise in recreational athletes. J Nutr Sci Vitaminol (Tokyo) 2009; 55: 120-125.

30) Han JR, Deng B, Sun J, Chen CG, Corkey BE, Kirkland JL, Ma J, Guo W. Effects of dietary medium-chain triglyceride on weight loss and insulin sensitivity in a group of moderately overweight free-living type 2 diabetic Chinese subjects. Metabolism 2007; 56: 985-991.

31) Augustin K, Khabbush A, Williams S, Eaton S, Orford M, Cross JH, Heales SJ, Walker MC, Williams RS. Mechanisms of action for the medium-chain triglyceride ketogenic diet in neurological and metabolic disorders. Lancet Neurol 2018; 17: 84-93.

32) Lambrechts DAJE, de Kinderen RJA, Vles HSH, de Louw AJ, Aldenkamp AP, Majoie MJM. The MCT-ketogenic diet as a treatment option in refractory childhood epilepsy: A prospective study with 2-year follow-up. Epilepsy Behav 2015; 51: 261-266.

33) Jain S, Rai R, Singh D, Vohora D. Octanoic acid a major component of widely consumed medium-chain triglyceride ketogenic diet is detrimental to bone. Sci Rep 2021; 11: 1-7.

34) Li Q, Zhong W, Qiu Y, Kang X, Sun X, Tan X, Zhao Y, Sun X, Jia W, Zhou Z. Preservation of Hepatocyte Nuclear Factor-4α Contributes to the Beneficial Effect of Dietary Medium Chain Triglyceride on Alcohol-Induced Hepatic Lipid Dyshomeostasis in Rats. Alcohol Clin Exp Res 2013; 37: 587-598.

35) Ronis MJ, Baumgardner JN, Sharma N, Vantrease J, Ferguson M, Tong Y, Wu X, Cleves MA, Badger TM. Medium chain triglycerides dose-dependently prevent liver pathology in a rat model of non-alcoholic fatty liver disease. Exp Biol Med 2013; 238: 151-162.

36) Zhong W, Li Q, Xie G, Sun X, Tan X, Sun X, Jia W, Zhou Z. Dietary fat sources differentially modulate intestinal barrier and hepatic inflammation in alcohol-induced liver injury in rats. Am J Physiol Liver Physiol 2013; 305: G919-G932.

37) Zhang M, Lv X-Y, Li J, Xu Z-G, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res 2008; 2008: 704045.

38) Sung M-H, Liao F-H, Chien Y-W. Medium-Chain Triglycerides Lower Blood Lipids and Body Weight in Streptozotocin-Induced Type 2 Diabetes Rats. Nutrients 2018; 10: 963.

39) Nagarchi K, Ahmed S, Sabus A, Saheb SH. Effect of streptozotocin on glucose levels in albino Wister rats. J Pharm Sci Res 2015; 7: 67.

40) Mostafavinia A, Amini A, Ghorishi SK, Pouriran R, Bayat M. The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of typel diabetes mellitus and mortality rate in rats. Lab Anim Res 2016; 32: 160-165.

41) Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-

dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res 2005; 52: 313-320.

42) Subhasree N, Kamella A, Kaliappan I, Agrawal A, Dubey GP. Antidiabetic and antihyperlipidemic activities of a novel polyherbal formulation in high fat diet/streptozotocin induced diabetic rat model. Indian J Pharmacol 2015; 47: 509-513.

43) Shah ND, Limketkai BN. The use of medium-chain triglycerides in gastrointestinal disorders. Pr Gastroenterol 2017; 41: 20-28.

44) Traul KA, Driedger A, Ingle DL, Nakhasi D. Review of the toxicologic properties of medium-chain triglycerides. Food Chem Toxicol 2000; 38: 79-98.

45) Gawad SA, Fikry H, Amin MM, Elmahdi AR, Elaziz DA. Effect of apitherapy on the pancreas and liver of streptozotacin induced diabetic rats: a biochemical and histological study. Eur J Pharm Med Res 2016; 3: 555-565.

46) Bancroft JD, Gamble M. Theory and practice of histological techniques. Churchill Livingstone. London, Engl. Published online 2008.

47) Al-Ani IM, Al-Mishadani NS, Muslih RK, Hamoodi SR. Histological liver changes in streptozotocin induced diabetic mice. Int Med J Malaysia 2009; 8.

48) Sumida Y, Murotani K, Saito M, Tamasawa A, Osonoi Y, Yoneda M, Osonoi T. Effect of luseogliflozin on hepatic fat content in type 2 diabetes patients with non-alcoholic fatty liver disease: A prospective, single-arm trial (LEAD trial). Hepatol Res 2019; 49: 64-71.

49) Cusi K, Bril F, Barb D, Polidori D, Sha S, Ghosh A, Farrell K, Sunny NE, Kalavalapalli S, Pettus J, Ciaraldi TP. Effect of canagliflozin treatment on hepatic triglyceride content and glucose metabolism in patients with type 2 diabetes. Diabetes Obes Metab 2019; 21: 812-821.

50) Mantovani A, Sani E, Fassio A, Colecchia A, Viapiana O, Gatti D, Idolazzi L, Rossini M, Salvagno G, Lippi G, Zoppini G. Association between non-alcoholic fatty liver disease and bone turnover biomarkers in post-menopausal women with type 2 diabetes. Diabetes Metab 2019; 45: 347-355.

51) Canfora EE, Meex RCR, Venema K, Blaak EE. Gut microbial metabolites in obesity, NAFLD and T2DM. Nat Rev Endocrinol 2019; 15: 261-273.

52) Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol 2019; 16: 411-428.

53) Abbasi J. Interest in the ketogenic diet grows for weight loss and type 2 diabetes. Jama 2018; 319: 215-217.

54) Zhu H, Bi D, Zhang Y, Kong C, Du J, Wu X, Wei Q, Qin H. Ketogenic diet for human diseases: the underlying mechanisms and potential for clinical implementations. Signal Transduct Target Ther 2022; 7: 1-21.

55) Zhang M, Lv XY, Li J, Xu ZG, Chen L. The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. Exp Diabetes Res 2008; 2008: 704045.

56) Correia-Santos AM, Suzuki A, Anjos JS, Rêgo TS, Almeida KCL, Boaventura GT. Indução de Diabetes Tipo 2 por dieta hiperlipídica e baixa dose de estreptozotocina em ratas wistar. Med (Ribeirão Preto) 2012; 45: 436-444.

57) Zhang J-G, Liu Q, Liu Z-L, Li L, Yi L. Antihyperglycemic activity of Anoectochilus roxburghii polysaccharose in diabetic mice induced by high-fat diet and streptozotocin. J Ethnopharmacol 2015; 164: 180-185.

58) Galmes-Pascual BM, Martínez-Cignoni MR, Moran-Costoya A, Bauza-Thorbrügge M, Sbert-Roig M, Valle A, Proenza AM, Llado I, Gianotti M. 17β-estradiol ameliorates lipotoxicity-induced hepatic mitochondrial oxidative stress and insulin resistance. Free Radic Biol Med 2020;150:148-160.

59) Song JD, Alves TC, Befroy DE, Perry RJ, Mason GF, Zhang XM, Munk A, Zhang Y, Zhang D, Cline GW, Rothman DL. Dissociation of muscle insulin resistance from alterations in mitochondrial substrate preference. Cell Metab 2020; 32: 726-735.

60) Fu Z, Wu J, Nesil T, Li MD, Aylor KW, Liu Z. Long-term high-fat diet induces hippocampal microvascular insulin resistance and cognitive dysfunction. Am J Physiol Metab 2017; 312: e89-e97.

61) Chalkley SM, Hettiarachchi M, Chisholm DJ, Kraegen EW. Long-term high-fat feeding leads to severe insulin resistance but not diabetes in Wistar rats. Am J Physiol Metab 2002; 282: e1231-e1238.

62) Banerjee A, Sharma D, Trivedi R, Singh J. Treatment of insulin resistance in obesity-associated type 2 diabetes mellitus through adiponectin gene therapy. Int J Pharm 2020; 583: 119357.

63) Zhang L, Yang J, Chen XQ, Zan K, Wen XD, Chen H, Wang Q, Lai MX. Antidiabetic and antioxidant effects of extracts from Potentilla discolor Bunge on diabetic rats induced by high fat diet and streptozotocin. J Ethnopharmacol 2010; 132: 518-524.

64) Mansor LS, Gonzalez ER, Cole MA, Tyler DJ, Beeson JH, Clarke K, Carr CA, Heather LC. Cardiac metabolism in a new rat model of type 2 diabetes using high-fat diet with low dose streptozotocin. Cardiovasc Diabetol 2013; 12: 1-10.

65) Elsherbiny NM, Said E, Atef H, Zaitone SA. Renoprotective effect of calycosin in high fat diet-fed/STZ injected rats: Effect on IL-33/ST2 signaling, oxidative stress and fibrosis suppression. Chem Biol Interact 2020; 315: 108897.

66) Zhang S, Xu H, Yu X, Wu YI, Sui D. Metformin ameliorates diabetic nephropathy in a rat model of low-dose streptozotocin-induced diabetes. Exp Ther Med 2017; 14: 383-390.

67) Kim YJ, Kim YA, Yokozawa T. Pycnogenol modulates apoptosis by suppressing oxidative stress and inflammation in high glucose-treated renal tubular cells. Food Chem Toxicol 2011; 49: 2196-2201.

68) Carlson SJ, Nandivada P, Chang MI, Mitchell PD, O'Loughlin A, Cowan E, Gura KM, Nose V, Bistrian BR, Puder M. The addition of medium-chain

triglycerides to a purified fish oil-based diet alters inflammatory profiles in mice. Metabolism 2015; 64: 274-282.

69) Zhang H, Chen Y, Li Y, Yang L, Wang J, Wang T. Medium-chain TAG attenuate hepatic oxidative damage in intra-uterine growth-retarded weanling piglets by improving the metabolic efficiency of the glutathione redox cycle. Br J Nutr 2014; 112: 876-885.

70) Zhou S, Wang Y, Jiang Y, Zhang Z, Sun X, Yu L. Dietary intake of structured lipids with different contents of medium-chain fatty acids on obesity prevention in C57BL/6J mice. J Food Sci 2017; 82: 1968-1977.

71) Pujol JB, Christinat N, Ratinaud Y, Savoia C, Mitchell SE, Dioum EHM. Coordination of GPR40 and Ketogenesis Signaling by Medium Chain Fatty Acids Regulates Beta Cell Function. Nutrients 2018; 10: 473.

72) Otunola GA, Afolayan AJ. Antidiabetic effect of combined spices of Allium sativum, Zingiber officinale and Capsicum frutescens in alloxan-induced diabetic rats. Front life Sci 2015; 8: 314-323.

73) Ahmed SK, Mohammed SA, Khalaf G, Fikry H. Role of bone marrow mesenchymal stem cells in the treatment of CCL4 induced liver fibrosis in albino rats: a histological and immunohistochemical study. Int J stem cells 2014; 7: 87.

74) Fikry H, Gawad SA, Baher W. Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver injury induced by Schistosoma mansoni: A histological study. Int J Stem Cells 2016; 9: 96-106.

75) Yuan J, Peng J, Yin K, Wang J. Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. Mol Nutr Food Res 2011; 55: 150-165.

76) Lieber CS, Cao Q, DeCarli LM, Leo MA, Mak KM, Ponomarenko A, Ren C, Wang X. Role of medium-chain triglycerides in the alcohol-mediated cytochrome P450 2E1 induction of mitochondria. Alcohol Clin Exp Res 2007; 31: 1660-1668.

77) Clegg ME. Medium-chain triglycerides are advantageous in promoting weight loss although not beneficial to exercise performance. Int J Food Sci Nutr 2010; 61: 653-679.

78) Iranloye B. Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. J Diabetes Mellit 2013; 3: 221.

79) Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci 2015; 16: 26087-26124.

80) Wollin SD, Wang Y, Kubow S, Jones PJH. Effects of a medium chain triglyceride oil mixture and α-lipoic acid diet on body composition, antioxidant status, and plasma lipid levels in the Golden Syrian hamster. J Nutr Biochem 2004; 15: 402-410.

81) Pahua-Ramos ME, Garduño-Siciliano L, Dorantes-Alvarez L, Chamorro-Cevallos G, Herrera-Martínez J, Osorio-Esquivel O, Ortiz-Moreno A. Reduced-calorie avocado paste attenuates metabolic factors associated with a hypercholesterolemic-high fructose diet in rats. Plant foods Hum Nutr 2014; 69: 18-24.