Effect of camel milk protein hydrolysates against hyperglycemia, hyperlipidemia, and associated oxidative stress in streptozotocin (STZ)-induced diabetic rats

Bhanu Priya Kilari, Priti Mudgil, Sheikh Azimullah, Nidhi Bansal, Shreesh Ojha, and Sajid Maqsood

ABSTRACT

This study investigated the effect of camel milk protein hydrolysates (CMPH) at 100, 500 and 1,000 mg/kg of body weight (BW) for 8 wk on hyperglycemia, hyperlipidemia, and associated oxidative stress in streptozotocin-induced diabetic rats. Body weights and fasting blood glucose levels were observed after every week until 8 wk, and oral glucose tolerance test (OGTT) levels and biochemical parameters were evaluated after 8 wk in blood and serum samples. Antioxidant enzyme activity and lipid peroxidation in the liver were estimated, and histological examination of the liver and pancreatic tissues was also conducted. Results showed that CMPH at 500 mg/kg of BW [camel milk protein hydrolysate, mid-level dosage (CMPH-M)] exhibited potent hypoglycemic activity, as shown in the reduction in fasting blood glucose and OGTT levels. The hypolipidemic effect of CMPH was indicated by normalization of serum lipid levels. Significant improvement in activity of superoxide dismutase and catalase, and reduced glutathione levels were observed, along with the attenuation of malondialdehyde content in groups fed CMPH, especially CMPH-M, was observed. Decreased levels of liver function enzymes (aspartate aminotransferase and alanine aminotransferase) in the CMPH-M group was also noted. Histology of liver and pancreatic tissue displayed absence of lipid accumulation in hepatocytes and preservation of β-cells in the CMPH-M group compared with the diabetic control group. This is the first study to report anti-hyperglycemic and anti-hyperlipidemic effect of CMPH in an animal model system. This study indicates that CMPH can be suggested for its therapeutic benefits for hyperglycemia and hyperlipidemia, thus validating its use for better management of diabetes and associated comorbidities.

Key words: camel milk, protein hydrolysates, diabetes mellitus, streptozotocin, hypoglycemia

INTRODUCTION

Diabetes is an exponentially increasing global health concern. It is also known as a lifestyle-related disease and a complex metabolic disorder, mainly due to insulin resistance, and begins with postprandial hyperglycemia (Patil et al., 2015). According to the International Diabetes Federation’s Diabetes Atlas (eighth edition), the Middle East and North African regions have the second highest occurrence of diabetes globally (Williams et al., 2020). Deaths related to diabetes are frequently caused by complications such as oxidative dysfunction, organ failure, hypertension, hyperlipidemia, and cardiovascular disease (Sayed et al., 2017). Consistent elevated blood glucose levels result in the production of reactive oxygen species (ROS) that leads to oxidative stress, causing β-cell damage and reduced insulin sensitivity within the pancreas (Ma et al., 2017). Therefore, the primary challenge for medical practitioners and scientists worldwide is to manage and normalize blood glucose levels to avert diabetes-associated complications. Various therapies exist for diabetes management, consisting of nonpharmacological (diet, exercise, and weight loss) and pharmacological approaches (insulin secretagogues, insulin sensitizers, and α-glucosidase inhibitors). However, people with diabetes often prefer complementary and alternative medications, considering their minimal or negligible side effects (Grossman et al., 2018). Among the numerous complementary and alternative approaches, the American Diabetes Association has primarily suggested nutritional approaches in the prevention and management of diabetes mellitus (Aligita et al., 2019). Functional foods represent one...
of the most interesting areas of research and innovation in the food industry, and their possible role in the management of diabetes has been regularly cited (Venkatakrishnan et al., 2019). Among various food components, a significant amount of scientific literature demonstrates the existence of biologically active peptides derived from food proteins with tremendous beneficial effects to human health, specifically diabetes (Nongonierma and FitzGerald, 2016; Nongonierma et al., 2019). Milk proteins are known as the most prominent source of biologically active peptides with potential antidiabetic effects (Sultan et al., 2018). Among the numerous milk varieties, camel milk is known for its superior health effects and is traditionally used in the treatment and prevention of various ailments, especially diabetes (Agrawal et al., 2011; Dubey et al., 2016). In addition, camel milk also shows protective effects against infection, allergies, diabetes, hypercholesterolemia, hypertension, and cancer (Alhaj et al., 2017). It has been reported that naturally existing insulin-like protein and small-sized immunoglobulins in camel milk help in the regeneration of β-cells and also regulate glucose levels (Malik et al., 2012), and therefore camel milk has been proposed as an adjunct to antidiabetic drugs for the management of type 1 and 2 diabetes (Agrawal et al., 2011; Ejtahed et al., 2015). Consumption of camel milk concentrate was effective in maintaining normal serum glucose levels and securing optimal renal and hepatic function in streptozotocin-induced diabetic rats (Ismail et al., 2018). Moreover, camel whey protein supplementation in a streptozotocin-induced type 1 diabetic mouse model has been reported to have beneficial antidiabetic effects by decreasing the free radicals, enhancing antioxidant levels, and orchestrating redox status, hence restoring blood glucose and insulin levels. Recent investigations conducted on camel milk protein hydrolysates indicated their potential in inhibiting 2 major metabolic enzymes (dipeptidyl peptidase-IV and α-amylase) that regulate insulin secretion and carbohydrate digestion, possibly by lowering blood glucose levels (Kamal et al., 2018; Mudgil et al., 2018; Nongonierma et al., 2019). In vitro antidiabetic effects (via inhibition of key metabolic enzymes such as dipeptidyl peptidase-IV, α-amylase, and α-glucosidase) of camel milk protein hydrolysates have been reported to be manifold higher compared with those of intact camel milk proteins (Mudgil et al., 2018), indicating higher efficacy of protein hydrolysates in managing diabetes compared with intact proteins. Alcalase enzyme, also known as subtilisin, is a group of serine proteases that has been extensively used for generation of bioactive peptides, including milk, fish, and soy proteins (Corrêa et al., 2014; Osman et al., 2016). Based on the results of our previous study (Mudgil et al., 2018), camel milk protein hydrolysates generated with alcalase for 9 h displayed potent in vitro antidiabetic properties (Supplemental Table S1; https://data.mendeley.com/datasets/rhf73mzx64/1) and, therefore, was further selected for in vivo studies. To the best of our knowledge, no study has been conducted to evaluate the efficacy of camel milk protein hydrolysates in controlling or managing diabetic complications in an in vivo model. Strong scientific evidence using animal-based model systems is needed to provide further proof of the antidiabetic effects of camel milk proteins and their hydrolysates. Therefore, this study was conducted with an aim of increasing our understanding of the potential contributions of camel milk protein hydrolysates against diabetes and its related complications in streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Chemicals and Reagents

Alcalase (from Bacillus licheniformis), streptozotocin, reduced glutathione (GSH) kit, Trizma base, formalin, xylene, and ethanol were procured from Sigma-Aldrich (St. Louis, MO). Cholesterol assay kit (ab65390) was purchased from Abcam (Cambridge, MA); superoxide dismutase (SOD) and catalase (CAT) estimation kits (catalog nos. 706002 and 707002) were obtained from Cayman Chemical (Ann Arbor, MI). Malondialdehyde (MDA) assay kit (catalog no. MDA01) was purchased from North West Life Sciences Specialties (Vancouver, WA). All other chemicals needed for the study were procured from Sigma-Aldrich.

Production of Camel Milk Protein and Hydrolysates

Camel milk protein hydrolysates (CMPH) were generated through alcalase-driven hydrolysis of skimmed camel milk, according to the method described by Mudgil et al. (2018). For this purpose, raw camel milk from one-humped Arabian camels (Camelus dromedaries) was procured from local camel farms in Al-Ain, United Arab Emirates. The collected milk was immediately refrigerated and brought to the laboratory under chilled conditions. The raw camel milk was skimmed twice by centrifugation at 4,255 × g for 15 min at 10°C, and the upper fat layer was removed manually. Enzymatic hydrolysis of whole camel milk proteins was conducted in 3 different batches using alcalase at an enzyme-to-substrate ratio (wt/wt) of 1:100 at 50°C (pH 8) under constant stirring at 100 rpm for 9 h in a water bath (Clifton NE5-28D, Nickel-Electro Ltd., North Somerset, UK). The enzymatic reaction was terminated by heat inactivation at 100°C for 10 min,
after which the samples were centrifuged at 10,000 × g for 15 min at 4°C, and the supernatants were collected and freeze-dried. The unhydrolyzed skimmed camel milk was also lyophilized, which served as a control sample. The degree of hydrolysis was determined using o-phthalaldehyde method, as described in our previous study Mudgil et al. (2018), and was found to be 81.01%.

**Diabetes Induction**

Animals were acclimatized on a standard diet [American Institute of Nutrition, 1993, maintenance (AIN-93M)], which included the following ingredients: corn starch (46.5%), casein (14.0%), sucrose (10.0%), soybean oil (4.0%), fiber (5.0%), mineral mix (3.5%), vitamin mix (1.0%), L-cysteine (0.18%), choline bitartrate (0.25%), and tert-butylhydroquinone (0.8%). After acclimatization, hyperglycemia was induced by a single intraperitoneal injection of freshly prepared STZ (0.01 M citrate buffer with pH 4.5; 60 mg/kg of BW) in all groups except the normal control group, which was injected with a similar amount of citrate buffer (0.01 M; pH 4.5). After 3 d of injection, the animals with blood glucose levels >250 mg/dL were randomly sorted into 5 groups of 8 animals per group.

**Experimental Design**

A total of 48 rats were randomly allocated into 6 groups (n = 8) as follows:

- **Group 1:** Nondiabetic rats designated as normal control (NC).
- **Group 2:** STZ-induced diabetic control (DC).
- **Group 3:** Diabetic rats administered camel milk protein (CMP) at a dose of 500 mg/kg of BW for 8 wk through oral gavage.
- **Groups 4, 5, and 6:** Diabetic rats respectively administered CMPH at a low dose of 100 mg/kg of BW (CMPH-L), medium dose of 500 mg kg of BW (CMPH-M), and a high dose of 1,000 mg/kg of BW (CMPH-H) orally once daily through gavage for 8 wk.

The present study was designed based on results of our previous study, wherein we reported in vitro antidiabetic activities of CMPH through inhibition of α-amylase and dipeptidyl peptidase-IV (Mudgil et al., 2018; Supplemental Table S1). Camel milk protein hydrolysates generated via alcalase treatment for 9 h demonstrated potential in vitro antidiabetic properties.

The results of our previous study encouraged us to investigate the antidiabetic properties of CMPH under in vivo conditions, to provide supportive evidence related to the role of CMPH in antidiabetic mechanisms of camel milk. During the 8 wk of experimental period, changes in BW and the development of any other symptoms among the experimental rats were recorded weekly. After completion of the experimental period, the rats were decapitated to avoid stress. Blood was collected from all rats, and the clotted blood was centrifuged at 2,054 × g for 30 min to separate the serum, and serum was stored at −80°C for further analysis. Liver tissue samples were collected from each rat and homogenized at 4°C in Tris-buffered saline (50 mM Tris-HCl, 150 mM NaCl, pH 7.4), and the supernatant was collected to determine the biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), total triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Subsequently, the liver and pancreas were excised and stored in 10% formalin for histological examinations.

**Measurement of Fasting Blood Glucose Levels**

Fasting blood glucose (FBG) levels were monitored 3 times to confirm sustained hyperglycemia during the experiment at 0, 4, and 8 wk, using a blood glucometer (Accu-Check, Roche Diagnostics Middle East, Dubai, UAE). Blood was drawn from the tail vein using a sterilized needle, and rats with FBG levels >250 mg/dL were considered diabetic (Ghanbari et al., 2016).

**Oral Glucose Tolerance Test**

An oral glucose tolerance test (OGTT) was performed at the end of the experiment (after 8 wk). Rats in all the experimental groups were orally administered glucose (2 mg/kg of BW) after overnight fasting. Glucose was given orally to the rats after 30 min of administration of CMP and CMPH. Blood glucose levels were measured at 0.5, 1.0, 1.5, and 2 h after gavage, with blood drawn from the tail vein using the One-Touch blood glucometer (Accu-Check, Roche Diagnostics
Middle East) to assess the efficacy of CMP and CMPH on glucose tolerance (Tanaka et al., 2009).

**Estimation of Liver Function Markers**

Levels of AST and ALT in liver and serum samples were determined according to the method previously described (Ismail et al., 2018) using an autoanalyzer (Vet Test Chemistry Analyzer, Idexx Laboratories Inc., Westbrook, ME).

**Assessment of Serum Lipid Profile**

Serum levels of TC, HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C) were measured calorimetrically using diagnostic reagent kits (Cholesterol Assay Kit, ab65390, Abcam) following the instructions described in the manual. Triglyceride levels were estimated according to the Friedewald formula: LDL-C (mg/dL) = TC (mg/dL) − HDL-C (mg/dL) − TG (mg/dL)/5 (Friedewald et al., 1972).

**Determination of Antioxidant Levels**

Liver tissue was excised from all the rats in experimental groups upon sacrifice, washed immediately with cold saline to remove blood, and homogenized at 4°C in Tris-buffered saline (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) buffer using an IKA homogenizer (T 25 Digital Ultra-Turrax, IKA, Königswinter, Germany) followed by centrifugation at 11,000 × g for 15 min at 4°C. The resulting supernatant was collected for calorimetric evaluation of antioxidant levels, including SOD, CAT, and reduced GSH, using commercial kits following the manufacturer’s instructions (SOD and CAT kits were procured from Cayman Chemical; GSH kit from Sigma-Aldrich). The extent of lipid peroxidation was determined by measuring MDA levels in the liver tissue, using the commercial kit as per the manufacturer’s instructions (Northwest Life Science Specialties, Vancouver, WA).

**Histological Studies**

Liver and pancreatic tissues isolated from the experimental rats were washed and fixed immediately in 10% formalin solution. Histopathological studies were conducted by dehydration of formalin-fixed tissues in a graded series of ethanol, clearing in xylene, and embedding in paraffin wax. The paraffin blocks were sectioned into 5-µm thickness using a microtome (model no. RM2125 RTS, Leica Biosystems, Wetzlar, Germany). The sections were fixed on gelatin-coated slides and finally stained with hematoxylin and eosin. Finally, the pathological changes in the tissues were observed under a light microscope [model no. DM3000 LED, Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland] according to a previously described method (Ismail et al., 2018).

**Statistical Analysis**

Data are presented as mean ± standard error of means (SEM) obtained from 8 rats per group to evaluate variances between the groups. The statistical analysis was performed using SPSS Statistics 25.0 software (IBM Corp., Armonk, NY) by one-way ANOVA, followed by Tukey’s post hoc test. Significance was considered at P < 0.05.

**RESULTS**

**Effects of CMP and Hydrolysates on FBG Levels**

The administration of CMP and its hydrolysates at 3 doses resulted in a significant improvement in BW compared with that in the diabetic control group (Supplementary Table S2). Please see supplementary material (https://data.mendeley.com/datasets/rhf73mzx64/1) for more details on changes in BW upon administration of CMPH.

Table 1 shows the results of glucose metabolism monitored in the normal and experimental rat groups on wk 0, 4, and 8 of the experiment. Overall, no significant (P < 0.05) changes in FBG values were detected in wk 0 of the experiment (after initiation) among the different treatment groups. A continuous increase in blood glucose levels was observed in the DC group from the first day, which continued throughout the experimental period (412.50 mg/dL on d 0 to 526.63 mg/dL at wk 8). Administration of STZ led to a significant increase in FBG levels compared with those of the NC group. However, in the groups administered CMP and CMPH, FBG levels began to decrease from wk 4 of the experiment. A significant reduction in blood glucose levels was found in the CMPH-M (500 mg/kg of BW) group compared with those of the NC group. However, in the groups administered CMP and CMPH, FBG levels began to decrease from wk 4 of the experiment. A significant reduction in blood glucose levels was found in the CMPH-M (500 mg/kg of BW) group compared with those in the DC group (P < 0.05) at the end of the experiment. The mean FBG levels in the CMPH-M group were 410.13 ± 3.65 mg/dL at the start of the experiment and progressively declined to 183.75 ± 2.92 mg/dL at the end of the final experimental day. A similar pattern of FBG-level reduction was also observed in the CMPH-H group, although the FBG levels at the end of the experiment remained slightly higher (199.6 ± 3.53 mg/dL) than those in the CMPH-M group. These results indicated that CMPH-M has the ability to reduce blood glucose levels, implying its antihyperglycemic effects.
Effects of CMP and CMPH on OGTT

The results of OGTT, expressed as blood glucose levels in mg/dL, are presented in Table 2. Overall, rats in the NC group exhibited a peak in blood glucose levels after 60 min of glucose loading, which remained within the prescribed limits (i.e., <140 mg/dL) at all time intervals. However, blood glucose levels in the DC group remained very high between 30 and 120 min and did not return to basal levels even after 120 min. The administration of CMP and CMPH at different doses led to reduction in glucose levels from 60 min onward. The blood glucose levels in the CMP-administered group also showed improvements in terms of glucose tolerance, in which the blood glucose levels increased from an initial level of 237.50 to 332.50 mg/dL after 60 min of glucose loading and declined to 274.25 mg/dL after 120 min of glucose loading. Similarly, in the CMPH-L group, blood glucose levels peaked at 60 min (345.25 mg/dL) and returned to near basal levels (265.38 mg/dL) after 120 min. Administration of CMPH at the mid-level dose resulted in the greatest effects, where the maximal reduction in blood glucose levels were found at 120 min (197.63 mg/dL), compared with other groups. Overall, the blood glucose levels at the end of the experiment (120 min) were found to be 95.75 ± 2.45, 530 ± 2.33, 274.25 ± 4.59, 265.38 ± 13.11, 197.63 ± 3.04, and 220.63 ± 6.10 mg/dL in the NC, DC, CMP, CMPH-L, CMPH-M, and CMPH-H groups. Among these groups, CMPH-M showed a steady decline in blood glucose levels, restoring them to normal values. Therefore, these results indicated that CMP and CMPH had a positive effect against diabetes.

Effects of CMP and CMPH on Serum and Hepatic Enzyme Markers

To assess the effects of STZ on liver function markers, serum AST and ALT were analyzed (Figure 1A). The levels of AST and ALT were elevated in the DC group compared with those in NC group. Upon administration of CMP and CMPH to diabetic rats, the AST and ALT levels were reduced significantly (P < 0.05) compared with those in the DC group. The serum AST levels were 46 ± 1.50, 145.88 ± 5.24, 103.38 ± 2.04, 97.75 ± 2.59, 61.38 ± 3.08, and 72.75 ± 2.30 IU/L in the NC, DC, CMP, CMPH-L, CMPH-M, and CMPH-H groups. The serum ALT levels were 28.88 ± 2.10, 89 ± 3.50, 53.13 ± 3.08, 52.25 ± 2.30, 40.88 ± 1.98, and 47.89 ± 2.40 IU/L in the NC, DC, CMP, CMPH-L, CMPH-M, and CMPH-H groups, respectively. Administration of CMPH-M for 8 wk was most effective in reducing the serum AST and ALT levels to the values closest to those of the NC group.
Levels of AST and ALT in the liver were higher in the rats belonging to the DC group than in the NC and treatment groups ($P < 0.05$; Figure 1B). Oral administration of CMP and CMPH at 3 different doses reduced these levels significantly compared with DC rats ($P < 0.05$). Reduction in hepatic enzymes was observed in the CMPH-M group, for which levels were closest to those found in the NC group, where the AST value was $94.63 \pm 2.98$ IU/L and ALT was $59.88 \pm 2.14$ IU/L. Thus, among all the treatment groups, the CMPH-M group demonstrated the greatest effectiveness in reducing both serum and liver enzyme levels.

**Hypolipidemic Effects of CMP and CMPH**

The results of lipid profile activities such as TC, TG, LDL, and HDL cholesterol analyzed in the serum are presented in Table 3. Overall, a significant ($P < 0.05$) elevation in serum levels of TC, TG, and LDL was observed in the DC group compared with the NC group. The oral administration of CMP and CMPH resulted in reduced TC, TG, and LDL levels compared with the DC group ($P < 0.05$). The greatest reductions in TC, TG, and LDL levels were observed in the CMPH-M group (500 mg/kg BW) compared with the CMPH-L and CMPH-H groups ($P < 0.05$). By contrast, the level of HDL-C showed a significant decline in DC rats ($24.13 \pm 0.63$ mg/dL), whereas the administration of CMP and CMPH over 8 wk showed significant increase in HDL levels compared with the DC group ($P > 0.05$). The highest HDL levels were found among the CMPH-M group (500 mg/kg BW). These results demonstrated that CMPH-M exhibited potent antihyperlipidemic effect in diabetes-induced rats, compared with other doses.

**Effects of CMPH on Oxidative Stress Markers**

The activities of antioxidant enzymes such as SOD and CAT, and the levels of GSH and MDA measured in the liver are presented in Figure 1 C, D, E, and F, respectively. The levels of SOD, CAT, and GSH were significantly ($P < 0.05$) reduced in the STZ-induced DC group compared with the NC group. The administration of CMP and CMPH at different doses for 8 wk to STZ-induced diabetic rats substantially increased the levels of SOD, CAT, and GSH in the groups, in a dose-dependent manner. In particular, the maximum effect was seen in the CMPH-M group (500 mg/kg of BW), with a significant ($P < 0.05$) increase in SOD, CAT, and GSH levels.

The oral administration of CMP and CMPH at 3 different doses resulted in an improvement in SOD activity (Figure 1C), with the CMPH-M group (46.25 ± 3.35 U/mg of protein) demonstrating a significant ($P < 0.05$) increase in SOD levels compared with the DC group (29.88 ± 3.18 U/mg of protein). Similarly, CAT levels were elevated in the groups treated with CMP and CMPH at different doses (Figure 1D). A significantly ($P < 0.05$) higher activity was detected in the CMPH-M group (172.5 ± 2.59 µU/min per mg of protein) compared with the DC group (93.13 ± 4.07 µU/min per mg of protein). The values for SOD and CAT in the CMPH-M group fed were not significantly different than the values found in the NC group, indicating the effectiveness of CMPH-M in reducing oxidative stress in the experimental animals.

Compared with the DC group (29.75 ± 2.46 mg/100 g of tissue), GSH levels in the liver were improved when rats were administered with CMPH-M (59.13 ± 3.40 mg/100 g of tissue). The ameliorative effects of CMPH-M were more distinct in this group than in all other groups (Figure 1E), which appeared to be an augmented effect and to be even better than in the NC group (57.50 ± 2.42 mg/100 g of tissue). Similarly, levels of the lipid peroxidation product MDA were quantified in the liver tissue homogenate, revealing a substantial increase in DC group at a level of $5.4 \pm 0.34$ nmol/g of tissue, indicating an approximately 2-fold increase in lipid peroxidation compared with the NC rats.
± 0.15 nmol/g of tissue). The MDA levels in the liver tissue were attenuated in the group administered either CMP or CMPH compared with those in the DC group (Figure 1F). Interestingly, a significant (P < 0.05) decrease in MDA levels was observed in the CMPH-M rats (2.78 ± 0.36 nmol/g of tissue) compared with the DC rats. These results indicate effectiveness of CMPH in reducing the marker related to lipid peroxidation.

**Effects of CMPH on Liver and Pancreas Histopathology**

The liver histopathological examination was performed using hematoxylin and eosin staining, and the results are presented in Figure 2. Normal hepatic lobules with central veins, sinusoids, and portal tracts were seen in NC rats (Figure 2A). Rats in the DC group...
exhibited hepatocyte degeneration associated with the vacuolation of cytoplasm and the presence of inflammatory cells and necrosis (Figure 2B). Oral administration of CMP shows a slight improvement in the hepatic architecture, along with lipid droplet accumulation and existence of inflammatory cells (Figure 2C). Administration of CMPH at 3 different doses also improved the normal hepatic architecture, with few inflammatory cells (Figure 2D and F). Reduced liver tissue changes, ranging from severe to moderate, and considerable ameliorating effects on hepatic damage and restoration of histological alterations were observed in the CMPH-M group (Figure 2E).

Rats in the NC group exhibited normal architecture of pancreatic sections, with evenly organized β-islet cells (Figure 3A). Sections from the DC group revealed damaged β-cells with irregular shape and reduced numbers, infiltration of lymphocytes, necrosis, and damaged globules (Figure 3B). The pancreatic sections prepared from the CMP-administered group showed necrotic changes with reduced β-cells and partially deranged structure (Figure 3C). However, after administration of CMPH-L, the sections exhibited minimal necrosis of β-cells, accompanied by slight progress in the pancreatic structure compared with those in DC (Figure 3D). Both the CMPH-M and CMPH-H groups demonstrated preservation of pancreatic islets, with an increase in their number and size along with attenuation of the histological changes from severe to mild (Figure 3E and F). Complete recovery in the architecture, along with the inhibition of β-cell loss and no evidence of necrosis, was observed in the CMPH-M group.

**DISCUSSION**

Streptozotocin is a genotoxic agent that is used to mimic the diabetic model in laboratory animals in a generic and cost-effective manner. This study is based on STZ-induced diabetes in albino Wistar rats and its various biological effects on different biochemical and oxidative stress markers, as well as histopathological changes in the liver and pancreas. The effects of CMP and enzymatic CMPH at different doses were investigated against diabetes induced in the albino Wistar rats.

Increased FBG levels are a major indication in diabetes mellitus, and accordingly, a trend of significantly elevated FBG levels was found in the DC rats, which continued until the end of the experimental period of 8 wk. These results were comparable to those of a previous study, where a single injection of STZ triggered an elevation of blood glucose levels in diabetic rats compared with control rats (Ghanbari et al., 2016). In the present study, the CMPH-M group displayed significantly lower (4× lower) FBG levels compared with DC and other treatment groups (P < 0.05). These results are also comparable to those of other previous studies (Ismail et al., 2018; Korish et al., 2020), where the increased blood glucose levels in STZ-induced diabetic rats were significantly reduced after treatment with camel milk (Ismail et al., 2018; Korish et al., 2020). Similar results were reported by Manaer et al. (2015), wherein feeding diabetic rats with the fermented camel milk product shubat resulted in improvements in FBG levels. Our results agree with reports demonstrating that the presence of insulin-like molecules in camel milk contributes to its hypoglycemic activities (Agrawal et al., 2011; Ismail et al., 2018; Korish et al., 2020). Consequently, our findings revealed the ability of CMPH-M to improve the potential hypoglycemic effects against STZ-induced diabetes, due to the presence of insulin-like molecules that contribute to hypoglycemic activity by lowering FBG levels. The antidiabetic property of camel milk is considered a very complex phenomenon, with the involvement of various molecular and cellular pathways. Moreover, the role of camel milk on glucose metabolism and transport, as well as on insulin synthesis and secretion, is considered as a mechanism for the antidiabetic action of camel milk (reviewed by Ayoub et al., 2018). At molecular or cellular level, camel milk may have direct effects on insulin receptor function and may play a role in glucose transport in the insulin-sensitive tissues, may have possible direct or indirect

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**Table 3. Serum lipid profiles in normal rats, streptozotocin-induced diabetic rats, and different treatment groups administered camel milk proteins (CMP) and camel milk protein hydrolysates (CMPH)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal control</th>
<th>Diabetic control</th>
<th>CMP</th>
<th>CMPH-L</th>
<th>CMPH-M</th>
<th>CMPH-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>98.24 ± 2.20a</td>
<td>168.14 ± 3.23c</td>
<td>132.63 ± 0.69b</td>
<td>142.37 ± 2.97d</td>
<td>108.35 ± 0.60b</td>
<td>126.30 ± 1.64c</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>31.55 ± 2.11b</td>
<td>158.25 ± 3.15c</td>
<td>41.90 ± 1.26b</td>
<td>38.90 ± 1.97b</td>
<td>20.05 ± 2.08a</td>
<td>84.55 ± 3.20c</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>49.46 ± 0.90a</td>
<td>24.13 ± 0.63b</td>
<td>36.58 ± 0.79b</td>
<td>29.72 ± 1.03b</td>
<td>40.39 ± 0.71d</td>
<td>39.34 ± 0.49c</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>42.57 ± 2.96a</td>
<td>158.25 ± 3.15d</td>
<td>87.67 ± 0.74b</td>
<td>104.87 ± 1.27b</td>
<td>63.95 ± 1.41b</td>
<td>84.55 ± 3.20c</td>
</tr>
</tbody>
</table>

a Values not sharing a common superscript in each parameter within the same row differ significantly at P < 0.05. bCMPH-L = low dose (100 mg/kg of BW), CMPH-M = mid-level dose (500 mg/kg of BW), CMPH-H = high dose (1,000 mg/kg of BW). All values are expressed as mean ± SEM, n = 8 rats/group. Data were analyzed via one-way ANOVA.

The anticaseinogenic and antitumor activity of CMPH may play a role in glucose transport in the insulin-sensitive tissues, may have possible direct or indirect

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effects on insulin secretion by pancreatic β-cells, and might also affect the survival, growth, and overall activity of pancreatic cells (Ayoub et al., 2018).

The OGTT is considered a more precise technique for the detection of variations in glucose regulations than any other method, and it prevents false positive

**Figure 2.** Histopathological sections of liver. Green arrows indicate hepatocyte; black arrows indicate fatty degeneration. (A) Normal control group rat, showing normal structure of hepatocytes and central vein. (B) Diabetic control group rat, showing altered structure, with hepatocyte degeneration, vacuolation of cytoplasm and epithelial cells, congested central vein, and presence of fat globules and inflammatory cells. (C) Camel milk protein (CMP)-treated group, showing morphology similar to that of diabetic rats, with slight improvement in the architecture. (D) Group receiving CMP hydrolysate at low dose (CMPH-L), showing necrotic areas (NCA), presence of hepatocytes with damaged architecture, and inflammatory cells. (E) Group receiving CMP hydrolysate at mid-level dose (CMPH-M), showing very good recovery of liver morphology, with well-formed central vein and healthy hepatocytes. (F) Group receiving CMP hydrolysate at high dose (CMPH-H), showing few inflammatory cells, with normal liver architecture. S = sinusoids, CV = central vein, BD = biliary duct, H = hepatocytes. Hematoxylin and eosin staining, 200× magnification.
Figure 3. Histological sections of pancreas, stained using hematoxylin and eosin (200× magnification). (A) Normal control rat group, showing granulated cytoplasm of islet cells with small, dark nuclei on the periphery (α-cells), or with light and large nuclei (β-cells). (B) Streptozotocin (STZ)-induced diabetic rat, showing pathological and degenerative changes in cells, especially in center of the islet. Swollen acinar cells and small vacuoles were observed. Islet β-cells show irregular outlining and are almost entirely lost in STZ-treated rats. Green arrow indicates islets of Langerhans with regions of fibrosis, indicating loss of β-cells. (C) Camel milk protein (CMP)-treated group, showing similar morphology to the diabetic group, with slight improvement in the architecture. (D) Group receiving CMP hydrolysate at low dose (CMPH-L), showing islets, degranulated cytoplasm of most cells compared with normal control. Wider interlobular (indicated by blue arrow) and intralobular (indicated by red arrow) ducts were observed. (E) Group receiving CMP hydrolysate at mid-level dose (CMPH-M), showing nearly normal outline of an islet with apparently intact appearance of cells. (F) Group receiving CMP hydrolysate at high dose (CMPH-H), showing increased islet cell density, with presence of inflammatory cell infiltration. AC = acinar cells, I = islets of Langerhans, C = congestion, V = vacuolation, NCC = necrotic change.
results (Singleton et al., 2003). Overall, our results showed reduction in blood glucose levels among the rats administered different doses of CMPH (CMPH-L, CMPH-M and CMPH-H) compared with those in DC rats. We detected a constant and significant \( P < 0.05 \) improvement in OGTT levels in the CMPH-M group from 0.5 to 2 h, compared with those in other treatment groups with increased glucose tolerance. These results were consistent with the findings of Ben Slama-Ben Salem et al. (2018), who showed that Octopus vulgaris protein hydrolysates possess antihyperglycemic activity, evidenced by the effective reduction in blood glucose levels at 2 h compared with a group treated with nonhydrolyzed octopus muscle proteins, with the results being comparable to those achieved by acarbose (a standard antidiabetic drug). Similarly, analysis of glucose tolerance upon the administration of goat, camel, cow, and buffalo milk to STZ-induced diabetic rats showed that camel milk resulted in an effective drop in blood glucose levels compared with milk from other species, indicating the superiority of camel milk in restoring insulin levels (Meena et al., 2016). Overall, OGTT results of the present study indicated that CMPH at the mid-level dose (CMPH-M) was the most optimal among the tested doses at reviving insulin levels following the maintenance of glucose hemostasis, which is due to the presence of insulin nanoparticles in camel milk that have traits similar to those of human insulin (Malik et al., 2012; Mansour et al., 2017).

Diabetes and liver disease are interrelated and pose a risk for hepatic dysfunction or nonalcoholic fatty liver disease in the diabetic population. Aminotransferases such as AST and ALT serve as marker enzymes of liver damage. Injection of STZ causes hepatic injury, which is known to be a primary feature in diabetes mellitus, as evidenced by high serum levels of AST and ALT. These cytosolic enzyme levels are usually low in serum, but their levels are increased because of liver damage. An elevation in these enzyme levels is used as a key factor in monitoring diabetes (Ismail et al., 2018). In the present study, liver damage from STZ injection was estimated via both liver and serum samples. Elevation in the levels of AST and ALT in the liver and serum samples of DC rats revealed liver dysfunction. The administration of CMP and CMPH at different doses appears to ameliorate AST and ALT values to near normal levels in liver and serum samples, specifically at the mid-level CMPH dose (CMPH-M), indicating effective hepatoprotective efficiency. A considerable number of studies have shown reduced levels of AST and ALT in rats treated with whole milk (Khan et al., 2013; Mansour et al., 2017; Ismail et al., 2018). However, only a few studies have been conducted on milk protein hydrolysates; in particular no such study on CMPH has been reported. To the best of our knowledge, our study is the first to report improved liver functions resulting from the administration of CMPH in vivo. The present results showed that administration of camel milk to diabetic rats for 8 wk lowers the activities of liver function marker enzymes (AST and ALT) to near normal levels, which signifies the ability of camel milk peptides to protect against liver damage. These results are consistent with the results of a study by Abdel-Hamid and colleagues (2020), who reported that papain-hydrolyzed buffalo milk protein hydrolysates could exhibit significant hepatoprotective effects against carbon tetrachloride–induced oxidative stress. Similarly, significant reductions in liver tissue degeneration, apoptosis and necrosis, inflammation, steatosis, and fibrosis were reported with the administration of fermented camel skim milk in hypertensive rats (Yahya et al., 2017). Altogether, results from the present study revealed that CMPH, especially at the mid-level dose, exerted hepatoprotective effects against STZ-induced diabetes. These hepatoprotective effects of CMPH-M were further confirmed by histopathological examinations of the liver.

Hyperlipidemia, hypercholesterolemia, and hypertriglyceridemia are abnormalities generally associated with diabetes mellitus, eventually increasing the risk for cardiovascular disease. In the present study, induction of diabetes led to an abrupt increase in TC, TG, and LDL-C levels and a steep decline in HDL levels among rats in the DC group. These results are in accordance with those reported by other researchers who have showed abnormal lipid profiles with diabetes mellitus (Mansour et al., 2017). During our experimental period, a decline in serum levels of TC, TG, and LDL-C occurred among diabetic rats fed with CMP and CMPH. This reduction was observed in a dose-independent manner, whereby the mid-level CMPH dose displayed the maximum optimal activity, along with normalization of HDL-C levels. These results indicated that CMPH, especially at the mid-level dose (CMPH-M), displayed potent lipid-regulating properties; this could be due to the presence of more compelling bioactive peptides in this hydrolysate, which appear most effective at the mid-level dose. Our results were in accord with those published by Meena et al. (2016), who found that feeding whole camel milk to diabetic rats for 4 wk resulted in restoration of TC, TG, LDL-C, and HDL-C levels to normal values compared with goat, cow, or buffalo milk treatments. Similarly, treatment of diabetic rats with camel milk reduced the levels of TC, TG, and LDL-C, with increased HDL cholesterol levels (Al-Numair, 2010). Such a regulation in lipid profile was also observed with camel milk administration in both type 1 and type 2 diabetes patients, as indicated by decreased
TC and TG levels and increased HDL levels (Ejtahead et al., 2015). The hypocholesterolemic mechanism of camel milk is uncertain. However, various hypothesis indicate that interactions between bioactive peptides generated from CMP culminate in cholesterol reduction. This confirms that the presence in camel milk of orotic acid, which is considered to play a crucial role in reducing cholesterol levels (Kumar et al., 2016).

The STZ-induced diabetic model gives a classic example of oxidative stress and lipid peroxidation caused by excessive generation of ROS in major organs such as the liver and kidney cells (Vinothiya and Ashokkumar, 2017). Injection of STZ produces a significant effect on antioxidant enzymes by increasing the production of ROS in the liver tissue, which is evident in our studies by the decreased activities of SOD, CAT, and GSH in DC rats. These results are consistent with those of previous studies, where the activities of liver SOD, CAT, and GSH were drastically decreased and MDA levels amplified in DC rats compared with control rats (El Saïd et al., 2010; Badr, 2013). In the present study, the levels of SOD in the liver were higher in the CMPH-M followed by the CMPH-H group, and these levels were similar to the levels found in the NC group (\(P > 0.05\)). We observed that CMPH-M resulted in a significant increase in catalytic activity that was similar to levels found in the NC group, compared with the DC group. Reduced GSH acts against ROS and is also a co-factor for glutathione peroxidase activity. The GSH levels in the livers of diabetic rats were found to be lower, whereas oral administration of CMPH to diabetic rats restored GSH levels, with the CMPH-M group demonstrating the highest GSH level. These levels were higher in the CMPH-M group than those found in NC rats. The reduced activities of SOD, CAT, and GSH are accountable for the oxidative stress associated with diabetes, which deteriorates the islet cells by altering the insulin level, resulting in hyperglycemia (Nua et al., 2018). The high level of MDA is an indication of oxidative stress due to an upsurge in free radicals leading to an excess generation of MDA, a final product of lipid peroxidation. Our results confirmed the higher production of MDA in the DC group, whereas these levels were found to be significantly lower in the CMPH-M group rats compared with DC as well as other treatment groups. The administration of CMPH at the mid-level dose resulted in effective antioxidant activity by reducing hyperglycemia. Studies on diabetic animal models have demonstrated the correlation between decreased oxidative stress and improvement in hyperglycemia (Mansour et al., 2017). Likewise, the antioxidant activity of casein in camel milk has shown the ability to scavenge free radicals by deteriorating ROS (El Saïd et al., 2010). Furthermore, administration of camel milk to diabetic rats has been found to reduce MDA levels and increase CAT, SOD, and GSH levels (Meena et al., 2016). The antioxidant effects of CMPH, as reflected by lower MDA content, are attributable to the decrease of the oxidative stress caused by STZ-induced diabetes. Moreover, CMPH might also play a protective role in preventing damage to the liver caused by oxidative stress.

Histological observations of liver tissue from the DC rats revealed degenerative changes such as hepatic necrosis, sinusoidal hemorrhages, and vacuolation in the hepatocytes, consistent with previous studies (Huang et al., 2014). These changes were reversed in the diabetic rats administered CMPH, wherein the hepatocytes appeared normal, with no accumulation of lipid droplets, along with less necrosis and improved liver architecture. However, the improvement in liver architecture was not observed in the CMP group, demonstrating the effectiveness of CMPH in preventing hepatic damage. Furthermore, fatty liver (formation of fat droplets in hepatocytes) is commonly found in diabetic conditions, and it was noted in the present study as well as in previous research (Merzouk et al., 2000). Ben Slama-Ben Salem et al. (2018) reported that the administration of octopus (\(O.\ vulgaris\)) hydrolysates to diabetic rats exhibited substantial influence in restoring the hepatic architecture without lipid deposition in hepatocytes. Thus, the histopathological findings support the results of our biochemical studies observed in the liver markers.

We also evaluated the STZ-induced alterations in the rat pancreatic architecture and the protective effects of CMP and CMPH against STZ-induced damage through hematoxylin and eosin staining. Results showed that the DC rats exhibited loss in structural architecture and decreased numbers of pancreatic islet and \(\beta\)-cells, changes caused by STZ injection as shown in previous reports (Ismail et al., 2018). However, the administration of CMP and CMPH to diabetic rats, especially at the medium dose (CMPH-M), restored \(\beta\)-cell architecture, along with improving the number of cells compared with the DC group. These histological findings were consistent with previous reports (Anjani et al., 2018) regarding the renewal of \(\beta\)-cells, as the pancreas contains quiescent cells that have the capacity to regenerate. The revitalization capacity of pancreatic islet and \(\beta\)-cells in chemically induced diabetic models is an alternative approach to treat diabetes mellitus (Risbud and Bhonde, 2002). Interestingly, the mid-level CMPH dose (CMPH-M) resulted in attenuation of the islet and functional \(\beta\)-cells, with improvement in insulin resistance, with the second-strongest effects shown by the high dose (CMPH-H). Similar changes in histology of the pancreas were reported in STZ-induced diabetic rats that were fed on shubat, probiotic-fermented camel
milk, where rejuvenation of pancreatic islet cells was observed (Manaer et al., 2015). Similarly, camel whey protein treatment in diabetic rats has demonstrated restoration of the islet structure (Sayed et al., 2017). A previous study conducted on STZ-induced diabetic rats fed with camel milk for 2 mo showed re-establishment of the pancreatic β-cell structure (Mansour et al., 2017). Moreover, Ismail et al. (2018) reported that diabetic rats treated with evaporated camel milk exhibited mild recovery in pancreatic β-cells, with less atrophy compared with rats treated with pasteurized and powdered camel milk. In the present study, microscopic examination of the pancreatic tissue showed no altered or pathological observations, and the regenerative capacity was pronounced in the liver and pancreatic tissues in the CMPH-M and CMPH-H groups. However, in the low-dose group (CMPH-L), the restorative capacity of tissue damage was very low, and CMP results were lower still. This strongly indicates that CMPH-M possesses protective effects against the depletion of β-cells, with reduced pancreatic oxidative stress and increased insulin production. However, the exact mechanism involved in the restoration of insulin secretion or β-cells in diabetic rats is yet to be explored.

CONCLUSIONS

The therapeutic benefits of camel milk have garnered the attention of the scientific community worldwide. In this study, we demonstrated that camel milk proteins constitute an interesting source of bioactive peptides that displayed effective antidiabetic, antihyperlipidemic, and antioxidant properties in STZ-induced diabetic rats. Alcalase-generated camel milk protein hydrolysate administration at a daily dose of 500 mg/kg of BW (CMPH-M) exhibited potent antihyperglycemic activity, decreasing the blood glucose levels as measured by FBG and OGTT. Regulation of the serum lipid profile after administration of CMPH-M for 8 wk demonstrated its antihyperlipidemic properties. Overall, we conclude that CMPH-M has the ability to prevent STZ-induced oxidative stress by inhibiting lipid peroxidation and increasing the activities of antioxidant enzymes. Histological examinations also revealed the protective effects of CMPH-M against STZ-induced cellular changes in liver and pancreatic tissues. This study demonstrated that oral administration of CMPH at the mid-level dose (500 mg/kg of BW) resulted in protective effects against STZ-induced diabetes and associated oxidative changes, due to the presence of insulin-like protein, minerals, immunoglobulins, and orotic acid, which contribute to hypoglycemia, hypolipidemia, and antioxidant activities. The plausible mechanism for the action of these hydrolysates may be attributable to the presence of bioactive peptides with potent antidiabetic activities, which appear most effective at the mid-level dose. Further studies are required to characterize the compounds, to elucidate the underlying mechanisms for the antidiabetic activity, and to further investigate the dose-response effect in both in vitro and in vivo studies.

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