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Ameliorative Effect of Ketogenic Diet on High Fat Diet Induced Metabolic Syndrome in Rats Via GLP-1R and PGC-1α

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Abstract

Metabolic syndrome (MetS) is a kind of metabolic disorder, including abdominal obesity, hyperglycemia, dyslipidemia, hypertension, etc. Dietary intervention was thought to be one of the lifestyle strategies, along with others like nutritional intervention, psychological intervention, etc., that might effectively treat MetS. the ketogenic diet (KD) is a high-fat diet that has been shown to be helpful in a variety of diseases such as polycystic ovarian syndrome, acne, cancer, and respiratory distress. The study was conducted to investigate the therapeutic potential of a ketogenic diet on rat MetS models. In this study, 40 males' rats were divided into four groups, for a period of 24 weeks, the rats were received KD. Some biochemical, molecular parameters and histopathology of liver tissues were determined. Our results demonstrated that compared to rats in the MetS group, the KD group and MetS +KD group reduced glucose, insulin, HOMA-IR, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) levels and malondialdehyde (MDA), while the KD group and MetS +KD group increased glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) levels ($P < 0.05$). An upregulation was found in the gene expression levels of the homogenate of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and the Glucagon-like peptide-1 receptor (GLP-1R) in control group, KD group, and MetS +KD group compared with Mets group. In conclusion based on these results, KD can be suggested as a healthy weight loss diet with positive metabolic and hepatic benefits.

KEYWORDS
Ketogenic diet, Metabolic syndrome, High-fat diet, GLP-1R; PGC-1α

INTRODUCTION

Multiple systemic metabolic abnormalities contribute to the complex homeostatic problems that characterize metabolic syndrome (MetS). Inactivity and a diet high in simple sugar carbs and saturated fat are major contributors to the development of MetS (Ahmed *et al*., 2023). The incidence of MetS is rapidly increasing, making it a major global public health concern in many nations. Since lifestyle management has been shown to be the most desired and effective approaches to treating MetS, the interest in alternative diets has resurged in response to the growing prevalence of this condition. Dietary intervention was thought to be one of the lifestyle strategies, along with others like nutritional intervention, psychological intervention, etc., that might effectively treat MetS (Khalil *et al*., 2018; Du *et al*., 2023).

Dietary changes are only regarded as a medical treatment when at least two other drugs have been tried without success. The consumption of health functional meals that help regulate biological activities has been on the rise all over the world in recent years. These foods provide basic nourishment in addition to gratifying the palate. In particular, the needs of consumers have been met through the development of diet therapy into a variety of forms as food appropriate for a healthy diet culture. One diet that shows promise in treating MetS is the ketogenic diet (KD) (Sacchetti *et al*., 2022). Among the many lifestyle therapies used to treat MetS, dietary interventions have proven particularly helpful. Since the KD is a nutritional intervention that may have the ability to alleviate risk factors of MetS, its influence on MetS has garnered growing interest (Angelico *et al*., 2023).

Reports indicate that KD provide significant health effects, such as weight loss/maintenance, and improved glycemic management, which has piqued the interest of academic and public health disciplines. They've also seen a rise in popularity among fitness fanatics (Basolo *et al*., 2022). Common to all KD is their lack of carbohydrates, which can cause the body to respond in ways similar to those seen during bouts of energy deficiency. Ketones are produced when the liver's mitochondria metabolize fatty acids and certain amino acids in the absence of sufficient carbohydrates and insulin. Ketogenesis is a metabolic process that uses fat instead of glucose to feed the body's peripheral tissues (the heart, the brain, the muscles, etc.). Knowing how hepatic metabolic pathways adjust to a KD and how this sort of diet affects exercise adaptations is crucial due to the liver's crucial role in gluconeogenesis and ketogenesis (Huang *et al*., 2020).

Identifying individuals at high risk for metabolic problems (Hatami *et al*., 2016), requires an evaluation of the oxidative status in MetS, as this disease is linked to an oxidative/antioxidant imbalance. Also, MetS is linked to atherogenic hyperlipemia and

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abnormal lipid metabolism (Zafar *et al*., 2018). Given the foregoing, it is clear that weight loss is the single most important factor in improving all MetS characteristics and that it is therefore essential to regulate MetS through lifestyle management.

Glucagon-like protein 1 (GLP-1), an antiobesogenic hormone that stimulates beta cell insulin secretion in response to glucose and inhibits alpha cell glucagon production, GLP-1 has additional functions beyond the pancreas. Other than its role in glucose metabolism, GLP-1 regulates the upper gastrointestinal tract, gastric motility, acid secretion, increases satiety in the coordination of the gut and the brain, promotes thermogenesis in the adipose tissue, and exerts anti-inflammatory effects (Müller *et al*., 2019). Widespread acceptance has led to extensive research into GLP-1 and GLP-1R analogs as a therapeutic target in obesity and diabetes. However, there is still a lot to learn about GLP-1R and metabolic illnesses including diabetes and obesity. In obesity and diabetes, studies on animals and humans show conflicting findings on the secretory response of GLP-1 to meal or glucose intake (Shen *et al*., 2021).

Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), characterized by controlling lipid metabolism and controls mitochondrial DNA replication and cellular oxidative metabolism by modulating the expression of mitochondrial genes (Cheng *et al*., 2018). PGC-1α has a strong association with the formation of MetS and its main consequences, such as obesity, cardiovascular disease, and hepatic steatosis, and is highly expressed in tissues with high energy demands (Mahmoud *et al*., 2021). Therefore, this study aimed to evaluate the possible effect of KD on high fat diet (HFD) induced MetS in rat model via modulating GLP-1R and PGC-1α expression levels.

MATERIALS AND METHODS

Diets

The standard diet was as follows: 15% protein, 7% palm oil, 6% fibre, 7% calcium, 0.7% phosphor and 52.2% corns). The standard ketogenic diet (SKD) was as follows: 5% vitamin mixture, 5% fibers/flaxseeds, 15% protein/casein, 70% fat/buffalo fat, and 5% carbs/sucrose. Both diets ingredients were purchased from El-Gomhoria Company in Cairo, Egypt.

Animals and study protocol

Forty adult male albino rats (165-170 g) were obtained from the experimental animal house of the Faculty of Veterinary Medicine, Zagazig University, Egypt. The study was approved by committee of ZU-IACUC with approval number (ZU-IACUC/1F/130/2021).

The animals were divided into four groups (n=10), Control group: rats were untreated to obtain the normal control references. MetS group: rats were received HFD every day for 8 weeks. KD group: Rats were received a SKD with a ratio of 70% fat, 15% protein, and only 5% carbs for 16 weeks. Rats were fast for 24 h before being placed on the diets. MetS + KD group: Mets were induced in rats as in group 2 for 8 weeks then receives SKD as in group 3 for another 16 weeks.

Sampling and tissue preparation

All of the rats were anaesthetized with pentobarbital sodium at the end of the trial, after they had fasted for 12 hours. The liver was removed surgically, and blood was drawn from the orbital venous plexus and, blood samples were centrifuged (3000 rpm/15 minutes) to separate serum, the latter was frozen at -20°C until further analysis. To preserve tissue for histological analysis, parts of the liver were placed in 10% neutral buffer formalin, while the rest parts were frozen in liquid nitrogen, the other tissue parts were collected in1 ml of thiazole and stored at -80°C for analysis of the gene expression.

Assessment of serum biochemical and antioxidant parameters

Serum Glucose (MyBioSource, USA) and insulin (MyBioSource, USA) levels were measured. The homeostasis model assessment for IR (HOMA-IR) index were calculated by multiplying blood glucose level (mg/dl) by insulin level (ng/ml) and then dividing on 405. Total bilirubin was evaluated colorimetrically using kits from Diamond (Cairo, Egypt). Additionally, albumin was evaluated using kits from Stanbio Laboratory (TX, USA). Alkaline phosphatase (ALP) level in the blood was evaluated using reagents manufactured by ELITech (Paris, France), and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were calculated using kits manufactured by (Wiesbaden, Germany). The MDA (MyBioSource, USA), CAT (MyBioSource, USA), GSH (Biodiagnostic, USA), and SOD (Abcam, USA) were evaluated following the manufacturer's instructions.

Hepatic gene expression

Isolation of total liver RNA was performed according to the manufacturer's instructions using TRIZOL reagent (Invitrogen, Germany), quantification was performed using a NanoDrop® ND-1000 spectrophotometer, and the RNA was used in a cDNA synthesis reaction using a Maxima first strand cDNA synthesis kit from Thermo Fisher Scientific (MA, USA). Real-time quantitative PCR was performed on a Stratagene (MX3005P, USA) using the QuantiTect SYBR Green PCR Master Mix (Qiagen). Primers for detecting GLP-1R and PGC-1 are included in Table 1. Using the Stratagene MX3005P programme, we determined the amplification curves and cycle threshold (CT) values. Fold change in gene expression was reported as as $2^{-\Delta\Delta CT}$ relative to control after normalization to GADPH as a housekeeping gene.

Histopathological investigations

Liver sections were fixed in 10% neutral buffered formalin for 48 hours, followed by alcoholic dehydrated at concentrations

Table 1. Primers sequences for the real time PCR.

Gene	Sequence	pb	Gene Bank accession number
PCG-1α	F 5′-TTCAGGAGCTGGATGGCTTG-3' R 5'-GGGCAGCACACTCTATGTCA-3'	70	NM_031347.1
GLP-1R	F 5′- TCCTGTTAAAGCTGCAAGGC-3' R 5'- TTGTCCGAGAGGAAGGCTG-3'	232	NM_012728
GADPH	F 5′-GCATCTTCTTGTGCAGTGCC-3' R 5'-GGTAACCAGGCGTCCGATAC-3'	91	NM_017008.4

ranging from 70% to 100%, then embedded in paraffin, sectioned at 5 µm were used for microscopic analysis. Histopathological sections stained with H&E.

Statistical analysis

The GraphPad Prism 8 (GraphPad software Inc., San Diego, CA 18940, USA) was used to analyses the data. The acquired information is presented as mean values ± SD. The Shapiro-Wilk test was used to check for data normality. One-way analysis of variance (ANOVA) was used for group comparisons. The Tukey post-hoc test was used to analyses the data, and differences between groups were declared significant when the p-value was more than 0.05.

RESULTS

Effect of KD on the levels of blood glucose, serum insulin, and HOMA-IR in HFD induced MetS rat model

Results in Figure 1 revealed that the levels of blood glucose (mg/dL), serum insulin (µIU/mL) and HOMA-IR were significantly increased in Mets group compared with other groups. While in the KD group the levels of blood glucose, serum insulin and HOMA-IR were significantly decreased compared with the Mets group and the KD group was the most significant group. The control group and KD group showed that the levels of blood glucose were non-significant to each other, but the insulin levels in Mets group and Mets+ KD group were non-significant to each other. On the other hand, the control group and KD group showed that the HOMA-IR levels were significantly decreased compared with the Mets group, but they were non-significant to each other.

Effect of KD on the serum levels of albumin and T. bilirubin in HFD induced MetS rat model

Figure 2 illustrated that the serum levels of total bilirubin were significantly increased in Mets group compared with the control group and KD group but non-significant with Mets+ KD group. While in the KD group the level of total bilirubin was significantly decreased compared with the Mets group and Mets+ KD group. The KD group was the most significant group. The control group and KD group showed that the levels of total bilirubin were non-significant to each other. It was revealed that the albumin level in Mets group was significantly decreased compared with other groups. On the other hand, the control group and KD group showed that the albumin levels were significantly improved compared with the Mets group and Mets+ KD group, but they were non-significant to each other.

Effect of KD on the serum levels of liver enzymes in HFD induced MetS rat model

Serum ALT, AST, and ALP levels were represented in Figure 3.

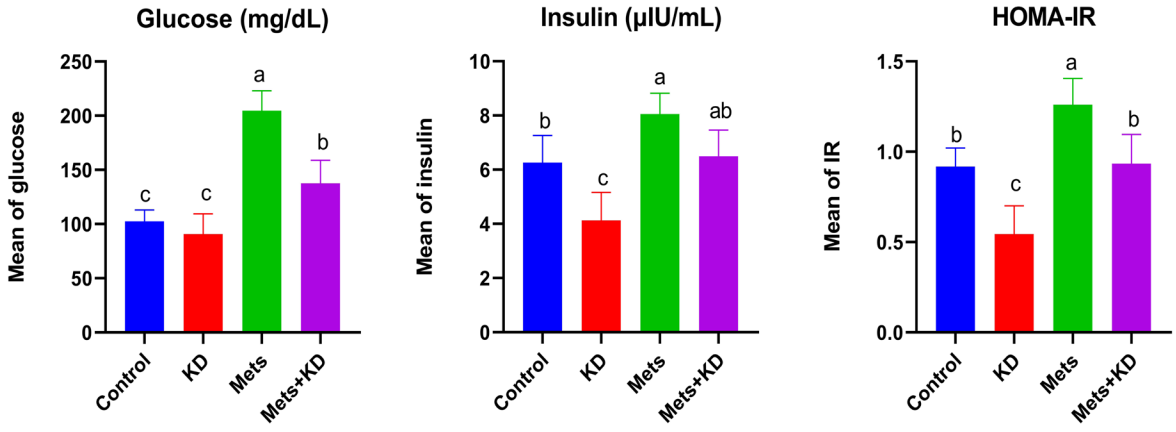

Fig. 1. Effect of KD on the levels of blood glucose (mg/dL), serum insulin (µIU/mL), and HOMA-IR in HFD induced MetS rat model.

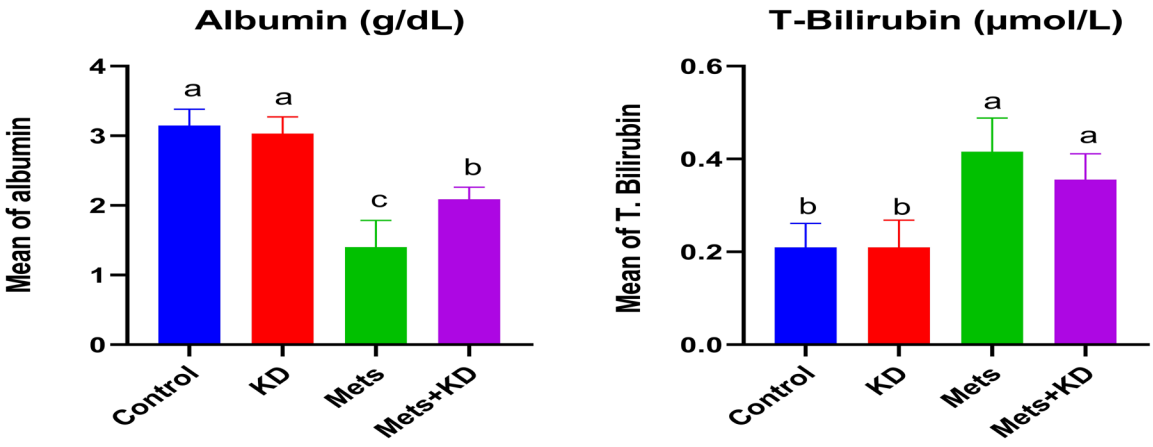

Fig. 2. Effect of KD on the levels of serum albumin (g/dL), and total bilirubin (µmol/L) in HFD induced MetS rat model.

ALT, AST, and ALP levels in serum were significantly increased in Mets group compared with other groups. While in the KD group, ALT, and ALP levels were significantly decreased compared with the Mets group and Mets+KD group, while they were non-significant to each other. The KD group was the most significant group.

Effect of KD on the serum levels of oxidant stress markers in HFD induced MetS rat model

Figure 4 demonstrated that MDA activity in serum was significantly increased in Mets group compared with other groups. MDA levels showed significant improvements in their levels in control group, KD group, and Mets+KD group, but their levels were non-significant to each other. CAT, and SOD activities were higher in control group, KD group, and Mets+KD group compared with Mets group, but their levels were non-significant to each other. The GSH levels were significantly decreased in Mets group and Mets+KD group compared with other groups. The KD group was the most significant dose in GSH level.

Determination of KD on the mRNA expression of genes in HFD induced MetS rat model

PGC-1α and GLP-1R mRNA expression in control group, KD group and Mets+KD group were more significant than the Mets group. The Mets+KD group was the lowest significance level.

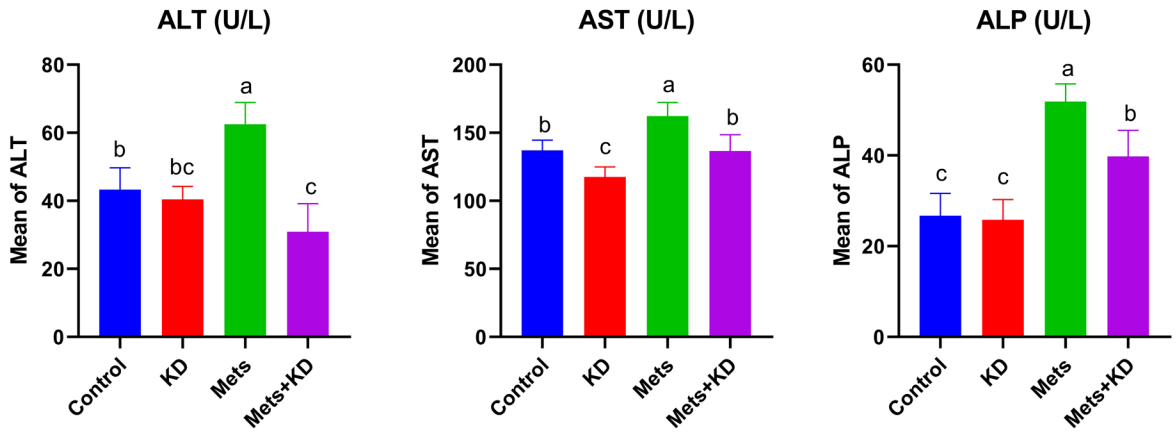

Fig. 3. Effect of KD on the levels of serum ALT (U/L), AST (U/L), and ALP (U/L) in HFD induced MetS rat model.

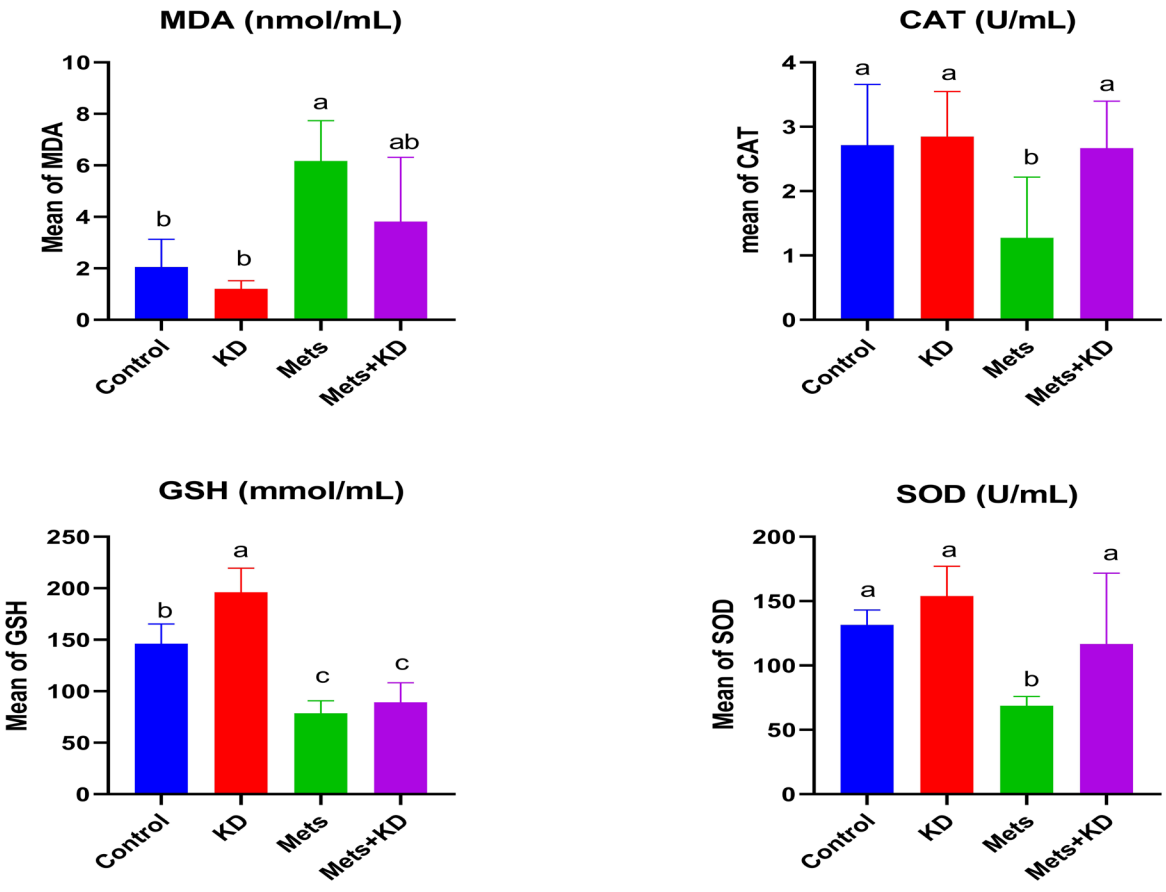

Fig. 4. Effect of KD on the serum levels of MDA (nmol/mL), CAT (U/mL), GSH (mmol/mL), and SOD (U/mL) in HFD induced MetS rat model.

mRNA expression of PGC-1α were non-significant in between control group and KD group, also in between KD group and Mets+KD group (Figure 5).

Histopathological results

Histopathological results in liver sections in different groups illustrated at figure 6. Control group showed normal histological structure of liver section (Figure 6A). In KD treated group liver showed normal appearance of the central veins, radiating cords of hepatocytes and laso portal area (Figure 6B). MetS group showed several changes in comparison to the control one. It reveals dilated central veins, abnormal portal area of vacuolated cells (Figure 6C). In MetS+KD group liver showed a central vein and radiating cords of hepatocytes with narrow blood sinusoids. Numerous hepatocytes have rounded vesicular nuclei and pale acidophilic cytoplasm while, other have vacuolated cytoplasm. Few hepatocytes show darkly stained nuclei and two vesicular nuclei (Figure 6D).

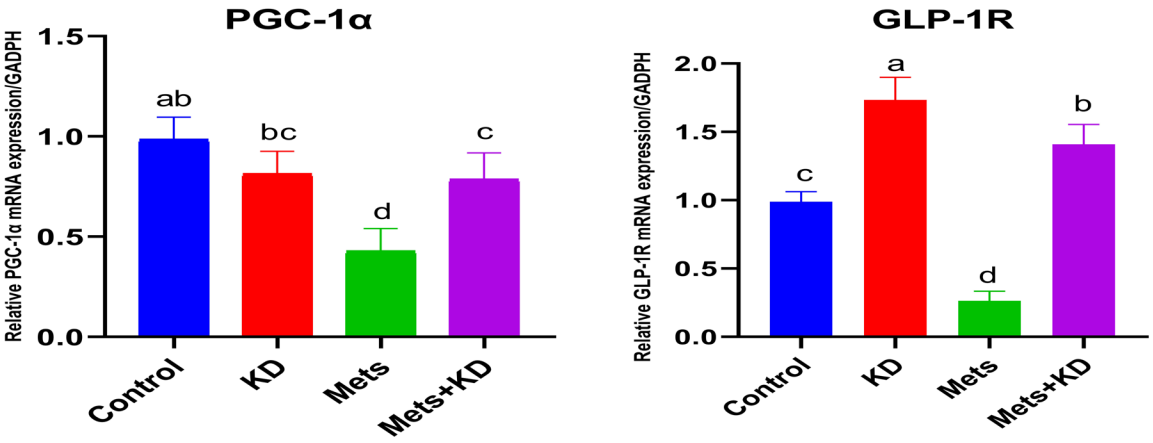

Fig. 5. Effect of KD on the mRNA expression levels of PGC-1α, and GLP-1R (Fold change/ ref. gene) in HFD induced MetS rat model.

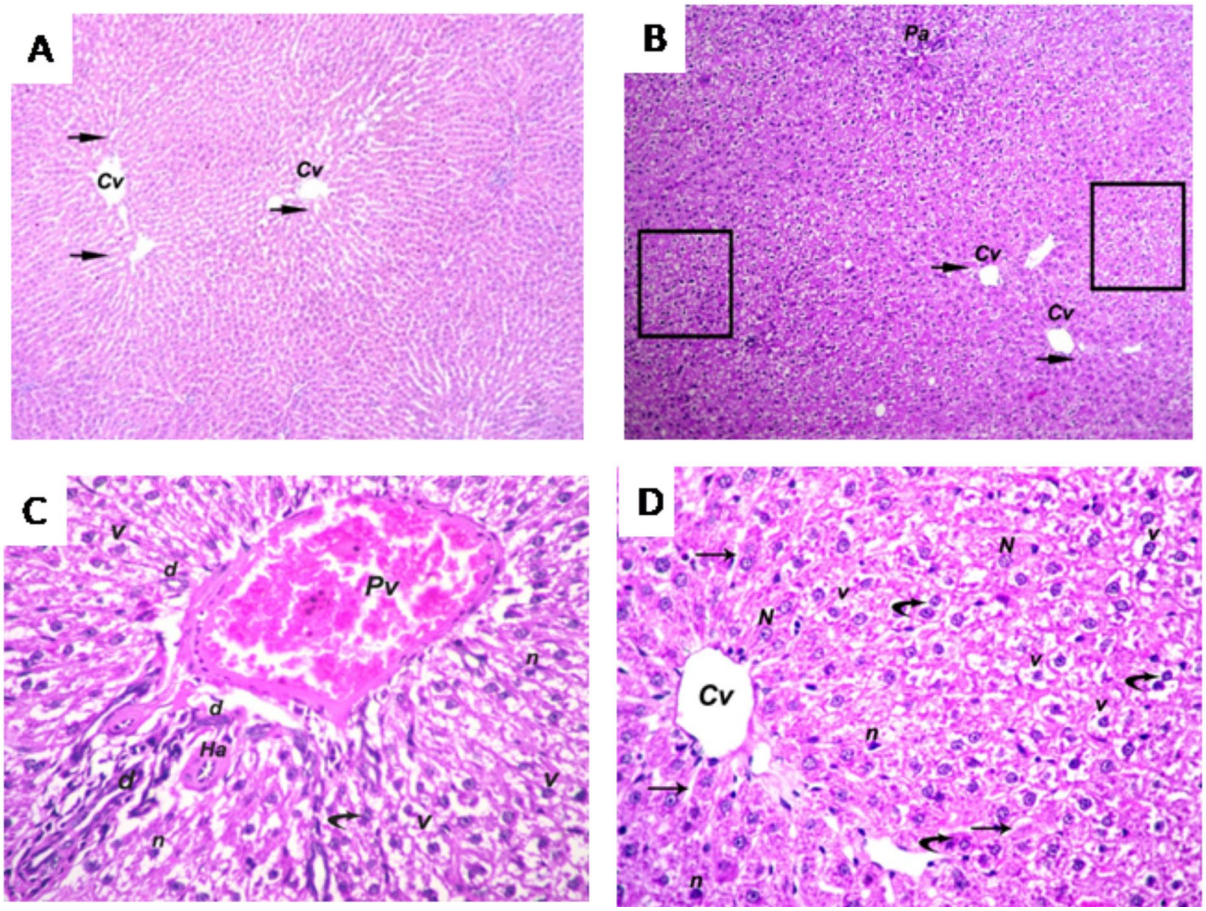

Fig. 6. Histopathological results in liver. (A) Liver of control rat shows normal histological picture, Central veins (Cv) with radiating cords of hepatocytes (arrow) are observed, H&E, X 100. (B) Liver of KD group showing normal appearance of the Central veins (Cv), radiating cords of hepatocytes (arrow) and laso portal area (Pa). Notice, areas of vacuolated cells (square), H&E, X 100. (C) Liver of Mets induced rat showing several changes in comparison to the control one. It reveals dilated Central veins (Cv), abnormal portal area (Pa) of vacuolated cells (square), H&E, X 100. (D) Liver of Mets+KD group showing the central region of the hepatic lobules. A central vein (Cv) and radiating cords of hepatocytes with narrow blood sinusoids (arrow) are observed. Numerous hepatocytes have rounded vesicular nuclei and pale acidophilic cytoplasm (N) while, other have vacuolated cytoplasm (v). Few hepatocytes show darkly stained nuclei (n) and two vesicular nuclei (curved arrow), H&E, X 400.

DISCUSSION

The MetS is characterized by abdominal obesity, dyslipidemia, low high-density lipoprotein cholesterol levels, hypertension, and insulin resistance (IR). Although lifestyle modifications, especially dietary adjustments, are the major therapeutic strategy for the treatment and management of MetS, the most beneficial dietary pattern for the management of MetS has not been identified (Castro-Barquero *et al*., 2020).

Weight gain, cardiovascular disease, and type 2 diabetes are all symptoms of MetS, although they can be prevented or delayed with a healthy lifestyle in those who are at risk. Several suggestions have been made that should aid patients and physicians in learning and applying the best methods for making lifestyle adjustments to prevent MetS and boost cardiometabolic health. As a result, there are several diets that can lessen the severity of symptoms or prevent them from appearing altogether. One such diet is the KD, for which there is a growing body of research demonstrating its effectiveness in treating and preventing obesity and its related health problems (Charlot and Zoll, 2022).

Our findings corroborated those of Zhang *et al*. (2018), who observed that the high-fat high-fructose diet (HFFD) induced metabolic changes in glucose, insulin, IR, liver function, liver histology, total bilirubin, and albumin in MetS rats, including hepatic dysfunction and bilirubin metabolism problems as evidenced by elevated blood ALT, AST, ALP, and total bilirubin concentrations. Similar results were seen in HFFD-fed rats by Park *et al*. (2020). The oversupply of fatty acids to the liver caused by an HFFD may be connected to these diseases through oxidative stress and inflammatory pathways (Khlifi *et al*., 2020).

We found that the MetS group had significantly lower levels of SOD, GSH, and CAT, indicating that oxidative damage is a major contributor to the progression of MetS and its accompanying complications. In accordance with our results, Reda *et al*. (2022) reported that rats with MetS were found to have elevated levels of liver damage indicators, dyslipidemia, hyperglycemia, and IR. Hepatic antioxidant indicators (SOD, GSH, and CAT), anti-inflammatory cytokines concentration were all significantly reduced in the MetS group, whereas the oxidative stress marker MDA was significantly increased.

Several studies found that the level of oxidative stress in organisms is reflected by the amount of MDA caused by HFD. Accumulated free radicals and elevated oxidative stress were reflected in significantly decreased SOD, GSH, and CAT levels and an elevated MDA level in the model group of obese mice (Wu *et al*., 2021).

Gancheva *et al*. (2015), found that liver microvesicular steatosis, portal and lobular inflammation, and lipid droplets of varying sizes were also observed in H&E- and ORO-stained sections from the MetS group, lending further histological support to the present biochemical findings. Increases in HOMA-IR, insulin, and blood glucose due to MetS suggest the onset of IR. Consumption of HFFD has been connected to the development of MetS, which is characterized by the IR, hepatic oxidative stress, and insulin signalling transduction.

The obtained results revealed that in Mets group, PGC-1α and GLP-1 RA showed significant downregulation compared with KD treated groups.

There is still much to learn about the integrated molecular mechanisms involved in the regulation and mode of action of PGC-1α, but it has the potential to be a very appealing therapeutic target with significant advantages for a wide spectrum of metabolic disorders. PGC-1α is overexpressed in tissues that have a high need for energy, and it has been linked to the development of MetS and its major consequences such obesity, T2DM, heart disease, and hepatic steatosis. Inflammation is often accompanied by metabolic abnormalities, and both are made worse by PGC-1α dysregulation. Reduced levels of PGC-1α during inflammation are associated with an increase in oxidative stress, mitochondrial antioxidant gene downregulation, and nuclear factor kappa B activation. MetS is characterized by chronic low-grade inflammation, and PGC-1α dysregulation modifies the metabolic properties of tissues by changing mitochondrial function and increasing development of reactive oxygen species (Rius-Pérez *et al*., 2020).

Insulin production from pancreatic β-cells is increased by incretions, but glucagon secretion is decreased. Among the several types of incretins, GLP-1 and glucose-dependent insulinotropic polypeptide are the most frequent. Intestinal L-cells release GLP-1, which binds to the GLP-1R on pancreatic -cells to maintain constant glucose levels. Activation of GLP-1R in β-cells and skeletal muscle mitochondria has been associated to enhanced insulin production and glucose tolerance. Furthermore, it has been shown that GLP-1R activation stimulates mitochondrial biogenesis, thereby converting them white adipocytes into brown that are more reliable in energy expenditure (Pal *et al*., 2019). Consistent with the vast majority of studies (Costa *et al*., 2022), findings showed a decrease in GLP-1R in the stomach and intestine of the HFD group. For instance, one study reported lower GLP-1R expression in the stomachs of patients with poor glucose metabolism compared to healthy people (Broide *et al*., 2014).

The impacts of KD on the management of blood glucose levels in diabetic and obese patients were met with conflicting findings. Ketogenesis has also been suggested to help fatty liver disease. However, a different study found that KD increased fat in the liver of mice (El-sayed *et al*., 2023). This disagreement prompted us to investigate whether KD mitigate the metabolic and hepatic alterations brought on by experimentally produced HFD in male albino rats. Our study demonstrated that KD treated group had improved the biochemical and histopathological disturbance occurred by HFD, these results were in agreement with the following reports.

Our findings corroborated with Crabtree *et al*. (2021), who found that liver enzymes were unaffected, or very slightly affected, by an increase in HFD. Hypoalbuminemia is related with liver fibrosis and ALP is typically high in fatty liver disease, suggesting that the modest but significant rise in albumin and persistent decrease in ALP that we found may give some amount of hepatoprotection. The appropriate formulation of the experimental meals may also account for the little albumin increase, which was observed primarily in the KD diet groups. Also damage to hepatocytes causes the liver index to rise and causes cellular morphological abnormalities. Since elevated levels of ALT, AST, and ALP can sensitively indicate liver impairment, they were employed as the primary indicators of liver function in this investigation (Wu *et al*., 2021).

Antioxidant enzymes (CAT, SOD, and GSH) and MDA are useful biomarkers for assessing cellular redox state. SOD removes harmful superoxide anion from cells and prevents membrane lipid peroxidation. In order to neutralize H2O2, SOD must form a conjugate with either CAT or GSH. It is also possible that the KD upregulates and/or induces antioxidant enzyme synthesis, which would explain the observed increases in antioxidant enzyme activities and explain why the KD was able to effectively neutralise the circulating free radicals and, in turn, reduce oxidative stress (Kayode *et al*., 2020), and these results were in agreement with our data.

Researchers hypothesized that eating a normal-protein KD (NPKD) would have a multiplicative and synergistic effect on the liver's metabolic pathways; they found evidence for both hypotheses. In mice fed NPKD, PGC-1α levels were shown to be reduced (Huang *et al*., 2020). To the best of our knowledge, this is the first research demonstrating the effect of KD on MetS by adjusting the mRNA expression levels of PGC-1α and GLP-1R. In conclusion, this investigation shows that HFD created MetS in rats characterized by hyperinsulinemia, IR, hepatic oxidative stress, and related alterations in hepatic function and hepatic histopathology.

CONCLUSION

This study revealed that MetS induction cause dysfunction alteration of redox status, reduced GSH, CAT, SOD levels and

increased insulin, glucose, liver function and MDA. In contrast, post treatment with KD improves the rat biochemical parameters as well as the liver functional indices and upregulates the gene expression levels of PGC-1α and GLP-1R. Together, findings support the prospect of KD to restore and improve HFD- induced MetS in rats. More research is necessary, nevertheless, to quantify KD's metabolic effects and therapeutic merits in the management of MetS.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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