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Therapeutic evaluation of the anti-inflammatory and anti-oxidative protective effects of the ketogenic diet on Wister rats.

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ABSTRACT

Background: Recent weight loss trends have favored the ketogenic low-carb diet. Due to conflicting studies, the carbohydrate restriction needed to induce ketosis is unknown. Ketogenic low-carbohydrate diets reduce weight and improve triglyceride and high-density lipoprotein levels better than low-fat diets.

Study Design: This study separated animals into four groups. The control group ate a usual diet of 24% protein, 58% carbs, and 18% fat for 10 weeks. The second group had a four-week high-fat diet (HFD. The third and fourth groups followed the ketogenic diet for four and six weeks, respectively. After dissection, liver weights were assessed, homogenates were produced, and oxidative stress biomarkers, liver function tests, and pro-inflammatory cytokines were analyzed using an enzyme-linked immunosorbent assay.

Result: There was a reduction in liver weight observed in the animals following the administration of KD. Additionally, a decrease in liver enzyme levels was observed in both Groups GIII and GIV. Moreover, there is a significant disparity in oxidative stress between the corresponding group and the GI and GII groups. Furthermore, the levels of proinflammatory cytokines (IL-6 and TNF-α) exhibited a decrease. It has been scientifically proven that the ketogenic diet possesses anti-inflammatory advantages.

Conclusion: The hepatoprotective effects of the ketogenic diet were demonstrated by reduced fat accumulation, anti-inflammatory effects, increased antioxidant defense, and weight loss potential in Wister Rats.

Keywords: Ketogenic Diet; liver Function; Obesity; Oxidative Stress; Inflammatory Markers.

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INTRODUCTION

The ketogenic diet (KD) is a high-fat, low-protein, and very low-carbohydrate diet. In recent years, low-carbohydrate ‘‘ketogenic’ diets have received much attention as a means of rapid weight loss. KD mimics the metabolic effects of fasting without large calorie deprivation. It has received extensive interest because of its beneficial effects on a number of diseases, such as type 2 diabetes mellitus , neurological disorders, obesity, cancer, and intestinal disorders **(Zhu et al., 2022) .**

Clinical research has demonstrated that mitochondrial metabolism can aid in the ketogenic diet's underlying mechanisms. Beta-hydroxybutyrate (BHB) and acetoacetate are the most common ketones. They are created when the liver oxidizes fat more efficiently, and they are precursors to acetyl CoA, the first step in the citric acid cycle **(Alhamzah et al., 2023).**

There is substantial evidence that the ketogenic diet is associated with improved mitochondrial function and a substantial reduction in oxidative stress. Oxidative stress, which is characterized by an imbalance between free radicals and antioxidants, produces reactive oxygen species (ROS) and free radicals. ROS and free radicals, which are remnants of cellular energy production, are neutralized and eliminated by antioxidants **(Reuter et al., 2010).**

In addition, It is a fact that oxidative stress plays a significant role in the development of a number of acute and chronic diseases, such as cardiovascular disease, cancer, and pulmonary problems and this is a fact that is well accepted. The ketogenic diet affects oxidative stress by inhibiting the synthesis of reactive oxygen species and increasing endogenous antioxidant capacity. Thus, the ketogenic diet arises as a promising dietary strategy with potential therapeutic implications for mitigating oxidative stress-related pathologies **(Poff et al., 2013).**

Recent clinical studies have shown KD to be effective for the treatment of obesity and insulin resistance **(Long et al., 2023)**. In addition, researchers reported the ketogenic diet's anti-inflammatory effect **(Rhyu and Cho, 2014).**

The impact of the ketogenic diet (KD) on inflammation is a multifaceted process, intricately influenced by the interplay of several hormones and metabolites, primarily at the peripheral level. Central to this mechanism is the production of ketone bodies, which serve as a key mediator in eliciting physiological responses. Specifically, the activation of the ventromedial nucleus of the hypothalamus emerges as a critical driver of the diet's effects on inflammation, as it directly relates to the regulation of satiety. Importantly, this activation varies in response to the intake of dietary fats. As a result, the ketogenic diet induces alterations in body composition, leading to weight loss. Particularly, in the context of chronic inflammatory conditions like obesity, the physiological state of cells undergoes changes that disrupt the production of adipokines **(Sherrier and Li, 2019).** This study set out to examined the impact of ketogenic diet (KD) and BHB salt supplementation on liver oxidative stress and mitochondrial function, hypothesizing that increasing endogenous antioxidants would decrease tissue oxidative stress indicators.

MATERIALS AND METHODS

Chemicals:

Phosphate buffer (PBS, pH 7.4), Chloroform and all other chemicals used were purchased from Sigma-Aldrich, Co., Ltd. (St.Louis, MO, USA). Kits for assessment of liver function (ALT, AST, GGT) and Albumin were purchased from Bio-Diagnostics, Egypt. Also, Kits for assessment of superoxide dismutase (SOD), Malondialdehyde (MDA), Nitric oxide (NO), and catalase (CAT) were purchased from Bio-Diagnostics, Egypt.

Study design

The study employed forty male Wistar rats (9 weeks old) weighing 300-320 g, purchased from the laboratory animal, facility at the School of Science at Al-Kufa University. The animals were kept in breeding cages at a room temperature of 22 ± 2°C with 12 h light/dark cycles under 50–60% relative humidity. After one week of adaptation, the rats were divided into four equal main groups, each comprising 10 rats, as follows:

Group I (Control Group): These rats were fed an ordinary diet consisting of 24% protein, 58% carbohydrate, and 18% fat. Throughout the entire 10-week experimental period, they did not receive any specific treatment.

Group II: Thirty rats were fed a high-fat diet (HFD) for the entire 4-week experimental period then 20 rats out of 30 were subdivided to represent Group III and Group IV as shown in Figure 1.

Group III: Starting from week 5 and continuing for 4 weeks, these rats were administered a keto supplement (KD) in their diet.

Group IV: These rats were treated with the KD diet for the entire 6-week experimental period, starting from week 5 until week 10.

N.B: The doses were adjusted weekly based on the mice's weight gains or losses so that the dose per kilogram of rat body weight remained constant across the whole trial.

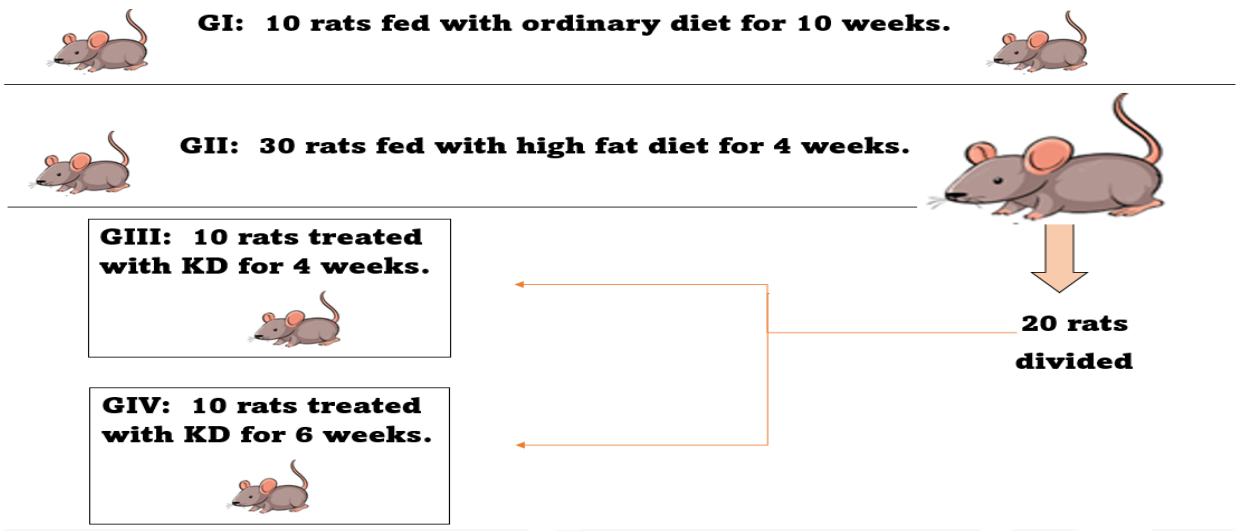

Figure 1 shows the animal experimental study design.

Sample collection

At the end of the experiment, the rats were anesthetized using a local pharmacological anesthetic known as "Inhaler Anesthetic Liquid for Inhale Solution. Following the experimental procedures, the animals were euthanized, and both blood and liver tissues were meticulously collected for analysis. The blood samples underwent centrifugation at 5000 rpm for 10 minutes to obtain serum, which was then stored at -20°C to facilitate further biochemical investigations. Additionally, liver homogenates were meticulously prepared and utilized for the assessment of hepatic parameters.

Ethical approval:

The present study was approved according to the Biomedical-Ethics Committee at the College of Medicine, Wasit University, Iraq. With an ethical approval number (UW.Med.2023.082).

Preparation for oxidative stress analysis

One gram of liver was homogenized in 0.1M phosphate buffer (PBS pH 7.4) at ice cold temperatures, and the resulting 10% (w/v) homogenates were centrifuged at 4 degrees Celsius (12,000 revolutions per minute) for 10 minutes before being stored at -80 degrees Celsius.

Biochemical assays

Liver function tests and Oxidative stress biomarkers

The enzyme activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (γGT) and the measurement of albumin were done in accordance with the steps suggested by the commercial kits (Bio-diagnostic, Egypt). Superoxide dismutase (SOD), malondialdehyde (MDA), Nitric oxide (NO) and catalase (CAT) levels were measured according to the guidelines of the commercial kits used (Bio-diagnostic, Egypt, CAT no.SD-2521 and MD-2529, NO-25 33, CA-25 17 respectively).

Proinflammatory Cytokine Levels by Enzyme-Linked Immunosorbent Assay.

Enzyme-linked immunosorbent assay (ELISA) kits, as directed by the protocols (R & D Systems), were used to measure the levels of proinflammatory cytokines (IL-6, TNF-). Liver from all groups was removed at the end of the experiment and stored at -80°C. All the tissues were homogenized on ice and centrifuged before the measurements were taken. In all samples, the supernatant was gathered, and the total protein concentration was assessed using the Biuret protein assay. Then, using the appropriate kits, the levels of IL-6 and TNF- were determined.

Statistics and Data Analysis

The present study employed SPSS version 25.0 software to perform statistical analyses on the acquired data. The results were presented as the mean ± standard deviation (SD). The alterations in biomarker data were subjected to a one-way ANOVA to enable multiple comparisons. A significance level of $p < 0.05$ was considered the threshold for statistical significance.

RESULTS AND DISCUSSION

RESULTS

After administration of KD, comparing the control and KD groups, there was a statistically significant difference in body weight. ($p< 0.05$, Table 1). In comparison to the control group, the 4 week and 6-week KD treatment groups experienced a significant reduction in body weight gain ($p< 0.05$). In KD-exposed groups, there were also significant differences in liver weight.

Organ index (g/g BW) = Organ absolute weight (g)/Body weight (g) × 100%.

Table 1 shows the body weight, liver weight, and liver weight to body weight ratio for each group of animals.

Groups n=10	Body weight g	Liver weight g	Organ index %
GI	315±6.1	10.74±0.47	3.40±0.04
GII	365±5.17[a]	12.65±0.22[a]	3.46±0.07
GIII	354.2±3.2 [a,b]	12.40±0.19 [a,b]	3.50 [a, b]±0.02
GIV	338.5±3.9 [a,b]	12.35±0.5 [a,b]	3.64 [a, b]±0.05

Indicated data are means and standard deviation. A substantial difference between the corresponding group and the GI and GII groups is denoted by the small letters (a,b). $p < 0.05$

Table 2 displays the data as means and standard deviations.

Group	ALT IU/L	AST IU/L	γ-GT IU/L	ALB g/dL
GI	50.7±3.6	94.7 ±2.2	32.60±0.72	3.3±0.51
GII	56.5±1.2[a]	96.3±3.4[a]	33.07±0.47[a]	3.1±0.13
GIII	45.1±3.2 [a,b]	94.3±3.9 [a,b]	31.2±3.1 [a,b]	3.3±0.26
GIV	42.4±3.7 [a,b]	91.2 ±4.4 [a,b]	30.2±1.3 [a,b]	3.8±0.02 [a,b]

The small letters (a) and (b) signify that the corresponding group has significantly changed from the GI and GII groups, respectively. $p < 0.05$

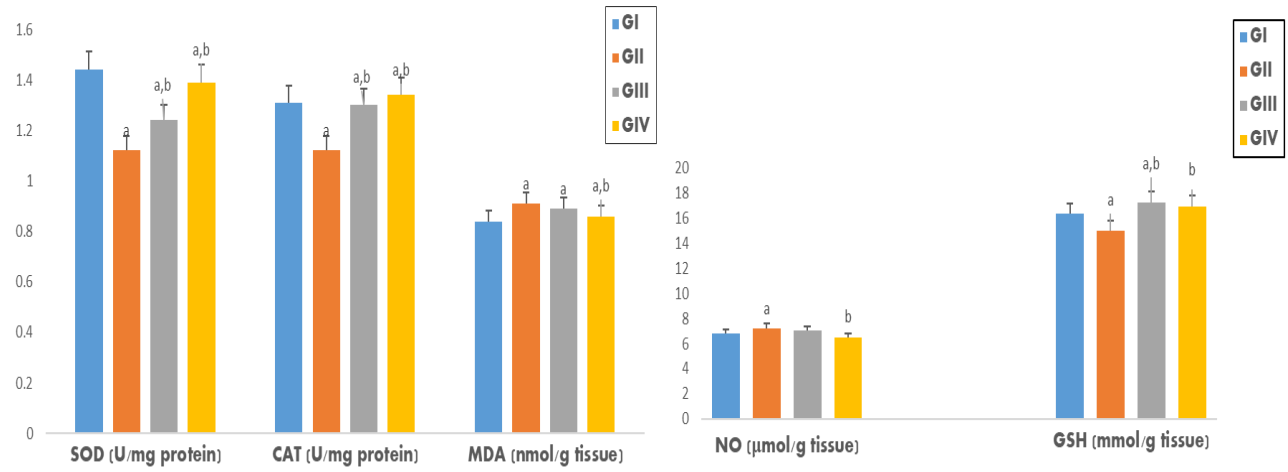

Figure 2 levels of indicators for oxidative stress in experimental groups. A substantial difference between the corresponding group and the GI and GII groups is denoted by the small letters (a & b). The ANOVA test was used to compare the group means.

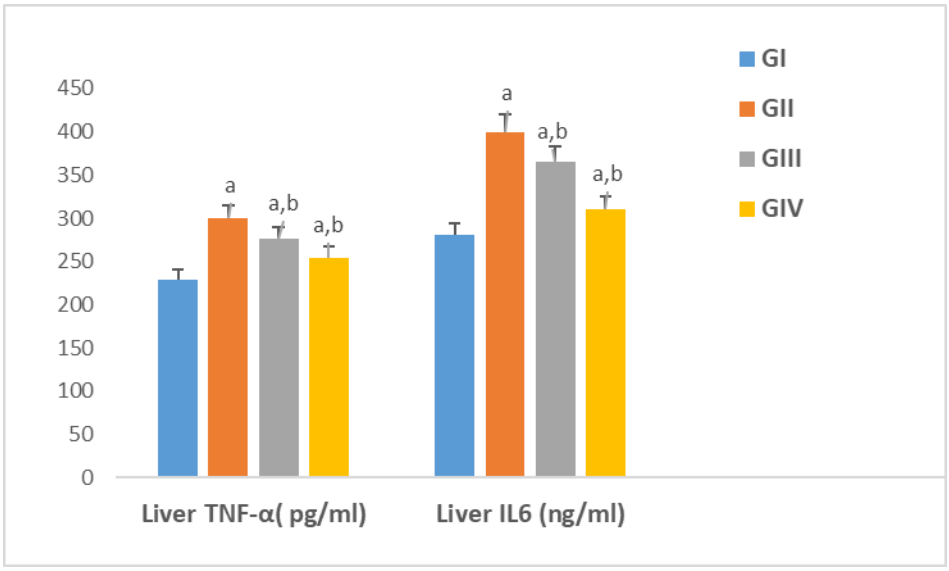

Figure 3, The levels of proinflammatory cytokines (IL-6, and TNF-α)

DISCUSSION

Our study purpose was to examine the anti-inflammatory and anti-oxidative protective effects of the ketogenic diet on Wister rats livers by measuring different parameters. Chronic inflammation and oxidative stress are now recognized as two important causes of the emergence of numerous diseases. A bulk of publications have shown that the ketogenic diet (KD) enhances cellular metabolism and mitochondrial function, inducing a shift in energy metabolism **(Pinto et al., 2018).**

According to our results, KD administration results in significant weight loss. The GIV group demonstrates a 92% decrease from the GII group after six weeks, with hepatic weight loss also noted (Table 1). This finding was consistent with the hypothesis that KD has garnered considerable attention as a potential weight-loss strategy. It was hypothesized that hepatic oxygen consumption for gluconeogenesis and triglyceride-fatty acid recycling would increase during KD consumption **(Basolo et al., 2022, Abbasi, 2018). Mohorko et al.,** (2019) reported that KD cause the loss of body weight without any significant negative effects on metabolic, physiological parameters **.**

Ketogenic diets provide improvement and aid in the management of some chronic disease symptoms **(Crosby et al., 2021).** Restricting carbohydrate intake leads to nutritional ketosis, which has been shown to minimize or eliminate insulin needs in type II diabetics and shows promise in helping patients lose weight and correct metabolic syndrome symptoms **(Kalra et al., 2018),** lower inflammation, enhance lipid profiles, modify the microbiome, and enhance epigenetic profiles **(Hussain et al., 2012),** support cancer treatments **(Klement, 2017),** and brain function **(Dowis and Banga, 2021, Dąbek et al., 2020).**

One of the most notable protective effects attributed to ketone bodies is their ability to mitigate the generation of reactive oxygen species (ROS) by mitochondria. Specifically, β-hydroxybutyrate (BHB), a type of ketone body, has been identified as a cell-protective agent against oxidative damage. Research studies have demonstrated that the treatment of cells with BHB leads to a reduction in the cytosolic [NADP+]/[NADPH] ratio and an increase in reduced glutathione, a crucial low molecular weight antioxidant present in the cell. Furthermore, BHB has been found to inhibit the activity of NF-κB (nuclear factor-kappa B) by promoting the translocation and degradation of IκB-α, a key regulator of the inflammatory response. NF-κB governs the expression of numerous pro-inflammatory genes, including iNOS, COX-2, TNF-α, IL-1β, and IL-6. Consequently, the administration of BHB to cells effectively attenuates the pro-inflammatory response, showcasing its anti-inflammatory properties **(Dąbek et al., 2020, Bendridi et al., 2022).**

Considering that ketosis decreases overall oxidative stress, our data observed a significantly higher reduction in MDA and NO levels after KD administration in contrast to enzymes (SOD, CAT) Fig 1. In agreement with these results, several support the idea that KD improves oxidative status.**(Wallace et al., 2021, Rius-Pérez et al., 2020).**

Although the exact mechanisms are still not understood, KD improves the redox status of the cell in multiple different ways; one of these methods is that BHB, which has a hydroxyl group as part

of its structure, functions as a scavenger for hydroxyl radicals (OH) **(Haces et al., 2008).** On the other hand, the beneficial effects of KD on oxidative stress have been well documented both in vivo and in cellular models. These benefits manifest themselves as a rise in the NAD+/NADH ratio. In addition to this, KD ought to be recognized as an activator of a variety of signalling pathways that influence the dynamics of mitochondria, the amount of energy expended, and the stability of DNA**(Paoli and Cerullo, 2023).**

The relationship between the inflammatory process and KD has recently drawn considerable attention. Proinflammatory cytokine levels, TNF-a and IL-6, are elevated in obesity **(Matsubara et al., 2012).** TNF can increase the number of diseases and be associated with the inflammatory response to infection, cancer and various autoimmune disorders **(van Loo and Bertrand, 2023).**

TNF and IL-6 markedly decreased throughout KD (Fig. 2). It has been demonstrated that the ketogenic diet has anti-inflammatory benefits. Previous research on the impact of KD on systemic inflammation showed alterations in cytokine levels and a general decline in peptides associated with inflammation with KD treatment **(Dahlin et al., 2022).**

CONCLUSION

To summarize, we have identified a positive effect of the ketogenic diet in cell metabolism association. Treatment with the ketogenic diet lowers both oxidative stress and inflammation-related variables. Our study confirms the KD has a good effect on energy states.

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