Immunostimulatory effect of ketogenic diet in cyclophosphamide-induced immunosuppression in adult albino rats

Rasha A.K. Elsisy[a], Marwa M. Mona[b], Sanad S. Elkholy[c]

Departments of [a]Anatomy, [b]Medical Biochemistry, [c]Physiology, Faculty of Medicine, Kafr Elsheikh University, Kafr Elsheikh, Egypt

Correspondence to Rasha A.K. Elsisy, MD, Department of Anatomy, Faculty of Medicine, Kafr Elsheikh University, Kafr Elsheikh, Postal Code: 33516, Egypt. Tel: +20 127 433 7050; e-mail: rasha_saleh2014@med.kfs.edu.eg

Received: 1 January 2020
Accepted: 2 February 2020
Published: 18 February 2022

Egyptian Journal of Obesity, Diabetes and Endocrinology 2021, 7:25–32

Background
The ketogenic diet (KD) is effective to fight obesity and has therapeutic effects on various body systems.
Aim/objectives
This study aimed to evaluate the role of KD in improving immune response against cyclophosphamide (CTX)-induced immunosuppression in rats.
Methods
Young adult albino rats (21 male) were divided into three groups: G1 (Cnt), normal control fed on a basal diet; G2 (CTX), injected with CTX and fed on a basal diet; and G3 (Keto), injected with CTX and fed on a ketogenic diet for 4 weeks.
Results
This study revealed that treatment with CTX decreased serum levels of total protein, albumin, globulin. Administration of CTX also resulted in a significant decrease in catalase (CAT), superoxide dismutase (SOD) levels and a significant increase in the levels of malondialdehyde (MDA) in the spleen. Histopathological examination revealed that CTX caused lymphocyte depletion in the spleen and thymus. Molecularly, CTX significantly downregulated the expression of interferon-gamma (IFN-γ), while it upregulated interleukin 1 beta (IL1b) in the spleen. Co-administration of the ketogenic diet was able to normalize the antioxidant status and most of the biochemical and immunological parameters.
Conclusion
With these findings, we could conclude that feeding on ketogenic diet could improve the immunity.

Keywords:
antioxidant, cyclophosphamide, immunosuppression, ketogenic diet

Egypt J Obes Diabetes Endocrinol 7:25–32

2356-8062

Introduction

Obesity is a worldwide health concern as it increases the risk of several diseases like cardiovascular diseases, diabetes, and cancers [1]. Ketogenic diet (KD) is effective in fighting obesity, at least in the short to medium term, in addition to hyperlipidemia and some cardiovascular risk factors [2,3]. KD is high in fat of ~90%, has adequate protein of ~8%, and has very low amounts of carbohydrate of ~2%. The name KD is derived from the formation of ketones, mainly beta-hydroxybutyrate and acetoacetate, which occur owing to increased fatty acid oxidation in the liver, which shifts energy sources to fat instead of carbohydrate [4–6]. The weight loss caused by KD was suggested to be induced by losing energy through excretion of ketone bodies [7]. Other reports suggested that the use of energy from protein in KD needs more calories [8]. Some authors explain this weight loss owing to reduction in appetite as the proteins have high satiety [9] or to some effects on appetite control hormones [10]. Other authors suggest a possible direct appetite-suppressant action of the ketone bodies [11]. KD may have beneficial effects on mood in overweight patients [12] and also improved glycemic control, hemoglobin A1c, and lipid markers, in addition to decrease the use or withdrawal of insulin and other medication in many cases [13,14]. Furthermore, it is used in the treatment of cardiomyopathy with glycogen storage disease type III [15,16]. Abdelwahab *et al.* [17] observed that administration of KD with radiation could improve the survival. Moreover, KD has therapeutic effects on the central nervous system. It has a role in the treatment of medication-intolerant epilepsy [18]. The effect of KD on the treatment of this immune-mediated status epilepticus is owing to systemic and metabolic effects of KD on the immune system [19]. KD has been shown to retard tumor growth in many types of cancer through anti-angiogenic, anti-

inflammatory, and proapoptotic mechanisms [20]. KD feeding has also been shown to decrease reactive oxygen species and hydrogen peroxide production in rodent tissues by increasing uncoupling protein-mediated proton conductance and/or increasing glutathione biosynthesis [21].

It was demonstrated that the KD reduces activation of the pro-inflammatory transcription factor nuclear factor kappa B and reduces the expression of cyclooxygenase-2 [22,23], both of which have been involved in hypoxia-driven immunosuppression [24]. KD may work as an immune stimulant in the glioma microenvironment by reducing immune suppression and promoting Th1-type immune responses against the tumor [25].

Cyclophosphamide (CTX) is known to possess antitumor and immunomodulatory properties [26]. CTX is also known to suppress immune responses by modulating lymphocytes [27]. CTX and its metabolites are commonly used as antineoplastic and immunosuppressive drugs [28]. According to reports, CTX can cause a decrease in body weight, splenocyte proliferation, organ index, macrophage phagocytosis, and natural killer cell activity when used at high doses and over longer durations [29]. Most importantly, immunosuppression was one of the major adverse effects of using high doses of CTX. Therefore, CTX was often used to construct murine models of immunosuppression [30].

This study aimed to evaluate the role of KD in improving immune response against CTX-induced immunosuppression in rats.

Materials and methods

Animals and experimental design

Healthy male albino rats (n=21) of similar weight (180 ±10 g) were kept in a controlled temperature (25±2°C) and light (12-h light/dark cycle). Standard clean rodent food and sterilized water were supplied *ad libitum*. The animals were acclimatized to laboratory condition for at least 12 days before experiments. Animal-management procedures were undertaken in accordance with the requirements of the Animal Care and Ethics Committee of the Faculty of Medicine, Kafr Elsheikh University.

The animals were divided into three groups (G) (n=7/group): G1, control rats fed a basal diet for 4 weeks and received 1 ml of PBS intraperitoneally for 4 days (from day 1 to the day 4); G2, rats received 1 ml of CTX (50 mg/kg/day, intraperitoneally; Sigma, St Louis, California, USA) for four successive days (from day 1 to the day 4) [31] and fed on a basal diet for 4 weeks; and G3, rats received CTX as previously mentioned and fed on KD for 4 weeks. Table 1 shows the nutrient composition of basal and KDs [32].

Table 1 Nutrient composition of basal and ketogenic diets

Nutrients	Basal diet (g/kg)	Ketogenic diet	
		g/kg	%
Protein (casein) 20%	200	142.090	16.6
Carbohydrate (starch)	665	4.887	3.4
Fat (corn oil)	40	737.83	80
Vitamin mixture	10	4.611	
Minerals mixture	35	44.472	
Fiber	50	66.087	
Choline bitartrate	2.5		
L-cysteine	1.8		

On day 29, blood samples were collected using orbital bleeding and sera were obtained by centrifugation at 3000 rpm for 5 min. Then, the animals were killed. Thymus and spleen were excised, homogenized for biochemical assay, and snap-frozen in liquid nitrogen for real-time PCR or fixed in 10% formalin for histopathology.

Biochemical analysis

Total protein and albumin were measured using Spinreact kits (Spain) according to the manufacturer's instructions. The serum globulin level was calculated by subtracting the obtained serum albumin concentration from the obtained serum total protein concentration. Lipid profile [triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein] was determined spectrophotometrically using commercially available kits. The homogenized spleen supernatants were used to determine the levels of the lipid peroxidation biomarker malondialdehyde (MDA) and the activity of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) using commercial kits (Bio-Diagnostics Co, 29 El-Tahrer St., Dokki, Giza, Egypt) according to the manufacturer's instructions.

Histopathological examination

After fixation in 10% formalin, spleen and thymus specimens were dehydrated through alcohols, cleared in xylene, and then embedded in paraffin wax. Sections (5 μm) were stained with either hematoxylin and eosin. Micrographs were performed under a microscope with an automatic camera (Olympus, Japan).

Real-time PCR analysis

Total RNA was isolated using a commercial kit (Gene JET RNA Purification Kit) following the manufacturer's protocol (# K0731; Thermo Scientific, Applied Biosystems, Waltham, Massachusetts, United States). The cDNA was synthesized by reverse transcription using a commercial kit (RevertAid H Minus Reverse Transcriptase) as described in the manufacturer's instruction (# EP0451; Thermo Scientific). Based on the published rat primers' sequences, specific primers for interferon-gamma (IFN-γ) (Fw 5′-GATCC AGCACAAAGCTGTCA-3′ and Rv 5′-GACTC CTTTTCCGCTTCCTT-3′), interleukin (IL)-1b (Fw 5′-CACCTCTCAAGCAGAGCACAG-3′ and Rv 5′-GGGTTCCATGGTGAAGTCAAC-3′), and β-actin (Fw 5′-AAGTCCCTCACCCTCC CAAAAG-3′ and Rv 5′-AAGCAATGCTGTCA CCTTCCC-3′) genes were used. QPCR was performed using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) in a StepOnePlus real-time thermal cycler (Applied Biosystems, Life Technology, USA). PCR was performed in the following thermal condition: 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s for 40 cycles after initial denaturation (95°C, 10 min). The quantities of critical threshold (Ct) of the target gene were normalized with quantities (Ct) of the housekeeping gene (*β-actin*) by using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

One-way analysis of variance and the post-hoc test Tukey's Honestly Significant Difference (Tukey's HSD) were used to compare means using Graph Pad Prism 5 software (San Diego, CA, USA). The obtained data were presented as mean±SEM, and significance was set at *P* value less than 0.05.

Table 2 Alterations in serum parameters after different treatments

Parameters	Control	CTX	Keto
Total protein (g/dl)	6.98±0.40[a]	5.32±0.25[b]	6.54±0.37[a]
Albumin (g/dl)	4.09±0.12[a]	3.30±0.14[b]	4.02±0.18[a]
Globulin (g/dl)	2.90±0.12[a]	2.02±0.10[c]	2.52±0.10[b]
TG (mg/dl)	127.52±3.40[a]	120.66±3.28[a]	101.80±3.00[b]
TC (mg/dl)	112.37±2.90[a]	118.81±2.85[a]	96.17±2.26[b]
HDL (mg/dl)	50.42±1.49[a]	53.05±1.62[a]	61.24±1.57[b]
LDL (mg/dl)	24.74±0.75[a]	25.19±0.80[a]	20.00±0.46[b]

Data are presented as mean±SEM, *n*=5. CTX, cyclophosphamide; HDL, high-density lipoprotein; Keto, Keto diet; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglycerides. Values with different superscript letters in the same row are significantly different at ($P<0.05$).

Results

Serum parameters

Serum levels of total proteins, albumin, and globulin of CTX-treated rats were significantly decreased ($P<0.05$) compared with the control animals (Table 2). CTX-treated animals fed on a KD showed a significant ($P<0.05$) improvement in the total protein, albumin, and globulin compared with the CTX-treated rats. No significant difference was noticed in TG, TC, and HDL between CTX and control groups. However, in the keto group, animals had significantly lower TG, TC, and low-density lipoprotein and significantly higher HDL as compared with other groups.

Oxidative stress and antioxidant status

CTX treatment resulted in a significant ($P<0.05$) reduction in the level of the antioxidant enzymes (CAT and SOD) activities associated with a significant increase in the levels of MDA in the rat spleen as compared with the control group (Table 3). CTX-treated animals fed on a KD showed a significant ($P<0.05$) improvement in the antioxidant enzyme activities and MDA levels in the rat spleen compared with CTX-treated animals.

Gene expression in the spleen

Animals injected with CTX and fed on basal diet showed significant downregulation in the *INFg* gene and a significant upregulation of the *Il1b* gene in their spleen as compared with the normal control animals (Fig. 1). However, CTX-treated animals fed on the KD had significantly upregulated expression of *INFg* and significantly downregulated expression of *Il1b* as compared with CTX-treated animals and fed on basal diet. On the contrary, all treated animals had expressions of lower *INFg* and higher *Il1b* than that of the normal control group.

Histopathological changes in spleen and thymus

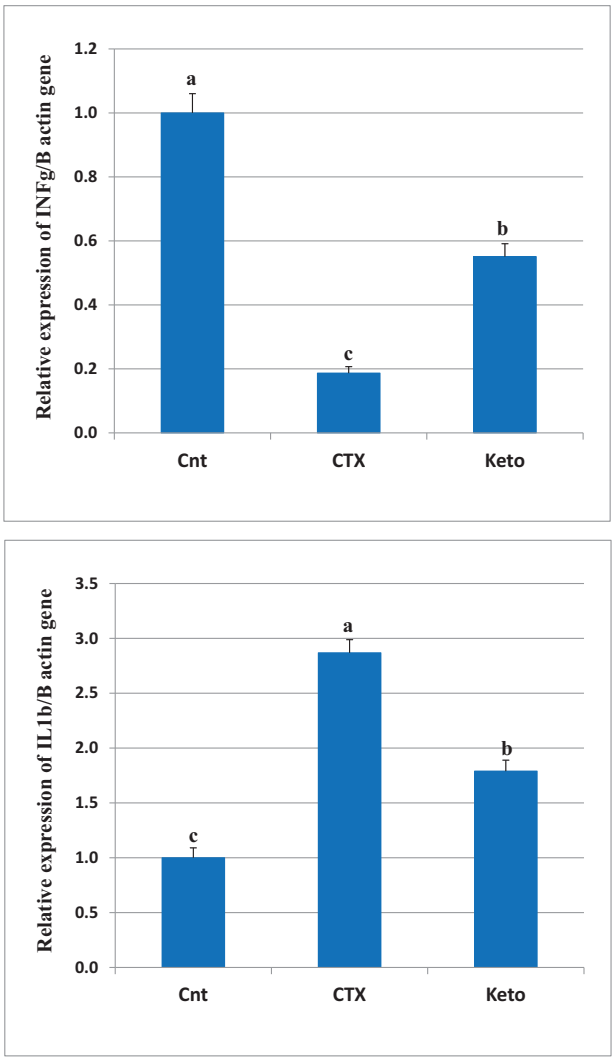
Spleen of the control group showed normal lymphoid structure (Fig. 2). Spleen of CTX-treated animals fed

Table 3 Changes in antioxidant enzyme activities and malondialdehyde levels of rats' spleen following different treatments

Parameters	Control	CTX	Keto
Catalase (U/g tissue)	8.48±0.40[a]	4.82±0.22[c]	6.06±0.23[b]
SOD (U/g tissue)	51.08±1.65[a]	32.11±0.81[c]	43.29±0.64[b]
MDA (nmol/g tissue)	24.17±0.50[c]	40.67±1.25[a]	31.48±0.54[b]

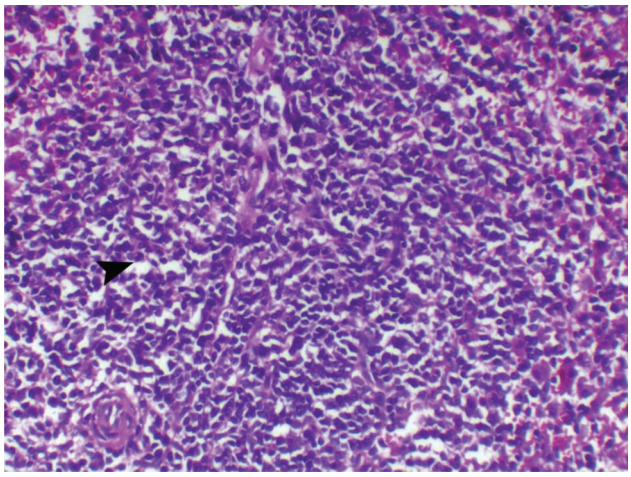
Data are presented as mean±standard error of mean (SEM), *n*=5. CTX, cyclophosphamide; Keto, Keto diet; MDA, malondialdehyde; SOD, superoxide dismutase. Values with different superscript letters in the same row are significantly different at ($P<0.05$).

Figure 1



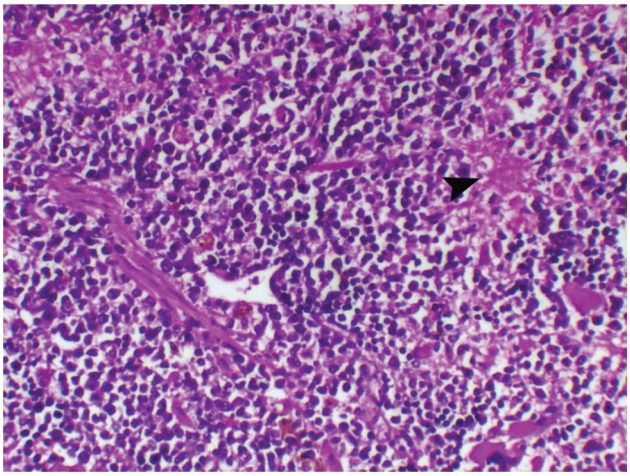
Quantification of *INFg* and *IL1B* gene expression in the spleen using real-time PCR. B-actin was used as an internal reference. Data are presented as mean±SEM, *n*=5. Columns with different letters are significantly different at *P* value less than or equal to 0.05. IL1B, interleukin 1 beta; INFg, interferon-gamma.

Figure 2



Spleen of normal control animal showing normal splenocytes (arrowhead), hematoxylin and eosin, ×200.

Figure 3



Spleen of CTX-treated animal fed on basal diet showing a marked degree of lymphoid depletion associated with lymphoid necrosis (arrowhead), hematoxylin and eosin, ×200. CTX, cyclophosphamide.

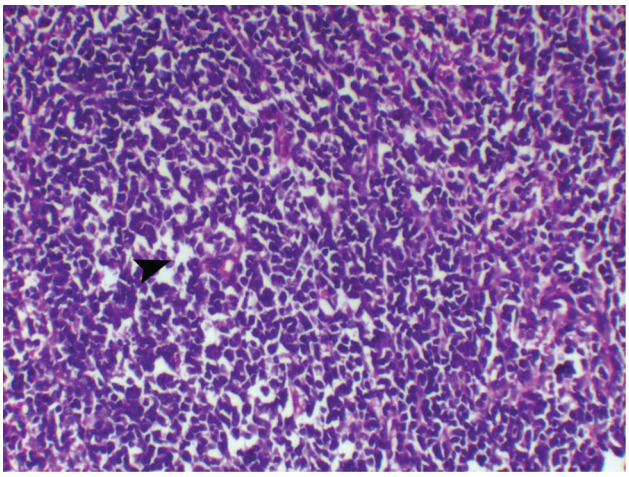
on basal diet revealed a marked degree of lymphoid depletion associated with lymphoid necrosis (Fig. 3). Spleen of CTX-treated animals fed on a KD exhibited a mild degree of lymphoid depletion (Fig. 4).

Thymus of normal control animals showed normal thymic follicles filled with normal thymocytes (Fig. 5). Thymus of CTX-treated animals fed on basal diet exhibited a marked thymic follicle depletion (Fig. 6). Thymus of CTX-treated animals fed on KD revealed a moderate degree of thymic follicle depletion (Fig. 7).

Discussion

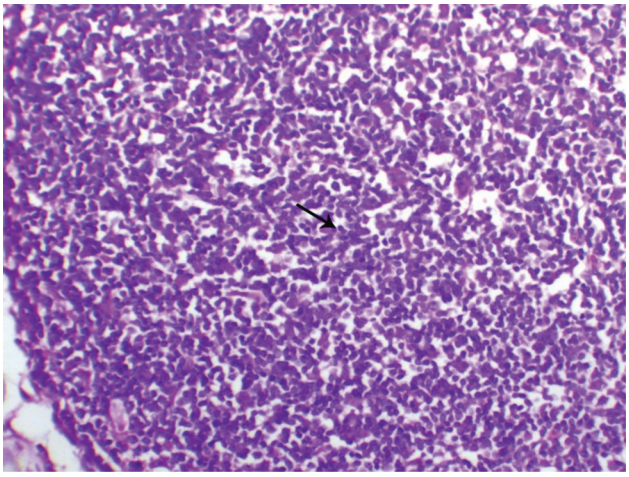
KD is effective in fighting obesity, hyperlipidemia, and some cardiovascular risk factors [3]. Previous studies demonstrated the immunostimulant role of KD to treat medication-intolerant epilepsy [19] and an immune stimulant in the glioma microenvironment by reducing immune suppression and promoting Th1-type immune responses against the tumor [25]. CTX is mainly used as chemotherapeutic agents for various tumors, cancers, and marrow transplantation [26]. CTX is an alkylating cytotoxic agent that disrupts DNA replication by crosslinking DNA strands, inhibiting cell propagation, and inducing apoptosis [33]. CTX is also known to suppress immune responses by modulating lymphocytes [27]. So, CTX and its metabolites are commonly used as antineoplastic and immunosuppressive drugs [28]. The main objective of this study was to investigate

Figure 4



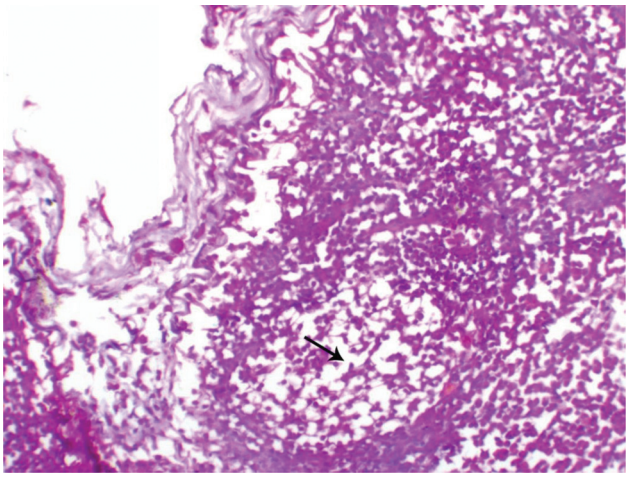
Spleen of CTX-treated animal fed on ketogenic diet showing a mild degree of lymphoid depletion (arrowhead), hematoxylin and eosin, ×200. CTX, cyclophosphamide.

Figure 5



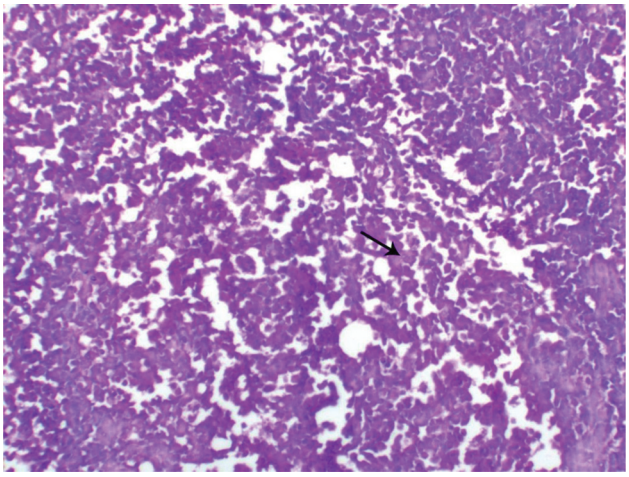
Thymus of normal control animal showing normal thymic follicle filled with normal thymocytes (arrow), hematoxylin and eosin, ×200.

Figure 6



Thymus of CTX-treated animal fed on basal diet showing marked thymic follicle depletion (arrow), hematoxylin and eosin, ×200. CTX, cyclophosphamide.

Figure 7



Thymus of CTX-treated animal fed on ketogenic diet showing a moderate degree of thymic follicle depletion (arrow), hematoxylin and eosin, ×200. CTX, cyclophosphamide.

the immunostimulatory effects of a KD in the CTX immunosuppressed rat model.

CTX at high doses suppresses the immune system. As a result, CTX is frequently employed to create immunosuppression models in rats [30,34,35]. The injection of 40 mg/kg CTX to normal immunocompetent mice for four days resulted in immunosuppression [36]. In the present study, the rats received 50 mg/kg CTX for 4 days to produce a state of immunosuppression. Serum levels of total proteins, albumin, and globulin of CTX-treated rats were significantly decreased compared with the control animals. CTX-treated animals fed on a KD showed a significant improvement in the total protein, albumin, and globulin compared with the CTX-treated rats. The current results agreed with Ibrahim *et al.* [31] and Mohamed [37] who showed a significant reduction in total protein, -globulin, and -globulin levels after CTX treatment. These findings could be due to a reduction in protein synthesis as a result of CTX-induced immunosuppression and liver damage [37,38].

MDA is commonly considered to be an index for oxidative stress severity, as it is the most important component among reactive aldehydes originating from lipid peroxidation [39]. MDA level is an indicator of the severity of damage caused by free radicals, whereas the activity of SOD and GPx in the body can reflect the

scavenging capacity of free radicals [40]. In the present work, administration of CTX resulted in a significant decrease in CAT and SOD levels associated with a significant increase in the levels of MDA in the spleen. This was related to excessive production of CTX-alkylating metabolites, which mediated oxidative stress and cellular lipid peroxidation [41]. These results agreed with El-Sebaey *et al*. [42] Who reported that CTX caused oxidative damage in the liver, as seen by an increase in hepatic MDA and a decrease in hepatic antioxidants enzyme values (CAT, SOD, and GSH). Duggina *et al*. [43] observed a significant elevation of the hepatic MDA level in CTX-administrated rats. Moreover, Wang *et al*. [40] found that the activities of CAT, SOD, and GPx in serum significantly decreased and the MDA levels markedly increased when the mice were treated with CTX. In the current work, KD-treated group showed a normal level of the antioxidant enzymes CAT and SOD and a decrease in the level of MDA in the spleen. This result indicates that the antioxidant system of CTX-treated rats tends to be normalized under the protection of KD.

Cytokines are produced by multiple immune cells and regulate immune responses, such as immune cell differentiation, inflammation, and host defense against bacterial infection [44]. INF-γ is produced primarily by T-helper cells [45]. INF-γ is a mediator of cellular immunity, which is one of the major immunoregulatory molecules. IFN-γ can induce immune responses against bacteria and exogenous infectious agents [46–48]. So, the induction of cytokine synthesis is one of the methods to evaluate the augmentation activity of innate immunity. In the present study, CTX-treated group showed significantly downregulated expression of *IFN-γ* gene in the spleen. This agrees with Cheng *et al*. [36] who stated that CTX-treated rats showed a decrease in the expression of *IFN-γ* in the spleen. Furthermore, Chen *et al*. [49] confirmed the same result in the serum. CTX-treatment significantly reduced the secretion of *IFN-γ*.[19,34,43].

The nuclear factor kappa B signaling pathway would be activated to release a variety of cytokines such as TNFα and IL1β of the host's innate immune response [50–52]. IL1β could promote immune cell functions and can increase the immunity of the body [53]. In the current study, the CTX-treated group showed upregulated *IL1β* in the spleen. El-Sebaey *et al*. [42], Kim *et al*. [54], and Song *et al*. [55] found the same result in the serum of the CTX-treated group. On the other hand, Gao *et al*. [34] and Chen *et al*. [49] mentioned that there was a decrease in the level of IL1β in the serum of CTX-treated group.In the present study, KD-treated group showed upregulation of expression of *IFN-γ* and downregulation *IL1β* in the spleen. These results indicate that KD exhibited regulatory effects on the immune function by promoting the expression of various cytokines and maintaining the cellular immune system.

The host immune response is made up of innate and adaptive immunity, which depends on some important immune organs like the thymus and spleen [56]. Thymus and spleen are two important immune response organs that unite the innate immune system with the adaptive immune system. In the present study, the histological structure of the thymus and spleen of the control group showed normal lymphoid structure, whereas the spleen and thymus of CTX-treated animals fed on basal diet showed a marked degree of lymphoid depletion associated with lymphoid necrosis. This result agreed with Zhou *et al*. [30], who mentioned that the spleen of CTX-treated group showed intermixed red pulp and white pulp and reduced lymphocytes. Furthermore, ElSebaey *et al*. [42] discovered that the CTX-treated group's spleen revealed severe congestion and lymphoid depletion, as well as decreased lymphoid follicle size. Moreover, Yun *et al*. [57] found obvious intercellular space dilatation and necrotic areas in CTX-treated animals. Moreover, a decrease in the lymphoid cells was noticed in the thymic cortex of CTX-treated group [58]. Moreover, Zhang *et al*. [59] found lymphocytes depletion and vacuoles in the thymus cellular structure, representing the marked cell death that appeared in CTX-treated mice. Gao *et al*. [34] and Chen *et al*. [49] also found that CTX-treated mice had much lower thymus and spleen indices, reflecting worse immune activity. These histological changes in the spleen and thymus indicated their destruction because of immunosuppression induced by CTX [36,60]. In the current study, spleen and thymus of CTX-treated animals fed on a KD showed a mild to moderate degree of lymphoid depletion, indicating that the KD had immunostimulatory activity.

Conclusion

According to the findings of our investigation, KD ingestion was able to restore antioxidant status and improve the majority of biochemical and immunological markers that had been detoriorated by CTX. KD could therefore have immunomodulatory and antioxidant effects.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. Lancet 2011; 378:815–825.

2 Al-Khalifa A, Mathew TC, Al-Zaid NS, Mathew E, Dashti HM. Therapeutic role of low-carbohydrate ketogenic diet in diabetes. Nutrition 2009; 25:1177–1185.

3 Bueno NB, de Melo ISV, de Oliveira SL, da Rocha Ataide T. Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. Br J Nutr 2013; 110:1178–1187.

4 Bielohuby M, Menhofer D, Kirchner H, Stoehr BJ, Müller TD, Stock P, *et al.* Induction of ketosis in rats fed low-carbohydrate, high-fat diets depends on the relative abundance of dietary fat and protein. Am J Physiol Endocrinol Metab 2011; 300:E65–E76.

5 McPherson PAC, McEneny J. The biochemistry of ketogenesis and its role in weight management, neurological disease and oxidative stress. J Physiol Biochem 2012; 68:141–151.

6 Paoli A. Ketogenic diet for obesity: friend or foe? Int J Environ Res Public Health 2014; 11:2092–2107.

7 Atkins RC. Dr. Atkins' diet revolution; the high calorie way to stay thin forever. Bantam edition, published in the united States and Canada, 1972; 5:41–50.

8 Feinman RD, Fine EJ. Nonequilibrium thermodynamics and energy efficiency in weight loss diets. Theoret Biol Med Model 2007; 4:1–13.

9 Westerterp-Plantenga M, Nieuwenhuizen A, Tome D, Soenen S, Westerterp K. Dietary protein, weight loss, and weight maintenance. Annu Rev Nutr 2009; 29:21–41.

10 Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, Proietto J. Ketosis and appetite-mediating nutrients and hormones after weight loss. Eur J Clin Nutr 2013; 67:759–764.

11 Johnstone AM, Horgan GW, Murison SD, Bremner DM, Lobley GE. Effects of a high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. Am J Clin Nutr 2008; 87:44–55.

12 Brinkworth GD, Noakes M, Clifton PM, Buckley JD. Effects of a low carbohydrate weight loss diet on exercise capacity and tolerance in obese subjects. Obesity 2009; 17:1916–1923.

13 Al-Khalifa A, Mathew T, Al-Zaid N, Mathew E, Dashti H. Low carbohydrate ketogenic diet prevents the induction of diabetes using streptozotocin in rats. Exp Toxicol Pathol 2011; 63:663–669.

14 Volek JS, Phinney SD, Forsythe CE, Quann EE, Wood RJ, Puglisi MJ, *et al.* Carbohydrate restriction has a more favorable impact on the metabolic syndrome than a low fat diet. Lipids 2009; 44:297–309.

15 Brambilla A, Mannarino S, Pretese R, Gasperini S, Galimberti C, Parini R. Improvement of cardiomyopathy after high-fat diet in two siblings with glycogen storage disease type III. JIMD Reports 2014; 17:91–95.

16 Francini-Pesenti F, Tresso S, Vitturi N. Modified Atkins ketogenic diet improves heart and skeletal muscle function in glycogen storage disease type III. Acta Myol 2019; 38:17.

17 Abdelwahab MG, Fenton KE, Preul MC, Rho JM, Lynch A, Stafford P, Scheck AC. The ketogenic diet is an effective adjuvant to radiation therapy for the treatment of malignant glioma. PLoS One 2012; 7:e36197.

18 D'Andrea Meira I, Romão TT, Pires do Prado HJ, Krüger LT, Pires MEP, da Conceição PO. Ketogenic diet and epilepsy: what we know so far. Front Neurosci 2019; 13:5.

19 Yu N, Liu H, Di Q. Modulation of immunity and the inflammatory response: a new target for treating drug-resistant epilepsy. Curr Neuropharmacol 2013; 11:114–127.

20 Wright C, Simone NL. Obesity and tumor growth: inflammation, immunity, and the role of a ketogenic diet. Curr Opin Clin Nutr Metab Care 2016; 19:294–299.

21 Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, *et al.* Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. Ann Neurol 2006; 60:223–235.

22 Stafford P, Abdelwahab MG, Preul MC, Rho JM, Scheck AC. The ketogenic diet reverses gene expression patterns and reduces reactive oxygen species levels when used as an adjuvant therapy for glioma. Nutr Metab (Lond) 2010; 7:1–11.

23 Woolf EC, Curley KL, Liu Q, Turner GH, Charlton JA, Preul MC, Scheck AC. The ketogenic diet alters the hypoxic response and affects expression of proteins associated with angiogenesis, invasive potential and vascular permeability in a mouse glioma model. PLoS One 2015; 10:e0130357.

24 Bruzzese L, Fromonot J, By Y, Durand-Gorde JM, Condo J, Kipson N, *et al.* NF-κB enhances hypoxia-driven T-cell immunosuppression via upregulation of adenosine A2A receptors. Cell Signal 2014; 26:1060–1067.

25 Lussier DM, Woolf EC, Johnson JL, Brooks KS, Blattman JN, Scheck AC. Enhanced immunity in a mouse model of malignant glioma is mediated by a therapeutic ketogenic diet. BMC Cancer 2016; 16:1–10.

26 Ahlmann M, Hempel G. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. Cancer Chemother Pharmacol 2016; 78:661–671.

27 Strauss G, Osen W, Debatin KM. Induction of apoptosis and modulation of activation and effector function in T cells by immunosuppressive drugs. Clin Exp Immunol 2002; 128:255–266.

28 Matalon ST, Ornoy A, Lishner M. Review of the potential effects of three commonly used antineoplastic and immunosuppressive drugs (cyclophosphamide, azathioprine, doxorubicin on the embryo and placenta). Reprod Toxicol 2004; 18:219–230.

29 Nafees S, Rashid S, Ali N, Hasan SK, Sultana S. Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in Wistar rats: role of NFκB/MAPK pathway. Chem Biol Interact 2015; 231:98–107.

30 Zhou Y, Chen X, Yi R, Li G, Sun P, Qian Y, Zhao X. 2018. Immunomodulatory effect of tremella polysaccharides against cyclophosphamide-induced immunosuppression in mice. Molecules 23:239.

31 Ibrahim HM, Mohammed-Geba K, Tawfic AA, El-Magd MA. Camel milk exosomes modulate cyclophosphamide-induced oxidative stress and immuno-toxicity in rats. Food Funct 2019; 10:7523–7532.

32 Nylen K, Likhodii S, Abdelmalik PA, Clarke J, Burnham WM. A comparison of the ability of a 4: 1 ketogenic diet and a 6.3: 1 ketogenic diet to elevate seizure thresholds in adult and young rats. Epilepsia 2005; 46:1198–1204.

33 Brode S, Cooke A. Immune-potentiating effects of the chemotherapeutic drug cyclophosphamide. Crit Rev Immunol 2008; 28:109–126.

34 Gao X, Qu H, Gao Z, Zeng D, Wang J, Baranenko D, *et al.* Protective effects of Ulva pertusa polysaccharide and polysaccharide? iron (III) complex on cyclophosphamide induced immunosuppression in mice. Int J Biol Macromol 2019; 133:911–919.

35 Zhu ZY, Meng M, Sun H, Li Y, Ren YY, Zhang Y. Immunostimulatory activity of glycopeptides from Paecilomyces sinensis under normal and cyclophosphamide induced immunosuppressive conditions in mice models. Food Funct 2016; 7:3566–3576.

36 Cheng D, Wan Z, Zhang X, Li J, Li H, Wang C. Dietary Chlorella vulgaris ameliorates altered immunomodulatory functions in cyclophosphamide-induced immunosuppressive mice. Nutrients 2017; 9:708.

37 Mohamed WAM. Can lactoferrin modulate the immunostimulant activity of levamisole in rats immunosuppressed by cyclophosphamide?. J Clin Exp Invest 2014; 5:48–53.

38 Senthilkumar S, Devaki T, Manohar BM, Babu MS. Effect of squalene on cyclophosphamide-induced toxicity. Clin Chim Acta 2006; 364:335–342.

39 Niki E, Noguchi N, Tsuchihashi H, Gotoh N. Interaction among vitamin C, vitamin E, and beta-carotene. Am J Clin Nutr 1995; 62:1322S–1326S.

40 Wang M, Meng XY, Le Yang R, Qin T, Wang XY, Zhang KY., *et al.* Cordyceps militaris polysaccharides can enhance the immunity and antioxidation activity in immunosuppressed mice. Carbohyd Polym 2012; 89:461–466.

41 Alabbassi MG. Melatonin ameliorates hepatic damage induced by cyclophosphamide in rats. J Tech 2010; 23:1–7.

42 El-Sebaey AM, Abdelhamid FM, Abdalla OA. Protective effects of garlic extract against hematological alterations, immunosuppression, hepatic oxidative stress, and renal damage induced by cyclophosphamide in rats. Environ Sci Pollut Res 2019; 26:15559–15572.

43 Duggina P, Kalla CM, Varikasuvu SR, Bukke S, Tartte V. Protective effect of centella triterpene saponins against cyclophosphamide-induced immune and hepatic system dysfunction in rats: Its possible mechanisms of action. J Physiol Biochem 2015; 71:435–454.

44 Annunziato F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. J Allergy Clin Immunol 2015; 135:626–635.

45 Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? Immunology 2008; 123:326–338.

46 Blankenstein T, Qin Z. The role of IFN-γ in tumor transplantation immunity and inhibition of chemical carcinogenesis. Curr Opin Immunol 2003; 15:148–154.

47 Raymond CR, Wilkie BN. Th-1/Th-2 type cytokine profiles of pig T-cells cultured with antigen-treated monocyte-derived dendritic cells. Vaccine 2004; 22:1016–1023.

48 Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-γ: an overview of signals, mechanisms and functions. J Leukoc Biol 2004; 75:163–189.

49 Chen D, Chen G, Ding Y, Wan P, Peng Y, Chen C, *et al.* Polysaccharides from the flowers of tea (Camellia sinensis L.) modulate gut health and ameliorate cyclophosphamide-induced immunosuppression. J Funct Foods 2019; 61:103470.

50 Han L, Meng M, Guo M, Cheng D, Shi L, Wang X, Wang C. Immunomodulatory activity of a water-soluble polysaccharide obtained from highland barley on immunosuppressive mice models. Food Funct 2019; 10:304–314.

51 Tao JH, Duan JA, Zhang W, Jiang S, Guo JM, Wei DD. Polysaccharides from Chrysanthemum morifolium ramat ameliorate colitis rats via regulation of the metabolic profiling and NF-κ B/TLR4 and IL-6/JAK2/STAT3 signaling pathways. Front Pharmacol 2018; 9:746.

52 Wang YQ, Mao JB, Zhou MQ, Jin YW, Lou CH, Dong Y, *et al.* Polysaccharide from Phellinus Igniarius activates TLR4-mediated signaling pathways in macrophages and shows immune adjuvant activity in mice. Int J Biol Macromol 2019; 123:157–166.

53 Hebel K, Rudolph M, Kosak B, Chang HD, Butzmann J, Brunner-Weinzierl MC. IL-1β and TGF-β act antagonistically in induction and differentially in propagation of human proinflammatory precursor CD4+ T cells. J Immunol 2011; 187:5627–5635.

54 Kim SH, Lee IC, Ko JW, Moon C, Kim SH, Shin IS, *et al.* Diallyl disulfide prevents cyclophosphamide-induced hemorrhagic cystitis in rats through the inhibition of oxidative damage, MAPKs, and NF-κB pathways. Biomol Ther 2015; 23:180.

55 Song Y, Zhang C, Wang C, Zhao L, Wang Z, Dai Z, *et al.* Ferulic acid against cyclophosphamide-induced heart toxicity in mice by inhibiting NF-κB pathway. Evid Based Compl Altern Med 2016; 2016:1261270.

56 Zhang M, Wang G, Lai F, Wu H. 2016. Structural characterization and immunomodulatory activity of a novel polysaccharide from Lepidium meyenii. J Agric Food Chem 64:1921–1931.

57 Yun L, Wu T, Li Q, Zhang M. Dietary supplementation with purified wheat germ glycoprotein improve immunostimulatory activity in cyclophosphamide induced Balb/c mice. Int J Biol Macromol 2018; 118:1267–1275.

58 Yoon HS, Kim JW, Cho HR, Moon SB, Shin HD, Yang KJ, *et al.* Immunomodulatory effects of Aureobasidium pullulans SM-2001 exopolymers on cyclophosphamide-treated mice. J Microbiol Biotechnol 2010; 20:438–445.

59 Zhang H, Yu R, Liu X, Guo X, Zeng Z. The expression of PAC1 increases in the degenerative thymus and low dose PACAP protects female mice from cyclophosphamide induced thymus atrophy. Peptides 2012; 38:337–343.

60 Wei YY, Hu TJ, Su ZJ, Zeng Y, Wei XJ, Zhang SX. Immunomodulatory and antioxidant effects of carboxymethylpachymaran on the mice infected with PCV2. Int J Biol Macromol 2012; 50:713–719.