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Original article

Hypocholesterolemic effects of diets containing different levels of kishk as a dried fermented milk–whole wheat mixture in experimental rats

Rehab F.M. Ali*

Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, Egypt

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ABSTRACT

Background: Kishk is a popular traditional functional food in Egypt. This study was performed to investigate the effects of different levels of *kishk* as a dried fermented milk/whole wheat mixture on growth performance, relative weight of organs, lipid profile, and some biochemical parameters in rats fed a cholesterol-rich diet.

Methods: Forty male rats were assigned to five groups, each consisting of eight rats. The first one presents the negative control group that received the basal diet, while the second group that serves as the positive (+) control group received a high-cholesterol diet (HCD). The last three groups received HCD supplemented with 10%, 20%, and 30% of *kishk*.

Results: Rats fed diets containing various levels of *kishk* for 8 weeks had significantly ($p < 0.05$) lower body weights compared with the rats of both negative and positive groups. The liver/body weight ratio significantly increased in rats fed HCD compared with the control rats. Incorporation of *kishk* into the HCD at levels of 10%, 20%, and 30% significantly ($p \leq 0.05$) decreased the change of liver/body weight ratio by 14.46%, 17.51%, and 18.78%, respectively, when compared with the HCD group. Results also indicate that rats fed HCD had a state of dyslipidemia, compared with the negative control group. Administration of HCD supplemented with various levels of *kishk* for 8 weeks significantly ($p < 0.05$) attenuated the increases in serum cholesterol, low-density lipoprotein cholesterol, triglyceride concentration, and atherogenic indices, and increased high-density lipoprotein cholesterol in a dose-dependent manner compared with the HCD group. Activities of liver enzymes (alanine transferase and aspartate transferase) as well as kidney function parameters (urea, uric acid, and creatinine) were elevated in the HCD group compared with the negative control group.

Conclusion: Consumption of HCD supplemented with various levels of *kishk* for 8 weeks induced a significant protective effect reflected in the reductions of the serum levels of aspartate transferase and alanine transferase, as well as kidney functions (uric acid, urea, and creatinine).

1. Introduction

Hypercholesterolemia is the presence of high levels of cholesterol in the blood [1]. It is a form of “hyperlipidemia” (elevated levels of lipids in the blood) and “hyperlipoproteinemia” (elevated levels of lipoproteins in the blood) [1]. Hypercholesterolemia has emerged as a strong risk factor for cardiovascular disease (CVD). CVD is a severe problem in developed and developing countries. The World Health Organization (WHO) estimated that 17.3 million people died from CVD in 2008 and warned that 23.6 million people will die annually from CVD by 2030. CVD is a disease mainly caused by atherosclerosis [2]. One of the risk factors of atherosclerosis is hypercholesterolemia [3,4], and low-density lipoprotein cholesterol (LDL-C) is the major cause of onset of the atherogenic process [2]. Total cholesterol (TC) can be broken down into a diagnostic lipoprotein profile, including high-density lipoprotein (HDL), LDL, intermediate-density lipoproteins, very-low-density lipoprotein, chylomicron remnants, and triglycerides (TG). Patients at an increased risk of coronary artery disease frequently exhibit an

* Corresponding author. Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza 12613, Egypt.
E-mail address: malk_anany@yahoo.com.

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atherogenic lipoprotein phenotype characterized by elevated plasma levels of both TG-rich lipoproteins and small, dense LDL and low concentrations of HDL-C. Recently, in a large observational study, the calculated non-HDL plasma cholesterol concentration (the sum of the cholesterol contents of LDL, intermediate-density lipoprotein, and very-low-density lipoprotein) was a stronger predictor of cardiovascular events than plasma cholesterol alone [5,6]. Improvement in the predictability of coronary artery disease on inclusion of very-low- and intermediate-density lipoprotein cholesterol emphasizes the proatherogenic nature of TG-rich lipoproteins and their remnant particles. Control of cholesterol levels through therapeutic drugs have significantly reduced the risk of developing atherosclerosis and associated CVDs [7–10]. Notably, statins, a class of cholesterol-lowering drugs inhibiting cholesterol synthesis, have most widely been prescribed for treating hypercholesterolemia and reducing CVDs [8,10]. However, adverse effects associated with therapeutic drugs, such as myopathy, liver damages, and potential drug–drug interaction, have been reported [11–16]. Therefore, development of additional therapies for controlling cholesterol levels is warranted, especially for those with better safety profiles. Functional foods have played an important role in the food industry during the past decade. These foods provide basic nutrition as well as health benefits such as disease prevention, or may delay the evolution of chronic disorders. Components in these foods can produce physiological benefits or remove compounds that may pose a health risk [17]. Recent researches have focused on the search for functional foods for combating chronic diseases such as cancer, CVDs, and Type 2 diabetes [18]. Fermented foods are an important part of diet in many parts of the world and are known from ancient times. Traditional dried fermented milk/cereal foods are widely used in the diet of people in the Middle East, Asia, Africa, and some parts of Europe [19]. *Kishk* is one of the traditional food products in Egypt. It is a fermented milk–wheat mixture stored in the form of dried balls in Egypt [20]. *Kishk* is a balanced food with excellent preservation quality, richer in B vitamins than either wheat or milk, and well adapted to hot climates by its content of lactic acid, and it has a therapeutic value [20–22]. *Kishk* product is a highly nutritious food, having a protein content of about 25.3%. It is highly digestible and of high biological value [23]. The main objective of the present study was to evaluate the hypocholesterolemic effect of diets containing different levels of *kishk* as a dried fermented milk/cereal mixture in rats.

2. Materials and methods

2.1. Ethics statement

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85–23, revised 1996). Experimental design and animal handling procedures were approved by the ethical committee of the Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. Every effort was made to minimize the number of animals and their suffering.

Kishk was purchased from the local market in Giza, Egypt. *Kishk* was ground in an electric grinder (Braun Model 1021), passed through a 150 μm mesh sieve, and stored in glass containers at 4°C for further use. The chemical composition and energy of *kishk* as determined by the Standard Association of Official Analytical Chemists (AOAC) methods (2000) are shown in Table 1. Cholesterol and cholic acid were purchased from Sigma Chemical Co., Ltd (St Louis, MO, USA). The kits of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Triglyceride (TG), transaminase enzymes [aspartate transferase (AST) and alanine transferase (ALT)], and kidney function parameters (creatinine, urea, and uric acid) were obtained from Biodiagnostic Co. (Dokki, Giza, Egypt). All chemicals used were of analytical reagent grade.

Table 1
Proximate composition and calorie content of *kishk*.

Component	*Kishk*	
	%	Energy (kcal/100 g)
Moisture	8.97	—
Protein	18.64	74.56
Ash	7.30	—
Crude fiber	7.57	—
Fat	5.19	46.71
Total carbohydrate*	61.30	245.2
Total energy	366.47	

* By difference.

2.2. Animals and experiment design

Forty male Albino rats with an average weight of 190–200 g were obtained from experimental animals of the Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. The animals were pair housed in plastic cages at 25 ± 2°C and a humidity of 55 ± 6% with a 12-hour light–dark cycle. Food and water were provided *ad libitum* throughout the study. All the rats were acclimated to the basal diet for 2 weeks to stabilize the metabolic conditions before carrying out feeding experiments. The basal diet was formulated according to the AOAC methods [24], and consisted of casein (15%), corn oil (10%), cellulose (5%), salt mixture (4%), vitamin mixture (1%), and starch (65%). Casein is characterized by its low content of sulfur-containing amino acids; hence, L methionine was added to the basal diet at the level of 4.6 g/kg diet. The composition of the vitamin and salt mixtures used was similar to that reported by AOAC [24] and Reeves et al [25], respectively. The rats were randomly divided into five groups ($n = 8$). The first one presents the negative control group, which was fed only the basal diet, while the second group, which serves as the positive (+) control group, was fed a high-cholesterol diet (HCD) containing 98.75% basal diet, 1% cholesterol, and 0.25% cholic acid. The third, fourth, and fifth groups were fed HCD supplemented with 10%, 20%, and 30% of *kishk* respectively. The animal experiments conducted according to the recommendations of the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH Publication No. 85–23, revised 1996). Experimental design and animal handling procedures were approved by the ethical committee of the Food Technology Research Institute; Agricultural Research Center, Giza, Egypt. Every effort was made to minimize the number of animals and their suffering.

2.3. Biological evaluation

Daily food intake and weekly body weights were recorded during the experimental period (8 weeks). At the end of the experiment, total food intake, body weight gain, and food efficiency ratio were calculated.

2.4. Relative organ weight (g) of experimental rats

At the time of sacrifice, the hearts, livers, and kidneys of the experimental rats were identified, removed, rinsed with physiological saline solution, and dried by tissue papers. Weights of