Bulgarian Chemical Communications, Volume 52, Special Issue D (pp. 60-67) 2020

Antiobesity, antioxidant and hepatoprotective properties of aqueous infusion of *Kochia scoparia* seeds in rats with diet-induced metabolic syndrome

S. Gancheva[1], M. Tzaneva[2], S. V. Valcheva-Kuzmanova[1]*, M. D. Zhelyazkova-Savova[1]

[1]*Department of Pharmacology and Clinical Pharmacology and Therapeutics, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria, 55 Marin Drinov Str., Varna 9002, Bulgaria*
[2]*Department of General and Clinical Pathology, Forensic Medicine and Deontology, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria, 1 Hristo Smirnenski Blvd., Varna 9010, Bulgaria*

Received February 13, 2020; Accepted April 16, 2020

Metabolic syndrome (MS) is a disorder of energy homeostasis associated with oxidative stress. *Kochia scoparia* (KS) is used in traditional Chinese medicine to alleviate obesity, dyslipidemia, and other ailments. KS fruits contain triterpenoid and quercetin glycosides with anti-oxidant and anti-inflammatory activities. The seeds of KS are less widely studied.

The aim of this study was to determine the effects of aqueous infusion of KS seeds on energy metabolism and oxidative stress in rats with diet-induced MS. Fifty male Wistar rats were allocated into 5 groups: a control group, a MS group, and 3 groups of MS rats treated with an aqueous infusion of KS containing 1.5 g, 3 g, and 6 g seeds per 100 ml water (MS+1.5KS, MS+3KS, and MS+6KS, respectively). Control rats received regular rat chow diet, and all MS groups were fed high-fat high-fructose diet. KS-treated animals received the infusions as drinking water. After 12 weeks of dieting and treatment, the energy metabolism was evaluated, liver samples were examined histologically, and oxidative stress was determined by the levels of thiobarbituric acid reactive substances (TBARS). Rats from the MS group developed visceral adiposity, dyslipidemia, insulin resistance and TBARS elevation. The histological examination revealed liver steatosis and apoptotic bodies. KS treatment reduced visceral adiposity and improved dyslipidemia. TBARS levels were decreased in a dose-dependent manner. The highest strength of the infusion alleviated the histological liver impairment. The results demonstrate that the aqueous infusion of KS seeds possess antiobesity, anti-oxidant, and liver protective effects in rats with diet-induced metabolic syndrome.

Keywords: Rat, metabolic syndrome, oxidative stress, liver impairment, *Kochia scoparia* seeds, aqueous infusion

INTRODUCTION

Metabolic syndrome (MS) is a disorder of energy utilization and storage with high social impact. According to the International Diabetes Federation (IDF) it affects about 20–25% of the world adult population with increased prevalence in advanced ages [1]. At least one component of the syndrome is found in 26.9–41.2% of the adults at young age (18–30 years old) [2]. IDF defines MS as a cluster of metabolic abnormalities, including central obesity and at least two of the following: hypertriglyceridemia, reduced HDL-cholesterol, hypertension, and raised fasting plasma glucose. Insulin resistance and visceral adiposity are the major features in MS development [1]. In addition to the abnormalities of the glucose and lipid metabolism, MS is associated with increased level of oxidative stress [3]. According to some authors, oxidative stress can be considered as an additional main component of the syndrome [4]. MS is a risk factor for the development of cardiovascular diseases, type-2 diabetes, neuropsychiatric and other disorders. The rate of occurrence of MS and the severity of its complications determine the condition as one of the most important burdens to the healthcare system worldwide.

Kochia scoparia (KS) is a large annual plant of the family Amaranthaceae, native to Central and Eastern Europe and Asia. KS reproduces by seeds. The mature plant grows round and bushy. KS flowers are small, greenish, and inconspicuous at the end of branches. The fruits are star-shaped and clustered on the brunches. Each fruit contains a single teardrop-shaped brown to black seed. The commonly used name of KS is fireweed or burning bush because of the flaming red color that the plant takes on in autumn. KS is used as an attractive ornamental plant, as forage for cattle, sheep and horses, and for manufacture of brooms. In Japan, the seeds are used as a food garnish called Tonburi or field caviar. In traditional Chinese medicine they are used to treat a variety of diseases including obesity, dyslipidemia, and other ailments. In traditional Bulgarian medicine, the tea prepared from the seeds alleviates chronic liver disorders.

Regardless of the wide traditional use of KS, experimental data is scarce. There are several experimental studies finding beneficial effects of KS fruits in mouse models of dermatitis [5, 6], and asthma [7], as well as *in vitro* studies demonstrating

* To whom all correspondence should be sent:
E-mail stefkavk@yahoo.com

anti-cancer activity in cell cultures [8, 9].

One experimental study demonstrated the antiobesity effect of an ethanol extract of KS fruits in high-fat fed mice [10]. KS fruits contain mainly the triterpenoid saponin, momordin Ic [11]. A number of flavone glycosides are also isolated from the fruits [12]. Triterpenoid saponins produce a variety of effects through their anti-inflammatory, antioxidant, hypocholesterolemic, and hypoglycemic properties [13].

The seeds of KS are less widely studied. A recently performed research reports on two phenolic compounds found in the seeds – hydroxytyrosol and morin hydrate, as well as several unknown compounds that should be determined further [14].

The aim of the current study was to determine the effects of aqueous infusion of KS seeds on the energy metabolism and oxidative stress in rats with diet-induced MS.

EXPERIMENTAL

Experimental animals

We used 50 male Wistar rats provided by the Vivarium of Varna Medical University. They were kept at a temperature of 20–25°C and 12-h light-dark cycle and had free access to food and drinking water. The study was ethically approved by the Bulgarian Food Safety Agency in the Ministry of Agriculture, Foods and Forestry. It was conducted in agreement with the national policies and the international guidelines (EU Directive 2010/63/EU for animal experiments).

Dieting and treatment

The animals were allocated into five experimental groups of 10 rats each: a control group (C), a metabolic syndrome group (MS), and 3 groups of MS rats treated with aqueous infusion of KS seeds (MS+1.5KS, MS+3KS, and MS+6KS).

Control rats received a standard rat chow diet and tap water. With each 100 g food consumed, the animals had a caloric intake of 279 kcal. According to the producer's recipe, the 100 g chow contains 20.48 g protein (29% kcal), 3 g fat (10% kcal), carbohydrates (61% kcal) in the form of starch (38.3 g) and sugars (4.32 g). This chow, utilized in the Vivarium for the normal animal feeding, was purchased from the Bulgarian forage producer TopMix. All other groups received a diet prepared by adding lard (20% w/w) and fructose (20% w/w) to the standard rat chow, and their caloric intake was 427 kcal/100 g food. The lard provided 42% kcal, and the added fructose accounted for 19% kcal as compared to 6% from simple sugars in the regular chow.

Throughout the experiment, the rats from the MS group were drinking tap water, while the rats from groups MS+1.5KS, MS+3KS and MS+6KS were drinking the corresponding aqueous infusions of KS seeds *ad libitum*. The infusions were prepared by soaking of 1.5 g, 3 g and 6 g seeds, respectively, in 100 ml boiling water for 15 min and straining after cooling down.

The duration of the study was 12 weeks.

Biological parameters

The food and water/infusions consumption per 5 rats in a cage was monitored daily and was calculated as a mean value per rat per day. The caloric intake was calculated. Body weight of animals was measured once weekly and weight gain was calculated. After animal sacrifice, the right retroperitoneal fat pads and the livers were dissected on ice and weighed. The corresponding organ indices were calculated as a ratio to the body weight $\times 10^3$. Liver samples were preserved in 10% neutral formalin for histopathological evaluation.

Insulin tolerance test (ITT)

ITT was performed in the last week of the experiment. After 6 hours of fasting, the animals were injected intraperitoneally with regular insulin (ActRapid), dissolved in saline, at a dose of 0.75 UI/kg. Blood sugar was measured by a glucometer (ACCU-CHEK Performa). Blood samples were taken by incision of the distal part of the tail [15] immediately before the injection of insulin (at time 0) and on the 30^{th}, 60^{th} and 90^{th} minute thereafter. The initial value of blood glucose level of each animal was referred to as fasting blood glucose.

Biochemical measurements

At the end of the experiment, blood was taken from the sublingual veins of the animals under ether anesthesia. The blood was centrifuged, and the serum samples were stored at -20°C for biochemical analyses. Serum triglyceride, cholesterol and alkaline phosphatase levels were measured on a spectrophotometer (AURIUS 2021, Cecil Instruments Ltd.) using colorimetric kits of BioMaxima S.A., Poland for the lipids and BioSystems S.A., Barcelona, Spain for alkaline phosphatase according to the instructions of the producers. Thiobarbituric acid reactive substances (TBARS) were determined as a marker of oxidative stress in serum. The method measures the color obtained as a result of the reaction of thiobarbituric acid with the lipid peroxides [16].

Histopathological examination

After 24-hour fixation, the samples were cut into appropriate portions and placed in embedding cassettes. The process continued with dehydration in progressively more concentrated ethanol baths, followed by a clearing agent (xylene) and finally molten paraffin wax infiltrated the samples. Next steps were to embed tissues into paraffin blocks, cut 5 μm slices and mount them on glass microscope slides. Ultimately, sections were stained with hematoxylin and eosin.

Statistical analysis

Results are presented as means ± SEM. The data were analyzed by one-way ANOVA followed by Dunnet's post-test to compare the MS group with all the other groups. The food and water consumption, the caloric intake, and the ITT were analyzed by two-way ANOVA with a column factor "animal treatment" and a row factor "time". Bonferroni's multiple comparison post-test was used to assess differences between groups. A level of $p < 0.05$ was considered significant. GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA) statistical software was used.

RESULTS

Biological parameters

The consumption of food and water/KS infusion, as well as the caloric intake of the animals are presented in Table 1. The rats receiving high-calorie diet consumed significantly less amount of food and water than the rats on regular rat chow diet ($F(4,420)=48.92$, $p=0.0003$ for the food intake and $F(4,420)=8.104$, $p=0.0207$ for water/KS infusion intake). Despite of the lower food consumption of the MS groups, all of them had a higher intake of calories daily ($F(4,420)=27.37$, $p=0.0014$). The amount of the infusions drunk by groups MS+1.5KS, MS+3KS and MS+6KS did not differ from the amount of water drunk by group MS. KS infusions did not affect the food and caloric intake, either. In general, all the MS groups received similar amounts of food, liquids and calories.

Table 2 presents some of the biological measures. At the end of the study, the body weight did not differ significantly between the groups. However, the weight gain of the animals differed ($F(4,43)=4.709$, $p=0.0031$), and the rats from group MS had higher weight gain compared to the control group ($p<0.05$). There were no differences between the groups in respect to liver weight and liver index.

Lipid metabolism

The parameters characterizing visceral obesity are presented on Fig. 1. The weight of the retroperitoneal fad pads (Fig. 1A) was affected by the treatment ($F(4,43)=6.339$, $p=0.0004$), and the post-test demonstrated that it was significantly increased by the high-calorie diet in the group MS compared to group C ($p<0.001$) and reduced by the 3KS infusion compared to the MS rats ($p<0.05$). The fat index (Fig. 1B) was affected in the same way ($F(4,43)=7.118$, $p=0.0002$), and the post-test revealed the same effects of the diet and 3KS infusion ($p<0.001$ group MS vs. group C and $p<0.05$ group MS+3KS vs. group MS).

Table 1. Food and liquid consumption and caloric intake of experimental animals

	C	MS	MS+1.5KS	MS+3KS	MS+6KS
Food intake [g/rat/day]	22.79±0.20	18.58±0.19	16.88±0.18	18.6±0.12	18.49±0.16
Liquid intake [ml/rat/day]	34.35±0.27	29.03±0.32	26.87±0.28	29.72±0.27	27.49±0.28
Caloric intake [kcal/rat/day]	63.59±0.56	79.32±0.82	72.09±0.77	79.43±0.53	78.97±0.70

Table 2. Biological measures. ** $p<0.01$ (one-way ANOVA); $^{\#}$ $p<0.05$ *vs.* C (Dunnett's multiple comparison post-test)

Biological measures	C	MS	MS+1.5KS	MS+3KS	MS+6KS
Initial body weight [g]	241±4.42	239.8±5.97	239.2±4.48	239.4±4.24	240.2±8.24
Final body weight [g]	383.6±7.9	419.8±14.39	394.4±10.71	398.6±14.9	428.8±17.42
Weight gain [g] **	142.6±7.19	180±11.09$^{\#}$	146.7±8.14	146.4±19.41	188.6±14.3
Liver weight [g]	9.58±0.24	9.53±0.27	9.84±0.3	9.36±0.4	10.37±0.37
Liver index	2.602±0.06	2.394±0.06	2.55±0.07	2.39±0.05	2.502±0.07

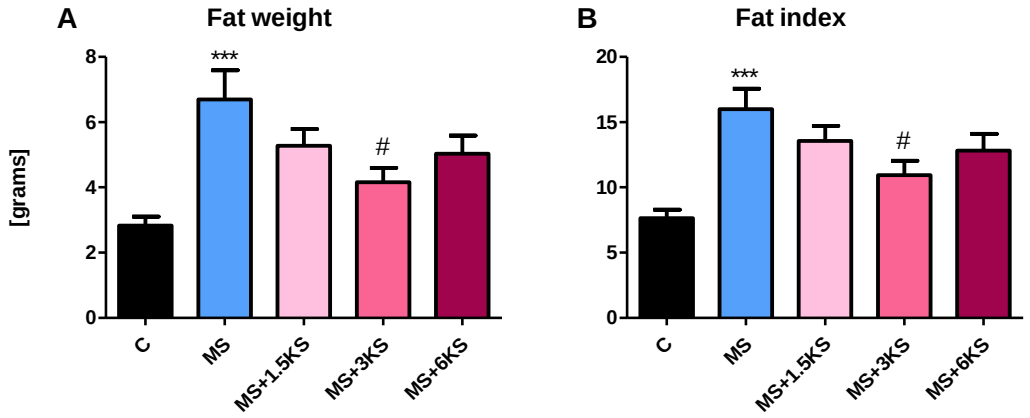

Fig. 1. Evaluation of visceral adiposity. Panel A: Weight of the right retroperitoneal fat pad. Panel B: Fat index. C – control rats; MS – rats receiving high-caloric diet; MS+1.5KS, MS+3KS and MS+6KS – rats receiving high-caloric diet and aqueous infusion of KS seeds of increasing strength (1.5KS, 3KS and 6KS, respectively); *** $p<0.001$ *vs*. C, # $p<0.05$ vs. MS (Dunnett's multiple comparison post-test)

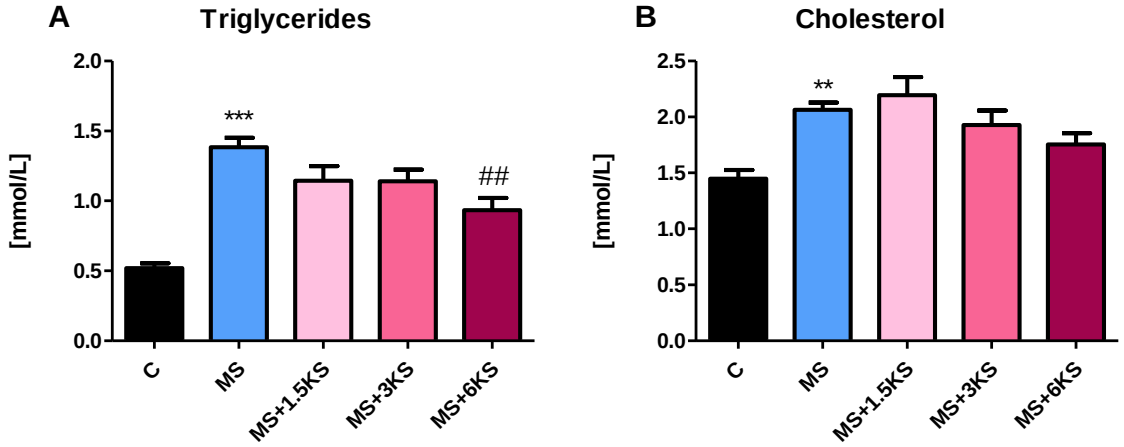

Fig. 2. Lipid profile. Panel A: Serum triglycerides. Panel B: Serum cholesterol. C – control rats; MS – rats receiving high-caloric diet; MS+1.5KS, MS+3KS and MS+6KS – rats receiving high-caloric diet and aqueous infusion of KS seeds of increasing strength (1.5KS, 3KS and 6KS, respectively); ** $p<0.01$, *** $p<0.001$ vs. C; ## $p<0.01$ vs. MS (Dunnett's multiple comparison post-test)

Table 3. Insulin tolerance test. * $p<0.05$ *vs*. C (Bonferroni post-test)

	30^{th} min [% of initial value]	60^{th} min [% of initial value]	90^{th} min [% of initial value]
C	59.83±2.02	44.29±3.04	43.86±2.36
MS	78.13±5.48*	56.13±5.38	52.88±5.29
MS+1.5KS	67.5±6.96	49.13±4.95	46.5±6.41
MS+3KS	89.75±4.50	57.75±2.56	53±3.85
MS+6KS	75.2±3.37	52.4±2.13	44.6±2.25

Serum lipids are presents on Fig. 2. The results from one-way ANOVA analyses showed a significant effect of the treatment on serum triglycerides ($F(4,42)=16.41$, $p<0.0001$) and cholesterol ($F(4,43)=5.864$, $p=0.0007$). The post-test confirmed that the rats from group MS had higher triglycerides ($p<0.001$) and cholesterol ($p<0.01$) compared to the control group, and that the strongest infusion reduced the serum concentration of triglycerides ($p<0.01$ vs. group MS).

Glucose metabolism

The results from the ITT are presented on Table 3. No differences were observed in the fasting blood glucose level between the groups. The ITT demonstrated lower sensitivity to insulin in the MS

group, significant at the 30th min after insulin administration, as revealed by the post-test following the two-way ANOVA analysis. KS infusions did not affect insulin resistance.

Oxidative stress

Serum concentrations of TBARS are presented on Fig. 3. One-way ANOVA revealed a significant difference between groups ($F(4,40)=2.918$, $p=0.0329$). The post-test showed an increased level of oxidative stress in group MS ($p<0.05$ *vs.* C). KS infusions demonstrated anti-oxidant properties in a dose-dependent manner with significant effect of the strongest infusion ($p<0.05$ *vs.* MS).

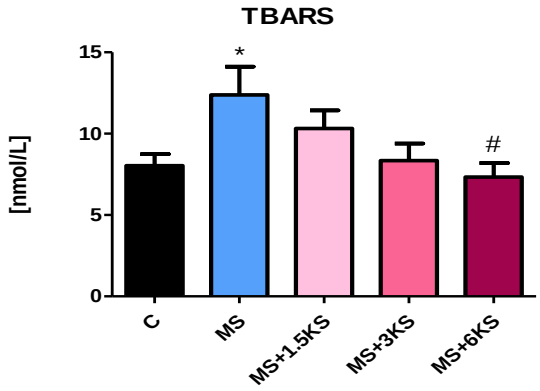

Fig. 3. Serum concentrations of thiobarbituric acid reactive substances (TBARS). C – control rats; MS – rats receiving high-caloric diet; MS+1.5KS, MS+3KS and MS+6KS – rats receiving high-caloric diet and aqueous infusion of KS seeds of increasing strength (1.5KS, 3KS and 6KS, respectively); * $p<0.05$ *vs.* C, # $p<0.05$ *vs.* MS (Dunnett's multiple comparison post-test.

Liver morphology and function

Fig. 4 presents the serum concentrations of alkaline phosphatase. One-way ANOVA revealed a significant difference between groups ($F(4,31)=5.838$, $p=0.0013$) and the post-test showed that alkaline phosphatase was elevated in rats from group MS ($p<0.05$ *vs.* C). The highest strength of KS infusion reduced alkaline phosphatase level, although insignificantly.

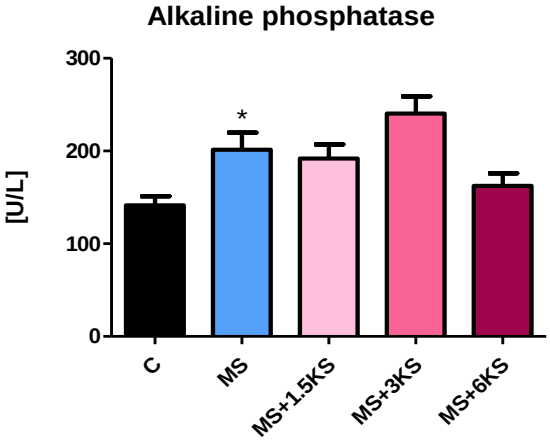

Fig. 4. Serum concentration of alkaline phosphatase. C – control rats; MS – rats receiving high-caloric diet; MS+1.5KS, MS+3KS and MS+6KS – rats receiving high-caloric diet and aqueous infusion of KS seeds of increasing strength (1.5KS, 3KS and 6KS respectively); *$p<0.05$ *vs.* C (Dunnett's multiple comparison post-test)

The histopathological examination of the liver samples is presented on Fig. 5. Compared to normal morphology of the liver samples of control rats, the liver tissue of the MS group displayed focal both macrovesicular and microvesicular steatosis in all rats. In most of the samples, there were apoptotic bodies and unspecific granulomas. The strongest KS infusion alleviated the signs of steatosis. In 4 of the animals from group MS+6KS the liver tissue displayed normal morphology. In the remaining 6 rats, there was only microvesicular steatosis.

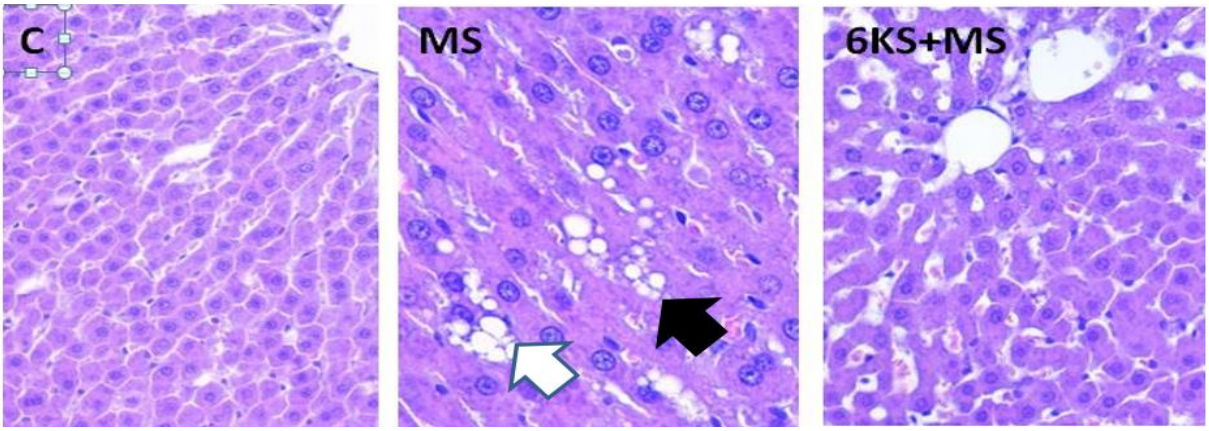

Fig. 5. Histopathological examination of liver tissue. The white arrow points to macrovesicular steatosis and the black arrow points to microvesicular steatosis (Hematoxylin-eosin, magnification ×100).

DISCUSSION

Experimental models of MS are a commonly used tool for studying the pathogenesis, prevention and therapy of the condition. In humans, obesity and MS usually result from non-rational nutrition with high caloric intake. Therefore, the dietary induced models, relying on increased intake of animal fats and simple sugars, are considered to mostly mimic the pathogenesis of the syndrome in humans. In our previous study, we demonstrated the ability of high-caloric diets to induce many features of MS in rats [17]. In the current study, we confirmed that the enrichment of rat chow with lard and fructose was able to produce visceral adiposity, hypertriglyceridemia, hypercholesterolemia, and insulin resistance, as shown by the experimental animals from group MS. In addition, they displayed the presence of oxidative stress, demonstrated by elevated serum concentration of TBARS, and signs of hepatosteatosis.

In the current study, the rats receiving high-fat high-fructose diet consumed less amount of food compared to control animals, probably as a result of the satiety effect due to the high lipid content of the food and/or because of stimulation of gut hormones [18]. The lower food intake in MS groups was expected as we have had already observed this previously [17]. Despite the lower food consumption of MS rats, all of the MS groups had higher daily intake of calories due to the higher calorie content of the diet. KS did not change food consumption, thus showing no effect on appetite. We measured the liquid intake during the experiment in order to monitor the consumption of KS infusions. The rats receiving high-caloric diet consumed less fluids daily compared to the control rats, an effect already described by Kaunitz *et al.* [19]. The type of liquid was not a factor determining the consumption: all rats given KS infusions drank similar amounts and these did not differ from the MS group.

Although KS infusion did not affect food and caloric intake, it did improve many of the symptoms of MS induced by the high fat high fructose diet. KS infusion prevented the diet-induced weight gain in the MS+1.5KS and MS+3KS groups. All strengths of KS infusion tended to reduce the retroperitoneal fat weight and the corresponding fat index, but this antiobesity effect was significant only in the MS+3KS group. Han *et al.* reported similar results [10]: they have treated mice subjected to high-fat diet with ethanol extract of KS fruits for 9 weeks and have observed reduction of fat storage and prevention of diet-induced weight gain. According to the authors, momordin Ic, which is the principal saponin constituent of KS fruits, is responsible for the antiobesity effect. This triterpenoid glycoside, as well as the total KS saponins inhibit the pancreatic lipase activity (an effect determined *in vitro*), thus reducing the intestinal absorption of dietary fat.

In our study, the action of KS on the visceral fat was accompanied by a beneficial effect on serum lipid profile. All KS-treated animals had lower serum triglyceride concentrations compared to the KS-untreated animals receiving the same diet. The effect was significant with the strongest KS infusion. This group also displayed the lowest cholesterol levels among the MS groups, although the effect was not statistically significant.

Increased level of oxidative stress is an important characteristic of MS. In diet-induced MS, increased levels of glucose and free fatty acids contribute to the generation of free radicals because of the enhanced oxidation of these energy substrates. On its turn, the increased level of reactive oxygen species induces cellular insulin resistance [20]. In addition to the increased production of free radicals, fructose-enriched diet is associated with reduced antioxidant defense [21]. It appears that oxidative stress is both a trigger and a consequence of obesity and metabolic syndrome. In addition, it serves as a link between the disturbances in energy metabolism and other conditions, such as cardiovascular diseases [3] or neuropsychiatric disorders [22]. In our study, all KS infusions reduced the serum concentration of TBARS. The antioxidant effect was dose-dependent but significant only at the strongest strength. The antioxidant capacity of KS has been studied by Wang *et al*. using a protein oxidation model [23]. The authors reported that fruit extracts of KS effectively scavenge different free radicals and related these antioxidant properties to the presence of momordin Ic in the extracts.

Nonalcoholic fatty liver disease is another disorder that is typically associated with obesity, insulin resistance and MS in humans and experimental models [24, 25]. In the current study, the liver weight and liver index did not differ between the groups. However, livers from the MS group displayed histopathological signs of focal macro- and micro-vesicular steatosis. The serum level of alkaline phosphatase was also increased thus demonstrating impaired liver function. Insulin resistance and increased amount of free fatty acids in hepatocytes play a key role in the origin and maintenance of hepatic steatosis creating the basis for triglyceride production. The excess of free fatty acids induces a rise in mitochondrial β-oxidation, production of reactive oxygen species and oxidative stress, as well as elevated lipid peroxidation [25]. In our experiment, the strongest infusion of KS entirely prevented the development of hepatic steatosis or alleviated the histopathological signs of the diet-induced liver injury. Alkaline phosphatase levels tended to be reduced by the 6KS infusion. The same strength of the infusion demonstrated the highest antioxidant activity, suggesting a possible mechanism for the liver protection. Kim *et al.* studied the hepatoprotective effect of momordin Ic and oleanolic acid obtained from KS fruits in a rat model of carbon tetrachloride-induced hepatotoxicity and concluded that the observed beneficial effects were produced by enhancing the hepatic antioxidant defense system [26].

Most of the published research on KS, including the above mentioned studies, focuses on the effects of the plant fruits. Momordin Ic, the principal glycoside isolated, is usually considered the major carrier of the activity. In all mentioned studies, the described antiobesity, antioxidant, and hepatoprotective effects of KS fruits are thought to be due to the actions of this triterpenoid saponin. As for the seeds of KS, however, their composition has not been fully established. Morin hydrate

and hydroxytyrosol have been found in KS seed extract, as well as five additional unidentified substances [14].

Morin hydrate is a polyphenolic compound with structure representing an isomeric form of quercetin, which is regarded as one of the flavonoids with the highest antioxidant potential. Morin acts as preventive antioxidant by inhibiting xanthine oxidase and as a curative antioxidant by scavenging reactive oxygen radicals [27]. Morin function is tested in many experimental settings [27-30]. In some of them, its antioxidant potential is evaluated by TBARS concentration [28, 29]. Thus, it is plausible to consider that morin could be responsible for the dose-dependent antioxidant effect of KS infusions demonstrated in our study. In addition to its antioxidant properties, morin hydrate has showed a hepatoprotective potential in different experimental models of hepatotoxicity, such as ischemia-reperfusion [27] and ethanol-induced liver damage [29]. These studies base the liver protective effects of morin on its antioxidant action.

Hydroxytyrosol is another potential carrier of the antiobesity, antioxidant and hepatoprotective activities of KS seeds. It is a phenolic alcohol with a strong antioxidant activity through free-radical scavenging. In addition, hydroxytyrosol increases the endogenous defense antioxidant systems by activating different cellular signaling pathways [31]. The antiobesity effect of hydroxytyrosol has been demonstrated in diet-induced experimental models of metabolic syndrome [32, 33]. It inhibits lipogenesis, suppresses the triglyceride accumulation and expression of adipogenesis-stimulating factors, and promotes lipolysis [34]. Hydroxytyrosol prevents liver steatosis in diet-induced obesity by reducing hepatic inflammation and oxidative stress [35, 36].

In this experiment, we demonstrated a parallel in the dose-dependent antioxidant and liver protective effect of KS infusion. A weakness of the study is the lack of information on the active principles present in the seed infusion that we used. Thus, it is not known, whether it is momordin Ic, morin, hydroxytyrosol, or any other component of the KS infusion, that is responsible for the observed benefits in the diet-induced metabolic and liver impairments. Such a tempting hypothesis remains speculative until it is proven. Therefore, an important future task is to investigate the composition of the aqueous infusion of KS, as it represents an easy to use approach to prevent or treat metabolic and liver disorders.

In conclusion, this study demonstrates that the aqueous infusion of KS seeds possesses antiobesity, antioxidant, and liver-protective effects in rats with diet-induced metabolic syndrome. In general, the strongest infusion exerted the highest beneficial effects – it improved the lipid profile, reduced the level of oxidative stress and alleviated the diet-induced liver impairment. The antioxidant effect was dose-dependent. The infusions of lower-to-moderate strength tended to prevent the diet-induced weight gain and reduced visceral obesity, but did not improve the dyslipidemia and liver steatosis.

REFERENCES

1. The IDF consensus worldwide definition of the metabolic syndrome (2006).
2. P. B. Nolan, G. Carrick-Ranson, J. W. Stinear, S. A. Reading, L. C. Dalleck, *Prev. Med. Rep.*, **7**, 211 (2017).
3. C. Roberts, K. Sindhu, *Life Sci.*, **84**, 705 (2009).
4. E. Hopps, D. Noto, G. Caimi, M. Averna, *Nutr. Metab. Cardiovasc. Dis.*, **20** (1), 72 (2010).
5. S. Jo, J. Ryu, H. Y. Han, G. Lee, M. H. Ryu, H. Kim, *Mol. Med. Rep.*, **13** (2), 1695 (2016).
6. M. Y. Lee, I. S. Shin, H. S. Lim, C. S. Seo, H. Ha, H. K. Shin, *Inhal. Toxicol.*, **23** (14), 938 (2011).
7. Y. Y. Choi, M. H. Kim, J. Y. Lee, J. Hong, S. H. Kim, W. M. Yang, *J. Ethnopharmacol.*, **154** (2), 380 (2014).
8. H. Y. Han, H. Kim, Y. H. Son, G. Lee, S. H. Jeong, M. H. Ryu, *Pharmacogn. Mag.* **10** (Suppl 3), S661 (2014).
9. H. Y. Han, H. E. Lee, H. J. Kim, S. H. Jeong, J. H. Kim, H. Kim, M. H. Ryu, *J. Ethnopharmacol.* **192**, 431 (2016).
10. L. K. Han, R. Nose, W. Li, X. J. Gong, Y. N. Zheng, M. Yoshikawa, K. Koike, T. Nikaido, H. Okuda, Y. Kimuraet, *Phytother. Res.*, **20** (10), 877 (2006).
11. H. Matsuda, Y. Dai, Y. Ido, S. Ko, M. Yoshikawa, M. Kubo, *Biol. Pharm. Bull.*, **20** (10), 1086 (1997).
12. Y. H. Xu, H. Huang, N. Zhang, D. Y. Kong, M. L. Hua, *J. Asian Nat. Prod. Res.*, **16** (2), 141 (2014).
13. M. Marrelli, F. Conforti, F. Araniti, G. A. Statti, *Molecules*, **21** (10), 1404 (2016).
14. H. D. Cho, J. H. Kim, J. K. Park, S. M. Hong, D. H. Kim, K. I. Seo, *Pharm. Biol.*, **57** (1), 684 (2019).
15. M. Fluttert, S. Dalm, M. Oitzl, *Lab. Anim.*, **34**, 372 (2000).
16. H. Ohkawa, N. Ohishi, K. Yagi, *Anal. Biochem*., **95** (2), 351 (1979).
17. S. Gancheva, M. Zhelyazkova-Savova, B. Galunska, T. Chervenkov, *Scri. Sci. Med.*, **47** (2), 14 (2015).
18. R. A. Samra, in: Taste, Texture, and Post Ingestive Effects, J. P. Montmayeur, J. le Coutre (eds.), Boca Raton (FL): CRC Press/Taylor & Francis, 2010. https://www.ncbi.nlm.nih.gov/books/NBK53541/
19. H. Kaunitz, C. A. Slanetz, R. E. Johnson, J. Guilmain, *J. Nutr.*, **60** (2), 221 (1956).
20. J. W. Eriksson, *EFBS Letters*, **581** (19), 3734 (2007).
21. A. Girard, S. Madani, F. Boukortt, M. Cherkaoui-Malki, J. Belleville, J. Prost, *Nutrition*, **22**, 758 (2006).
22. S. Gancheva, B. Galunska, M. Zhelyazkova-Savova, *Int. J. Exp. Pathol.*, **98** (5), 296 (2017).
23. J. Wang, L. Yuan, B. Cheng, W. Li, C. Xiao, Y. Wang, X. Liu, *Qual. Assur. Saf. Crop*, **6** (4), 383(2014).
24. L. A. Adams, P. Angulo, K. D. Lindor, *Can. Med. Assoc. J.*, **172** (7), 899 (2005).
25. J. Willebrords, I. V. Pereira, M. Maes, S. C. Yanguas, I. Colle, B. Van Den Bossche, T. C. Da Silva, C. P. de Oliveira, W. Andraus, V. A. Alves, B. Cogliati, M. Vinken, *Prog. Lipid Res.*, **59**, 106 (2015).

26. N. Y. Kim, M. K. Lee, M. J. Park, S. J. Kim, H. J. Park, J. W. Choi, S. H. Kim, S. Y. Cho, J. S. Lee, *J. Med/ Food*, **8** (2), 177 (2005).
27. T. W. Wu, L. H. Zeng, J. Wu, K. P. Fung, *Life Sci.*, **53** (13), PL213 (1993).
28. K. S. Al-Numair, G. Chandramohan, M. A. Alsaif, C.Veeramani, A. S. El Newehy, *Afr. J. Tradit. Complement Altern. Med.*, **11** (3), 14 (2014).
29. S. G. Shankari, K. Karthikesan, A. M. Jalaludeen, N. Ashokkumar, *J. Basic. Clin. Physiol. Pharmacol.*, **21** (4), 277 (2010).
30. S. Kitagawa, H. Sakamoto, H. Tan, *Chem. Pharm. Bull.*, **52** (8), 999 (2004).
31. A. K. Marković, J. Torić, M. Barbarić, C. J. Brala, *Molecules*, **24** (10), 2001 (2019).
32. K. Cao, J. Xu, X. Zou, Y. Li, C. Chen, A. Zheng, H. Li, H. Li, I. M. Szeto, Y. Shi, J. Long, J. Liu, Z. Feng, *Free Radic. Biol. Med.*, **67**, 396 (2014).
33. H. Poudyal, N. Lemonakis, P. Efentakis, E. Gikas, M. Halabalaki, I. Andreadou, L. Skaltsounis, L. Brown, *Pharmacol. Res.*, **117,** 32 (2017).
34. B. Stefanon, M. Colitti, *Exp. Biol. Med. (Maywood)*, **241** (16), 1796 (2016).
35. C. Pirozzi, A. Lama, R. Simeoli, O. Paciello, T. B. Pagano, M. P. Mollica, F. Di Guida, R. Russo, S. Magliocca, R. B. Canani, G. M. Raso, A. Calignano, R. Meli, *J. Nutr. Biochem.*, **30**, 108 (2016).
36. R. Valenzuela, F. Echeverria, M. Ortiz, M. Á. Rincón-Cervera, A. Espinosa, M. C. Hernandez-Rodas, P. Illesca, A. Valenzuela, L. A. Videla, *Lipids Health Dis.*, **16**, 64 (2017).