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Homocysteine metabolism in rats with metabolic syndrome and the impacts of nigella sativa oil on some biochemical parameters

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Abstract

The high level of fructose taken in the diet is one of the reasons for the increased prevalence of metabolic syndrome, which is increasing day by day globally in association with the effects of genetic and environmental factors. In the study, 21 male Sprague-Dawley rats of 220±20 gr body weight were used. The rats were assigned to 3 groups as the control group, metabolic syndrome group, and the group where *Nigella sativa* oil was administered. The serum homocysteine levels were increased in the metabolic syndrome group compared to the control group but without statistical significance ($p>0.05$). Homocysteine levels decreased significantly after *Nigella sativa* oil compared to metabolic syndrome group. LDH ($p <0.001$) and uric acid ($p <0.05$) levels which were higher in metabolic syndrome group were decreased in *Nigella sativa* oil group. Hyperhomocysteinemia is a risk factor for endothelial dysfunction. In our study, the treatment of the metabolic syndrome and regulation of the increased levels of homocysteine with *Nigella sativa* oil in metabolic syndrome were discussed. Some biochemical parameters and improvements in homocysteine levels with *Nigella sativa* oil has been identified. In this study, we have concluded that the occurrence of elevated levels of plasma homocysteine are closely associated with the development of inflammation, cellular adhesion, hepatic dysfunction, and cell proliferation and that the reduction in the serum levels of homocysteine by the administration of *Nigella sativa* oil will lead to favorable out comes.

Keywords: Homocysteine, Metabolic syndrome, *Nigella sativa* oil, Vanillylmandelic acid

Introduction

Metabolic syndrome (MetS), is a complex condition leading to several disorders, including sympathetic activation, oxidative stress, systemic inflammation, hypercoagulability, endothelial dysfunction, and hyperleptinemia (Scott 2004; Reaven 1988; Fulop et al. 2006). It is a serious health problem which starts with insulin resistance and becomes a combination of several disorders such as obesity or increased waist circumference, hyperglycemia, atherogenic dyslipidemia, hypertension, and proinflammatory and prothrombotic disorders (Iannucci et al. 2007; Brent 2004). Gerald M Reaven described the resistance as the stimulation of glucose intake by insulin. He called the entire combination of the following findings as "Syndrome X", including hyperinsulinemia, glucose intolerance, decreased HDL-cholesterol levels, elevated VLDL-cholesterol levels, hypertension, and the increased risk for ischemic cardiac diseases (Reaven 1988). The definition of this table has become broader day by day with a variety of different definitions introduced by several scientists. "Syndrome X plus" defines the additional four findings called "the fatal four", which are upper body obesity; glucose intolerance, hypertriglyceridemia, and hypertension. The inclusion of erythrocytosis and elevated uric acid levels into the clinical table is called "fatal six" (Sencer 2001). In this way, the definition of the syndrome has been improved day by day, being named with a variety of different terms along with the quality and

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comorbidity of various factors.

It is well known that the risks for developing Type 2 diabetes mellitus (DM) and the cardiovascular disease are remarkably high in MetS patients (Özgen 2006). The prevalence of this major public health issue, MetS, is on the rise gradually all around the world due to the contributions of increasing rates of obesity and sedentary life, leading to the increasing rates of several diseases, especially Type 2 DM and cardiovascular diseases (Carr and Brunzell 2004).

In addition, another cause of the increased prevalence of MetS is the intake of high-level dietary fructose, which is another individual risk indicator (Ford et al. 2002; Pyorala et al. 2000; Bruce and Byrne 2009). Fructose is a natural sugar found in honey and several fruits. Today, it has a wide spread use as a sweetener in the form of corn syrup in the food and industrial sector (Miller and Adeli 2008). The corn syrup rich in fructose contains 55-90% fructose, and being an ingredient as a sweetener in ready-made foods, it is the major source of dietary intake of fructose. Due to its low cost and easier miscibility, the corn syrup high in fructose is very commonly used in all sweetened ready-to-drink beverages (fruit-flavored sodas, ice tea, fruit juices etc.), in a variety of confectionery types, jam, marmalade, chocolate, cake, crackers, and other food products with a jelly content. Because of this increase in its use, fructose consumption per capita (excluding the natural consumption of fructose found in fruits and vegetables) was less than 0.5 g per day in the 1970s, however, today this figure has become 85-100 g per day with an astonishing increase (Hanson et al. 2002; Gaby 2005; Korkmaz 2008; Reddy et al. 2009).

Today, the use of medicinal plants against diseases and associated research has gained importance in European countries. In this study, we used the oil of *Nigella sativa* plant which has an important place in traditional medicine. *Nigella sativa* plant of the Ranunculaceae family has a remarkable history as it has been referred to in a number of references on history and religion (Salem 2005). The main origin of *Nigella sativa* is Egypt and it has been well known since ancient times. It has been popular in the Eastern and European countries as a medicinal plant rich in hundreds of active ingredients. *Nigella sativa* is known to be commonly used in for many years in folk medicine as a traditional medicine for the treatment of hypertension, rheumatismal disorders, gastrointestinal diseases, burns, cutaneous diseases, liver and kidney diseases, diabetes, constipation, asthma, bronchitis, diarrhea, dyspepsia, headache, dizziness, jaundice, and fever in Middle and far East (Hajhashemi et al. 2004; Ramadan 2007). Today, it is suggested by the medical community that the oil extracted from the black cumin "*Nigella sativa*" grown naturally in some parts of Egypt shows favorable effects in the treatment of cancer and several other diseases. In this study, we aimed to determine the change of homocysteine, vanillylmandelic acid and some serum parameters after fructose-induced metabolic syndrome before and after *Nigella sativa* oil administration.

Materials and Methods

This study was carried out in accordance with standard experimental animal studies after the approval of the Ethics Committee (Firat University Ethics Committee of Animal Experiments; 06.09.2017 date, protocol number 2017/83 and decision number179).

Approved by the Ethics Committee, the study was conducted in compliance with the ethical principles applied to the standard experimental animal research. In this study, 21 eight-week-old Sprague-Dawley male rats were used, each weighing 220±20 grams on average. Standard pellet feed and drinking water were supplied to the animals. The rats were assigned to three groups, each group consisting of 7 rats. During the study, the standard rat diet, fructose, and *Nigella sativa* Oil (NSO) were administered orally. A standard pellet diet and % 10 fructose added into the tap water was administered to the groups, but the control group, to induce MetS (Sánchez-Lozada et al. 2007).

Group 1 (n=7): Control group

Group 2 (n=7): MetS group

Group 3 (n=7): NSO group

Mode of administration of the oil of *Nigella sativa*

After inducing MetS in group 3, by adding fructose of % 10 into the drinking water for a period of 10 weeks, the rats in this group were administered 0.1 ml NSO daily by oral gavage for four weeks (Perveen and Hainder 2013). The rats in group 1 and group 2 were decapitated 10 weeks after the start of the experiment and the rats in group 3 were decapitated 14 weeks after the start of the experiment.

Laboratory Analyses

The blood samples were collected into plain biochemistry tubes containing gel. These samples were centrifuged at 4000 rpm for 5-10 minute sob taining the sera. Pinkish red or red serum, which is a sign of hemolysis, has not been observed in the study samples. The levels of urea, creatinine, uric acid, inorganic phosphorus, alkaline phosphatase (ALP), sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), lactate dehydrogenase (LDH), creatine kinase (CK), and CK-MB (creatine kinase-muscle/brain) were determined by the colourimetric method using a autoanalyser (Advia 2400 Chemistry Analyzer, Siemens Healthcare, Germany) device and appropriate kits. In order to collect urine samples to be analyzed for vanillylmandelic acid (VMA), funnels of appropriate size were placed under the floors of metabolic cages. The volumes of each of the 24-hour urine samples were documented individually. From each animal individually, a urine sample of approximately 2 ml (mixture) was collected into the sample tubes and then centrifuged at 3000 rpm for 10 minutes. The elicited supernatants were transferred to another group of clean and dry tubes and stored at -20°C until the time of analysis with high-performance liquid chromatography (HPLC system, Shimadzu Corporation, Kyoto, Japan).

The amount of VMA in urine was determined using a commercial kit and the HLPC method at a column temperature of 25°C, flow velocity of 1.0 ml/min, and an injection volume of 10 µL.

Serum homocysteine levels were determined with HPLC device Agilent, 1200 series, with fluorescence detectors by means of a reverse-phase C18 ODS column and a mobile phase column for tri-n-butyl phosphyne, dimethylformamide, 7-floro-2,1,3-bensoxadiasole-4-sulfonamide and acetonitrile

(Sridhar et al. 2016).

Histopathological analysis

Liver tissue samples were fixed in % 10 formalin and embedded in paraffin. 5-µm-thick paraffin sections were cut from the paraffin-embedded tissue blocks and stained with haematoxylin and eosin and picrosirius red F3BA (% 0.5 saturated picric acid solution). The paraffin sections were deparaffinized by immersing in xylene and rehydrated through a series of graded alcohols (100%, 95% and 75%), for 15 min each. The slides were stained with haematoxylin and eosin as well as picrosirius red F3BA and mounted with coverslip using distyrene plasticizer and xylene (Maulik et al. 2012). The slides were examined under light microscope by a pathologist blinded to the study groups. Images were taken at magnification × 20.

Statistical Evaluation

The data collected in the study have been presented in means±standard deviation. KruskalWallis test was used to evaluate the data in the groups and Mann Whitney-U test was used for binary comparisons between groups. The level of statistical significance was accepted at a level of $p<0.05$. SPPS statistical package program (IBM SPSS Version 22.0) was used for the analysis of data.

Results and Discussion

Determination of biochemical parameters

The levels of serum homocysteine, urinary VMA, and the results of the biochemical tests were compared statistically (Table 1 and 2). It was observed that the elevated levels of homocysteine after the occurrence of MetS were decreased statistically significantly by the administration of NSO ($p<0.05$) (Table 1 and figure 2).

The serum levels of LDH, CK-MB, uric acid, and Ca statistically significantly increased in the MetS group compared to the control group and those levels were decreased by the administration of NSO similarly ($p<0.05$) (Table 2).

Effect of NSO on histopathological studies

Haematoxylin and eosin-stained liver tissue Figure 1: 1a control group with normal histology, 1b MetS group showing micro- and macrovesicular fatty change in hepatocytes and 1c NSO group with normal histology. In the MetS group caused micro- and macrovesicular fatty changes of the hepatocytes. No infiltration of inflammatory cells, necrosis or fibrosis was observed in the MetS group. NSO group led to protection of micro- and macrovesicular fatty changes of hepatocytes caused by MetS (Figure 1).

Table 1. The Levels of Homocysteine and VMA by group

Parameters	Groups			p
	Group 1	Group 2	Group 3	
Homocysteine (Mmol/L)	9.64±0.25^{ab}	11.06±0.55^{a}	9.51±0.42^{b}	0.033 or *
VMA (µg/L)	21.53±0.99	20.31±0.87	20.56±1.50	0.737 or NS

The data are presented as means and standard deviation. a, b, c: There is a statistically significant difference between the measured levels of the parameters when they are marked with different letters on the same line. Different letters in the rows represent statistically significant difference *p<0.05; **p<0.01; ***p<0.001; NS: Not significant.

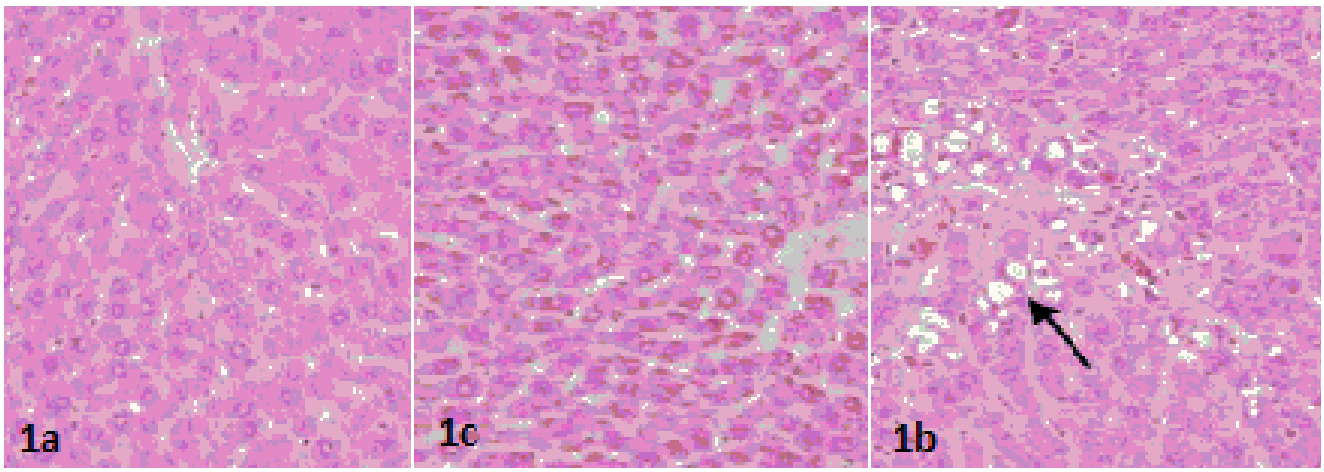
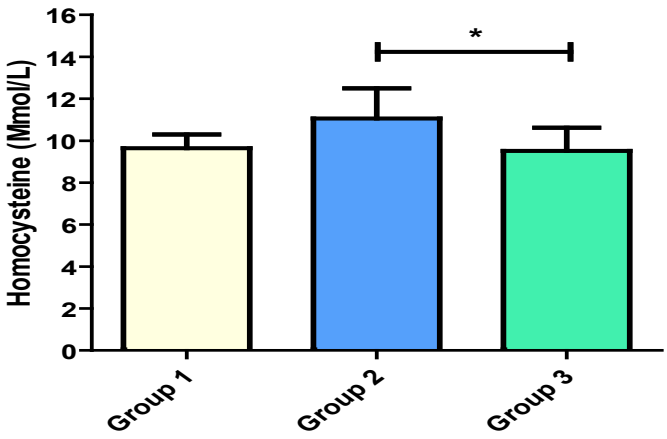


Figure 1 (1a, 1b, 1c). Haematoxylin and eosin-stained liver tissue

Table 2. Biochemical Parameters

Parameters	Groups			p
	Group 1	Group 2	Group 3	
Urea (mg/dL)	45.29±2.12[a]	36.86±1.30[b]	43.57±2.66[ab]	0.026 or *
Creatinine (mg/dL)	0.33±0.03	0.35±0.01	0.39±0.02	0.242 or NS
Uric acid (mg/dL)	1.33±0.05[b]	1.97±0.27[a]	1.31±0.07[b]	0.016 or *
Inorganic P (mg/dL)	7.19±0.16	7.43±0.53	7.37±0.17	0.869 or NS
ALP (mg/dL)	155.86±6.49	175.86±15.06	141.71±5.96	0.081 or NS
Na (mEq/L)	145.86±0.83[a]	145.00±0.87[a]	141.57 ± 0.53[b]	0.002 or **
K (mEq/L)	7.30±0.12	7.77±0.22	7.27±0.19	0.113 or NS
Cl (mEq/L)	107.57±0.37[b]	104.43±0.78[c]	110.14±0.34[a]	0.000 or ***
Ca (mg/dL)	9.44±0.04[b]	9.67±0.04[a]	9.43±0.08[b]	0.010 or *
LDH (u/L)	1787.57±27.83[c]	2786.71±192.27[a]	2292.43±119.06[b]	0.000 or ***
CK-MB (u/L)	1713.71±13.36[b]	2304.71±147.32[a]	1782.00±146.74[b]	0.005 or **
Ck (u/L)	13780.00±225.55[b]	26375.86±319.99[a]	12203.43±263.96[c]	0.000 or ***

The data are presented as means and standard deviation. a, b, c: There is a statistically significant difference between the measured levels of the parameters when they are marked with different letters on the same line. Different letters in the rows represent statistically significant difference *p<0.05; **p<0.01; ***p<0.001; NS: Not significant.



Different letters in the rows represent statistically significant differences (* P < 0.05).

Figure 2. Plasma homocysteine levels by group.

It is known that drinking water rich in fructose results in the occurrence of oxidative stress, which is the major factor involved in the progression of the cardiovascular disease (Busserolles et al. 2002). Studies demonstrate that a 6-week administration of fructose leads to oxidative stress due to the emergent imbalance between the ROS production and antioxidant capacity (Panda et al. 2015). In another study, it was reported that chronic consumption of fructose might lead to increased ROS production and oxidative stress (Sreeja et al. 2014).

It is known that fructose intake might induce hypertriglyceridemia and lipogenesis (Basciano et al. 2005). Fructose is absorbed in the intestines by the glucose transporter GLUT 5 and then it is delivered to the blood vessels by GLUT 2. In contrast to glucose, the absorption of fructose from the intestinal lumen does not require ATP hydrolysis and it is independent of sodium absorption. Fructose uptake by the liver is followed by its conversion into the following molecules in a row, which are fructose 1-phosphate, and then dihydroxyacetone phosphate and glyceraldehyde via aldolase respectively. The metabolites produced by these processes consequently lead to the synthe-

sis of triglycerides. Exposure of liver to excessive amounts of fructose induces lipogenesis and accumulation of triglycerides leading to decreased insulin sensitivity.

The levels of VMA and homocysteine provides a unique overview of the progression of the disease, which may lead to the development of new treatments. VMA and homovanillic acid (HVA) are the biomarkers of sympathetic system activity and can easily be determined in the urine. The metabolic end-product of epinephrine and norepinephrine is VMA. The levels of VMA provide information on the general activity of the sympathetic nervous system. VMA is usually found in small quantities in the urine and its levels are elevated shortly after the exposure of the body to stressors (Csaba 2014). When the levels of VMA were compared (Table 1), no significant differences were observed between the groups ($p>0.05$).

It is known that homocysteine is the degradation product of the amino acid methionine. Hyper homocysteinemia is an independent risk factor for the development of cardiovascular diseases. High plasma homocysteine levels can lead to insulin resistance or can be involved in atherogenesis actively (Hayden and Tyagi 2004). Fructose-fed rats are reported to develop significant hyper homocysteinemia compared to normal rats (Panda et al. 2017). Homocysteine should be converted to methionine by remethylation and to cysteine by trans sulfuration. Water-soluble vitamin B preparations are known to affect these pathways significantly and decrease the levels of homocysteine. In our study, it was observed that the elevated homocysteine levels in the fructose-fed rats were reduced significantly by NSO (Figure 2). We are of the opinion that the elevated but not statistically significant levels of homocysteine in MetS, compared to the levels in the control group, might have occurred due to the stress which was experienced by all rats during the experiment (Table 1).

When there is a cardiac injury, the levels of LDH and CK-MB, which are abundantly found in the heart, increase and these enzymes are released into the bloodstream. Therefore, the high levels of these enzymes, released into the bloodstream indicate a myocardial necrosis. In this model, the emergent hyperglycemia after the fructose overload causes the over production of ROS, leading to developing damages in the myocardial membrane (López and Hernández, 2013). Therefore, it is reported that the activities of LDH and CK-MB increase in the serum of the fructose-fed rats (Panda et al. 2017). Our study findings support the reports in the literature (Table 2). Because, the reduction in the elevated levels of LDH and CK-MB enzymes in fructose-induced MetS after NOS administration indicates that this plant has a significant cardio protective activity.

A study has reported that the serum levels of uric acid, urea, creatinine, and lactate dehydrogenase increased significantly in the rats treated with fipronil (FPN) compared to the control group. The occurrence of the oxidative damage after FPN-induced changes in all study parameters was alleviated with thymoquinone, probably by enhancing the tissue antioxidant defense system (Ohamed et al. 2018). In our study, it was observed that the elevated levels of uric acid and LDH were reduced after NSO administration (Table 2). Severity sympathetic in metabolic syndrome it is a fact that the system is activated and increases the level of plasma catecholamines (Cornier et al. 2008). Muscle contraction in a study performed by applying catecholamine level of exercise type, the severity of and duration was reported to be important (Cornier et al. 2008). In a study with animals, treadmill after the exercise, epinephrine level increased 2-fold was determined (Jobidon et al. 1985). Literature There was no statistically significant difference between VMA results.

Conclusions

In this study, we have concluded that the occurrence of elevated levels of plasma homocysteine are closely associated with the development of inflammation, cellular adhesion, hepatic dysfunction, and cell proliferation and that the reduction in the serum levels of homocysteine by the administration of NSO will lead to favorable out comes. Since there was not a significant difference in the VMA levels, we have been prevented from having an opinion whether the sympathetic system activity and MetS were associated. Supporting the reports in the literature, the elevated levels of LDH, CK-MB, and uric acid occurring after the development of MetS were favourably reduced by the administration of NSO. Our study findings may provide important information about the pathophysiology and the course of the disease after the development of MetS.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Experimental procedures were approved by Animal Experiments Local Ethics Committee of Fırat University (Decision No: 179, Protocol No: 2017/83, Date: 06.09.2017).

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Data availability

Not applicable.

Consent for publication

Not applicable.

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