Original Article

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The Protective Effects of Virgin Coconut Oil on High-Fat Diet Induced Rat Liver

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ABSTRACT

BACKGROUND AND OBJECTIVE: A high-fat diet can disrupt the antioxidant system and damage the liver. One of the most important ways to control non-alcoholic fatty liver is to use natural compounds with antioxidant properties. Therefore, this study was performed to evaluate the effect of coconut oil on cholesterol, triglyceride and antioxidant status in high-fat diet- induced rat liver.

METHODS: In this experimental study, 30 male rats were randomly divided into 6 groups of 5 including: 1) control group, 2 and 3) control+10% and 8% virgin coconut oil, 4) only receiving high fat diet, and 5 and 6) receiving high fat diet+10% and 8% virgin coconut oil. Coconut oil was prepared daily and mixed with animal food. At the end of the study, the rats were anesthetized and liver tissue was isolated and used for antioxidant tests.

FINDINGS: In this study, the levels of triglyceride (159±11.5) in the high-fat diet group increased significantly compared to the control (64±4.2) ($p<0.001$). Treatment of high-fat diet group with coconut oil at doses of 8% (104.5±9.1) and 10% (97.5±8.2) was able to reduce triglyceride levels ($p<0.05$) significantly. Cholesterol (118±6.7) in the high-fat diet group increased significantly compared to the control (60±6.6) ($p<0.001$). Treatment of high-fat diet group with coconut oil at a dose of 10% (94±5.3) was able to reduce cholesterol ($p<0.01$) significantly.

CONCLUSION: According to the results of this study, virgin coconut oil can be useful in the treatment of fatty liver by reducing lipids and increasing antioxidants.

KEY WORDS: *Coconut Oil, Fatty Liver, Rat.*

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Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is caused by a variety of factors, including insulin resistance, obesity, blood lipid disorders, diabetes, sedentary lifestyle, and a high-fat diet (1-4). The most important mechanisms involved in non-alcoholic fatty liver include changes in lipid metabolism, changes in insulin signaling, mitochondrial dysfunction, inflammation, and oxidative stress (5-8).

One of the most important ways to control non-alcoholic fatty liver is to use natural compounds with antioxidant properties (9-14). In this regard, vegetable oils such as coconut oil can be useful. Coconut oil is prepared in two ways: Copra Oil and Virgin Coconut Oil (VCO). Copra oil has been reported to have no positive effect on blood lipids and may even increase blood lipids. But the effective and therapeutic form of coconut oil is Virgin coconut oil. Virgin coconut oil is able to significantly reduce the concentration of cholesterol and levels of phospholipids, triglycerides and LDL (Low-Density Lipoprotein) and also increase the serum level of HDL (High-Density Lipoprotein) (15).

It has been reported that virgin coconut oil has beneficial effects on lipid parameters by reducing lipogenesis and increasing fatty acid catabolism (16). Other beneficial effects of virgin coconut oil include antioxidant, anti-inflammatory, analgesic, anti-aging, anti-bacterial and chemical protective activities. Coconut oil is known as a very useful nutrient that is rich in polyphenol compounds (17). These data suggest that virgin oil can be beneficial in improving fatty liver through its antioxidant and hypolipidemic properties. Therefore, the aim of this study was to investigate the antioxidant effects of virgin coconut oil on an animal model of non-alcoholic fatty liver.

Methods

In this experimental study, after approval by the ethics committee of Hamadan University of Medical Sciences with ethics code IR.UMSHA.REC.1397.550, male Wistar rats aged 8 weeks after two weeks of adaptation to the new environment (temperature of 22±2 °C with standard food and exposed to natural light/dark cycles and proper ventilation), were randomly divided into 6 groups of 5:

1-Healthy control group

2-Healthy control group+8% virgin coconut oil (VCO)

3-Healthy control group+10% virgin coconut oil (VCO)

4-High fat diet group (HFD)

5-High fat diet group (HFD)+8% virgin coconut oil (VCO)

6-High fat diet (HFD)+10% virgin coconut oil (VCO).

At the end of the eighth week of treatment and after overnight fasting, the animals were anesthetized through ketamine injection (Sigma-Aldrich, USA) and then blood samples were collected. In addition, the isolated liver was washed with PBS (Phosphate Buffer Saline) buffer and immediately frozen in liquid nitrogen and stored at -80 °C and finally used for antioxidant tests. Doses of 8% and 10% of virgin coconut oil were more effective than other doses, according to previous reports in rats. Therefore, in order to evaluate the antioxidant effects in this study, treatment with the mentioned doses was performed (18). Virgin coconut oil was prepared according to previous studies (19).

All of the tools were prepared from Sigma-Aldrich (USA). Blood chemistry factors were measured using biochemical kits of Pars Azmun Company according to the existing instructions (20, 21). This method is based on the ability of homogenized tissue to reduce ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. The regeneration process is performed in the presence of a substance called TPTZ (Tripyridyl-S-triazine, Sigma-Aldrich). The Fe^{2+}-TPTZ complex forms a color complex with a maximum absorption of 593 nm. TAC levels were reported in terms of nmol/mg protein (22-24). Lipid oxidation was measured according to previous studies. MDA was reported in terms of U/mg protein (25). The total oxidant content of homogenized liver samples was measured by oxidation of ferrous to ferric iron under moderate acidity using Xylenol orange dye.

TAC levels were reported based on nmol/mg protein (26). Descriptive statistics of mean, median, standard deviation and interquartile range as well as graphs of data were used to describe the collected data. To evaluate the effect of coconut oil and to compare the groups, one-way analysis of variance test based on Bootstrap method and Bayesian approach were used with respect to the appropriate previous distribution of response variables in Stan statistical software in R3.6.1 development environment, as well as the statistical packages of Ggplot2, BRMS and Quantreg. Furthermore, for abnormal data with abnormal distribution values, a non-parametric test was used and $p<0.05$ was considered significant.

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Results

In this study, the levels of triglyceride (159±11.5) in the high-fat diet group increased significantly compared to the control (64±4.2) ($p<0.001$). Treatment of high-fat diet group with coconut oil at doses of 8% (104.5±9.1) and 10% (97.5±8.2) was able to reduce triglyceride levels ($p<0.05$) significantly. Cholesterol (118±6.7) in the high-fat diet group increased significantly compared to the control (60±6.6) ($p<0.001$). Treatment of high-fat diet group with coconut oil at a dose of 10% (94±5.3) was able to reduce cholesterol ($p<0.01$) significantly (Table 1). Lipid peroxidation activity was significantly increased in the high-fat diet group compared to the control group ($p<0.001$). This marker showed a significant decrease in the group receiving high-fat diet+10% virgin coconut oil and also in the group receiving high-fat diet+8% virgin coconut oil compared to the group receiving high-fat diet ($p<0.05$) (Figure 1). There was a significant increase in TOS in the group receiving high-fat diet compared to the control group ($p<0.001$). The amount of TOS in the control group receiving coconut oil at a dose of 10% compared to the control group showed a significant decrease (Figure 2) ($p<0.05$). The amount of TOS in the group receiving high-fat diet+10% virgin coconut oil ($p<0.001$) and also the group receiving high-fat diet+8% virgin coconut oil ($p<0.01$) showed a significant decrease compared to the group receiving high-fat diet. In the group receiving high-fat diet compared to the control group, the amount of TAC was significantly reduced ($p<0.001$). The level of TAC in the control group receiving coconut oil at doses of 8 and 10% did not show a significant increase compared to the control group. The amount of TAC in the group receiving high-fat diet+10% virgin coconut oil ($p<0.05$) and also the group receiving high-fat diet+8% virgin coconut oil ($p<0.01$) showed a significant decrease compared to the group receiving high-fat diet (Figure 3).

Table 1. The effect of virgin coconut oil on cholesterol and triglyceride levels in different treatment groups

Study groups	Cholesterol (mg/dl)	triglyceride (mg/dl)
Healthy control group	$60±6.6^{a}$	$64±4.2^{A}$
Healthy control group+8% virgin coconut oil (VCO)	$62±4.7^{a}$	$62.5±7.2^{A}$
Healthy control group+10% virgin coconut oil (VCO)	$56±3.3^{a}$	$60±3.7^{A}$
High Fat Diet (HFD) Group	$118±6.7^{b}$	$159±11.5^{B}$
HFD+8% virgin coconut oil (VCO)	$104.5±9.1^{b*}$	$104.5±7.6^{B*}$
HFD+10% virgin coconut oil (VCO)	$94±5.3^{b*}$	$97.5±8.2^{B*}$

Non-similar Latin letters indicate significant differences and similar Latin letters indicate no significant differences between groups ($p<0.001$). (*) indicates a significant difference with the group receiving high-fat diet (HFD).

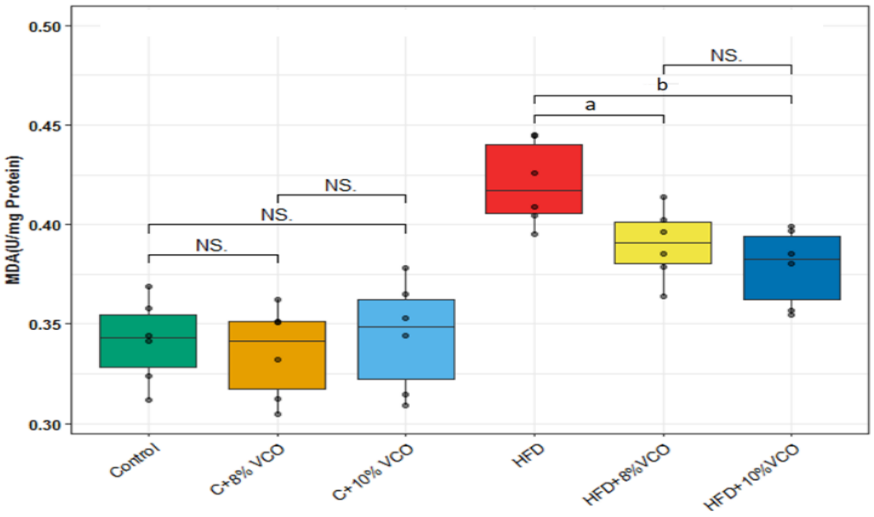

Figure 1. Effect of virgin coconut oil on malondialdehyde (MDA) in liver tissue. There was a statistically significant difference between the high-fat diet group and the groups of HFD+8% VCO and also the HFD+10% VCO, so that the mean MDA in the groups with VCO intervention was higher than the group of high-fat diet without intervention. C: Control, HFD: high-fat diet, VCO: virgin coconut oil, NS: no significance. Significance was shown as $p<0.05^{a}$ and $p<0.01^{b}$ in comparison with untreated HFD group.

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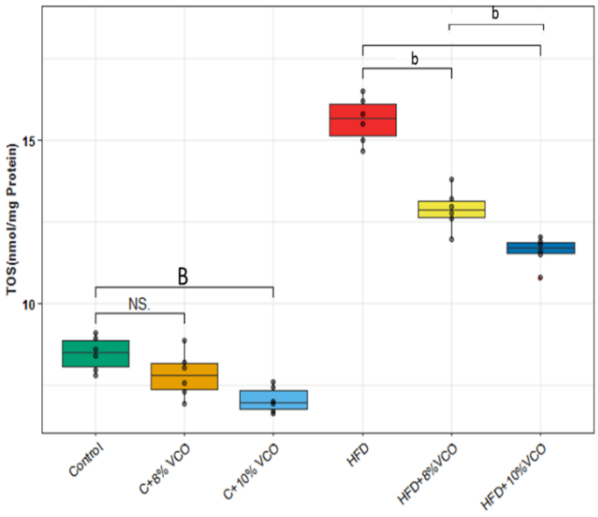

Figure 2. Effect of virgin coconut oil on total oxidative state (TOS) of liver tissue. **There was a significant difference between the control group and the control group treated with 10% VCO. Moreover, a significant difference was observed between the high-fat diet group and the groups of HFD+8% VCO and also the HFD+10% VCO, so that the mean total oxidation was reduced in the groups with VCO intervention compared to the high-fat diet group without intervention. NS: No significance. Significance was shown as $p<0.01^{b}$ and $p<0.001^{c}$ compared to untreated HFD group and $p<0.01^{B}$ compared to control.**

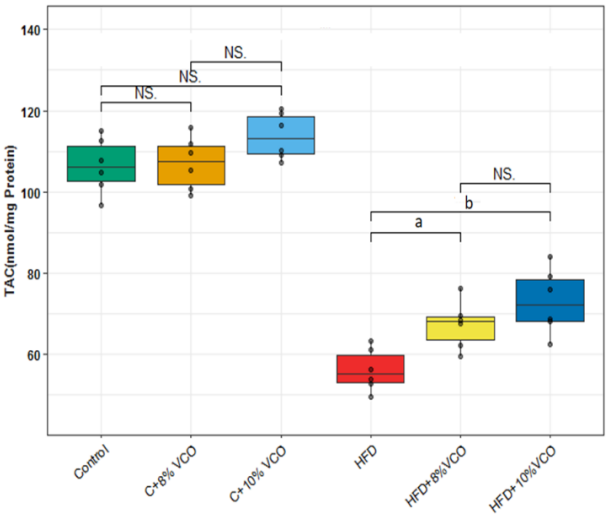

Figure 3. Effect of virgin coconut oil on total antioxidant capacity (TAC) in liver tissue. **There was a significant difference between the high-fat diet group and the groups of HFD+8% VCO and also the HFD+10% VCO, so that the mean total antioxidant capacity was increased in the groups with VCO intervention compared to the high-fat diet group without intervention. There was no statistically significant difference between the two groups of HFD+8% VCO and HFD+10% VCO. NS: No significance. Significance was shown as $p<0.05^{a}$ and $p<0.01^{b}$ in comparison with untreated HFD group.**

Discussion

The present study showed that HFD in rats increased oxidative factors including TOS and decreased TAC. Treatment of hyperlipidemic rats with virgin oil showed that it has a beneficial effect on the oxidant-antioxidant system and increased antioxidant factors such as TAC and decreased TOS. 10% virgin coconut oil was more effective.

In this study, cholesterol and triglyceride levels increased in the high-fat group, while it showed a significant decrease in rats receiving virgin coconut oil. This process can be effective in reducing the accumulation of fat in the liver. The biological effect of virgin coconut oil is probably due to its high polyphenols such as ferric acid, vanillic acid, p-coumaric acid, syringic acid, and caffeic acid (27). Feranil et al. in a cohort study administering coconut oil showed that this compound increased HDL levels (28). The hydroxyl group of phenolic compounds is able to trap free radicals produced in the process of non-alcoholic fatty liver and control oxidative stress (29-31).

Increased fat deposition in the liver increases oxidative stress by increasing the level of MDA, a marker of lipid peroxides (32). In this study, the amount of MDA was reduced by coconut oil. It has been shown that accumulation of fat and increased MDA in the liver due to consumption of high-fat diet, disrupts the expression of inflammatory factors and genes involved in fatty acid synthesis, which eventually progresses to non-alcoholic fatty liver, fibrosis, and cirrhosis (33). Newell-Fugate et al. showed that the administration of virgin oil reduced inflammation (34).

The present study showed that high-fat diet in rats increased oxidative factors such as TOS and decreased TAC. Treatment of rats with high-fat diet using virgin coconut oil showed that it has a beneficial effect on the oxidant-antioxidant system and increased TAC and decreased TOS. Consistent with the present study, Famurewa et al. showed that treatment of rats with virgin coconut oil improves antioxidant status, reduces TOS levels and increases TAC (35).

Ferulic acid is a phenolic compound with effective antioxidant activity and anti-inflammatory activity that is abundant in virgin oils. The strong anti-inflammatory and antioxidant properties of ferulic acid have been proven. This compound reduces lipid accumulation and is able to inhibit oxidative stress by binding to free radicals (36). Ferulic acid has been shown to be effective in improving fatty liver by inhibiting inflammatory pathways (37).

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P-coumaric acid is another compound in coconut oil that has high antioxidant power (38). Nevin et al. showed that administration of coconut oil increased antioxidant enzymes and decreased lipid peroxidation (17). Babu et al. also showed that virgin coconut oil due to its phenolic compounds increases antioxidant activity and decreases lipid index and blood pressure (39). The researchers reported that phenolic compounds significantly reduced inflammatory cytokines, increased antioxidant potency, and decreased interleukin-6 production (40, 41). Famurewa et al. showed that administration of virgin oil in normal rats reduces liver enzymes and increases antioxidant activity (35). Administration of coconut oil to male rats has been reported to increase antioxidant enzymes and decrease lipid peroxidation (17). The results of this study showed that this oil can be useful in the treatment and prevention of non-alcoholic fatty liver due to its anti-inflammatory, antioxidant and cholesterol lowering activity. Therefore, in this study, virgin coconut oil reduced lipid indexes and improved antioxidant status in non-alcoholic fatty liver.

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