

Carvone Prevents and Alleviates Hepatic Steatosis in Rat Model with Nonalcoholic Fatty Liver Disease

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Abstract

Aims: Nonalcoholic fatty liver disease (NAFLD) is known as condition in which fat significantly aggregates in the liver and the use of antioxidants can alleviate its adverse effects. This study aims to evaluate the effects of carvone on hepatic steatosis and NAFLD by investigation of the lipids in serum and liver.

Materials & Methods: Animals were grouped into four groups and studied for 42 days, including rats fed with 1) control diet (Control), 2) high fat diet (HF), 3) HF+50 mg/kg body weight of carvone (50 Carv) and HF+100 mg/kg body weight of carvone (100 Carv). Animals were killed, and blood and liver samples were obtained to evaluate the biochemical analyses including triglyceride, cholesterol, nonesterified fatty acids and thiobarbituric acid-reactive substances (TBARS).

Findings: Rats fed with HF diet showed higher levels for TBARS, triglycerides and cholesterol in comparison to control group ($p < 0.05$). Dietary inclusion of carvone, especially in the higher levels, could reverse effects of HF on TBARS, cholesterol and triglycerides ($p < 0.05$).

Conclusion: Carvone can alleviate hepatic steatosis in animals with NAFLD. It can be suggested to use of the carvone for patients with NAFLD.

Keywords: Carvone; High fat Diet; Lipid Oxidation; NAFLD Model

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is known as condition in which fat significantly aggregates in the liver of a patient lack a history of alcohol abuse [1].

NAFLD is grouped into two groups including simple steatosis and nonalcoholic steatohepatitis (NASH). Under NASH condition, steatosis, intralobular inflammation and hepatocellular are found in the progressive fibrosis [2]. Permanent NASH may be caused to liver cirrhosis, and hepatocellular carcinoma [3-5]. NAFLD not only increases risk for developing liver disease but is also one key component for metabolic syndrome, obesity, and type 2 diabetes [6]. NAFLD encompasses a broad range including simple fatty liver (intracellular lipids >5%) up to progressive NASH which is accompanied with lobular inflammation, fibrosis, and cirrhosis and increases the risk for hepatocellular carcinoma [7]. Increased aggregation of the triglycerides in hepatocytes is the indicator for NAFLD, which is severely related with hepatic insulin resistance [8]. Increased formation of the triglyceride is observed in fatty livers that are accompanied with obesity and type 2 diabetes mellitus [9]. Hepatic fat aggregation causes to hepatic insulin resistance through promoting gluconeogenesis and activation of the PKC- ϵ and JNK1 signaling pathways [10]. It is not approved agents available for treatment of NAFLD. Improvement of some factors such as weight reduction and dietary fat intake, are usually known as treatment modalities in NAFLD disease [11]. Studies showed that insulin sensitizers, such as thiazolidinediones, and some antioxidants could improve clinical conditions of NASH [12, 13]. Carvone (5-isopropenyl-2-methyl-2-cyclohexenone), is one monocyclic monoterpene ketone which is found in 70 different plants. It is one of main component in the caraway oil. It is known to have biological activities including antimicrobial [14], nematocidal [15], antitumor [16], and antioxidant [17] properties. It seems that carvone could improve prevents hepatic steatosis in rat model of NAFLD due to its antioxidant properties. This study was thus conducted to evaluate the effects of carvone on hepatic steatosis and NAFLD by preventing hepatic triglyceride formation and oxidative processes.

Materials and Methods

Materials

Carvone was purchased from Sigma Chemicals Company., St. Louis, MO, USA and kept in the 2-4 °C and protected from sunlight. Other chemicals were purchased from commercial suppliers.

Methods

Animal studies

A total number of 60 Wistar rats (180±10g) were used in this study. Control diet contained 12% of

total calories which is supplied from corn oil. The HF diet contained 60% of total calories which is supplied from corn oil, oleic acid and the saturated fatty acids palmitic and stearic. Animals were grouped into 4 groups and studied for 42 days, including rats fed with 1) control diet (Control), 2) HF diet (HF), 3) HF+50 mg/kg body weight of carvone (50 Carv) and HF+100 mg/kg body weight of carvone (100 Carv). After 42 days, 3 animals per group were fasted 16-18 h and killed, and blood and liver samples were obtained to evaluate the biochemical analyses. In addition, animals were weighted in initial and end of trial for body changes. To evaluate the regression studies, animals were first fed the HF diet for other 42 days up to induce hepatic steatosis. A number of seven animals in each group were randomized and fed either the HF diet or HF+100 mg/kg body weight of carvone (100 Carv).

Biochemical Analysis

Samples from total lipid of liver samples were extracted and TBARS, triglycerides and cholesterol were evaluated as reported by others [18]. The serum concentrations of nonesterified fatty acid (NEFA), triglycerides and cholesterol were evaluated by a commercial Kits (Abacam).

Statistical Analysis

The results are reported as mean \pm SD. A One-way ANOVA and Turkey's post-test were applied to compare of the data.

Findings

Effects of different levels of carvone on liver and body weight are shown in Figure 1. Results showed that rats in HF group showed higher body weight and liver weight in comparison to control group ($P < 0.05$). Rats fed with HF diets containing 50 and 100 mg/kg carvone showed lower weights in comparison to HF group ($P < 0.05$).

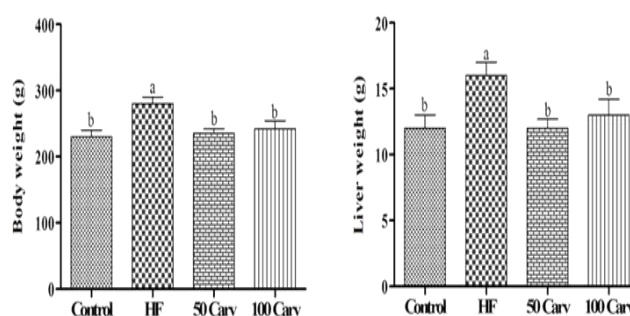


Figure 1) Effect of carvone on body weight and liver weight. Animals were fed 1) control diet (Control), 2) HF diet (HF), 3) HF+50 mg/kg body weight of carvone (50 Carv) and HF+100 mg/kg body weight of carvone (100 Carv) for 6 wk. Superscripts show significant differences at $P < 0.05$

Effects of carvone on serum and liver levels of triglycerides and cholesterol are shown in Figure 2. Results showed that rats fed with HF diet showed

higher levels for triglycerides and cholesterol in comparison to control group ($P<0.05$). Dietary inclusion of carvone, especially in the higher levels, could reverse effects of HF on cholesterol and triglycerides ($P<0.05$).

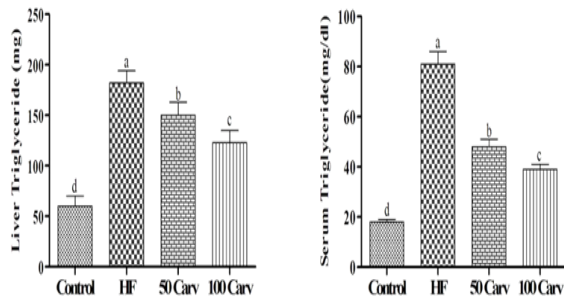


Figure 2) Effect of carvone on serum and liver triglycerides. Animals were fed 1) control diet (Control), 2) HF diet (HF), 3) HF+50 mg/kg body weight of carvone (50 Carv) and HF+100 mg/kg body weight of carvone (100 Carv) for 6 wk. Data were reported as means \pm SD. Superscripts show significant differences at $P<0.05$.

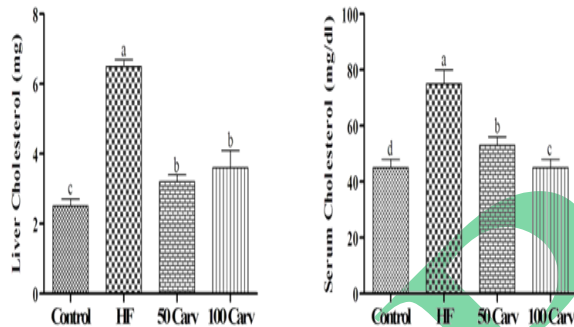


Figure 3) Effect of carvone on serum and liver cholesterol. Animals were fed 1) control diet (Control), 2) HF diet (HF), 3) HF+50 mg/kg body weight of carvone (50 Carv) and HF+100 mg/kg body weight of carvone (100 Carv) for 6 wk. Data were reported as means \pm SD. Superscripts show significant differences at $P<0.05$.

Effects of carvone on liver TBARS are illustrated in Figure 4. Results showed higher lipid peroxidation was significantly higher in the HF group compared with control group ($P<0.05$). Carvone supplementing could significantly alleviate adverse effects of HF ($P<0.05$) and the best response was observed in 100 mg/kg of the carvone.

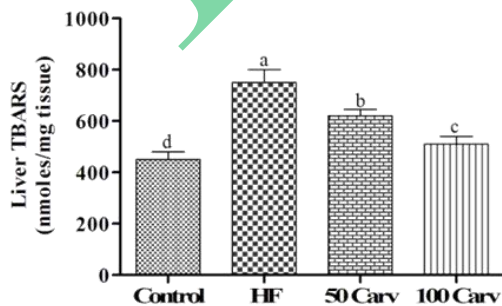


Figure 4) Effect of carvone on liver lipid peroxidation products. Animals were fed 1) control diet (Control), 2) HF diet (HF), 3) HF+50 mg/kg body weight of carvone (50 Carv) and HF+100 mg/kg body weight of carvone (100 Carv) for 6 wk. Data were

reported as means \pm SD. Superscripts show significant differences at $P<0.05$.

Following the induction of hepatic steatosis, liver weight and the serum concentrations of triglycerides and TBARS were significantly higher in HF group in comparison to control group ($P<0.05$) (Table 1) and the use of carvone alleviated effects of HF ($P<0.05$). Carvone did not have effects on serum NEFA ($P<0.05$).

Table 1) Effects of carvone on liver weight and some serum parameters

Parameters	Control	HF	100 Carv	P-values
Liver weight, g	14.01 \pm 1.20 ^b	19.35 \pm 1.30 ^a	15.52 \pm 0.75 ^b	0.025
Serum Triglycerides, mg/dl	38.50 \pm 3.21 ^b	95.85 \pm 11.30 ^a	42.51 \pm 2.75 ^b	0.001
Liver TBARS, nmol	421.21 \pm 21.21 ^b	752.15 \pm 35.23 ^a	469.21 \pm 45.75 ^b	0.001
Serum NEFA, mEq/l	0.59 \pm 0.03	0.51 \pm 0.08	0.54 \pm 0.05	0.0785

Animals were fed 1) control diet (Control), 2) HF diet (HF), 3) HF+50 mg/kg body weight of carvone (50 Carv) and HF+100 mg/kg body weight of carvone (100 Carv) for 6 wk. Data were reported as means \pm SD. Superscripts show significant differences at $P<0.05$.

Discussion

With regards to previous studies, animals fed with HF diets (50-75% calories derived from fat), 60% in the current present, progress hepatic steatosis and signs of initial NASH related with dyslipidemia, insulin resistance and changes in mitochondria which result in the increased oxidative stress [19-21]. However, HF diets cannot significantly develop severe steatohepatitis, but its pathophysiology resembles with human NAFLD [19-21]. The HF diet-induced NAFLD model is commonly used to evaluate the pathogenesis of NAFLD and in order to find the treatment strategies [22-24]. However, the use of carvone could alleviate the adverse effects of NAFLD. In addition to investigation of the steatosis, we also showed that carvone can regress of the preexisting steatosis, which could be found in the clinical conditions in the humans. Our results show that supplementation with carvone regresses of preexisting hepatic steatosis as assessed by biochemical liver triglyceride contents. Results also showed that carvone could significantly reduce body and liver weights. With regards to previous studies, it is also recommended to use of the treatment strategies for weight loss which improve hepatic steatosis [25-28]. In summary, carvone reduced the levels of cholesterol and triglycerides in the liver. It could be stated that the decreased both triglycerides and cholesterol in liver causes to increase the liver weight and reduced lipids may be the reason for reduction in liver weight. Since the both levels of carvone could improve the parameters and similar effects were observed in the both groups, it is possible that carvone in the lower doses can inhibit hepatic steatosis. We believe that carvone improves

levels of triglycerides and cholesterol by antioxidant mechanism and reduced cholesterol and triglycerides reduces liver weight. Our findings for antioxidant properties of carvone were approved by the data for TBARS which was significantly lower in carvone groups. A study reported antioxidant effect of D-carvone on DPPH• and ABTS+, D-carvone showed concentration-dependent antioxidant potential [28].

Conclusion

In summary, this study for the first time showed that carvone significantly inhibited and reversed hepatic steatosis. Clinical progressions of carvone formulations and carvone-related structures for the treatment of NAFLD will be significant in preparation of the need for the development of therapeutic agents for NAFLD/NASH and other forms of fatty liver disease.

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Authors' contributions: All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

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