Journal of Medicinal Herbs

journal homepage:**www.jhd.iaushk.ac.ir**

Thymol and its effects on the obesity parameters and fat tissue in NMRI mice

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ARTICLE INFO

Type: *Original Research*
Topic: *Medicinal Plants*
Received *July 08th2023*
Accepted *October 26th2023*

Key words:
- ✓ *Thymol*
- ✓ *obesity*
- ✓ *TNF-α*
- ✓ *adiponectin*
- ✓ *adipose tissue*

ABSTRACT

Background & Aim: Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. This study investigated the influence of thymol on obesity, liver enzymes and adipose tissue in mice fed a high fat diet (HFD).

Experimental: Male NMRI mice were divided into two groups; the control group that given normal rodent diet and obese group that received HFD for 8 weeks. The obese animals were divided into 3 groups: one group received thymol orally (12 mg/kg body weight) for a period of 56 days. Obese group didn't receive any treatment and the sham groups received thymol (12 mg/kg body weight) dissolved in grape seed oil**.** Alteration in body weight gain and serum biochemical markers were assessed and fat tissue was fixed in formalin in order to prepare microscopic slides. Finally, body weight, liver enzymes (ALT and AST), TNF-α, adiponectin, total antioxidant (TAC) and leptin levels were measured in obese mice compared with control mice.

Results: Thymol treatment resulted in increased serum adiponectin and TAC level, while significantly reduced TNF-α level ($P<0.05$). The leptin level decreased in HFD mice, but it was not significant ($P>0.05$). Serum ALT and AST levels were significantly decreased ($P<0.05$) in HFD mice, compare with control. The diameter of adipose cells were decreased in thymol-supplementation mice ($P<0.001$). This combination does not lead to significant reduction in body weight, but it can help to prevent weight gain.

Recommended applications/industries: The thymol was able to prevent HFD induced obesity in mice and attenuation of inflammation markers.

1. Introduction

The prevalence of being overweight, obesity, type-II diabetes, and metabolic syndrome has increased dramatically in the past 2 decades (Rubio *et al.*, 2019). Obesity is a multi-factorial disorder resulting from various causes, including genetic, medicinal, metabolic rate, endocrine function, nutritional, and other environmental factors which increases food intake, appetite and the risk of obesity for susceptible individuals (Hwalla *et al.*, 2020). Obesity is reported to induce systemic low-grade inflammation (Libby *et al.*, 2010). Predisposing to metabolic disorders including insulin resistance, diabetes, dyslipidemia, vascular diseases, arising from deregulated release of free fatty acids and inflammatory cytokines, such as tumor necrosis factor (TNF)-α, from adipose tissue (Wu *et al.*, 2020). The micronutrients play an important role in inflammation, metabolic syndrome and insulin resistance and could influence the weight loss. The screening of micronutrient deficiency should therefore be systematic in order to improve the management of obesity (Carpentier *et al.*, 2013). One of the weight loss strategies is the nutritional composition of the diet that can affect metabolic variables such as mitochondrial oxidation (Abete *et al.*, 2009) Many studies have

examined the impact of different dietary components, such as macronutrient distribution, meal frequency, n-3 fatty acids, or dietary TAC (Del *et al.*, 2011). Essential oils of the botanical families Lamiaceae and Myrtaceae were the most effective antioxidants. Thymol [5-methyl-2-(1-methylethyl) phenol] was the major terpene in all of the essential oils of the family Myrtaceae (Nunesb*et al.*, 2020). Supplementation of food with spices containing essential oils may counteract and retard the process of oxidative damage, lipid oxidation and elevate antioxidant activity of the final product. Thymol has revealed several biological properties, such as antibacterial, anti-inflammatory, and antioxidant (Villanueva *et al.*, 2015) and recognized safe for use in food as well as medical and cosmetic fields (Zeng *et al.*, 2020). The Environmental Protection Agency reported that thymol has no adverse effects on animals and human (Nagoorb *et al.*, 2017). Therefore, this study was conducted to determine the effects of supplementation of thymol on obese mice.

2. Materials and Methods

2.1. Animals

Male NMRI mice (six weeks old) weighing 25 ±5 g were purchased from the Razi Vaccine and Serum Institute, Karaj, Iran. Mice were exposed to a12-h light/dark cycle, and were housed in standard cages. The animal was maintained at a constant temperature of 25 °C. After one week of adaptation to the conditions, mice were randomly divided into two groups (n = 12 in each group): normal group which received standard rodent diet and obese group received HFD for eight weeks. The HFD was prepared from a mix of 15 g of mouse pellet standard chow, 10 g of roasted ground nut, 10 g of milk chocolate and 5 g of sesame crackers. To ten-fold of these ingredients 20 g roasted sesame was added, resulting into 18 KJ/g energy content. In addition, the obese group was fed 240g creamy biscuits (3644 KJ) weekly. The normal group was allowed access to standard chow, taking a total of 13 KJ/g weekly (Banakar *et al.*, 2021).

After eight weeks, all animals were given standard rodent diet, and the obese animals were divided into three groups: sham, obese and experimental group. The experimental group was treated with thymol (12 mg/kg/day) by oral gavage for eight weeks. The obese group did not receive any treatment and the sham groups received grape seed oil which was used as thymol solvent. Mice were weighed weekly during the 8 weeks of treatment. At the end of the experiment, the animals were anesthetized and sacrificed; blood samples were collected and immediately centrifuged at 1500 g for 10 min at 4 °C. Collected serums were then stored at 4 °C until further analyzes.

2.2. Biochemical parameters

Serum levels of ALT and AST were measured with the use of enzymatic methods (Pars Azmoon, Iran). Plasma adiponectin, TNF-α and leptin concentration were determined by ELISA (respectively R&D Systems, USA; Enzo life Science, USA and ALPCO, USA). Plasma total antioxidant capacity (TAC) was measured by a colorimetric method (Bio Vision, USA).

2.3. Histological studies

Adipose tissue samples were fixed in formalin. Smear microscopy of adipose tissue were prepared and stained with hematoxylin and eosin (H&E).

2.4. Statistical analyses

The data were statistically analyzed by SPSS with the use of ANOVA and Tukey's test. Values are reported as mean± SD and the level of significance considered at P< 0.05.

3. Results and discussion

3.1. Effect of thymol on plasma TNF-α and TAC level

Thymol-supplemented mice tended to have lower serum concentrations of TNF-α as an inflammatory factor (P<0.01)**,** this factor was higher in the untreated obese group, although not significantly (Figure 1).

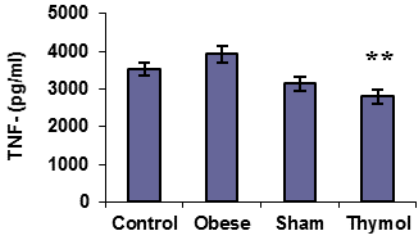

Figure 1. Effects of Thymol on serum TNF-α in mice (n=12 in each group) after eight weeks of treatment. Data are shown as mean ± SD.
**P<0.01 compared with the obese group.

The first clear link between obesity and chronic inflammation was reported as an increased expression of TNF-α in the adipose tissue of obese mice (Hotamisligil *et al.*, 1993). TNF-α is also overexpressed in the adipose tissue of obese humans (Tzanavari *et al.*, 2010). In the present study, TNF-α level increased in obese mice compared to control mice. Results showed that the thymol has a decreasing effect on serum levels of TNF-α in obese mice. Dhaneshwar *et al.* (2013) showed that thymol having anti-inflammatory properties and as co-drug can be effective in the treatment of arthritis. Bastard *et al.* (2006) demonstrated that leptin and TNF-α are factors that overproduced during obesity, also expression and plasma levels of adiponectin, an insulin-insulin-sensitizing effector, are down-regulated during obesity. Leptin could modulate TNF-α production (Bastard *et al.*, 2006).

Plasma TAC was found to be lower in untreated obese mice, although it was not significan (Figure 2). Thymol administration increased remarkably serum TAC (P<0.05), the sham group showed also increased TAC levels, but it was not significant compared to the obese group.

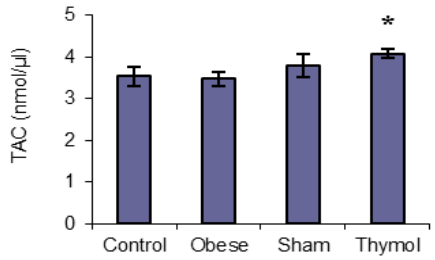

Figure 2. Effects of Thymol on serum TAC in mice (n=12 in each group) after eight weeks of treatment. Data are shown as mean ± SD.
*P<0.05 compared with the obese group.

Dietary total antioxidant capacity (TAC) is important to assess the quality of the diet (Lopez-Legarre *et al.*, 2013). The previous results confirmed that fat accumulation plays a key role in the pathogenesis of systemic oxidative stress (Faienza *et al.*, 2012). Increased plasma TAC has been associated with a high consumption of fruits and vegetables. TAC reflects the dietary intake of antioxidants and the levels of individual antioxidants in plasma (Wang, Yang *et al.*, 2012). Antioxidants have beneficial effects on weight and abdominal fat gain (Bahadoran, Golzarand *et al.*, 2012). The feed supplementation with thymol and carvacrol increased antioxidant enzyme activities (Hashemipour *et al.*, 2013). This study was in agreement with the recent report showing thymol and carvacrol are well known antioxidants found in the extract of the plants of thyme species (Beena, Kumar *et al.*, 2013). In this study, the antioxidant capacity decreased in obese mice, while thymol was able to significant increase the antioxidant capacity in the obese mice.

3.2. Effect of thymol on plasma adiponectin and leptin levels

Figures 3 and 4 shows plasma adiponectin and leptin levels in mice. After receiving HFD, the serum adiponectin level in obese mice was decreased, whereas leptin levels increased. However, adiponectin levels were increased in thymol – supplemented mice (P<0.05) (Figure 3).

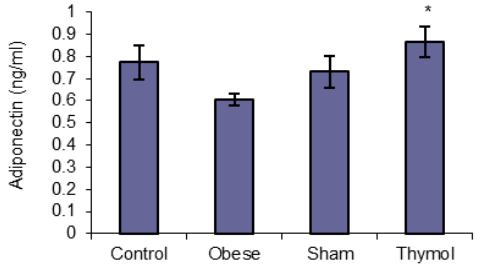

Figure 3. Effect of thymol supplementation on serum adiponectin in mice (n = 12 in each group) after eight weeks of treatment. Data are shown as mean ± SD.
*P<0.05 compared with the obese group.

Thymol -supplemented mice tended to have lower serum concentrations of leptin, but it was not significant compared to obese mice (P>0.05)(Figure 4).

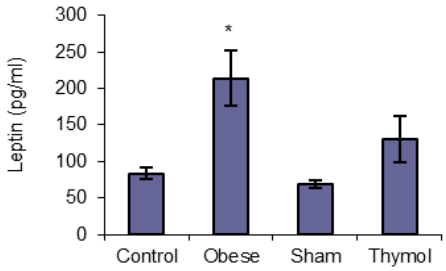

Figure 4. Effect of thymol supplementation on serum leptin in mice (n=12 in each group) after eight weeks of treatment. Data are shown as mean ± SD.
*P<0.05 compared with the control group

Obesity is associated with a chronic inflammatory response, characterized by abnormal adipokine

production, and the activation of some pro-inflammatory signaling pathways, resulting in the induction of several biological markers of inflammation (Ellulu *et al.*, 2017). Adiponectin is produced in the white adipose tissue and is known to have anti-inflammatory properties. Serum adiponectin levels depend on diet, physical activity, and inheritance (Nagaraju *et al.*, 2015). Thymol, a naturally occurring monocyclic phenolic compound, has been reported to exhibit anti-inflammatory properties in vivo and in vitro (Zhou *et al.*, 2014). The findings indicated that HFD decreased the sensitivity of adiponectin and increased the inflammation (Sakai *et al.*, 2013). The results of recent study showed that thymol has the significant increasing effect on adiponectin levels in the treated obese mice. Also, the leptin level was decreased by thymol, but not significantly**.**

3.3. Plasma liver enzymes levels, ALT and AST

After an HFD, the plasma ALT and AST levels in obese mice were increased, compare with control group. Upon treatment with thymol, the AST and ALT levels were significantly decreased, compare with obese group (Figure 5 and 6. P<0.05).

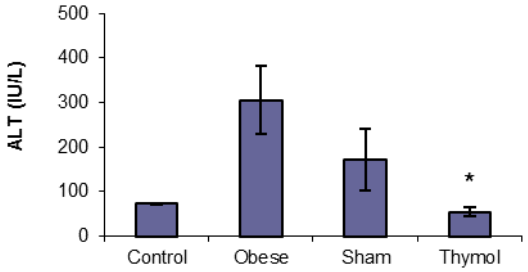

Figure 5. Effect of thymol supplementation on the serum ALT in mice (n=12 in each group) after eight weeks of treatment. Data are shown as mean ± SD.
*P<0.05 compared with the obese group.

The activity of liver function markers, including serum ALT and AST levels are clinically important indicators. As a consequence, to obesity and the subsequent liver disorder, ALT and AST levels may become elevated (Elizondo-Montemayor *et al.*, 2014). In recent study, ALT and AST levels significantly decreased by thymol treatment, these factors, increased in obese mice compared to control mice.

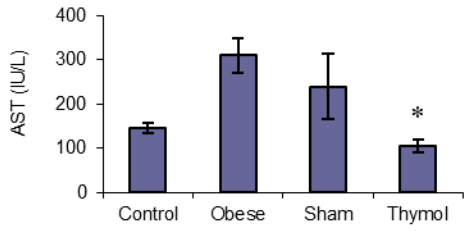

Figure 6. Effect of thymol supplementation on the serum AST (in mice (n = 12 in each group) after eight weeks of treatment. Data are shown as mean ± SD.
*P<0.05 compared with the obese group.

3.4. Body weight

During the second eight weeks of the experiment, treatment with thymol prevented the excessive weight gain (Figure 7), but not significantly (P>0.05).

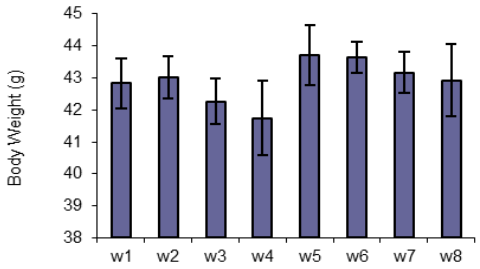

Figure7. Effect of thymol on body weight in obese animals in the second 8 - week study. Data are shown as mean ± SD.

This effect of thymol supplementation became apparent from the third week of treatment and was more pronounced at week 4. At the end of the treatment period by thymol, there were not significant reductions in body weight, but this compound prevented the excessive body weight in obese mice. The reduction in body weight is accompanied by a decrease or even a normalization of these biological parameters (van Dielen *et al.*, 2004). In the previous studies, it was demonstrated that thymol prevents HFD-induced obesity in murine model (Haque *et al.*, 2014). Ejaz *et al.* (2009) showed that polyphenols such as curcumin, may contribute to lower body fat and body weight gain.

3.5. Histological Studies

Hematoxylin- Eosin (H&E) staining of the adipose tissues taken from abdominal fat was shown in

Figure 8. After receiving HFD, adipocytes were larger than control mice. Interestingly, slides showed that thymol can affect fat tissue cells and they destroyed (Figure 8).

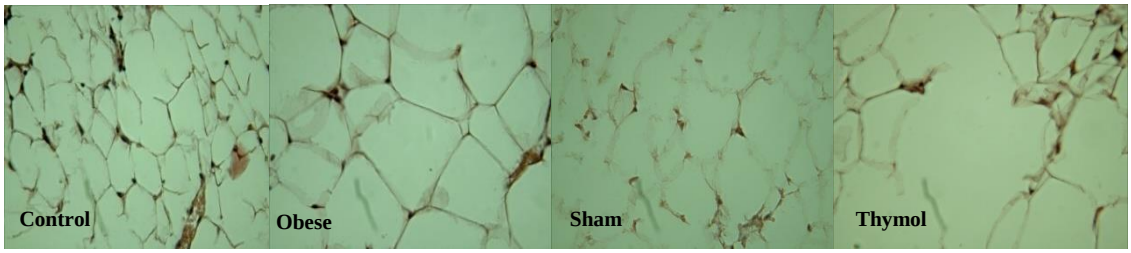

Figure 8. Thymol- supplementation on adipose tissue: representative stained sections of perinephric adipose tissue from Control, Obese, Sham and Thymol group at eight weeks of treatment.
Magnificent ×400.

Adipocytes diameter in HFD- mice were significantly higher compared to control mice ($P<0.001$), (Figure 9). Thymol-treated obese animals showed a significant reduction in fat cell size compared with obese mice ($P<0.001$). Obesity is a chronic metabolic disorder that is characterized by enlarged fat mass (Goossens, 2017). We demonstrated that thymol at concentrations of 12 mg/kg was capable to significant reduce of fat cell diameter and disruption of adipose tissue.

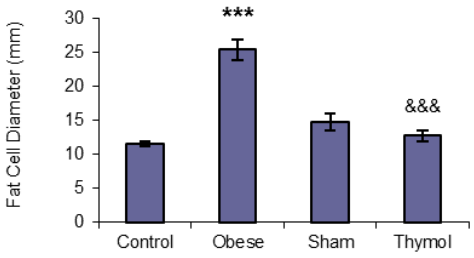

Figure 9. Effect thymol on fat tissue cells diameter.
Data are shown as mean ± SD.
***P<0.001 compared with the control group
&&&P<0.001 compared with the obese group.

4. Conclusion

In conclusion, our results demonstrated that thymol displays remarkable potential health benefits for prevention of obesity by disrupture in adipose tissue, improvement of inflammatory factors (TNF-α), leptin, liver enzymes, increase of serum TAC and adiponectin levels.

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