

## Effects of aqueous extract of lavender (*Lavandula officinalis*) on the liver tissue and blood fat in Balb/C adult female mice

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### Abstract

In addition to sedative and analgesic properties, aqueous extract of lavender is effective in the treatment of diabetes and rheumatism with antioxidant properties. However, the effects of extract have not yet been studied on the liver. In this study, effects of aqueous extract of lavender were studied on the liver tissue and blood fat in Balb/C adult female mice.

**Materials and methods:** Following preparation of aqueous extract, several doses of 500 (A), 300 (B), and 100 (C) mg/kg.bw were determined and injected intraperitoneally to 72 mice during 15 days. The mice were randomly divided into six groups, namely control (normal diet: 6 mice), control (fatty feed: 6 mice), sham (fatty feed and saline injection: 6 mice), and three experimental groups (each group with fatty feed and extract injection: 18 mice). Blood serum was obtained to measure triglyceride, cholesterol, HDL, and LDL levels. After dissection, the livers were removed and processed for sectioning. Data were analyzed using SPSS 21 software and also by Dunnett, Duncan, and Tukey tests providing  $P < 0.05$ .

**Findings:** According to the results, significant ( $P < 0.05$ ) accumulations of fat were observed in the livers of normal, sham, and Group C compared with the control group. A marked reduction ( $P < 0.05$ ) was also detected in the aggregation of fat liver in Group B. Group A displayed no accumulation of fat compared with the other groups but it showed significant drops ( $P < 0.05$ ) in triglyceride, LDL, and cholesterol levels.

**Conclusion:** In general, it can be concluded that high doses of lavender plant extract prevent accumulation of fat in the liver cells, hence, it could be used as a medicine for lowering blood and liver fats in the future.

**Key Words:** Lavender, liver, cholesterol, triglyceride, mice.

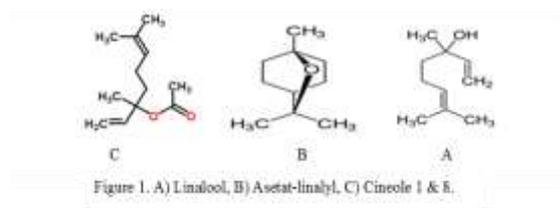
### Introduction

Hyperlipidemia or blood fat elevation is caused by metabolic problems arisen from multiple factors. Although lipid lowering agents are available, the disease is still one of the most important factors in aggravation of cardiovascular disease and also the first cause of death in industrialized countries. Factors such as patients' dissatisfaction of taking

common lipid-lowering drugs, side effects from long-term use of these medications, and the costs imposed on patients have caused increased tendency to alternative and traditional treatments (1, 2, and 3). Fortunately, extensive research has been conducted over the past 30 years on the effectiveness of medicinal plants used in traditional medicine in order to prove their effectiveness/ineffectiveness. Recent studies on dietary supplements, medicinal plants, and their compounds including dietary fibers, vitamins, flavonoids, sterols, and other antioxidant compounds have proven those compounds to be possibly effective in the treatment of many diseases through influencing the immune system and improving metabolism disorders of the body (5, 4).

Lavender has cumulative properties of analgesic, anti-Alzheimer's (memory booster), antiarrhythmic, anti-microbial, anti-convulsive, anti-inflammatory, anti-rheumatic, antiseptic, antispasmodic, anti-virus, anti-anxiety, carminative, anti-depressants, diuretic, menstrual stimulant, hypoglycemic, somniferous, digestive, sedative, and bile-inducing (6-12). It has also been studied in this examination because of the ease in collection and preparation of the extract. Lavender or *Lavandula* is a genus consisting of 30 different species from the family Lamiaceae (Labiataea) (13). The sedative effects of this plant have been confirmed in studies conducted by Woronuk et al. (2011) and Vanderploeg et al. (2010) (15, 14). Benbelaid et al. (2012) introduced the plant as a disinfectant (16) that can be used to disinfect wounds, burns, and insect bites. In veterinary medicine, it is used to kill lice and other parasites (17). Salehi Surmaghi (1999) stated that lavender tea could cause menstrual cycle in women due to an active ingredient called linalol (18). Issa Ala (2011) reported lavender to be effective in the control of diabetes. Lavender grows as a wildling in most parts of the world. Active ingredients of lavender are divided into different categories according to chemical structures, including essential oils, alkaloids, flavonoids, phenolic compounds, tannins, etc. (13). Among the most important ingredients of the essential oils are linalool (C<sub>10</sub>H<sub>18</sub>O), Asetat-linalyl (C<sub>12</sub>H<sub>20</sub>O<sub>2</sub>), and Cineole 1 & 8 (C<sub>10</sub>H<sub>18</sub>O) (Fig. 1).

Since the past to the present, this medicinal plant has been used to reduce blood fat. Therefore, the aim of this study was to determine the effects of lavender plant extract on fat accumulations in the liver and blood lipid metabolites (LDL, HDL, cholesterol, and triglyceride) in the mice of Balb/C strain.



## Method

**Preparing aqueous extract of lavender:** This plant species was identified at the Ornamental Plants Center of Koohsar Municipality in June 2014. The aerial parts were collected when they had flowering branches. The plant was first dried in a dark and non-moist place, powdered by an electric mill, and then an amount of 5.1 g of the powdered herb was added to 300 ml of boiling saline solution. The resulting solution was left for 10 min to allow brewing like that of tea followed by mild boiling on a magnetic hot plate (Ika, Germany) at 50 °C. The boiled mixture was then filtered through a sterile gauze and the filtrate was refined again with Wattman filter paper No. 4. The resultant solution was centrifuged (Hettich, Germany) in 4,000 rpm for 20 min. Afterwards, the solution was re-filtered and condensed at 50-60 °C to reach a final volume of 20 ml. In this condition, each ml of the prepared extract solution contained 1.5 g of the powdered plant.

**Preparation of fatty feed:** In order to raise blood fat in the experimental, sham, and control groups, a high-cholesterol diet was prepared with the following formulation. This meal contained 850 g of regular food powder per kg, 100 g of coconut oil, 20 g of cholesterol, 5 g of colic acid, and 25 g of wheat flour. After grinding, the ingredients were mixed with other ingredients of ready pellet food for mice, turned into a dough with saline serum, pelleted by a pastry funnel, and dried for two weeks, after which 4 kg of the high-cholesterol feed produced was fed to the mice *ad libitum*.

**Labomiceory model used:** The lab mice were purchased from the Razi Vaccine and Serum Research Institute (Hesarak, Iran) and were kept in the animal production room located at the Islamic Azad University, Karaj branch under controlled conditions of light, temperature, and humidity. The photoperiod, temperature, and humidity, respectively, were set as 12 hrs light/12 hrs dark, 22 °C, and 50 percent. The mice were

maintained in special mice cages in the animal room for two weeks in order to adapt to new environmental conditions. Each week, the cages were regularly washed and disinfected. The required experiments started after the mice had passed the two-week adaptation period under identical conditions. In this empirical study, 72 mice were randomly divided into six groups of control (normal diet: 6 mice), control (fatty feed: 6 mice), sham (fatty feed and saline injection: 6 mice), and three experimental groups (each group: fatty feed and extract injection: 18 mice). The first experimental group (Group C) was nominated as the one treated with a low-dose of lavender (100 mg/kg.bw) and fed fatty feed as the staple food. The second experimental group (Group B) was the group under lavender treatment with a moderate dose of 300 mg/kg.bw fed the fatty feed as the main diet. The third experimental group (Group A) was considered as the group under lavender treatment with a high-dose of 500 mg/kg.bw fed the fatty feed as the main diet.

**Experiments:** In all experiments, intraperitoneal injections were carried out periodically on both sides of the body (one day on the right and the next day on the left). In all the experiments conducted, the sham group was injected intraperitoneally the same amount of saline solution. All injections were performed after a fresh extraction during 15 consecutive days. After the mice had received the aqueous extract injections, they showed symptoms such as severe abdominal muscle contractions and severe leg muscle strains, especially in the mice injected a dose of 500 mg/kg.bw; the symptoms continued between 20 to 40 min following injection but then gradually declined and returned to normal conditions in about 1 h after injection. On the other hand, when the sham mice were injected the saline serum, they exhibited no such symptoms. During injection, the experimental mice had oily stool and the sham defecated constipated excreta. The next day of the last injection, the mice were weighed, dissected, their liver tissues removed carefully for further examinations, washed in physiological saline solution, and placed in 10% formaldehyde for fixation. After full fixation, the samples were undergone dehydration, transparency, smearing, and sectioning by microtome. Next, Oil Red staining was used to stain and study fat cells and the slides were prepared for examinations. During microscopy, changes in liver tissue was observed in the experimental mice. To measure changes in blood fat including triglyceride and cholesterol, blood samples were collected from the thorax and heart of the mice after they had been kept fasting for 12 h (to avoid errors in the experiments), and serum was isolated by centrifugation at 3000 rpm for 5 min. Serum

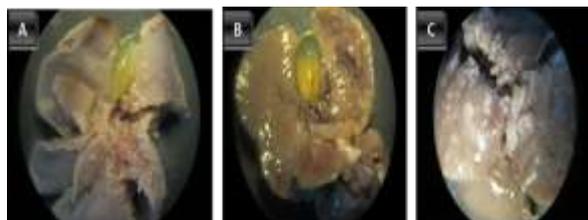
triglycerides, colorimetry (GPO-PAP), and total cholesterol, respectively, were measured by enzymatic methods, photometric procedures, and CHOD-PAP/Endpoint via a spectrophotometer (model UV- 2100).

**Statistical analyses:** Data were analyzed by SPSS 21 software through one-factor analysis, and through Dunnett, Duncan, and Tukey tests at significance levels of  $P < 0.05$  and  $P < 0.001$ .

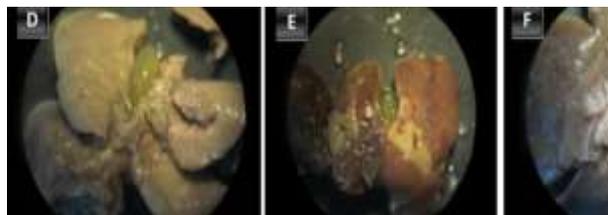
**Results**

**Liver macroscopic observations**

In the macroscopic examination of the liver, the mice were sacrificed, their livers isolated from the gastrointestinal tract, photographed in different groups, and their deformation evaluated qualitatively. Very little changes were observed in both the control and the sham groups with slight fat accumulations whereas no visible changes were obvious in the other groups.



**Figure 2.** Stereo photomicrographs of mice livers in groups A, B, & C (x 22)



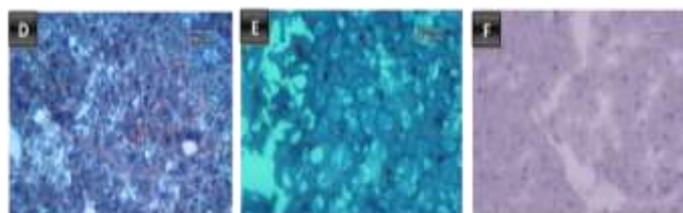
**Figure 3.** Stereo photomicrographs of mice livers in control (D), sham (E), & control (F) (x 22)

Based on the liver macroscopy, it can be stated that no fat cells were observed in the control group (D) because this group had a normal diet without excess fat. In the control group (F), a small number of fat cells were visible in the liver due to a high-fat diet. Sham group (E) displayed the highest fat cells as the control group fed high fatty feed and were injected saline serum, consequently, fat cells were seen everywhere in the liver of this group (Fig. 5.3). Fat cells were not observed in Group A because this group received fatty feed and high doses of extract leading to reduced amount of fat. In Group B, fat cells were noticeable but in very small quantities as this group fed fatty feed but was injected modemic doses of extract resulting in somewhat diminished levels of fat. A greater amount of fat cells was

seen in Group C than those in Group B treated with high-fat feed and a low dose of extract injection leading to decreased amount of fat (Figure 4.2).



**Figure 4.** Microscopic images of liver tissues from Group A without fat cells, Group B with very few fat cells, and Group C with low fat cells similar to the control group (Parabolic intersection; x 40)



**Figure 5.** Microscopic images of liver tissues from control without fat cells (D), sham (E) with high fat cells, and control (F) with a few fat cells (Parabolic intersection; x 40)

**Amounts of triglycerides, cholesterol, LDL, and HDL**

The levels of triglycerides, cholesterol, LDL, and HDL were measured after all the mice blood serum had been prepared. The results showed statistically significant differences among the four parameters (LDL, HDL, triglyceride, cholesterol) ( $P < 0.05$ )

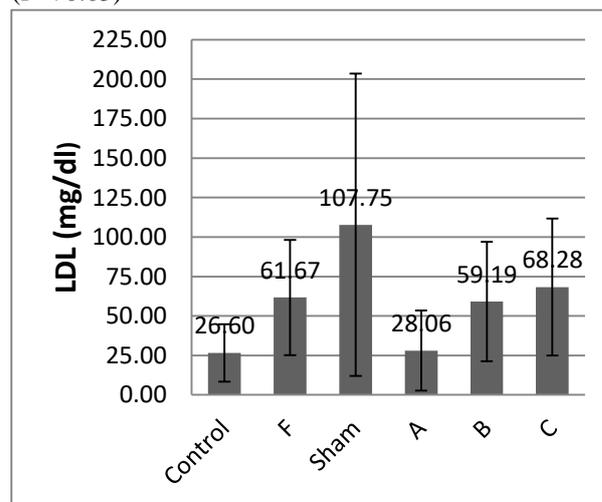


Chart 1. Amounts of LDL (mg/dl) in the normal, sham, control (F), and the experimental groups of C, B, & A

LDL changes in the C and sham groups significantly increased compared to the other groups. Group C received the least dose of extract in relation to groups B and A. Group A with the highest administered dose revealed a significant reduction ( $P < 0.001$ ) in the amount of LDL. A significant decline ( $P < 0.005$ ) was also recorded in Group B receiving a modemicee dose in comparison to Group C.

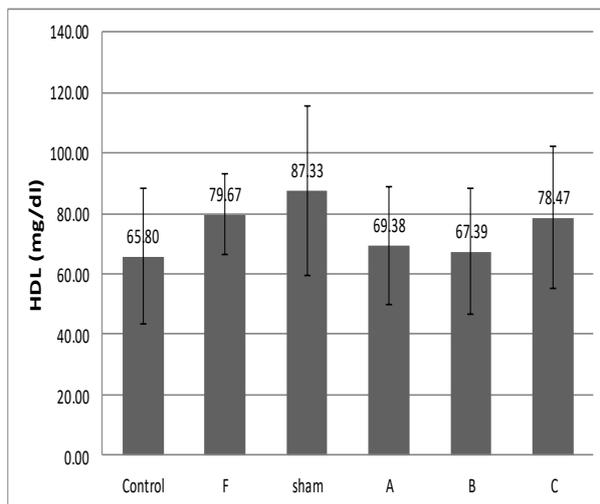


Chart 2. Amounts of HDL (mg/dl) in the control, sham, control (F), and the experimental groups of A, B, & C

Unlike LDL level, HDL changes in the C and sham were not significant compared to the other groups. However, HDL levels in groups A and B significantly decreased compared to Group C ( $P < 0.05$ ).

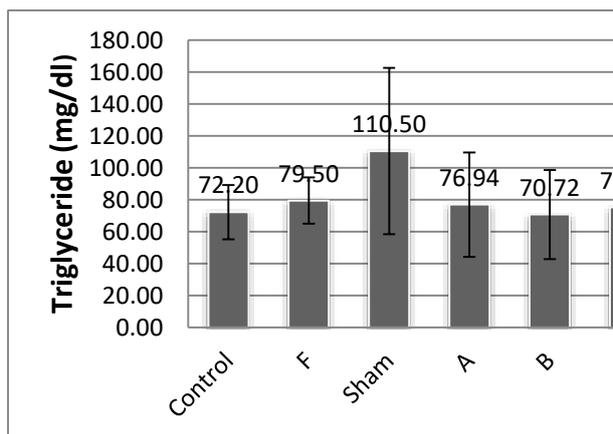


Chart 3. Amounts of triglyceride (mg/dl) in the control, sham, control (F), and the experimental groups of A, B, & C

The results of triglyceride indicate that none of the five groups studied were statistically different from the control group.

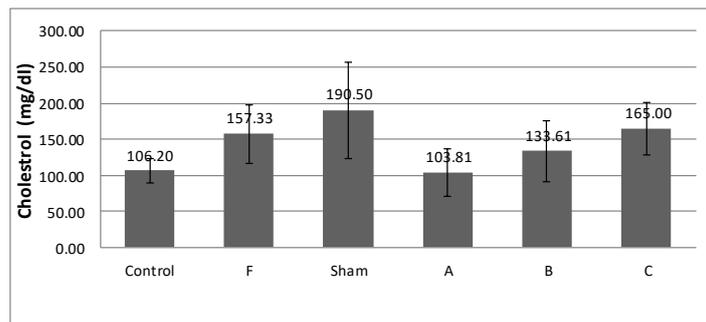


Chart 4. Amounts of cholesterol (mg/dl) in the control, sham, control (F), and the experimental groups of A, B, & C

The results showed that cholesterol levels in the control was not significantly different from the sham and C groups. But significant reductions ( $P < 0.001$  and  $P < 0.05$ , respectively) were found in cholesterol levels of groups A and B compared with the control.

**Discussion**

Lavender plant has lots of cumiceive effects on a variety of diseases and also possess antimicrobial and antioxidant properties. The main active ingredient of the plant is linalool, which is rich in phenolic substances (20, 19). This study investigated the effects of aqueous extract of lavender on the liver and blood fat of Balb/C mice. According to macroscopy of liver tissue and photometric serum measurements of the tested mice, valuable and acceptable results were achieved on the positive effects of this plant on the probable treatment of fatty liver and hyperlipidemia.

Examinations on the sections prepared from liver tissue demonstmiced that the control group fed normal diet developed no changes in the liver and liver cells being void of fat cells. In the control group fed fatty feed with no injections, a few fat cells were observed. The sham group fed a fatty meal with saline injections exhibited large numbers of fat cells. In the experimental groups, Group A with the highest extract dose did not show any fat cells similar to control group. Group B with a modemicee dose of extract displayed a small number of fat cells. And Group C with the lowest dose of extract presented the same numbers of cells as the control group.

Considering the diets given to the mice, the amount of LDL in Group A was much lower than the normal group and slightly higher than the control. Group B contained LDL levels slightly less than the normal group but higher than the control. Group C had greater amount of LDL compared to the control group. HDL level in Group A was somewhat lesser than the normal group but slightly greater than the control. HDL

levels in group B were lower than normal and slightly higher than the control in Group A. Group C showed slightly lower HDL concentrations than the normal group but slightly higher than the control.

Blood triglyceride levels in Group A were a little lower than the normal group and slightly greater than the control. Group B contained lower triglyceride levels than both the normal and control groups while this fat in Group C was slightly lesser than the normal and slightly higher than the control group. The amount of cholesterol significantly decreased in Group A so that it was much lower than even levels of cholesterol in the normal group but slightly lower than that in the control. In Group B, cholesterol levels were a little lower than the normal group and somewhat greater than the control. Group C displayed slightly higher cholesterol levels than the control group. As mentioned, the sham group had the highest cholesterol content even higher than the normal group. It can be explained that intraperitoneal injection of normal saline close to the intestine in the sham group (where digestion takes place) provided required water with the fats leading to increased blood fat in this group.

In this study, it was observed that consumption of high-fat feed elevated blood cholesterol and accumulation of fat in the liver but fat metabolites in the blood and liver tissue decreased following administration of different doses of the extract (Figs. 1, 2, and 3). These are similar to a study by Ramchoun et al., who reported that lavender's properties of antioxidant and free radicals absorption can be effective in reducing cholesterol and triglycerides (20). Dragland et al. pointed out that taking one gram of spices with antioxidant properties had significant impacts on health (21), which corresponds with the results of this research. High-phenol essential oils and extracts are significantly associated with antioxidant properties. In addition, ingredients such as dietary fiber, vitamins, flavonoids, sterols, and other antioxidant compounds contained in dietary supplements and herbs can influence metabolism reactions at different tissues through affecting lipid metabolism. The above studies somehow confirm the accuracy of our results, hence, the results obtained correspond to them.

According to the findings, a high dose of lavender extract yielded better consequences and resulted in significant decreases in cholesterol and LDL levels known as the dangerous fats. Although the extract was also effective in reducing triglycerides and HDL concentrations, these drops were not significant. On the other hand, the use of high doses of the extract can cause allergies as hair losses observed in the injection area in the mice received high doses of the extract during the

experiment, which need further research to be verified.

### **Conclusion**

The results of our observations suggest that high-dose aqueous extract of lavender had significant impacts on reducing blood LDL and cholesterol levels whereas it showed little impacts on decreasing triglycerides and HDL concentrations. It was also found that low doses of the extract, though not significantly, reduced both cholesterol and LDL. After all, high doses of the aqueous extract of lavender are more effective for reducing blood cholesterol but necessary precautions must be taken for the use of high doses of the extract to avoid resultant allergies.

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