

## Evaluation of antioxidant activity of *Citrus limon* L. essential oil and its effect on some blood parameters in Moghani sheep

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**Abstract:** Background and objectives: Plant food additives have received widespread attention in the recent years. Plants have different components such as essential oil. Citrus family is known as antioxidant resource. Citrus limon among citrus family contains limonoids, flavonoids and phenolic compounds. Regarding to this point that there are few researches about using Citrus limon essential oil in ruminant nutrition, the aims of this study were the evaluation of, antioxidant activity of Citrus limon and the effect of different levels of this essential oil on some blood parameters of Moghani sheep. Materials and methods: In this study, 6 lactating Moghani sheep were allocated to 3 × 3 Latin square design with three diets in twenty-one day periods, seventeen days for adaptation and four days for sampling. Dietary treatments included: Treatment 1: control diet, treatment 2: control diet with 150 mg essential oil per day and treatments 3: control diet with 300 mg essential oil per day. To evaluate the antioxidant effect of the essential oil, sustainable elimination of free radicals by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used. Blood profiles included glucose, blood urea nitrogen, albumin, total protein, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, triglycerides and very low density lipoprotein (VLDL) were also measured. Results: The antioxidant activity of essential oils at doses of 27.5, 55, 110, 220 and 480 mg/ml were 5, 8, 21, 49 and 85 percent, respectively. In this study, experimental treatments did not affect glucose, blood urea nitrogen, albumin, total protein, low density lipoprotein were, while increased the concentration of high-density lipoprotein. Cholesterol, triglycerides and very low density lipoprotein were decreased by our treatments significantly (P <0.05). Conclusion: According to the results of this research, the evaluation of antioxidant effect of Citrus limon using DPPH method showed that the antioxidant effect of Citrus limon in free radical removal is strong. In addition, this essential oil has decreasing effect on the blood plasma lipids.

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**Keywords:** Citrus limon, Antioxidant activity, Blood parameters, Sheep

### 1. Introduction

Plant food additives have received widespread attention in the recent years. Plants have different components such as essential oil. Essential oils, which are generally healthier food additive known as a safe alternative to antibiotics have been proposed (17). Essential oils of plant secondary metabolites that include aromatic compounds and volatile through steam distillation of some plants are obtained (26) and has multiple biological properties including antioxidant properties. Sretenovic et al. (2007) showed that adding antioxidants to the diet of dairy cows decreased milk somatic cells and breast infection and increase the quality of protein and milk fat (20). Velasco and Williams (2011) stated that adding extracts of oregano, sage and rosemary to animal feed can be fat oxidation during storage in the refrigerator or freezer time delay, and in this way to preserve meat quality (24).

Citrus family is known as antioxidant resource (13). Citrus limon among citrus family contains limonoids, flavonoids and phenolic compounds (2).

Numerous reports about the existence of a positive correlation between phenolic compounds and antioxidant activity of the extracts and essential oils provided, so that the antioxidant properties of fruits and vegetables significantly in the presence of high amounts of polyphenolic compounds increases (23). Phenols and flavonoids plant quell proxy radicals and restore the iron in enzymes inhibits peroxidation of lipids are salinity (21).

In a study on the antioxidant activity of the extract of lemon and progression of atherosclerosis in rabbits receiving high-cholesterol diet was evaluated. The results showed that taking the extract increased antioxidant capacity and levels of fatty streaks in the coronary arteries and the aorta is significantly decreased (4). In the process of atherosclerosis, where plaque inside the arteries layer is deposited, increasing the oxidation of low-density lipoprotein cholesterol concentration, the onset of the disease is essential. The disease can be stopped by preventing the oxidation of low-density lipoprotein. It has been shown that plant

extracts with antioxidant properties can prevent the oxidation of low-density lipoprotein (8).

Samadian et al. (2013) showed that adding meat to the diet of lemon essential oil reduces redness and yellowness of meat after slaughter is increasing, the use of this oil at a high level (150 mg per kg of diet) can significantly increase the brightness of the meat. The researchers stated that this occurred due to the antioxidant effect of the essential oils, because due to endogenous antioxidants discharge during storage of meat, fat and muscle proteins are sensitive to oxidative modification and factors affecting lipid oxidation in meat can affect color quality meat (15).

According to previous findings and the importance of finding viable alternative to antibiotics and the use of natural ingredients in the food chain animals and also due to the fact that limited research on the use of essential oils of lemon on feeding

ruminants has been made, the aim of this study was to evaluate the performance of antioxidant lemon essential oil to laboratory methods and Effects of essential oil of lemon on some blood parameters in Moghani sheep.

#### Materials and Methods:

In this study, six dairy Moghani sheep were considered and the average days of lactation  $42 \pm 5$  and BW  $56 \pm 5$  kg in Latin square design rotor  $3 \times 3$  with three dietary treatments in three rounds of twenty-one days of the seventeen days of adaptation and four days were dedicated sampling period. Experimental diet with a ratio of 64% forage and 36% concentrate and nutritional requirements based on the recommendations of NRC (2007) was adjust (Table 1).

Table 1. Nutrient composition of ingredients and diets (based on absolutely dry matter)

Treatments <sup>#</sup>	Case	1	2	3
Feed components (in terms of dry matter)				
Alfalfa		40	40	40
Corn silage		21	21	21
Barley		31	31	31
Wheat bran		4	4	4
Mineral-vitamin supplements		1	1	1
Dietary nutrient composition (in terms of dry matter)				
Metabolizable energy (megacalories/kg dry matter)		2.47	2.47	2.47
Total digestible nutrients (kg dry matter)		65.72	65.72	65.72
Neutral detergent fiber		39.07	39.07	39.07
Acid detergent fiber		27.1	27.1	27.1
Calcium		0.53	0.53	0.53
Phosphorus		0.34	0.34	0.34
Crude Protein (CP)		10.3	10.3	10.3

\* Dietary treatments included: Treatment 1: control diet, treatment 2: control diet with 150 mg per day of oil and treatment 3: control diet with 300 mg per day of oil.

Dietary treatments included: Treatment 1: control diet, treatment 2: control diet with 150 mg per day of oil and treatment 3: control diet with 300 mg per day of oil. Essential oil of lemon (Citrus limon) were obtained from Livestock. Throughout the trial period, the animals had free access to water and minerals were administered orally in the form of blocks. In order to determine the concentration of blood parameters of all sheep fed three hours after eating the morning meal of treatments, after disinfection blood samples from the jugular vein at a rate of 10 ml were taken. Blood serum samples using centrifugation at 2500 rpm for 20 min separated at 4 °C. The serum was isolated to measure blood metabolites were sent to the laboratory medical

university. Blood metabolites to include glucose, urea nitrogen, albumin, total protein, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol, triglycerides and very low density lipoprotein (VLDL), respectively.

Antioxidants remove free radicals to inhibit lipid oxidation accepted mechanism. Sustainable elimination of free radicals by 2,2-diphenyl-1-picrylhydrazyl (DPPH) to evaluate the antioxidant activity of specific combination or the extract is used in a short time (5). 2,2-diphenyl-1-picrylhydrazyl (DPPH) a stable radical combinations with purple, which has revitalized by the elements or hydrogen electron donor (antioxidant compounds) in diphenyl (DPPH) turns yellow. The ability of different

concentrations of hydrogen atoms or electrons by the way colorless or reduce the amount of UV absorbance of the solution is measured DPPH in methanol (6).

To study the antioxidant effect of the essential oils mentioned method 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used. For this purpose, DPPH dissolved in ethanol was prepared at a concentration of  $10^{-4}$   $\mu$ . One ml of different concentrations of the sample was mixed with three mL of DPPH solution and for one hour at room temperature and then the intensity of light absorption at 517 nm was determined and the percent inhibition of free radicals was calculated by the following formula (5):

$$\% I = (A_B - A_S) / A_B \times 100$$

Statistical analysis to examine the relationship between the experimental groups using one-way ANOVA followed by Duncan test was conducted and the difference in levels ( $p < 0.05$ ) were considered.

### Results and discussion

Antioxidant Effects of essential oil of lemon in using DPPH, the antioxidant activity of the extract in different concentrations, respectively, 27.5, 55, 110, 220 and 440 milligrams per ml was observed that the highest percentage of free radical elimination of the concentration of 440 milligrams per ml (85%) and the lowest concentration of 5.27 mg/ml (5%). Remove the free radicals treatments for 55, 110 and 220 milligrams per ml, respectively, 8, 21 and 49 percent, respectively. The results showed a significant

relationship ( $p < 0.05$ ) among the different amounts of essential oils (Figure 1).

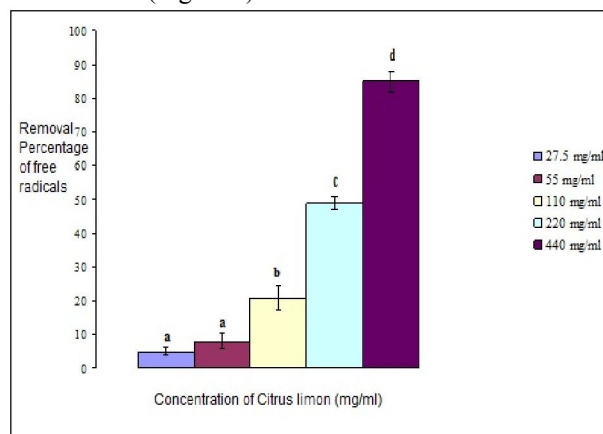


Figure 1. The antioxidant activity of different concentrations of the essential oil of lemon to the method of DPPH. Dissimilar letters in the columns indicate significant differences ( $p < 0.05$ ).

Table 2 compares the average of some blood metabolites of lemon essential oil has been shown in different treatments. The results show that lemon essential oil has a significant effect on serum glucose, urea nitrogen, albumin, total protein, low-density lipoprotein (LDL) ( $P > 0.05$ ). The essential oil of lemon increased the concentrations of high-density lipoprotein (HDL) whilst cholesterol, triglycerides and very low density lipoprotein (VLDL) significantly ( $p < 0.05$ ) decreased in between treatments (Table 2).

Table 2. Effect of lemon essential oil on blood parameters (in milligrams per deciliter)

Treatment				SEM	P
Case	1	2	3		
Cholesterol (milligrams per deciliter)	62.71 <sup>a</sup>	59.13 <sup>b</sup>	50.62 <sup>c</sup>	0.5	0.019
Triglycerides (milligrams per deciliter)	21.5 <sup>a</sup>	19.16 <sup>ab</sup>	19 <sup>b</sup>	0.98	0.014
High-density lipoprotein (milligrams per deciliter)	20.66 <sup>a</sup>	24.16 <sup>b</sup>	25.49 <sup>c</sup>	1.49	0.037
Low density lipoprotein (milligrams per deciliter)	36.7	33.66	35.2	2.14	0.25
Very low density lipoprotein (milligrams per deciliter)	4.3 <sup>a</sup>	3.83 <sup>ab</sup>	3.8 <sup>b</sup>	0.19	0.013
Glucose (milligrams per deciliter)	52.33	53.16	52.69	1.06	0.69
Urea nitrogen (milligrams per deciliter)	24.63	25.16	25.37	4.5	0.14
Albumin (milligrams per deciliter)	3.73	3.70	3.63	0.07	0.26
Total protein (milligrams per deciliter)	6.74	6.52	6.10	0.16	0.29

\* Dietary treatments included: treatment 1: control diet, treatment 2: control diet with 150 mg per day of oil and treatment 3: control diet with 300 mg per day of oil.

Dissimilar letters in each row indicate significant differences ( $p < 0.05$ ).

Based on the results of the present study was to evaluate the antioxidant activity of essential oil of lemon using DPPH showed strong antioxidant activity

of essential oil of lemon is the removal of free radicals, the property may be related to phenolic compounds and flavonoids found in lemons (11). The

antioxidant properties of essential oil of lemon has been confirmed in other studies (12). A study shows the power of Lemon approximately Lipid peroxidation inhibition by synthetic antioxidants BHA and BHT or more of power (19). In another study of lemon extract on lipid peroxidation and activity of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase has been studied in mice. The results of this study showed that lemon extract increased antioxidant enzymes and lipid peroxidation levels were significantly reduced (7).

In this study, experimental treatments on glucose, urea nitrogen, albumin, total protein, low-density lipoprotein (LDL) had no effect. The essential oil of lemon increased the concentrations of high density lipoprotein (HDL), whereas the concentration of cholesterol, triglycerides and very low density lipoprotein (VLDL) declined. Production control cholesterol and prevent its rise could be due to Trippine in Lime (22). Nobakhat and Amiri (2014) showed that the use of dried lemon pulp decreased low-density lipoprotein cholesterol in the turkey meat, also in this study, it was shown that the use of this waste will be significantly reduced abdominal fat turkeys (14). In another study it was shown that the addition of citrus juice (orange and lemon) broiler rations reduced total cholesterol in the blood (1). In the present study, similar results were obtained in total cholesterol in the blood of sheep.

In one study, the effects of essential oils of lemon in male Wistar rats suggests that low levels of essential oils on cholesterol and low-density lipoproteins, triglycerides lowering effect of weak, but the amount is not affected. Moderate and high levels of essential oils also significantly decreased total cholesterol, triglycerides and low-density lipoprotein (27). The results of another study showed liver and plasma cholesterol levels in the blood of mice that received essential oils of lemon peel, this reduction is due to lower flavonoid content of the oils (3).

Another is to add antioxidants to the diet of an animal can be considered as human dietary source of antioxidants and provide beneficial effects in gastrointestinal tissues and other tissues in the human body. Mutagenicity and carcinogenicity concerns about synthetic antioxidants have been proposed, the new increased attention to the use of natural antioxidants to replace synthetic antioxidants (16). Citrus fruits and juices are a very good source of bioactive compounds, including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectin, and of great importance in human nutrition are important. The antioxidant activity of phenolic compounds such as flavonoids and phenols found in lemons can improve in reducing the risk of cardiovascular disease (11).

Based on the results of research studies, herbs and antioxidants reduces the absorption of fats, stimulates the secretion of cholesterol via the bile and excretion of cholesterol through the feces. Also, some herbs inhibit lipoproteins, enzymes and proteins that are involved in the metabolism of lipids and lipoproteins, and thereby reduce the lipid profile (10). In a study in which the effect of lemon juice on blood plasma and liver fat mice, it was shown that the high fat and lemon polyphenols significantly to the receptor activator of peroxisome fat diet high and Low fat diet (that had no polyphenols lemon), in the liver increased. These receptors induces the expression of enzymes involved in the oxidation of fatty acids. The results showed that levels of triglycerides in mice fed a diet containing high fat and polyphenols lemon fed to mice fed diets high in fat and low fat diet (that had no polyphenols lemon ) significantly decreased. The level of serum phospholipids with high fat mice that were fed a lemon and polyphenols, compared to mice fed a high fat diet was significantly less. The researchers concluded that lemon polyphenols can prevent weight gain and accumulation of fat in the body by increasing the effects of beta oxidation of fats zoom proxy (9).

The results of the effect of lemon essential oil on nutrient digestibility and rumen fermentation of Holstein bull calves showed that treatment effects on glucose, high density lipoprotein, albumin, total protein, creatinine, triglycerides and urea means not significant but reduced serum cholesterol and beta-hydroxybutyrate concentrations (18) that some of the results of this study also corresponded with the findings. There are differences in the results of different studies in ruminants may be due to differences in the composition of essential oils used, diet and rumen fluid compatibility is to add a mixture of essential oils (25) the results of the study of this issue is no exception. In general, the results of this study showed that lemon essential oil has strong antioxidant properties and the effect of lowering the blood plasma lipids. Probably the essential oil of lemon with a decrease in plasma lipids and their antioxidant properties could play an important role in strengthening the antioxidant defense system of cattle. On the other hand we can extract oil or lemon or lime and other citrus pulp as an alternative to antibiotics used in ruminant nutrition. Because of lack of information and limited data on the use of herbal extracts and essential oils in ruminants to clarify the mechanism of action and mode of action and their applications in improving the performance of ruminants, further research seems necessary.

**Conclusion:**

Based on the results of the present study was to evaluate the antioxidant activity of lemon essential oil showed strong antioxidant activity using DPPH oil of lemon is the removal of free radicals. However, the essential lipid lowering effects on blood plasma. According to these results, this oil can be used to reduce the effects of oxidative stress induced by various stresses, strengthening the immune system and improve the performance used in ruminants.

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