Investigation of Changes in Biochemical Factors in the Serum of the Persian Shepherd Dogs due to Consumption of *Thymus daenensis* Extract

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Abstract

BACKGROUND: Recently, the researches about herbal medicines as substitutes for chemical drugs have risen in number.

OBJECTIVES: To add more aspects to this research area, this study evaluated the changes in biochemical factors of the blood serum due to the consumption of *Thymus daenensis* extract and the effect of the period of its consumption into the diet of herd dogs.

METHODS: Eight adult male dogs were chosen and fed with a fixed diet for 1 month. Then they were randomly assigned to two groups of four. The members of first (control) group were fed only with the fixed diet and the second group received 200mg/ml, administered as 1ml/kg of *Thymus daenensis* extract into their fixed diet. Finally, the dogs were bloodlet at 3 different times, namely before intervention, 7 and 14 days after the intervention to measure the factors of glucose, cholesterol, triglyceride, LDL and HDL, alanine aminotransferase and aspartate aminotransferases.

RESULTS: Comparing to the control group and the starting point of this experiment, levels of FBS, LDL, TGL, CHOL, AST factors significantly decreased 14 days after intervention, but the HDL factor increased after 7 and 14 days of intervention (P<0.05). The level of ALT factor did not change during period of the study (P> 0.05).

CONCLUSIONS: Consequently, oral consumption of *Thymus daenensis* extract led to decrease in the amounts of glucose and lipids of blood and this herb can be recommended for treating diabetics and persons suffering from high cholesterol.

KEYWORDS: Biochemistry, dog, serum, *Thymus daenensis*

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Introduction

Nowadays, many patients have resorted to the herbal medicine for treatment. On the other hand, new medicine despite its capabilities and merits, has problems in treating some diseases, especially chronic ones. In fact, there has been remarkable data on the use of herbal medicine in recent years. Generally speaking and disregarding exceptions, these medicines have less side effects than other drugs (Dolatkhahi et al. 2010). Diabetes mellitus whose prevalence –based on predictions– will increase in human beings' societies in the future is clinically one of the most common endocrine gland diseases. Failure to properly control diabetes would cause disorders such as Nephropathy, Retinopathy, Neuropathy, and heart disease (Nasri, Hajializadh, and Esmaeili-Mahani 2014). Although the use of insulin and chemical medicines are already the main and effective treatment for diabetes mellitus, these compounds have plenty of side effects such as increase of lipid storage (lipotrophy), loss of fat tissue in the injection site and outbreak of hypoglycemic shock, and they do not influence on the developmental process of diabetic disabling complications in the long term. Considering the growth of human's knowledge around the heterogeneity of this disease, the need of finding effective compounds with minimum side effects for the treatment of diabetes and its resulting disorders is felt (Musazadeh and Motavally 2009; Nasri, Hajializadh, and Esmaeili-Mahani 2014). Many drugs have also been used to prevent and treat hyperlipidemia in humans and in other species. They include Atorvastatin, fish oil containing omega-3 fatty acids, Gemfibrozil, Niacine and garlic. Among them Atorvastatin with a daily dosage of 5 mg/kg intake for 6 weeks has been significantly effective in decreasing total cholesterol, phospholipid and triglyceride levels in dogs.

Atorvastatin has anti-fatty effects in the blood through the competitive inhibition of the 3-hydroxy-3-methylglutaryl-coenzyme a reductase enzyme. This is an early stage in the biosynthesis of cholesterol. Hepatotoxic effects including increase in liver enzymes such as Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP), and also ceasing the outflow of bile are amongst the side effects of this drug in dogs (Mosallanejad et al., 2018). Unfortunately, the overuse of chemical drugs for treatment of these cases is the great disadvantage of modern medicine, therefore dangerous side effects are consequences of the frequent use of these drugs. For example, the most prevalent side effects of Atorvastatin in humans are namely digestive problems, dyspepsia, abdominal pain, headache and myopathic muscular complications, and hepatotoxic effects (increase in enzymes levels of liver such as ALT, AST) and less prevalent reported side effects are damage to optic nerves and intracranial hemorrhage (Haroun, Mahmoud, and Adam 2002). The herb we are taking into consideration in this study is *Thymus daenensis* Cleak. Thymus is a type of indigenous Iranian herb that is commonly found in the slope of hills and mountains or in gardens. This herb grows short and shrubby and has many medicinal and nutritional uses. All of the aerial parts of this herb –top branch trimmings in particular– have medicinal use. The most important species of thymus in Iran are *Thymus daenensis*, *Thymus serpllum, Thymus vulgaris, Zataria multiflura Boiss*. Thymus is a fragrant (aromatic) herb from the family of Lamiacea. *Thymus daenensis* is a lamia-

ceae herb and contains compounds of tannin, flavonoids, glycosides, caffeine and Rosemary acids (Lee et al. 2005; Mahmoudabadi, Dabbagh, and Fouladi 2007; Mokhberi et al. 2010) This herb has strengthening effect and digestive, antispasmodic, carminative, antifungal, antibacterial, antiseptic, anticonvulsant, vermicide, anti-rheumatic, expectorant, anti-rheumatism and antioxidant properties. Thymus extract has an extremely antioxidant effect which in addition to lowering blood lipids, can play a role in inhibiting LDL oxidation process. The thymus also reduces triglycerides and cholesterol levels of blood (Nazari et al. 2013).

Considering that studies on the hypoglycemic-hypolipidemic effects of *Thymus daenensis* cleak and on general changes in serum biochemical factors resulting from the use of its extract in the animal type samples (i.e. dogs) are limited; therefore, the present study aims at investigating the effects of thymus on the biochemical factors including Fasting Blood Sugar (FBS), Cholesterol (CHOL), Triglyceride (TGL), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST). The results are probably extendable to human beings.

Materials and Methods

To do this investigation, 8 adult male Persian Shepherd dogs with a weight range of 30-35 kg were used. During the experiment, the dogs were kept in separate cages under standard conditions at 25-30 °C and in the 12-hour light-dark cycle, so that they could easily access water and food. The dogs were fed with the same invariant diet (cooked chicken, i.e. chicken heads and paws) for 1 month. Also, dogs were given anti-parasitic

medications of Mebendazole and Praziquantel (given based on recommended dosage of them) two weeks before the start of the study to remove any digestive parasitic disease, they have also been cleansed of skin parasites through being injected twice with Ivermectin and their skin was washed with anti-parasitic shampoo. Then their health was thoroughly examined before starting the study. All of their vital signs, such as heart rate, respiratory rate, body temperature, and blood pressure, were checked and recorded. All of the dogs were examined at least twice a day and were kept in separate cages; the place they were kept was rinsed every day; its floor and the cages were disinfected once every 4 days and all of the reports were recorded (Mosallanejad et al. 2018; Vahid Dastjerdi, Jafarian Dehkordi, and Torkan 2017). This study was carried out at Veterinary Hospital of Islamic Azad University of Shahrekord, located in Kian, Iran. Dogs were randomly divided into two groups of four. The first group was a control group that did not receive any thing to eat other than water and food. The group 2 was the receiver of 200mg/ml and administered as 1ml/kg amount of *Thymus aenensis* Cleak extract twice a day (every time 100 mg/kg) for 7 and 14 days. The dosage assigned was based on the dose prescribed for lab rats rat (Yaghmaei, Heydarian, and Poorbahman 2012). After *Thymus daenensis* Cleak was provided for this research from Shaheed Fozveh research station located in Najaf Abad in Isfahan province, its type and species were identified and confirmed by the Agricultural and Natural Resources Research Center of Isfahan Province. Then its specimen was kept in Herbarium unit of the aforementioned center (Herbarium number 13713). The collected samples dried in the shadow were then powdered by a grind-

er. Thymus powder was poured into a glass container; 96% medical alcohol was added; its lid was tightly closed, following this the mixture was given 72 h to soak well. After the specified time passed, the extract of this mixture was taken through percolation method (extraction under pressure). It was condensed by Rotary Evaporator and then desiccated as much as possible by Desiccator. For use, some sterilized distilled water according to desired density was added to the solid extract. Finally, the animals under investigation were fed with a specified dose of the acquired extract twice during 24 h and for 14 days. To blood let, blood vessels of front paw (Cephalic veins) were used in this manner: the dogs were restrained; hairs covering the target place were shaved; then it was disinfected with some cotton and alcohol; and blood letting was done gently. Collecting blood specimens was done for each dog individually three times on the 1st , 7th and 14th day during the research using syringes of 5 cc blood capacity. Afterwards the specimens were transferred to the separate and marked test tubes. When the specimens had been collected, they were immediately transferred to the laboratory. They were centrifuged inside the laboratory, frozen after the serum separation by sampler and then transferred to separate and specific micro tubes. The intended factors —comprised of FBS, cholesterol (CHOL), triglyceride (TGL), low density lipoprotein (LDL), high density lipoprotein (HDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST)— were measured via an enzymatic and photometric method (Pars Azmon kits) using Italian Eco-Plus Autoanalyzer (2006) with serial number of GD100139, in a laboratory equipped with a spectrophotometer. Finally, the collected data were entered into SPSS (V20) software and because the results obtained from the Shaipro-Wilk test represented the normal distribution of the data, independent t-test was used in order to compare the means of blood parameters between the two groups at times, and ANOVA test was employed to compare the mean changes in blood parameters in each of the two groups after 14 days. In all stages of data analysis, the significance level less than 0.05 was taken into consideration

Results

In the present study, FBS level was not significantly different in two groups before intervention (P value > 0.05) but 14 days after the intervention, the average level of FBS (42.50 ± 9.27 mg/dl) in the group receiving the *Thymus daenensis* celak (treatment group) was significantly lower than its average level (59.50 ± 9.21 mg/dl) in the control group (P value = 0.045). In addition, after 14 days, the average level of FBS in the control group did not change significantly (P value = 0.760), but it had a significant decrease in the treatment group (decrease FBS mg/ml: 16.75; P value = 0.001, Table 1).

With respect to average levels of AST and ALT factors of the two groups under investigation, although there was no significant difference before the intervention (the starting point of this experiment) and 7 and 14 days after the intervention (P value > 0.05), there was a significant decrease in the AST level of the treatment group over 14 days (AST reduction: 18,000 u / l; P value = 0.035, Table 2).

Before intervention, there was no significant difference between the two groups regarding the levels of LDL and HDL factors (P value > 0.05); however, on the 14th day, the average level of LDL (37.50 ± 3.00 mg/

dl) in the group receiving *Thymus daenensis* celak (treatment group) was significantly lower than its average level in the control group (51.13 ± 3.56 mg/dl, P value = 0.001). Furthermore, on the 7th and 14th day, the average amounts of HDL level in the treatment group (216.25 ± 18.84 mg/dl and 193.25 ± 33.37 mg/dl respectively) were more than its average amounts in the control group (138.37 ± 12.46 mg / dl and 141.62 ± 27.52 mg/dl respectively,

P value > 0.05). On the other hand, while the changes in LDL and HDL levels over the period of 14 days were not significant in the control group (P value > 0.05), there was a significant decrease in the LDL level (decrease: 16; P value = 0.046) and there was a significant increase in HDL level (increase: 57.75; P value = 0.022) in the treatment group in this period compared to the time before intervention (starting point of this experiment).

Table 1. Comparing the average level of FBS between the two groups at different time intervals

Variable	Time	Control Group	Case Group	P value[*]
FBS(mg/dl)	Before the intervention	62.50 ±18.57	59.25± 19.82	0.819
	7 days after intervention	63.00± 22.32	56.25± 21.01	0.675
	14 days after intervention	59.50± 9.21	42.50 ±9.27	0.045
	P value[**]	0.760	0.001	

Data shown as mean ± SD.

[*]: Significant level of Independent Sample t-test for comparison between two groups

[**]: Significant level of repeated measure ANOVA for comparison by passing time in each group

Table 2. Comparing the average level of AST and ALT between the two groups under investigation at different time intervals

Variable	Time	Control Group	Case Group	P value[*]
AST(u/l)	(0) Before the intervention	49.75 ±14.57	50.25 ±17.35	0.966
	7 days after intervention	48.25± 15.32	35.00± 7.39	0.170
	14 days after intervention	51.13 ±13.56	32.25± 8.38	0.055
	P value[**]	0.769	0.035	
ALT(u/l)	(0) Before the intervention	27.25± 9.15	25.50± 10.25	0.807
	7 days after intervention	25.38± 12.53	21.50±8.96	0.632
	14 days after intervention	29.50± 10.87	15.00± 4.96	0.051
	P value[**]	0.812	0.123	

Data shown as mean ± SD.

[*]: Significant level of Independent Sample t-test for comparison between two groups

[**]: Significant level of repeated measure ANOVA for comparison by passing time in each group

Table 3. Comparing the average level of LDL and HDL between the two
groups under investigation at different time intervals

Variable	Time	Control Group	Case Group	P value*
LDL(mg/dl)	(0) Before the intervention	49.00 ±14.57	53.50± 8.96	0.617
	7 days after intervention	48.25± 15.32	49.25 ±14.56	0.928
	14 days after intervention	51.13± 3.56	37.50± 3.00	0.001
	P value**	0.628	0.046	
HDL(mg/dl)	(0) Before the intervention	140.25 ±26.81	135.50 ±37.86	0.844
	7 days after intervention	138.37 ±12.46	216.25± 18.84	<0.001
	14 days after intervention	141.62± 27.52	193.25± 33.37	0.028
	P value**	0.892	0.022	

Data shown as mean ± SD.

*: Significant level of Independent Sample t-test for comparison between two groups

**: Significant level of repeated measure ANOVA for comparison by passing time in each group

Finally, TG and CHOL levels were not significantly different between the two groups before intervention and seven days after intervention (P value > 0.05). However, on the 14th day after intervention, average levels of TG and CHOL in the treatment group being respectively 37.25 ± 2.75 mg/dl and 205.75 ± 48.32 mg/dl were remarkably and significantly lower than their average levels in the control group being 47.13 ± 3.54 mg/dl and 282.13 ± 18.64 mg/dl respectively (P value < 0.05). In addition, after 14 days passed, the changes in these two factors were insignificant in the control group (P value > 0.05). On the contrary, in the group receiving *Thymus daenensis* celak (treatment group), the significant decrease of these two factors was observed (P value < 0.05, Table 4).

Table 4. Comparing the average level of TGL and CHOL between the two
groups under investigation at different time intervals

Variable	Time	Control Group	Case Group	P value*
TG(mg/dl)	(0) Before the intervention	45.63±1.41	46.75±2.06	0.404
	7 days after intervention	43.25±2.98	42.50±3.11	0.739
	14 days after intervention	47.13±3.54	37.25±2.75	0.004
	P value**	0.237	0.027	
CHOL(mg/dl)	(0) Before the intervention	281.00±24.71	285.00±31.09	0.847
	7 days after intervention	283.88±17.56	240.50±56.10	0.190
	14 days after intervention	282.13±18.64	205.75±48.32	0.025
	P value**	0.455	0.005	

Data shown as mean ± SD.

*: Significant level of Independent Sample t-test for comparison between two groups

**: Significant level of repeated measure ANOVA for comparison by passing time in each group

It should be noted that no clinical symptoms of intoxication or drug intolerance (such as diarrhea, vomiting, loss of appetite, etc.) were perceived in the dogs investigated. All the information in the tables was expressed in terms of the average ± standard deviation. P values less than 0.05 were considered significant.

Discussion

The effects of thymus extract on reducing the level of glucose, lipid and liver enzymes have been approved by several studies. The results of present study demonstrated that the level of FBS parameter had a significant decrease subsequent to consumption of *Thymus daenensis* celak extract, so that in the treatment group, it was significantly lower on the 14th day after intervention than starting point (before the intervention, P value <0.05).

In a similar study conducted by Yaghmaie et al. (2011), the effect of thymus extract on hyperlipidemia was considered in male Wistar rats being adult and diabetic. They observed that thymus extract reduced glucose in blood serum and also it effectively reduced undesirable lipids in blood serum in those rats (Yaghmael, Heydarian, and Poorbahman 2012). Gholamhossienian et al. in another study (2008) regarding the hypoglycemic effect of four herbal extracts affecting α-glucosidase in diabetic rats stated *Zataria multiflora* caused lowering blood glucose level and was effective in controlling glucose in diabetic rats (Gholamhoseinian Najar, Fallah, and Sharififar 2015) Nasri et al. (2013), also in their study about the effect of thymus extract on reducing glucose in diabetic rats expressed that blood glucose levels in diabetic animals which received Thymus extract at the doses

of 150 and 100 mg/kg was reduced as compared to the pretreatment levels (P<0.05 and P<0.01, respectively)

As it is the case about other lamiaceae compounds, the effects of consumption of *Thymus daenensis* celak on lowering blood sugar was attributed to terpenoid and flavonoid compounds. So, considering all of the similar studies performed, we can conclude that they were consistent with the result of this study (Nasri, Hajializadh, and Esmaeili-Mahani 2014). Concerning LDL factor, on the 14th day, it was clear that it was significantly lower in the group receiving *Thymus daenensis* celak (treatment group) than the control group (P value = 0.001). Also, HDL level was higher in the treatment group than in the control group on 7th and 14th days (P value < 0.05). So, there was a significant decrease in LDL level and a significant increase in HDL level in the treatment group. Likewise, TG and CHOL levels in the treatment group were significantly lower than the control group on the 14th day after intervention (P value < 0.05). There was significant decrease in the group receiving the *Thymus daenensis* celak extract (treatment group, P value < 0.05). Illustrating more similar studies we refer to the study conducted by Nazari et al. (2013) on the effect of *Thymus daenensis* Cleak and Satureja Bakhtiarica on plasma's lipoproteins of gerbils fed with high-fat diet. They found that *Thymus daenensis* reduces LDL and TGL but it increases the HDL factor. They also expressed that the decrease in LDL in the recipient group was related to the compounds of this herb including flavonoids and phenols (Nazari et al. 2013)

According to a study done by Akhtar et al. in 2014 on evaluating the effect of *Thymus*

linearis Benth on reducing blood pressure in mice with normal and high blood pressure, it has been concluded that the thymus leads to reduction in AST, Chol, TG, LDL and increase in HDL (Akhtar et al. 2014). Zarei et al. (2011) in their study investigating the level of lipids in blood serum in rats which had high-cholesterol and consumed the extract of *Zataria multiflora* found that the extract of *Zataria multiflora* decreased blood lipids (Zarei, Eftekhary, and Aqhababa 2014). Therefore, the results of present study are consistent with most of the studies in this respect. Nonetheless, it does not conform to the study by Nik and colleagues (2012) who investigated the use of *Silybum marianum* and thymus and measured the blood parameter levels of broiler chicks. While they found that thymus increased LDL and decreased HDL in the blood serum of broiler chicks, we observed just the opposite in the present study: an increase in the HDL factor and decrease in LDL in blood serum (Nik et al. 2014).

The incidence of liver injuries due to herbal and dietary supplements have risen largely over the last decade probably in part due to their growing consumption (Maddukuri and Bonkovsky 2014). Thus, it is necessary to carefully determine the influence of various herbal medicines on liver function, in both normal and pathological conditions (Rašković et al. 2015). The extent of hepatocellular damage was assessed by measuring activities of AST and ALT in serum, in the present study . Ultimately, there was no significant difference between the two groups in terms of average levels of AST and ALT before intervention, on the seventh, and on the fourteenth days after the intervention (P value < 0.05) but the AST level had a significant decrease in the treat-

ment group over 14 days.

During the investigation of the effect of pharmaceutical formulation of a syrup containing thymus on liver injuries caused by carbon tetrachloride in gerbils, Raskovic et al. (2015) found that the Thymus syrup containing Thymol reduced AST in blood serum of gerbils (Rašković et al. 2015). While studying the effect of Caraway fruits and *Thymus vulgaris*' leaves or the combination of the two (containing 2% caraway and 10% *Thymus vulgaris*) on rats, Haroun et al. (2002) reached the conclusion that the combination reduced the AST factor in rats. This conclusion is in agreement with the results obtained from the present study (Haroun, Mahmoud, and Adam 2002).While Grespan et al. (2014) were examining the pretreatment effect of *Thymus vulgaris*' essential oil on the experimental model of liver injuries induced by Acetaminophen in rats, they relied on the hepatoprotective (TEO) effects of *Thymus vulgaris*' essential oil and found that the amount of AST has decreased due to the use of *Thymus vulgaris*. The results of this study are in agreement with those of the present study (Grespan et al. 2014). It can be said that regarding the ALT factor shown in Table 2 and the decreasing flow of the enzyme, if the duration of the intervention or dosage of the medicine was increased, the results of this parameter would probably become meaningful. In the same respect and in a similar study on rats receiving thyme syrup, Raskovic et al. in 2015 found a significant reduction of the AST parameter in the group taking carbon tetrachloride. Eventually, the researchers attributed the effects of thyme consumption on reduction of ALT and AST factors to compounds such as thymol and carvacrol.

Conclusion: Finally, considering the facts

that upon the consumption of the extract of *Thymus daenensis* Cleak there was a decrease in the amounts of FBS, LDL, TG, CHOL, and AST factors within 2 weeks, and there was an increase in the amount of the HDL factor, and the results are generally consistent with similar studies, then anti-diabetic, anti-atherosclerotic, anti-oxidant effects, as well as the absence of hepatotoxic effects (increased levels of liver enzymes such as AST, ALT) can be signaled. It should be noted that since the dose and duration of consumption of *Thymus daenensis* Cleak extract were specified, we recommend doing studies on this herb with different doses and longer duration of consumption. Considering that different reactions related to this herb in other samples of animals would be possible, it necessitates more thorough analysis of other blood factors including hematological and immunological parameters in different species, as well as studies to further analyze the compounds of this herb be done.

Acknowledgments

Conflict of Interest

The authors declare that there is no conflict of interest.

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بررسی تغییرات فاکتورهای بیوشیمیایی سرم ناشی از مصرف عصاره هیدروالکلی گیاه آویشن دنایی (*Thymus daenensis*) در سگ های نژاد پرشین شفرد

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چکیده

زمینه مطالعه: در سال‌های اخیر تحقیق در مورد داروهای گیاهی و جایگزینی آن ها به جای دارو های شیمیایی رو به افزایش است.

هـــدف: ارزیابی تغییرات فاکتورهای بیوشیمیایی سرم ناشی از مصرف عصاره گیاه آویشن دنایی و تاثیر مدت زمان مصرف در جیره سگ‌های گله بود.

روش کار: برای انجام این تحقیق از ۸ قلاده سگ بالغ نر استفاده شد و به مدت ۱ ماه با جیره ثابت تغذیه شدند سپس به صورت تصادفی به دوگروه چهارتایی تقسیم شدند گروه اول (کنترل) فقط با جیره ثابت تغذیه شدند و گروه دوم، عصاره گیاه آویشن دنایی را به میزان ۲۰۰ mg/ml یک سی سی به ازای هر کیلو وزن بدن همراه با جیره ثابت دریافت کردند در نهایت برای انجام آزمایشات، سگ ها در ۳ زمان مختلف یعنی قبل از مداخله، ۷ و ۱۴ روز بعد از مداخله خونگیری شدند. سپس نمونه‌ها جهت اندازه گیری فاکتورهای قند خون، کلسترول، تری گلیسرید، لیپوپروتئین های LDL و HDL، آلانین آمینوترانسفراز و آسپارتات آمینوترانسفر به آزمایشگاه مجهز به آزمایشگاه مجهز انتقال یافت.

نتایج: سطوح فاکتورهای CHOL، TGL، LDL، FBS و AST طی ۱۴ روز پس از مداخله در مقایسه با روز صفر و گروه کنترل به طور معنی داری کاهش یافتند. همچنین فاکتور HDL، طی ۷ و ۱۴ روز پس از مداخله نسبت به روز صفر و گروه کنترل افزایش یافت (۰/۰۵>P). همچنین میزان فاکتور ALT در طول مدت مطالعه تغییر نکرد (۰/۰۵<P).

نتیجه گیری نهایی: نتایج نشان داد مصرف خوراکی عصاره گیاه آویشن دنایی می‌تواند باعث کاهش قند و چربی خون شود. همچنین نشانه های هپاتوتوکسیک مشاهده نشد. لذا توصیه به استفاده از این گیاه برای درمان بیماران دیابتی و افراد با کلسترول بالا می‌شود.

واژه‌های کلیدی:

آویشن دنایی، بیوشیمی، سرم، عصاره

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