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Anti-aging Effects and Chemical Composition of *Dorema ammoniacum* Gum Essence and *Lonicera caprifolium* Extract

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ABSTRACT

The aging process is a multifactorial phenomenon that results from various diseases and abnormalities in living systems. Oxidative stress is a critical factor in aging, and it accelerates the aging process through different mechanisms. This study aims to explore the potential anti-aging effects of *Dorema ammoniacum* gum essence and *Lonicera caprifolium* plant extract in slowing down the aging process by using the antioxidant properties of these plants.

Following preparation, the plants underwent analysis to identify their compounds. 42 male mice aged between 6-8 weeks and weighing 22-30 grams were divided into seven groups of six and treated by gavage for six weeks. The control group received sesame oil and DMSO 10%, which were the solvents, D-galactose (500 mg/kg/day), Vitamin E (200 mg/kg/day), and D-galactose with E. The other groups received *D. ammoniacum* gum essence (200 mg/kg/day) and *L. caprifolium* extract (200 mg/kg/day) and their combination.

At the end of the treatment, the researchers measured the oxidative stress parameters such as malondialdehyde (MDA) level and glutathione content (GSH), catalase (CAT), and superoxide dismutase (SOD) activities, as well as sex hormones levels including testosterone and dehydroepiandrosterone sulfate (DHEA-S) and pro-inflammatory markers such as tumor necrosis factor-alpha (TNF-α) and Interleukin-1 beta (IL-1β) in serum by ELISA.

The use of D-galactose with a concentration of 500 mg/kg in 42 days induced aging. However, the group treated with *D. ammoniacum* gum essence alone or in combination with *L. caprifolium* extract showed a significant reduction in lipid peroxidation ($P<0.05$) and a significant increase in catalase enzyme activity ($P<0.05$). The group treated with the combination of these two plants also showed a significant increase in glutathione content ($P<0.05$). These plants are rich sources of antioxidant compounds and could potentially improve the aging process induced by D-galactose.

Keyword: *D. ammoniacum* gum, *L. caprifolium*, D-galactose, Anti-Aging, Antioxidant

INTRODUCTION

Aging is an inescapable process that comes with changes in cells and body tissues over time. Multiple theories have surfaced to explain this phenomenon, including genetic, immunological, free radical, and telomere shortening theories. The free radical theory of aging posits that free radical damage is one of the principal causes of aging. Most chronic diseases share a common pathology, namely oxidative stress, which arises from the production of oxygen species and their interaction with lipids, proteins, and nucleic acids due to their high reactivity and low stabilization. This leads to the formation of oxidized metabolites, and aging is the result of an imbalance between the damage caused by reactive oxygen species and the organism's antioxidant defense. The main biochemical features of aging are decreased sex hormones and increased oxidative and inflammatory stress parameters. Most plants have antioxidant properties, which reduce the production of free radicals that arise from abnormal metabolism [1-3].

Dorema ammoniacum, also known as Vosha, belongs to the Umbelliferae family and yields an oleo gum resin obtained by exudation from the stem of the flowering and fruiting plant, which is considered a pharmaceutical product [4]. It is used in Iranian, Indian, and Western medicine and is listed as an expectorant and antispasmodic in the British pharmacopeia. In Unani Medicine, it is used as a potent drug for various ailments, as indicated by Razi and Avicenna in their treatises [5].

The plant grows in the dry and semi-dry areas of Central Asia, including Pakistan, India, Afghanistan, and Iran, and is harvested during June and July. Its primary growth centers in Iran are Yazd, Isfahan, Kerman, Sistan and Baluchistan, Khorasan, and Semnan [4]. Several valuable studies have been conducted on the medicinal effects of the gum ammoniacum and its compounds. In one study, the antimicrobial effects of *D. ammoniacum* against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Shigella dysenteriae* were demonstrated [6]. Due to its high content of sesquiterpenes, coumarins/phenols, it has been shown to have diverse biological activities such as cytotoxic, antibacterial, antifungal, anticonvulsant, acetylcholinesterase inhibitory, antioxidant, analgesic, and anti-inflammatory activities [7]. In Iranian traditional medicine, the gum resin of the ammoniacum plant is used for various purposes, including the treatment of severe inflammatory diseases. In a 2018 study, it was found that local (skin) use of this plant had significant anti-inflammatory effects without erythema and edema [8].

Lonicera caprifolium, a member of the Caprifoliaceae family, is a popular plant used in the design of green spaces that play a significant role in enhancing the aesthetics of urban landscapes. Various parts of the *Lonicera caprifolium* plant are used in traditional herbal medicine for their antibacterial, antiviral, and antioxidant properties. The plant's decoction is used for gargling and as an astringent, while its essential oil is included in many herbal pharmaceutical and cosmetic preparations [9-11].

D-galactose, a monosaccharide sugar, is one of the substances used to induce aging in different models. Galactose oxidase oxidizes excessive levels of D-galactose into reactive aldehydes and hydrogen peroxide, which in turn causes an increase in hydrogen peroxide and decreased antioxidant enzymes (SOD). Moreover, galactose reductase reduces extra D-galactose to form galactitol, leading to osmotic stress. An uncommon accumulation of D-galactose in the body, as occurs in the injection of D-galactose-induced mimetic aging models, can cause harmful effects in the body [12]. D-galactose has been used in the current study to create artificial aging. Given the antioxidant effects of the two aforementioned plants and their use in traditional medicine, this study aims to assess the effects of D. *ammoniacum* gum essence and *L. caprifolium* extract, and the combination of both, on the aging process induced by D-galactose in mice, with the aim of developing a medicine to slow down the aging process.

MATERIAL AND METHODS

Chemicals and Reagents

The chemical D-Galactose (CAS 59-23-4) was obtained from Merck KGaA (Darmstadt, Germany). Vitamin E (CAS No. 10191-41-0) and DMSO (CAS No. 67-68-5) were supplied by Sigma-Aldrich (Sternheim, Germany), while all the chemicals and reagents used were of analytical reagent grade. ZellBio GmbH (Lonsee, Germany) provided the ELISA assay kits for MDA, GSH, Catalase, and SOD. The TNF-α ELISA assay kit was purchased from Karmania Pars Gene (Rafsanjan, Iran), and the Testosterone and DHEA-S ELISA assay kits were provided by Monobind (USA).

Plant Materials

The aerial parts of *Lonicera caprifolium* and *Dorema ammoniacum* gum were collected from Yazd, Iran in early July 2022. The species were identified by the supervisor of the Medicinal Plants Research Center of Shahid Sadoughi University of Medical Sciences (Yazd, Iran) and assigned herbarium codes SSU0070 and SSU0071 respectively.

Extraction of Essential Oil of *Dorema ammoniacum* gum by Means of a Clevenger Device

To extract the essential oil from *Dorema ammoniacum* gum, a hydro-distillation process using Clevenger apparatus was employed. First, 100g of dry ground gum was placed in a 1000 ml glass flask and 400 ml of distilled water was added. The sample was then fully immersed and the extraction process lasted for five hours.

The resulting essential oil was dehydrated using sodium sulfate and stored in a tightly sealed opaque glass tube in the refrigerator at 4 C until analysis. The yield obtained was 1.1 ml of essential oil from 100 grams of Vosha gum.

Methanol Extraction of *Lonicera caprifolium*

We harvested the aboveground parts of *L. caprifolium*, dried them out, and processed them into powder using an electric mill. Since we were primarily interested in analyzing polar compounds, we extracted the methanol from the plants using the maceration method. We poured 70 grams of the powdered plant material into a 500 ml beaker, added 350 ml of 80% methanol, and shook the mixture on an electric shaker for 72 hours. After filtering the solution through a filter paper, we left the obtained liquid in aluminum foil in the laboratory to dry for a week.

Analysis of *Dorema ammoniacum* Gum Essential Oil

The essential oil of *D. ammoniacum* gum was analyzed using Gas Chromatography coupled with mass spectrometry (GC-MS). The analysis employed a flame ionization detector (FID) and a Thermo-quest-Finnigan Trace GC instrument equipped with an FID and a silica capillary DB-5 column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Nitrogen was used as the carrier gas at a flow rate of 1.1 ml/min. The injector and detector temperatures were set at 280 °C and 250 °C, respectively. The oven temperature program started at 60 °C for 1 minute, followed by a heating rate of 4 °C per minute until reaching 250 °C, and finally held at 250 °C for 10 minutes. A 0.2 microliter injection volume was used, and a 1/50 split was employed. For GC-MS analysis, a Thermoquest-Finnigan Trace instrument and a TRACE mass detector were used, with helium as the carrier gas at a flow rate of 1.1 ml/min. The ionization voltage was set at 70 eV, and the pre-pressure from the column was 70 kPa. The ion source and interface temperatures were set at 200 and 250 °C, respectively. The mass range for copper ions was between 35 and 456 amu. The oven temperature program was based on the GC conditions used. The relative contribution of each constituent in the essential oil was determined by analyzing the area under the peak without calculating correction factors. The identification of compounds was carried out using Xcalibur 2.0 software, and a comparison of mass spectra patterns and retention indices (RI) was done by referring to the data from Adams and NIST mass spectra (Adams, 2017). The RI for each compound was calculated using a series of n-alkanes ranging from C8 to C24.

Analysis of *Lonicera caprifolium* Extract

High-Performance Liquid Chromatography (HPLC) was used to analyze the Methanolic extract of *L. caprifolium*. First, the dry extract, weighing 300mg, was dissolved in 1 mL of distilled water and filtered through a 0.22 µ PTFE membrane. Two microliters of the filtered solution were injected into the HPLC device, which utilized an automatic sampling injector into the Eurospher C18 column (4.6 × 150 mm, 3.5 µm) equipped with PDA detectors. The data was collected and integrated using Millennium32 software. The mobile phase consisted of methanol (A) and aqueous (B) TFA (Trifluoroacetic Acid) solution (0.02% v/v), with a flow rate of 0.5 ml/min. The gradient elution program started at 20% A:80% B and gradually changed to 30:70 over 10 minutes, then adjusted to 50:50 over the next 10 minutes and remained constant for 20 minutes. Finally, it changed to 100:0 over 2 minutes and held for 6 minutes. The program then returned to 20:80 over 7 minutes. Peaks were observed across a wavelength range of 200 to 600 nm. The injection volume of the extract was 20 microliters, and the temperature was maintained at 25 ° C. For quantitative analysis, an external standard calibration curve was employed, as outlined in Table 1.

Table 1 External calibration curve characteristics for simultaneous determination of compounds using the HPLC-DAD

Components	Equation of external calibration curve	Linearity range	R^2
chlorogenic acid	Y=6934.4X+12517	10–200 µg/ml	0.997
coumaric acid	Y=41760X+63852	10–200 µg/ml	0.987
rutin	Y=85957X-147985	10–200 µg/ml	0.992
rosmarinic acid	Y=163040X-587301	10–200 µg/ml	0.989
apigenin	Y=112833X-558841	10–200 µg/ml	0.991

Animals

This was an interventional experimental study conducted on 42 male mice aged 6-8 weeks and weighing 22-30 grams. The mice were sourced from the animal nest of Yazd Research and Clinical Center for Infertility and were kept under standard conditions of 12 hours of light and 12 hours of darkness, with a temperature of 23 ± 2 and humidity of 55 ± 5%. They had unrestricted access to food and water. All animal procedures were approved by the ethics committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.MEDICINE.REC.1400.080) and were carried out based on ethical guidelines for animal research. The mice were randomly divided into 7 groups of 6 and treated by the gavage method for 6 weeks. During the treatment period, one control group received sesame oil solvent, and the other control group received 15% DMSO solvent. The negative control received D-galactose (500mg/kg/day), and the positive control group received D-galactose for 14 days, followed by vitamin E (200mg/kg/day) for the remaining 28 days. The remaining groups also received D-galactose (500mg/kg/day) for 14 days, followed by the essence of *D. ammoniacum* gum and *L. caprifolium* extract (200 mg/kg/day) and their combination for the remaining 28 days. At the end of the sixth week, blood was collected from the hearts of the mice, and the blood samples were centrifuged. After separating the serum of the blood samples, they were transferred to microtubes and frozen at -80 ℃ immediately after sample collection until analysis.

Measurement of Oxidative Stress Parameters

Changes in glutathione (GSH), and malondialdehyde (MDA) levels (as lipid peroxidation indexes), as well as catalase, and superoxide dismutase enzyme activities were measured using ZellBio ELISA commercial kits.

Measurement of Sexual Parameters

Evaluation of changes in sex hormones DHEA-S and testosterone was done by ELISA method using a commercial Monobind kit.

Measurement of Inflammatory Parameters

The research analyzed variations in pro-inflammatory markers, specifically tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), through the application of the ELISA method. The TNF-α measurement was executed in conformity with the guidelines provided by the Karmania Pars Gene kit, while the IL-1β measurement was conducted in accordance with the directions of the Enzo commercial kit.

Statistical Analysis

The statistical data was presented as mean ± SEM and analyzed using one-way analysis of variance (ANOVA). In instances where ANOVA detected a significant difference, the treated groups were compared against the control groups using the Dunnett posttest. $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using GraphPad InStat software.

Ethical Considerations

Ethical principles have been heeded based on the laws of protection and care of laboratory animals with the code of IR.SSU.MEDICINE.REC.1400.080 received from the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

RESULTS

Analysis of the Plants Extracts

As shown in Table 2, the most abundant compounds in the *D. ammoniacum* gum essence, in descending order of magnitude include β-Maaliene13.1%, β- Bisabolene 8.59%, β-Selinene 8.09%, trans-β-Guaiene 6.59%, δ-Selinen 5.44%, Selina-3,7(11)-diene 5.31%, and Fenchyl acetate 4.4%. Thymol 1.51% and carvacrol 0.28% were also found in this extract.

According to HPLC/MS data, the methanolic extract of *L. caprifolium* contains 22% chlorogenic acid, 0.03% coumaric acid, 0.41% rutin, 0.42% rosmarinic acid and 0.02% apigenin (Table 3).

Table 2 Chemical composition of *D. ammoniacum* gum essence identified by GC/MS method

No	RI C	Area GCMS%	Compound
1	938	0.45	α-Pinene

2	955	0.75	Camphene
3	978	1.13	6-methyl-5-Heptene-2-one
4	983	0.18	β-Myrcene
5	1018	0.16	α-Terpinene
6	1025	0.14	p-Cymene
7	1029	1.75	Limonene
8	1032	0.24	β-Phellandrene
9	1047	0.17	2,6-Dimethyl-5-heptenal
10	1224	4.4	Fenchyl acetate
11	1231	1.51	Thymol, methyl ether
12	1235	0.46	Myrcenyl acetate
13	1241	0.28	Carvacrol, methyl ether
14	1291	0.12	Bornyl acetate
15	1380	13.1	β-Maaliene*
16	1386	3.26	α-Ylangene
17	1390	0.53	α-Copaene
18	1401	1.19	α-Gurjunene
19	1403	1.61	α-Cedrene
20	1416	3.72	β-Copaene
21	1421	1.3	β-Funebrene
22	1444	1.52	β-Gurjunene
23	1447	1.29	Neryl acetone
24	1450	2.42	α-Guaiene
25	1456	1.75	cis-Thujopsene*
26	1460	0.87	Aromadendrene
27	1467	0.47	alloAromadendrene
28	1474	8.09	β-Selinene
29	1486	0.85	ar-Curcumene *
30	1489	2.39	α-Selinene
31	1494	0.64	α-Muurolene
32	1495	5.44	δ-Selinene
33	1501	1	cis-Eudesma-6,11-diene*
34	1505	6.59	trans-β-Guaiene
35	1509	8.59	β-Bisabolene
36	1528	3.73	ϒ-Cadinene
37	1531	2.24	Selinene <7-epi-α->
38	1536	1.67	δ-Cadinene
39	1544	1.52	Cadina-1,4-diene <trans->
40	1549	1.44	α-Cadinene
41	1558	3.59	Muurola-4,9-diene
42	1565	5.31	Selina-3,7(11)-diene
43	1583	1.43	Germacrene B

Table 3 Chemical composition of *L. caprifolium* methanolic extract identified by HPLC/MS analysis

Peak	R.Time	% of total	Name
1	14.8	%22	Chlorogenic
2	22.6	%0.03	Comaric
3	27	%0.41	Rutin
4	28.59	%0.42	Rosmarnic Acid
5	38.6	%0.02	Apigenin

D-galactose Induced Aging Model

According to a study, administering D-galactose with a concentration of 500 mg/kg over a period of 42 days resulted in aging [13]. The use of D-galactose significantly raised levels of lipid peroxidation (MDA) and pro-inflammatory cytokines such as TNF-α and IL-1β ($P<0.05$). Additionally, there was a reduction in (GSH) content,

CAT, and SOD activities, as well as a decrease in the serum levels of sex hormones such as testosterone and DHEA-S ($P<0.05$).

Effect of Plants on Oxidative Stress Parameters

Figure 1A shows the effect of D-galactose, vitamin E, and the extracts on serum MDA as an index of lipid peroxidation. The results revealed a significant increase in MDA serum level in the group that received D-galactose compared to control groups 1 and 2 ($P < 0.01$). In comparison with the group that received D-galactose, MDA serum levels significantly decreased in groups that received vitamin E and a combination of extracts ($P < 0.01$), as well as the group that received the essence of *D. ammoniacum* gum ($P < 0.05$). The amount of serum MDA levels in control groups 1 and 2 were the lowest. The highest concentration belongs to the group received D-galactose (Figure 1. A).

The results of this study have shown that the amount of serum GSH significantly decreased in the group that received D-galactose compared to control groups 1 and 2 ($P < 0.01$). On the other hand, administration of vitamin E and a combination of extracts could increase the level of serum GSH significantly ($P < 0.05$). The amount of serum GSH was the highest in control groups 1 and 2, and its lowest concentration was in the group that received D-galactose (Figure 1. B).

Catalase enzyme activity decreased in all groups compared to the control group 1 and 2. The highest decrease in enzyme activity was observed in the D-galactose group ($P < 0.01$). According to the captured results, after administration of vitamin E, essence of *D. ammoniacum* gum, and a combination of two plant extracts to the mice who had received D-galactose before, the catalase enzyme activity increased significantly ($P < 0.05$) (Figure 1. C).

Superoxide dismutase enzyme activity reduced significantly in the D-galactose group compared to control groups 1 and 2 ($P < 0.05$). 05). No other significant difference was observed between the studied groups (Figure 1.D).

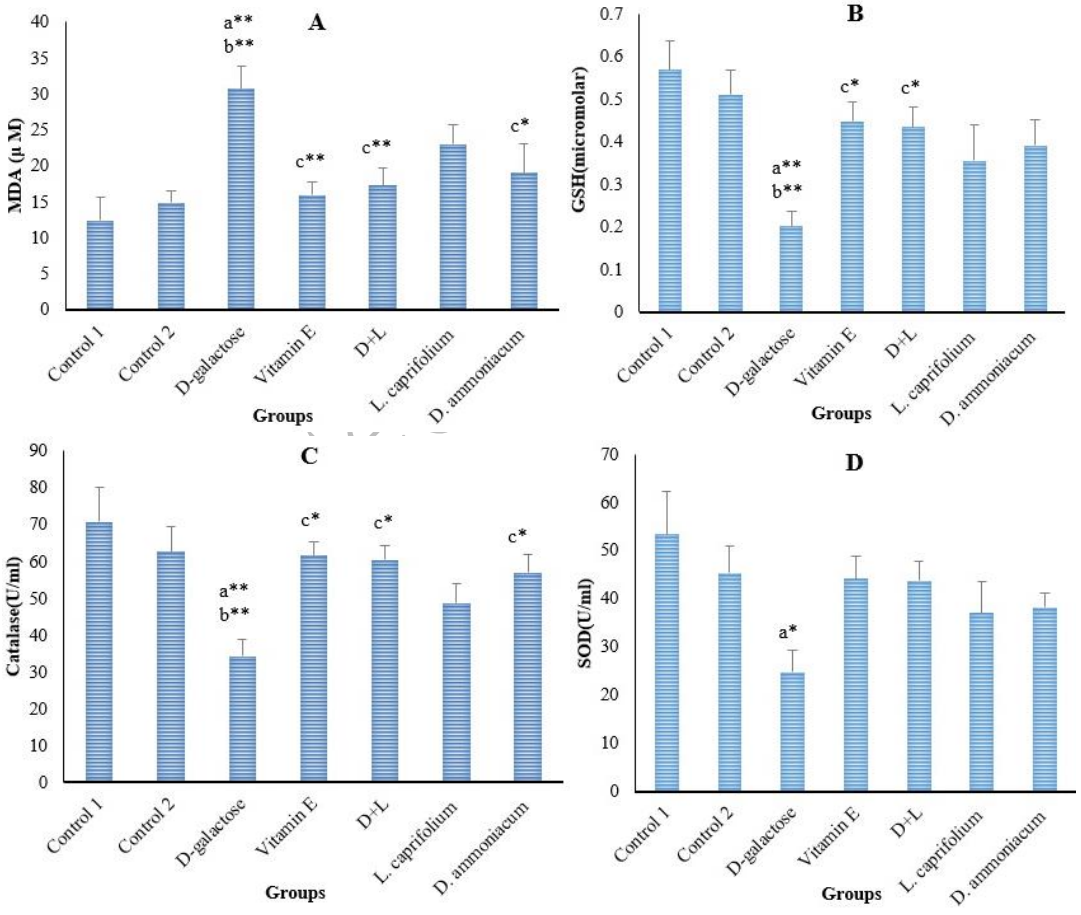

Fig. 1 Effects of different treatments on A) serum MDA, B) serum GSH contents, C) CAT activity, and D) SOD activity in mice at the end of the experiment. The control group1 received sesame oil, Control group 2 received DMSO 10% solvent, D-galactose group received 500 mg/kg/day D-galactose, Vitamin E group received 200 mg/kg/day vitamin E, *D. ammoniacum*: group treated with the essence of *D. ammoniacum* gum (200 mg/kg/day), *L. caprifolium*: group Treated with the extract of *L. caprifolium* (200 mg/kg/day), *D+L*: combined treatment group. Statistical analysis used one-way ANOVA with Dunnett's test. Values are expressed as means±SEM, n=6 for each group. **a**: Significantly different from control group

1, b: Significantly different from control group 2, **c**: Significantly different from D-galactose group. * ($P < 0.05$); ** ($P < 0.01$).

Effect of Plants on the Level of Pro-Inflammatory Markers; TNF-α and IL-1β

In Figure 2A, it is evident that the serum TNF-α levels are significantly higher in the group treated with D-galactose compared to Control Group 1. While the other treatments did not show a significant decrease in the cytokine levels as compared to the D-galactose group. Similarly, Figure 2B shows a noteworthy increase in the serum IL-1β levels in mice treated with D-galactose compared to Control Group 1 and 2 ($P < 0.01$ & $P < 0.05$). However, administering Vitamin E to mice was found to significantly decrease the levels of serum IL-1β in comparison to the group treated only with D-galactose ($P < 0.05$).

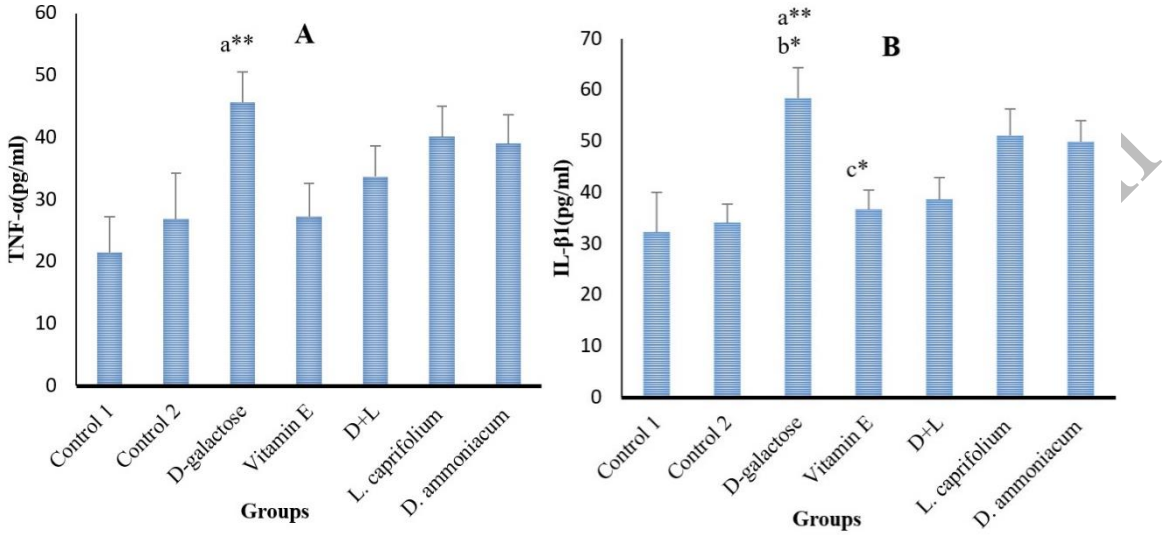

Fig. 2 Effects of different treatments on **A**) TNF-α serum levels, **B**) IL-1β serum levels in mice at the end of the experiment. The control group1 received sesame oil, Control group 2 received DMSO 10% solvent, D-galactose group received 500 mg/kg/day D-galactose, Vitamin E group received 200 mg/kg/day vitamin E, *D. ammoniacum*: group treated with the essence of *D. ammoniacum* gum (200 mg/kg/day), *L. caprifolium*: group Treated with the extract of *L. caprifolium* (200 mg/kg/day), *D+L*: combined treatment group. Statistical analysis used one-way ANOVA with Dunnett's test. Values are expressed as means±SEM, n=6 for each group. **a**: Significantly different from control group 1, b: Significantly different from control group 2, **c**: Significantly different from D-galactose group. * ($P < 0.05$); ** ($P < 0.01$).

Effect of Plants on the Level of Sex Hormones; Testosterone and DHEA

As seen in Figures 3A & B, the serum levels of Testosterone and DHEA in the group that only received D-galactose decreased significantly compared to the control group 1 and control group 2 ($P < 0.01$ and $P < 0.05$). However, there is no significant difference in the amount of sex hormones between other groups.

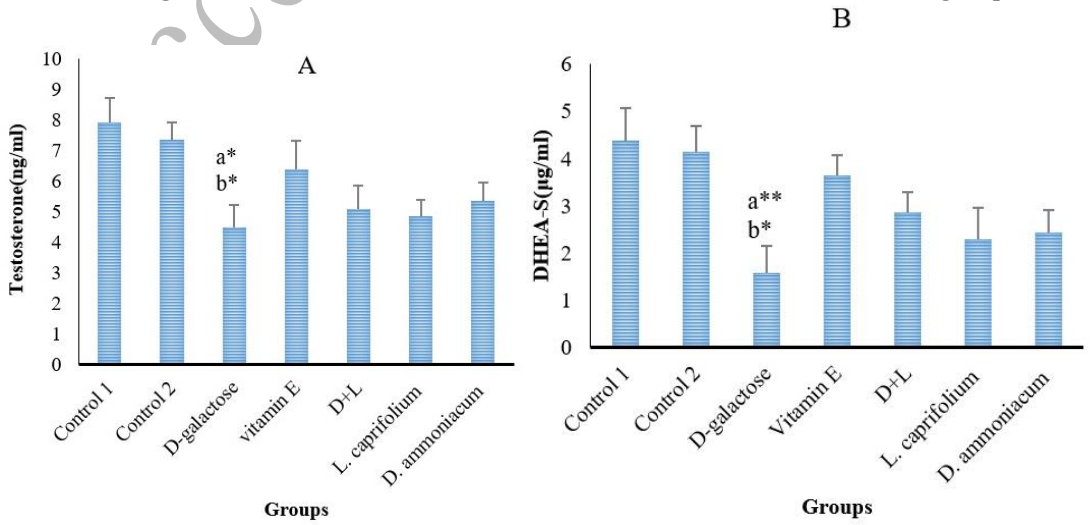

Fig. 3 Impacts of different treatments on **A**) Testosterone serum levels, **and B**) DHEA serum levels in mice at the end of the experiment. The control group1 received sesame oil, Control group 2 received DMSO 10% solvent, D-galactose group received 500 mg/kg/day D-galactose, Vitamin E group received 200 mg/kg/day vitamin E, *D. ammoniacum*: group treated with the essence of *D. ammoniacum* gum (200 mg/kg/day), *L. caprifolium*: group Treated with the extract of *L. caprifolium* (200 mg/kg/day), *D+L*: combined treatment group. Statistical analysis used one-way ANOVA with Dunnett's test. Values are expressed as means±SEM, n=6 for each group. **a**: Significantly different from control group 1, b: Significantly different from control group 2. * ($P < 0.05$); ** ($P < 0.01$).

DISCUSSION

The free radical theory of aging, later known as the oxidative stress theory of aging, hypothesizes that age-related dysfunction is caused by oxidative damage to macromolecules such as lipids, DNA, and proteins. The exact mechanism of aging caused by oxidative stress is not yet fully understood but it is believed that increased levels of reactive oxygen and nitrogen species lead to cellular aging. Cellular senescence is a mechanism that stops cellular proliferation in response to damage that occurs during replication. Senescent cells attain an irreversible senescence-associated secretory phenotype that comprises secretion of soluble factors such as chemokines, interleukins, and growth factors, degrading enzymes such as matrix metalloproteases, and insoluble proteins/extracellular matrix constituents. The prevalence of chronic diseases increases with age, and in most age-related diseases, people show a phase of chronic inflammation by inflammatory cells, including macrophages and pro-inflammatory cytokines. Many cytokines, including IL-1, IL-6, and TNFα, increase during the aging process and play a direct role in the pathogenesis of aging. The present study investigated the effects of *D. ammoniacum* gum essential oil and *L. caprifolium* methanolic extract, and a combination of both, on the aging process induced by D-galactose in mice. GC/MS analysis and compound determination in the essence of gum ammoniacum plant showed that β-Maaliene 13.1%, β-Bisabolene 8.59%, β-Selinene 8.09%, trans-β-Guaiene 6.59%, δ-Selinen 5.44%, Selina-3,7(11)-diene 5.31%, and Fenchyl acetate 4.4% had the highest amounts among the compounds found in the plant. These compounds are sesquiterpenes with high antioxidant properties. Thymol 1.51% and carvacrol 0.28% were among the other compounds observed in the essential oil of the *D. ammoniacum* gum. The antioxidant properties of thymol and carvacrol have been well-documented in various in vitro and in vivo studies. Both thymol and carvacrol possess ROS-scavenging activity, and it has been observed that they effectively neutralize free radicals. Their antioxidant activity is attributed to the presence of the hydroxyl group attached to an aromatic ring. In 2018, Güvenç et al. studied the effects of different doses of thymol and carvacrol on sperm quality, oxidative stress, and antioxidant system in rats. They concluded that administration of thymol and carvacrol decreased oxidative damage, increased antioxidant levels, and improved sperm quality parameters. However, the combined use of these two active components had a limited therapeutic effect on the mentioned parameters. Carvacrol is a monoterpene phenol with anti-inflammatory properties that could reduce the levels of inflammatory cytokines such as TNF-α and the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 in ischemic tissues. It has been shown in a study that carvacrol and thymol also improved the reduced level of glutathione in the liver, kidney, and spleen tissues and caused a decrease in the level of malondialdehyde in the spleen.

According to HPLC/MS data, the *L. caprifolium* extract contains 22% of chlorogenic acid, 0.03% of coumaric acid, 0.41% of rutin, 0.42% of rosmarinic acid, and 0.02% of apigenin. Chlorogenic acid acts as an antioxidant and is commonly present in the leaves and fruits of dicotyledonous plants as a plant polyphenol [21]. In recent years, many studies have shown that coumaric acid, found in fruits, vegetables, and grains, has antioxidant properties and represents anti-inflammatory and anti-tumor effects [22]. Another compound in honeysuckle extract is rutin, which has been reported to have antioxidant, anti-inflammatory, cardiovascular, neuroprotective, anti-diabetic, and anti-cancer properties. The extensive properties of rutin have made it widely used in medicine [23].

In recent years, an increasing focus on natural products as alternative treatments has led to a large number of pharmacological investigations into the biological activities of rosmarinic acid. These investigations have revealed its potential as an antioxidant, anti-inflammatory, anti-tumor, anti-diabetic, anti-viral, neuroprotective, and hepatoprotective agent [24]. Apigenin is a flavone belonging to the natural flavonoid category, found in significant amounts in fruits, vegetables, nuts, onions, oranges, and tea [25]. Apigenin has been found to possess

various beneficial health effects, such as antioxidant and anti-inflammatory properties. In mouse macrophages and human monocytes, apigenin reduces the expression of IL-1β and TNF-α [26]. An aging model with the aid of D-galactose was established by reducing the activity of superoxide dismutase and catalase enzymes compared to the control group. The extract of *Lonicera caprifolium*, *Dorema ammoniacum* gum essence, and their mixture could reduce the aging process by increasing the activity of the catalase enzyme. One of the most significant biomarkers of oxidative stress in serum is glutathione. Our study showed that the administration of D-galactose to mice significantly decreased the serum GSH level. Still, the treatment of D-galactose-exposed mice with vitamin E and a combination of plants increased the level of serum GSH significantly. This suggests that the combination of plants could inhibit aging by increasing the endogenous antioxidant agents, such as the reduced form of glutathione. A similar increase in glutathione levels was also reported in curcumin-treated mice exposed to D-galactose [27]. Antioxidants are essential to prevent the formation of free radicals and to inhibit some of the harmful effects of reactive oxygen species on lipids, DNA, and proteins. Malondialdehyde is a major reactive aldehyde formed from the peroxidation of membrane biological unsaturated fatty acids. This secondary product of lipid peroxidation is used as an indicator of tissue damage involving a series of chain reactions [28]. Our study results showed that D-galactose could significantly increase MDA serum levels as an index of lipid peroxidation, but treating D-galactose-exposed mice with vitamin E and a combination of extracts as well as the essence of *D. ammoniacum* gum reduced MDA serum levels. The amount of serum MDA levels in the control groups that received just vehicles was the lowest, while the highest concentration belonged to the group that received D-galactose. In agreement with our results, another study showed that after administration of carbon tetrachloride to induce toxicity in rats, MDA levels significantly elevated, and levels of GSH, vitamin C and E, and activities of CAT, SOD, and glutathione peroxidase decreased compared to normal rats. Administration of chrysin, a natural flavonoid, to CCl4-exposed rats resulted in GSH, vitamin C, and E levels elevation and enhancing the mean activities of CAT, SOD, and GPX and lowering the mean level of MDA in the tissue and hemolysate samples [29]. Circulating antioxidants, such as vitamins C and E, are non-enzymatic scavengers of free radicals. Vitamin E reacts with lipid peroxy radicals and acts as a lipid peroxidation chain terminator, protecting cellular structures against free radical attacks [30]. Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are steroids mainly secreted from the adrenal cortex. They are also secreted from the brain as neurosteroids. Their effects are mediated by their conversion into active sex hormones, such as testosterone and estradiol. Decreased serum levels of DHEA\DHEAS are related to aging and diseases caused by aging [31].

he declines in dehydroepiandrosterone (DHEA) levels has been linked to aging and cardiovascular diseases in men, as well as an increased risk of premenopausal breast and ovarian cancer, cardiac ischemia, osteoporosis, and reduced immune system efficiency in women. In contrast, high levels of DHEA have been associated with breast cancer after menopause [32].

Our findings indicate that administering D-galactose to mice resulted in a significant decrease in testosterone and DHEA serum levels. However, treatments with plants or vitamin E failed to significantly increase the amount of sex hormones in mice previously exposed to D-galactose. It seems that the change in hormone levels is due to a mechanism other than oxidative stress. In a similar study, Mohammadi et al. investigated the protective effects of crocin against aging caused by D-galactose in mice. The administration of crocin reduced D-galactose-induced aging by inhibiting oxidative stress (reducing levels of malondialdehyde and elevating glutathione content), reducing inflammation (declining in serum levels of tumor necrosis factor-alpha and interleukin-6), and elevating sex hormones (testosterone and DHEA) [33]. Numerous studies have shown that aging is accompanied by a pro-inflammatory state, as evidenced by increasing levels of inflammatory cytokines such as TNF-α, IL-6, and IL-1β. At the same time, aging is associated with a decline in serum testosterone levels. TNF-α, IL-6, and IL-1β inhibit testosterone secretion by their influence on the central (hypothalamic-pituitary) and peripheral (testicular) components of the gonadal axis [34]. In another study, Badibostan et al. investigated the protective effect of thymoquinone on aging caused by D-galactose in mice. Their research revealed that thymoquinone possesses significant anti-aging effects in the model of aging induced by D-galactose, particularly through enhancement of GSH content, decline of lipid peroxidation, and inhibition of inflammation. Our results indicated that D-galactose-induced aging is accompanied by a significant increase in serum levels of TNF-α and IL-1β, which can be considered indices of the inflammatory process. Although treatment of mice with Vitamin E could decrease

the serum levels of IL-1β significantly in comparison with the group that received only D-galactose, our extracts couldn't significantly reduce the levels of these cytokines. The aging process is associated with impaired immune system function, induction of inflammation, and increased pro-inflammatory cytokines. TNF-α plays an important role in the regulation of immunity, inflammation, and programmed cell death [36]. Pro-inflammatory cytokines, including TNF-α and IL-6, increase in aging. Administration of D-galactose to male BALB/c mice increased the levels of inflammatory factors such as TNF-α, IL-β, and IL-6 in blood samples significantly. In another study, a significant increase in plasma levels of TNF-α and IL-β was observed following the treatment of mice with D-galactose [37,38].

CONCLUSION

Taken together, our findings indicate that a combination of *Dorema ammoniacum* gum essence and *Lonicera caprifolium* methanolic extract possess anti-aging effects in the model of aging induced by D-galactose, especially through increase GSH content, enhancement of catalase activity and decline of lipid peroxidation.

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Authors' Contributions

Fatemeh Tavakoli, Mohammad Reza Lotfaliani and Azadeh Emami designed the research. Hamid Reza Moazzen and Mahtab Zarei Collected the plant and performed plant preparation for extraction. Azadeh Emami and Hamid Reza Moazzen performed experimental tests of this research. Fatemeh Tavakoli and Mohammad Reza Lotfaliani analyzed the data. Azadeh Emami and Fatemeh Tavakoli prepared the manuscript.

Declaration of Interest

The authors declare that there is no conflict of interest.

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