Full Length Research Article

Pharmacological and antioxidant activities of a lyophilized extract of *Salvia deserta* Schangin

https://doi.org/10.62940/als.v13i2.4101

Issue: Volume 13, Issue 2 (IN PROGRESS)

Received: 25-03-2026

Revised: 15-04-2026

Accepted: 03-06-2026

Published online: 16-06-2026

Keywords: Tartarian sage, Antinociceptive activity, Anti-inflammatory activity, Medicinal plant raw materials

Guldana Shoinbayeva[1],*, Bayan Sagindykova[1], Saltanat Imanalieva[1], Gnk Ganesh[2], Dithu Thekkekkara[3], Saravana Babu Chidambaram[3]

1. Department of Drug Technology and Pharmacognosy, South Kazakhstan Medical Academy, Shymkent, Republic of Kazakhstan
2. Department of Regulatory Affairs, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Ooty, Nilgiris, Tamil Nadu, India
3. Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru, India

* gulshoinbayeva@mymail.academy

ABSTRACT

Background: *Salvia deserta* Schangin (the Tartarian sage) is one of the little-studied species of the genus Salvia, despite the broad spectrum of biological activity of other representatives of this genus. The plant is common in Central Asia and is traditionally used in folk medicine, but its pharmacological properties have not yet been systematically investigated. The aim of the work was to experimentally study the pharmacological properties of the lyophilized extract of *Salvia deserta* Schangin, including the assessment of acute oral toxicity and analgesic, anti-inflammatory, and antioxidant activity.

Methods: The extract was obtained by ultrasonic extraction followed by lyophilization. Acute toxicity was determined according to OECD Guidelines No. 423. Analgesic activity was determined through the acetic acid writhing test in mice, and anti- inflammatory activity was tested in a model of carrageenan- induced paw edema in rats. Antioxidant properties were assessed using the DPPH and FRAP methods.

Results: The lyophilic extract of *Salvia deserta* Schangin showed low acute toxicity (LD_{50}> 2,000 mg/ kg), a pronounced analgesic effect comparable to that of diclofenac sodium, and a significant anti-inflammatory effect at a dose of 400 mg/kg. In DPPH and FRAP tests, the extract showed high antioxidant activity (EC_{50} = 11.85 µg/ml; IC_{50} = 10.4399 µg/ml).

Conclusion: The obtained results demonstrate the strong pharmacological effects of the Tartarian sage and prove it to be a promising source of biologically active compounds with analgesic, anti- inflammatory, and antioxidant properties. The study presents the first comprehensive assessment of the pharmacological activity of the lyophilized extract of *Salvia deserta* Schangin, which gives grounds to consider this species as a new promising object for the development of phytopreparations.

INTRODUCTION

Plant sources are an important object of pharmacological research due to their high content of biologically active compounds of various chemical categories. According to the World Health Organization, approximately 80% of the world's population utilizes medicinal plants for medicinal purposes. As of today, more than 35,000 plant species are used in pharmaceutical and clinical practice. Their therapeutic potential is determined by the content of secondary metabolites – phenolic compounds, terpenes, alkaloids, flavonoids, and essential oils with anti-inflammatory, analgesic, antioxidant, antimicrobial, and cytotoxic activity [1]. In this context, research into the pharmacological activity of new and understudied species of medicinal plants is of significant interest for the development of effective and safe phytopreparations.

Kazakhstan is home to about 6,000 plant species. According to the annotated list of medicinal plants, about 1,500 of these have medicinal properties. The pharmacological properties of many plants remain poorly understood and are of great interest for scientific research [2-5]. One such understudied species of the plant genus *Salvia* is the Tartarian sage (*Salvia deserta* Schangin).

The genus *Salvia* includes about 1,000 species worldwide. Sage is common in Central and South America (500 species), West Asia (200 species), and East Asia (100 species) [6]. Apart from that, it is cultivated in Spain, Italy, the Balkans, Germany, England, France, Malta, Turkey, the USA, Canada, and Argentina [7]. According to a taxonomic review, Central Asia has a total of 41 local species of sage, 24 of which are endemic.

The focus of the present study is owed to the natural, climatic, and floristic features of Kazakhstan, which has a wide variety of Salvia species and a high potential for their application in pharmaceutical practice. There are a total of 13 species of sage growing in the country, including one endemic species, yet the pharmacological properties of most of them remain underresearched [8]. Kazakhstan is characterized by rich medicinal flora, which gives reason to search for new natural sources of biologically active compounds. However, systematic pharmacological studies of endemic and adapted species are rather rare. In this connection, *Salvia deserta* Schangin, which grows in almost all regions of Kazakhstan, presents a promising object for the production of standardized plant extracts with potential analgesic, anti-inflammatory, and antioxidant effects.

Salvia deserta Schangin (the Tartarian sage) is a perennial species of the genus Salvia (Lamiaceae), common in the countries of Central Asia, the Caucasus, Russia, and China, including most regions of Kazakhstan [9-11]. Despite its wide distribution, there is little pharmacological research covering this species. Members of the genus Salvia have previously been proven to demonstrate a wide range of bioactive properties, including anti-inflammatory, analgesic, antioxidant, and cytostatic effects due to the high content of phenolic compounds, terpenoids, and essential oils [7,12].

Given the lack of scientific knowledge of the chemical composition and pharmacological potential of *Salvia deserta* Schangin, the purpose of this study was to conduct a comprehensive experimental assessment of its lyophilized extract, including the determination of acute oral toxicity and analgesic, anti-inflammatory, and antioxidant activity.

The presented study is the first wide-ranging pharmacological study of the lyophilized extract of the relatively less studied plant *Salvia deserta* Schangin, which is distributed throughout Central Asia. This study is relevant due to the demand for safe and effective plant origin pharmacological agents with anti-inflammatory and antioxidant properties. The findings will also help identify new natural sources of bioactive compounds with pharmaceutical applications.

METHODS

Plant material

The subject of the study was fresh samples of the aboveground part of *Salvia deserta* Schangin collected in the foothills of Kaskasu in southern Kazakhstan. The produced herbarium of the harvested plant raw material was transferred to the ethnobotanical collection of the botanical garden of the Institute of Botany and Phytointroduction of the Forestry and Wildlife Committee of the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan, where the

species was confirmed (No. 01-05/280, August 2, 2023). Plant collection was carried out from late June to mid-July, during the peak of the plant's flowering period, in accordance with the requirements of Good Agricultural and Collection Practices (GACP). The raw material was cleaned of foreign objects and dried in shade at room temperature (25°C±5°C) with ventilation. After drying, the raw material was ground to a size of 1–3 mm using an IKA M20 laboratory mill [13].

Obtaining the lyophilized extract

The pharmacological properties of the Tartarian sage were studied using ultrasonic extraction. Ground raw material in an amount of 100 g was subjected to extraction with a 40% water-alcohol solution in a 1:5 raw material to extractant ratio. Extraction was performed 3 times for 30 minutes in an ultrasonic bath (Elmasonic S100H, Germany) with a reflux refrigerator. The extracts were then filtered and refrigerated at +2– 8°C for 2 days to precipitate ballast. The resulting alcohol extract was evaporated in a rotary vacuum evaporator (IKA RV-10, Germany) to a thick extract and then lyophilized in a freeze dryer (Harvest Right, USA).

The obtained lyophilized extract of the Tartarian sage is a lyophilized dry powder, greenish-brown in color, with a slightly sweet, astringent taste and a specific aroma. The particles of the extract have an anisodiametric, plate-like shape, with an inhomogeneous and complex surface.

To study the pharmacological properties of the obtained lyophilized extract of Tartarian sage, non-clinical tests were conducted on laboratory animals.

Non-clinical studies of the pharmacological properties of lyophilized extract of Tartarian sage were carried out in the laboratory of the Centre for Experimental Pharmacology and Toxicology (CEPT) of the JSS Academy of Higher Education and Research (JSS AHER, JSS College of Pharmacy, Mysore), which was GLP certified (CPCSEA Central Animal Facility Registration Number 261/PO/ReBi/S/2000/CPCSEA).

Acute oral toxicity

Acute oral toxicity of the aqueous solution of the lyophilized extract was determined and analyzed according to OECD guidelines, test No. 423:2001, IDT, scheme 1 (GOST 32644-2014). Per the procedure, two groups of animals were used, and each group was divided into subgroups of 3 mice each. Since there was no prior information on the toxicity of the test substance, a dose of 500 mg/kg was used as the starting dose, and the maximum dose was 2,000 mg/ kg of body mass. The lyophilic extract was dissolved in purified water and administered to animals at a dose of 0.2 ml orally. The animals were clinically monitored prior to administering the substance, then 30 minutes, 1, 2, 3, and 6 hours after dosing, and at least once a day until the end of the study to assess toxicity and the general condition of the animals. Body weight was recorded before dosing, on day 7, and on day 14. The animals were observed for 14 days. After the studies were completed, the animals were euthanized to test for macroscopic anomalies of internal organs [14].

Antinociceptive properties

Test animals were divided into five groups of 6 mice. The control group was administered saline solution (10 ml/kg). An hour before the administration of acetic acid, a 100 mg/kg solution of diclofenac sodium was administered to the standard group. The experimental groups of animals received an aqueous solution of the lyophilized extract of Tartarian sage at a minimum dose of 100 mg/kg and a maximum dose of 400 mg/kg. All doses were calculated taking into account the weight of the mice and the volume of the injected solution. The preparations were administered by oral gavage. The total amount of writhing movements was calculated after intraperitoneal administration of 0.7% acetic acid solution in a volume of 0.2 ml. Twists and stretches were observed and counted for 15 minutes [15].

Anti-inflammatory activity

Anti-inflammatory activity was assessed by the method of carrageenan-induced paw edema. The tested animals were weighed and divided into five groups of 6 individuals each. The

lyophilic extracts and the reference preparation, diclofenac sodium, were dissolved in purified water and administered per os one hour before the injections. All doses were calculated based on the weight and volume of the therapeutic solution administered to rats. The preparations were administered by oral gavage. The carrageenan solution was administered through a subplantar injection (0.1 ml) to the inside of the right hind paw. Rats in group 1 received saline and served as negative controls. In group 2, the rats were injected with a 1% suspension of carrageenan in saline in the subplantar region of the right hind leg. Group 3 rats were administered diclofenac sodium (50 mg/kg of body mass), which was considered the standard. Increasing doses of the lyophilic extract of *Salvia deserta* Schangin (100 mg/kg, 400 mg/kg of body mass) were administered to rats in groups 4 and 5 [16].

Antioxidant activity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method

The DPPH test is based on the interaction of a stable 1,1-diphenyl-2-picrylhydrazyl free radical with a hydrogen donor. This test relies on the reduction of a radical solution with an antioxidant (AH) or a radical (R •), and the reaction proceeds as follows: DPPH• + AH → DPPH• -H + A•; DPPH• + R• → DPPH• -R [17]. During the reaction, the DPPH solution is discolored from violet, and the color change serves as an indicator of antioxidant activity. The absorbance of the test solutions is measured with an ultraviolet spectrophotometer (UV-1800 Shimadzu, Japan) at a wavelength of 517 nm. The antioxidant property determined by the DPPH reduction method (Sigma Aldrich, USA) is denoted as EC50, which is the concentration of extract consumed to absorb 50% of DPPH free radicals [18]. Methanol of 99.8% purity (Sisco Research Laboratories, India) was used as the solvent, and ascorbic acid solution (10 mg/ ml) was used as the reference standard. Antioxidant activity (AOA) was calculated [19]. Next, a diagram is plotted, and the EC50 is determined from the data of this figure (2) [20].

Determination of antioxidant activity by the Ferric Reducing Antioxidant Power (FRAP) method

The FRAP test is based on the reduction of the 2,4,6-tripyridyl-s-triazine complex (TPTZ) with iron chloride hexahydrate ($FeCl_3 \cdot 6H_2O$). Over the course of the reaction, they become colorless and change color to brown over time [17,18].

To conduct the study, a 10 mg/ ml aqueous- alcoholic solution of lyophilized extract was prepared. Aliquots of the extract solution (5, 10, 20, 40, 60, 80, and 100 µl) were transferred into test tubes and diluted to 1 ml with a 40% aqueous alcohol solution. Next, a phosphate buffer solution (0.2 M pH 6.6) and a 1% potassium ferricyanide solution were added and incubated in a water bath at 50°C for 30 minutes. A 10% trichloroacetic acid solution was added to the resulting solution and stirred in a centrifuge (6,500 rpm) for 5 minutes. To 2.5 ml of the resulting solution, 1 ml of purified water and 0.2 ml of 0.1% $FeCl_3$ solution were added, and the absorbance of the solution was measured with a spectrophotometer (UV-1800 Shimadzu, Japan) at λ = 700 nm. For comparison with the test solution, only the solvent was used in the control group. As a comparative reference group, an ascorbic acid solution (10 mg/ml) prepared in dimethyl sulfoxide (DMSO) was used. The reference standard was prepared in the same way, only using an ascorbic acid solution instead of the extracts [21]. Antioxidant activity and EC50 were determined in the same manner as described above [20].

RESULTS

Acute toxicity of the lyophilized extract of Tartarian sage

The determination of acute toxicity of medicinal substances on animals is conducted to test the safety and select the correct doses of the test substance for further pharmacological studies. Establishing the acute toxicity of the obtained extracts from medicinal plants is crucial, since plant products as part of traditional medicine can result in acute or chronic poisoning [22].

The results of the acute toxicity study of the lyophilized extract of Tartarian sage (*Salvia deserta* Schangin) over the observation period at the doses of 500 mg/kg and 2,000 mg/kg showed no significant changes in animal behavior, macroscopic tissue lesions, or weight loss and no signs of toxicity (changes in skin, coat, eyes, mucous membranes, or behavior, tremor, drooling, or stool change). In terms of body weight dynamics, in the group where the extract was administered at a dose of 500 mg/ kg, an increase in weight was recorded, while the group administered 2,000 mg/kg had no significant deviations in body weight (Table 1).

As a result, the study shows that the examined plant extract has a low risk of acute toxicity and belongs to toxicity class 5 (LD50 >2,000 mg/ kg of body mass). Body weight was monitored before extract administration and on days 7 and 14 of the observation period. No mortality, behavioral abnormalities, or visible signs of toxicity were recorded in any group.

Assessment of antinociceptive efficacy

The antinociceptive effect was determined through an acetic acid-induced writhing test, which is the most popular animal model to evaluate the analgesic effect of pharmacological substances.

Nociception caused by the intraperitoneal administration of acetic acid caused abdominal pain, which manifested itself in mice twisting their abdomen with a maximum reaction in the interval of 10– 15 minutes. In the acetic acid- induced writhing test, diclofenac sodium reduced the number of writhing movements compared with the control group (6.0 vs. 18.5), indicating a marked antinociceptive effect. In the study groups administered the lyophilized extract of Tartarian sage at the doses of 100 mg/ kg as the minimum (11.67) and 400 mg/ kg as the maximum (12.00), the results were positive compared to the control group (Figure 1). The number of writhing movements was decreased, and the nociceptive reaction was reduced after 15 minutes. The lyophilized extract produced a considerably reduced nociceptive response in both doses when compared to the control group, with no apparent increases in antinociceptic effectiveness between the two investigated doses of 100 and 400 mg/kg.

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Anti-inflammatory activity study

Carrageenan- induced hind limb edema is the most commonly used model for assessing the anti-inflammatory effects of pharmacological substances. In our studies, the anti-inflammatory activity of lyophilic extracts was tested by the method of carrageenan- induced paw edema in rats. The test was performed on Wistar albinos (weight 230±5 g).

Following the test, the percentage inhibition of inflammation was calculated [16]. The thickness of the carrageenan-inflamed hind paw was measured with calipers before and after the test at 0, 30, 60, 120, and 360 minutes. The paw thickness in the control group was 2▯16 ± 0▯15 cm at pre-treatment and 3▯61 ± 0▯17 cm at 360 min▯ The paw thickness in diclofenac sodium group (50 mg/kg) was 2▯02 ± 0▯20 cm at pre-treatment and 2▯46 ± 0▯27 cm at 360 min, and the paw thickness in 100 and 400 mg/kg extract treated groups was 2▯11 ± 0▯19 and 1▯94 ± 0▯1 cm at pre-treatment and 3▯27 ± 0▯19 and 2▯69 ± 0▯40 cm at 360 min respectively▯ The obtained data confirm the anti-inflammatory effect compared to controls. The measurement data are provided in Figure 2. The values are given in the inhibition percentage of the anti-inflammatory effect of the lyophilized extract of *Salvia deserta* Schangin at oral doses of 100 mg/ kg and 400 mg/ kg and of diclofenac sodium compared to the control in the model of carrageenan- induced paw edema.

At 360 minutes post- administration of carrageenan, the percentages of paw edema in the diclofenac sodium group, 100 mg/ kg extract group, and the 400 mg/ kg extract group were 68▯ 08%, 90▯ 41%, and 74▯ 47%, respectively▯ Notably, these data are percentages of the paw swelling relative to the control group, indicating the paw edema percent▯ Thus the lower values represent more anti-inflammatory activity and the group treated with the 400 mg/ kg dose was more active than the 100 mg/kg dose and was closer to the diclofenac sodium group (Figure 3).

The results of the antioxidant activity test using DPPH are expressed in the percentage of inhibition and EC50, which indicates the concentration of substance required to inhibit 50% of free radicals. The lower the EC50, the higher the antioxidant activity of the substance. The inhibition percentage is linked with a series of samples or standard concentrations to plot the curve, as shown in Table 2. According to the presented data, the lyophilized extract of Tartarian sage at concentrations up to 100 µg/ ml (0.1 mg/ ml) can reduce the absorbance of the DPPH radical solution. The lyophilized extract of Tartarian sage reduced absorbance by 35%. Based on

the DPPH assay results, calibration curves were constructed for ascorbic acid and the lyophilized extract. These curves were used to determine the EC_{50} values shown in Figure 4.

Regression equations for the standard ascorbic acid and the lyophilized extract of Tartarian sage are y = 6.6667x + 36.308 and y = 3.8462x + 3.4188, respectively. From this equation, we were able to find the EC50 for the control and the extract, which equal 2.05±0.02 and 11.85±0.2986, respectively. As a result, the standard group with ascorbic acid showed better inhibitory activity against DPPH compared to the lyophilized sage extract. However, the resulting difference in EC50 is not as significant, and it can be concluded that the lyophilized extract of Tartarian sage does have antioxidant activity. According to the scale of antioxidant activity (very strong (50 ppm), strong (50–100 ppm), moderate (101–150 ppm), and weak (>150 ppm)), the lyophilized extract of Tartarian sage has a very strong antioxidant effect, since the EC50 reaches 11.85±0.2986.

The study of antioxidant properties by the FRAP method established that the lyophilized extract of Tartarian sage does demonstrate antioxidant activity in concentrations under 100 µg/ml (0.1 mg/ml). As can be seen from the results of FRAP analysis (Table 3), the lyophilized extract of Tartarian sage showed antioxidant activity at a concentration of 10 µg/ml, reducing absorbance by 46.50% compared to the control. The reducing capacity of the lyophilized extract was assessed using the FRAP assay. The extract demonstrated increasing antioxidant activity with increasing concentration.

The results of the FRAP test, the IC50 of the standard and the lyophilized extract, are expressed in inhibition percentages (Table 3), which were then linked to a series of samples with increasing concentrations to plot the curve (Figure 5). Regression curves were plotted using the FRAP assay data to calculate the IC_{50} values for ascorbic acid and the lyophilized extract. The regression equations for ascorbic acid and the lyophilized extract of *Salvia deserta* Schangin are y = 5.944x + 37.879 and y = 4.452x + 3.5214, respectively. According to the classification of antioxidant activity, the lyophilized extract of *Salvia deserta* Schangin is a strong antioxidant, as the IC50 amounts to 10.4399 µg/ml.

Figures

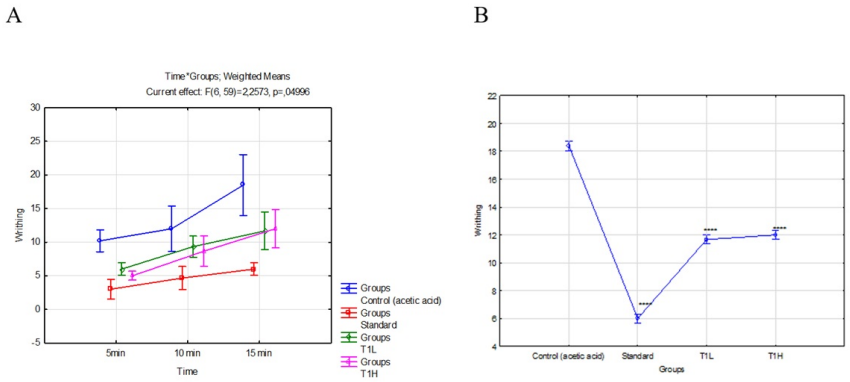

*Figure 1: A – two-way ANOVA for time-treatment curves; B – one-way ANOVA for dose-effect curves, followed by the Tukey-Kramer HSD test for unequal samples (spjotvoll/stoline). *P < 0.001 compared to the control and treated groups (p < 0.05) between groups. LSD = 4.6.*

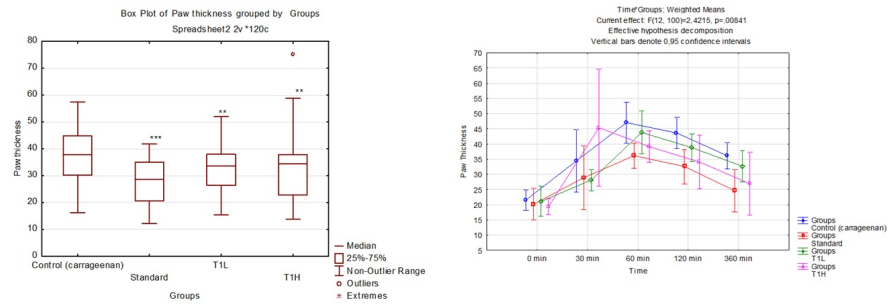

*Figure 2: Anti-inflammatory activity in the carrageenan-induced paw edema model. **P < 0.01 and ***P <*

0.001 compared to the control and treated groups. One-way ANOVA was used to analyze the dose-effect curve, followed by the Tukey-Kramer HSD test for unequal samples (spjotvoll/stoline). Two-way ANOVA was used to analyze the time-response curve.

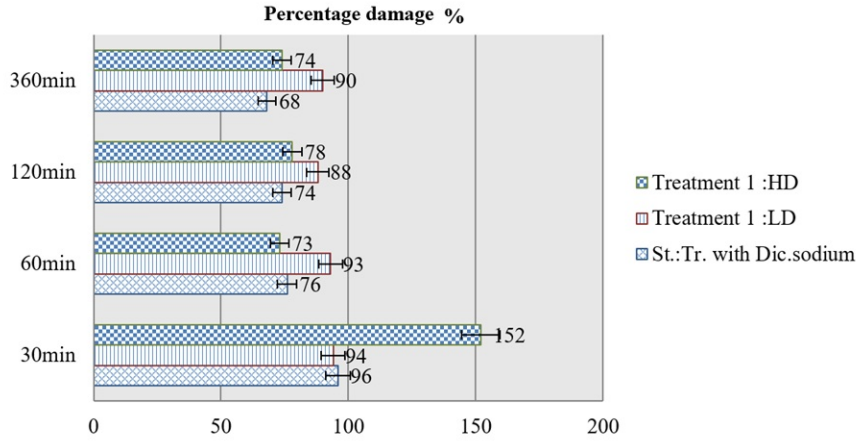

Figure 3: Percentage of carrageenan-induced paw edema over 360 minutes.

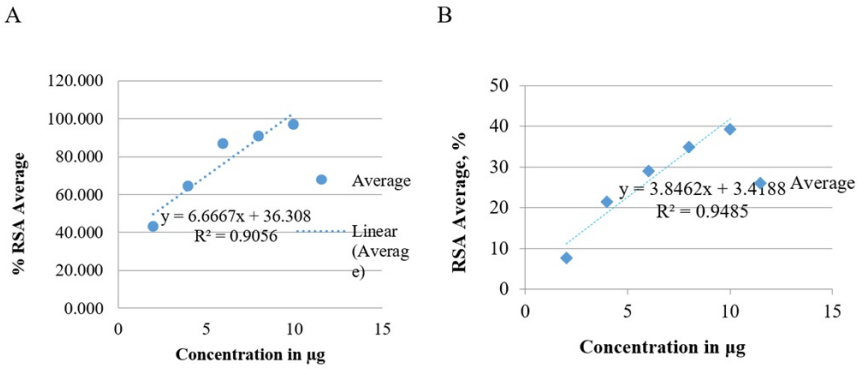

Figure 4: Antioxidant activity curves for (A) ascorbic acid and (B) the lyophilized extract of Salvia deserta Schangin by the DPPH method.

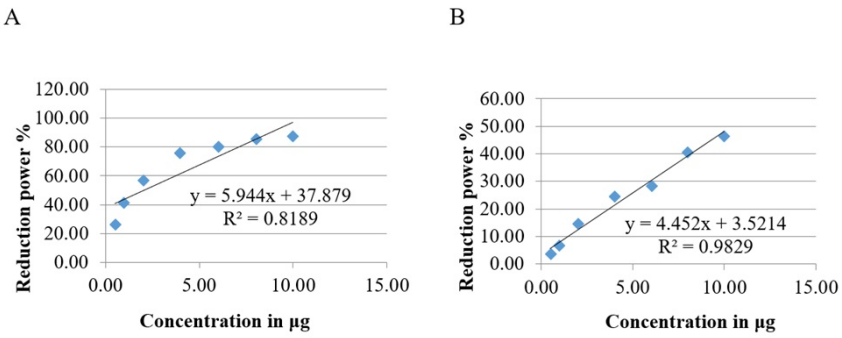

Figure 5: Calibration AOA curves for (A) ascorbic acid and (B) lyophilized extract of Salvia deserta Schangin by the FRAP method.

Tables

Group	Dose (mg/kg)	Day 0 (g)	Day 7 (g)	Day 14 (g)	Note
Control group (placebo)	0	29.20±1.5	30.1±1.4	31.05±1.6	No significant changes
Experimental group 1	500	29.30±1.6	30.30±1.5	32.00±1.4	Stable weight gain
Experimental group 2	2,000	29.33±1.7	29.03±1.6	30.13±1.5	Stable weight, no deviations

Table 1: Body weight changes after acute oral toxicity testing. Note: Data are presented as mean ± SD. The observation period was 14 days. The extract was administered orally at doses of 500 and 2,000 mg/kg.

Conc. µg/ml	Absorbance			
	Standard (ascorbic acid)	AOA, %	Tartarian sage (0.1 mg/ml)	AOA, %
Control	0.186±0.0084	-	0.117±0.0011	-
2	0.105±0.0084	43.369	0.108±0.0012	1.709
4	0.066±0.0003	64.158	0.092±0.0014	7.692
6	0.025±0.0005	86.559	0.083±0.0012	21.367
8	0.017±0.0003	90.681	0.076±0.0013	29.059
10	0.006±0.0001	96.774	0.071±0.0008	35.042

Table 2: Changes in the absorbance of the DPPH radical solution.

Conc. µg/ml	Absorbance			
	Standard (ascorbic acid)	AOA, %	Tartarian sage (0.1 mg/ml)	AOA, %
Control	0.238±0.0025		0.648±0.0114	
0.5	0.32±0.0026	25.86	0.673±0.0117	3.42
1	0.40±0.0035	40.94	0.6983±0.0006	6.92
2	0.56±0.0079	57.14	0.761±0.0015	14.59
4	0.97±0.0081	75.38	0.8593±0.0029	24.36
6	1.21±0.0360	80.27	0.91±0.0034	28.57
8	1.64±0.0209	85.49	1.093±0.3510	40.53
10	1.88±0.0108	87.31	1.215±0.0362	46.50

Table 3: Absorbance values of the standard and the lyophilized extract of Tartarian sage (FRAP).

DISCUSSION

Thus, our research proves that the lyophilized extract of *Salvia deserta* Schangin has pronounced antinociceptive, anti- inflammatory, and antioxidant properties and a low risk of acute toxicity. Its antinociceptive and anti-inflammatory effects may be due to the content of phenolic compounds [23] and are associated with a decrease in the release of secretory proinflammatory cytokines (IL-6 and TNF-α) in macrophages stimulated by lipopolysaccharides [24].

The conducted research provided a comprehensive characterization of the pharmacological properties of the lyophilized extract of *Salvia deserta* Schangin growing in Kazakhstan. Experimental data indicate pronounced analgesic, anti-inflammatory, and antioxidant properties of the extract with a low level of acute toxicity (LD_{50} > 2,000 mg/kg). A significant decrease in nociceptive activity was observed in the acetic acid writhing test, and in the model of carrageenan- induced inflammation, there was a significant decrease in paw swelling comparable to the effect of diclofenac sodium. The EC_{50} and IC_{50} values obtained in the DPPH and FRAP tests confirm the strong ability of the extract to neutralize free radicals.

The results of the study indicate that the pharmacological activity of *Salvia deserta* Schangin stems from the content of phenolic compounds, flavonoids, and terpenoids with anti-inflammatory and antioxidant potential. Given the wide distribution of the plant growth and its resistance to the climatic conditions of Kazakhstan, its extract can be a promising natural raw material for the development of analgesic and anti-inflammatory phytopreparations.

Nevertheless, some limitations of this study are acknowledged: the pharmacological responses were only evaluated in the acute experimental models and in a limited number of animals

Results should be interpreted with caution however and cannot be generalized to the chronic situation or clinical practice at this stage

The findings of this study may serve as a basis for further pharmacological and phytochemical investigations of *Salvia deserta* Schangin. In the future, this research could contribute to the development of standardized phytopreparations and the discovery of new bioactive compounds for the treatment of inflammatory and oxidative stress-related diseases.

This study demonstrated that the lyophilized extract of **Salvia deserta** Schangin possesses a favorable acute safety profile and significant pharmacological activity in the experimental models employed. Oral administration of the extract caused no animal mortality or visible signs of toxicity at doses up to 2000 mg/kg. In the acetic acid-induced writhing test, the extract reduced the nociceptive response in mice, while in the carrageenan-induced paw edema model, it exhibited an anti-inflammatory effect that was most pronounced at a dose of 400 mg/kg. Significant antioxidant activity was also observed: the EC50 value in the DPPH assay was 11.85 µg/mL, and the IC50 in the FRAP assay was 10.4399 µg/mL. Overall, the results indicate that **Salvia deserta** Schangin warrants further pharmacological and phytochemical investigation. Future studies should focus on identifying the extract's major active constituents, determining their contribution to the observed effects, and evaluating the extract's properties in additional toxicity and inflammation models before considering its potential use in standardized herbal preparations.

CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, collection and processing of plant raw material, as well as preparation of the lyophilized extract, were performed by Guldana Shoinbayeva, Bayan Sagindykova and Saltanat Imanalieva. Pharmacological experiments, including acute toxicity, antinociceptive and anti-inflammatory activity studies, were performed by Gnk Ganesh, Dithu Thekkekkara and Saravana Babu Chidambaram. Antioxidant activity testing, data collection and analysis were carried out by Guldana Shoinbayeva, Saltanat Imanalieva and Dithu Thekkekkara. The first draft of the manuscript was written by Guldana Shoinbayeva. Bayan Sagindykova and Saravana Babu Chidambaram supervised the study and critically revised the manuscript. All authors commented on previous versions of the manuscript and approved the final version. Guldana Shoinbayeva is the corresponding author of this manuscript.

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