Registered in Germany Europa Revue Journal
ISSN: 1862-6564 Vol. 6 No. 10 (2026) Published October 10, 2026

EFFECTS OF A WATER–ETHANOLIC EXTRACT OF *BOSWELLIA SACRA* RESIN ON THE CELL MEMBRANE

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Abstract

This study investigated the effects of a water–ethanolic extract of *Boswellia sacra* resin on cell volume regulation and hemolysis. The extract contained flavonoids and polyphenols and markedly inhibited regulatory volume decrease (*RVD*) in thymocytes in a concentration-dependent manner, with a half-maximal inhibitory concentration (C_{50}) of 33.0± 3.4μg/mL and a Hill coefficient (*h*) of 1.6±0.2, while also inducing hemolysis in human erythrocytes.

Keywords

Boswellia sacra, thymocytes, erythrocytes, flavonoids, polyphenols, hemolysis, cell volume regulation.

Introduction

Boswellia sacra is a perennial plant belonging to the family *Burseraceae* and is predominantly found in countries of the Middle East [1]. Its resin possesses anti-inflammatory, antibacterial, and wound-healing properties [2] and has traditionally been used in the treatment of gastric disorders, Alzheimer's disease, and liver diseases [1, 3]. Phytochemical studies have revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and phenolic compounds, with boswellic acids and their derivatives constituting major components of the extract [4]. Analysis of the essential oil isolated from its resin by gas chromatography–mass spectrometry (GC–MS) revealed the presence of

monoterpenes such as E-β-ocimene and limonene, as well as sesquiterpenes such as E-caryophyllene [5].

Cell volume regulation is a fundamental physiological process that is essential for maintaining osmotic homeostasis [6] and plays a critical role in key cellular processes, including proliferation, differentiation, and migration, as well as in the execution of apoptosis [7]. This study aimed to assess the effects of *Boswellia sacra* resin extract on thymocyte volume regulation and erythrocyte membrane integrity.

Materials and Methods

The *Boswellia sacra* resin used in this study was obtained by order from the website https://omanoutpost.bigcartel.com. Extraction was performed according to previously described methods [8–10]. Briefly, 0.5 g of dried and ground *Boswellia sacra* resin was mixed with 10 mL of 80% ethanol in a 50-mL round-bottom flask, shaken for 1 min, and then placed in an ultrasonic bath for 30 min. The resulting suspension was filtered through a paper filter. The total dry matter content of the extract was determined according to the requirements of the Russian Pharmacopoeia OFS.1.4.1.0021.15, with certain modifications. For this purpose, 2 mL of the liquid extract was transferred into a flat-bottomed glass vessel and dried at 102.5±2.5°C for 3 h. The vessel was then tightly covered, cooled to room temperature, and weighed until a constant mass was reached. The dry matter content was expressed as milligrams per milliliter of extract (mg/mL).

The total flavonoid content of the extract was determined according to [9, 10], with certain modifications, using the complexation method with aluminum chloride ($AlCl_3$). Briefly, 100 µL of the extract was added to a glass tube containing 400 µL of water, followed by 30 µL of 5% sodium nitrite ($NaNO_2$) solution. After 5 min, 30 µL of 10% aluminum chloride ($AlCl_3$) solution was added. After an additional 6 min, 200 µL of 1 M NaOH was added, and the final volume was adjusted to 1 mL with distilled water and thoroughly mixed. The flavonoid concentration was determined photometrically at 510 nm and expressed as quercetin equivalents.

The total polyphenol content of the extract was determined using the Folin-Ciocalteu method according to [9, 10], with certain modifications. Briefly, 0.5 mL of the extract or a standard solution of gallic acid (Cat. No. G7384; Sigma-Aldrich, St. Louis, MO, USA; 20–100 µg/mL) was placed in a glass tube. Then, 1.5 mL of Folin-Ciocalteu reagent (Cat. No. 47641; Sigma-Aldrich, St. Louis, MO, USA), diluted 1:10 with distilled water, was added, and the mixture was kept at room temperature for 5 min. Subsequently, 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added, and the

mixture was incubated in the dark for 90 min, with intermittent mixing every 15–20 min. The optical absorbance of the solutions was measured at 725 nm relative to the control sample. The total polyphenol content was expressed as milligrams of gallic acid equivalents per milliliter of extract.

In our experiments, the normal Ringer solution had the following composition (mM): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), and 5 glucose (pH 7.4; 290 mOsm/kg H_2O). The hypotonic solution (147 ± 2 mOsm/kg H_2O) was prepared by diluting the isotonic solution with H-buffer at a ratio of 3:4. The H-buffer had the following composition (mM): 5 KCl, 10 HEPES, 2 $CaCl_2$, 1 $MgCl_2$, and 5 glucose; pH 7.4 (40 ± 2 mOsm/kg H_2O). All reagents were of "chemically pure" or "analytical grade" quality.

The experiments were performed on 6–8-week-old outbred white rats maintained on a standard vivarium diet. All procedures were conducted in strict accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines (https://arriveguidelines.org), and were approved in advance by the Bioethics Committee of the Institute of Biophysics and Biochemistry (No. BEC/IBB-NUU/2019/01–1). Thymocytes were isolated using a standard method [9].

Changes in cell volume were recorded by measuring light transmission according to previously described methods [10, 11]. Changes in thymocyte volume were recorded in transmitted light using an МКМФ-1 microcolorimeter (Russia). A light filter with a maximum transmission at 610 nm was used. The output signal was amplified using a U5-11 amplifier (Russia), digitized using a GO! LINK USB Data Acquisition Interface analog-to-digital converter (Vernier, Beaverton, OR, USA), and recorded onto the computer hard drive using Logger Lite software (Vernier, Beaverton, OR, USA).

During the experiment, 900 μL of normal Ringer solution (control), hypotonic solution without extract (control), or solution containing the test extract at the indicated concentrations was placed in a glass cuvette. The cuvette was thermostated using a circulating bronze jacket connected to an LT Ecocool 100 water-circulating thermostat

(Grant Instruments, Cambridge, UK). After equilibration for 10 min, 100 μL of thymocyte suspension was added to the cuvette, yielding a final cell concentration of 10 million cells/mL. The regulatory volume decrease (RVD) was calculated using the following equation:

$$RVD = \frac{T_{\max} - T_{15}}{T_{\max} - T_0} \times 100\% \quad (1)$$

where T_0 and T_{max} are the initial and maximum values of light transmission, respectively, and T_{15} is the light transmission value measured 15 min after the onset of hypotonic stress.

The concentration dependence of the effects of the test substances on cell volume regulation was approximated using the following Hill equation:

$$RVD = RVD_{\min} + \frac{RVD_{\max} - RVD_{\min}}{1 + \left(\frac{C}{C_{50}}\right)^{h}} \quad (2)$$

where RVD_{min} and RVD_{max} are the minimum and maximum values of the regulatory volume decrease (%), respectively; C is the concentration of the substance (μg/mL); C_{50} is the concentration of the substance producing a half-maximal effect (μg/mL); and h is the Hill coefficient.

Experiments investigating the hemolytic activity of the *Boswellia sacra* resin extract were performed under isoosmotic conditions. Blood was collected from the cubital vein of healthy volunteers using heparin as an anticoagulant according to a generally accepted method [12]. All procedures involving the isolation of human erythrocytes were performed in accordance with the Declaration of Helsinki and the ARRIVE guidelines (https://arriveguidelines.org/) and were approved by the Bioethics Committee of the Institute of Biophysics and Biochemistry, National University of Uzbekistan (No. BEC/IBB-NUU/2019/01–2). Whole blood was diluted 1:10 with Ringer solution and centrifuged at 1000 g for 10 min. The erythrocytes were then washed three times with Ringer solution using the same procedure, and an 8% erythrocyte suspension was prepared. In the wells of a microtiter plate, 200 μL of pure Ringer solution (control) or Ringer solution containing the extract at the indicated concentrations was added. Subsequently, 200 μL of the prepared 8% erythrocyte suspension was added. The mixture was incubated at 37°C for 60 min, and the

hemoglobin content in the supernatant was determined from its optical absorbance at 540 nm. The percentage of hemolysis was calculated using the following equation:

$$\text{Hemolysis} = \frac{OD}{OD_{100}} \times 100\% \quad (3)$$

where OD is the optical density of the supernatant from the experimental sample, and OD_{100} is the mean optical density of the supernatants from three wells containing 1% Triton X-100 detergent. Three control wells were used in each experimental series to determine the background level of hemolysis. The data were analyzed using Origin 8 software (OriginLab, Northampton, MA, USA). All results are presented as the mean ± standard error for *n* experiments. Statistical analysis was performed using Student's *t*-test. Differences were considered statistically significant at $P<0.05$.

Results

Analysis of the *Boswellia sacra* resin extract revealed a dry matter content of 36.6 ± 0.8 mg/mL (n = 3). The total flavonoid concentration was 0.03 ± 0.01 mg/mL (n = 5), corresponding to 0.07% of the total dry residue mass, whereas the polyphenol content was 0.05 ± 0.01 mg/mL (n = 4), corresponding to 0.12%. In subsequent experiments, the liquid extract was used, and the applied concentrations were recalculated based on the dry matter content according to the values given above.

Under isotonic conditions (normal Ringer solution), thymocyte volume remained unchanged for 20 min. Upon transfer to a hypotonic medium, the thymocytes initially swelled rapidly and subsequently restored their volume to a level close to the initial value within 15–20 min. After 15 min of incubation under hypotonic conditions, the regulatory volume decrease (*RVD*) in the control group averaged 81.8 ± 1.6% (n = 3). The *Boswellia sacra* resin extract significantly inhibited the *RVD* process even at a concentration of 10 μg/mL, at which the *RVD* value was 71.7 ± 4.3% (n = 4) of the control value. Further increases in extract concentration progressively reduced the ability of thymocytes to restore their volume. At 150 μg/mL, the *RVD* process was almost completely blocked, with the *RVD* value reduced to approximately 1.8% (n = 4) (Fig. 1A). The concentration–response relationship was well described by the Hill equation (2), yielding a half-maximal inhibitory concentration (C_{50}) of 33.0 ± 3.4 μg/mL and a Hill coefficient (h) of 1.6 ± 0.2 (Fig. 1B).

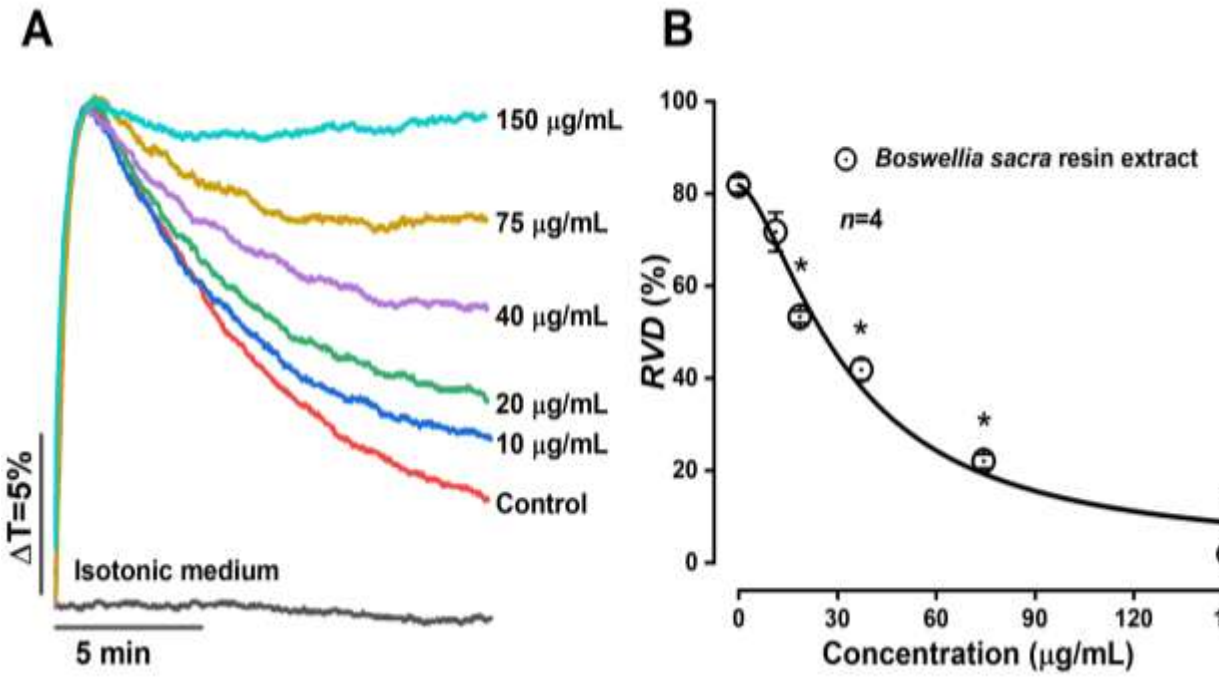

Fig. 1. Effect of *Boswellia sacra* resin extract on cell volume regulation in thymocytes under hypotonic conditions. (A) Representative original recordings of changes in light transmission of the cell suspension over time; (B) concentration dependence of the mean *RVD* values determined according to Eq. (1). An asterisk (*) indicates a statistically significant difference at $P<0.05$.

The hemolytic activity of the *Boswellia sacra* resin extract was evaluated in human erythrocytes under isoosmotic conditions. The effect of the extract was examined over a broad concentration range. The highest level of hemolysis was observed at 880 µg/mL and reached 18.5 ± 2.5% (Fig. 2).

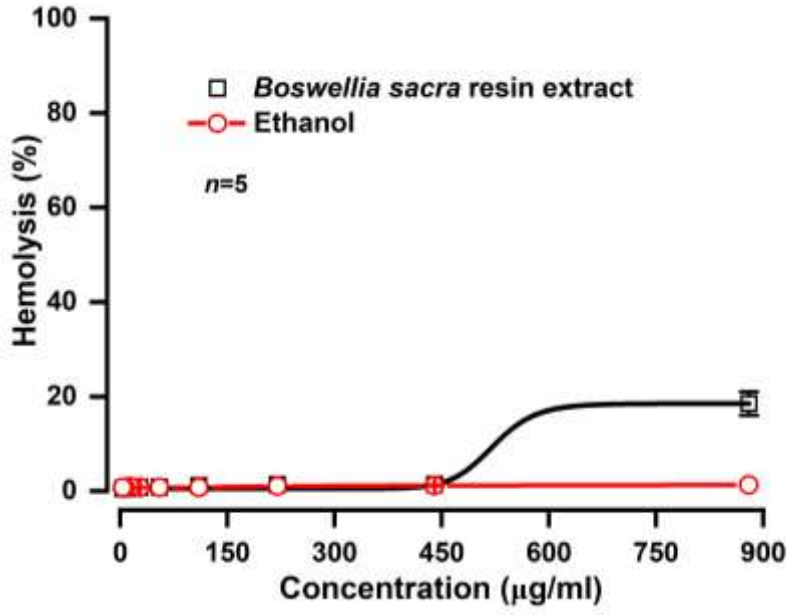

Fig. 2. Effect of *Boswellia sacra* resin extract on hemolysis of human erythrocytes. Dependence of the hemolysis level of human erythrocytes on the concentration of *Boswellia sacra* resin extract. *n* – number of replicates.

Discussion and Conclusion

The results of the present study demonstrate that the hydroethanolic extract of *Boswellia sacra* resin suppresses the regulatory volume decrease (RVD) response in thymocytes exposed to hypotonic stress in a concentration-dependent manner. Notably, the extract exerted a significant effect on cell volume regulation even at the relatively low concentration of 10 μg/mL, which may reflect the biological activity of its constituent compounds, including polyphenols, flavonoids, and terpenoids [4,5]. At the higher concentration of 150 μg/mL, the RVD response was almost completely abolished, which may result from stronger interactions of these bioactive constituents with the membrane lipid bilayer and a consequent impairment of membrane integrity [13,14]. Under hypotonic conditions, the efflux of K^+ and Cl^- constitutes a major component of the RVD response, with swelling-activated chloride channels playing a key role in this process [15]. The C_{50} value of 33.0 ± 3.4 μg/mL indicates a pronounced concentration-dependent activity of the extract and further supports the possibility that its bioactive constituents interfere with membrane-associated mechanisms involved in volume regulation, including ion channel function. Nevertheless, direct evidence for the individual effects of these constituents on ion channels is lacking, and further studies using isolated compounds and fractionated extracts are required to elucidate their specific contributions. In human erythrocytes, the *Boswellia sacra* extract exhibited relatively low hemolytic activity across the tested concentration range (0–880 μg/mL), with hemolysis reaching only 18.5 ± 2.5% at the highest concentration. This relatively limited hemolytic effect indicates that, under the experimental conditions employed, the extract did not cause substantial disruption of erythrocyte membrane integrity, even at its highest tested concentration.

The obtained results demonstrated that biologically active components of the *Boswellia sacra* resin extract inhibit cell volume regulation in a concentration-dependent manner and, owing to its relatively low hemolytic activity, may represent a promising source for the development of immunomodulatory agents.

Acknowledgments

This study was supported by grants F-OT-2021-157 and AL-9524115061 from the Ministry of Higher Education, Science and Innovation of the Republic of Uzbekistan.

Author Contributions

B.M. Kurbanbaev conducted the experiments. B.M. Kurbanbaev and D.D. Fayziev analyzed the data and wrote the manuscript. R.Sh. Kurbannazarova participated in the experimental design, data analysis, and manuscript preparation. R.Z. Sabirov supervised the experiments, analyzed the data, and edited the manuscript.

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