

Pharmacological potency of the different polarity fractions from the *Rosa damascena* Georgian cultivar oil waste aqueous extract.

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ABSTRACT

Damask rose (*Rosa x damascena* Herrm) is a well-known species within the genus *Rosa* L. cultivated in numerous countries due to its ornamental properties and especially for the valuable essential oil found in its flower petals. The latter has been utilized in perfumery, cosmetics, aromatherapy, and medicinal treatments for centuries. Overall, the studies on *R. damascena* provide promising insights into its diverse effects and applications. However, it is important to note that further research is needed to validate these findings and fully understand the mechanisms underlying the observed effects.

The present study aimed to assess the pharmacological features of the potency of the different polarity fractions RDF-H₂O, RDF-50% and RDF-100% derived from the aqueous extract of rose oil waste from the *R. damascena* Georgian cultivar.

In the carrageenan-induced acute inflammation model in rodents, it was established that all three fractions - RDF-H₂O, RDF-50% and RDF-100% have an anti-inflammatory effect of higher-than-average strength. Among them, RDF-50% was significantly more active and, caused the 62.5% inhibition of the edema. A similar efficacy trend was revealed when evaluating the analgesic properties in mice in "Hot plate" model, but the most powerful analgesic effect was shown by fractions RDF-50% and RDF-100%, the maximum effect of which (86.0 and 99.7%, respectively) was observed 1 hour after administration. As a result of the experiment on the gastric ulcer model caused by absolute ethanol in rodents, it was revealed that the samples RDF-H₂O and RDF-100% have a gastroprotective effect of medium strength (48 and 40%, respectively), whereas RDF-50% appeared less effective. The findings

of current study highlight the potential of further investigation of the above-mentioned fractions to identify individual constituents responsible for the observed pharmacological effects.

Keywords: *Rosa damascena* extract; anti-inflammatory; analgesic; gastroprotective

INTRODUCTION

Rosa x damascena Hermm, commonly known as Damask rose [32, 34], has been the subject of multiple studies investigating its effects in various domains, which highlight the potential benefits and applications of the plant [16, 35].

The historical significance of *R. damascena* as a medicinal herb is one aspect explored. For ages it has been traditionally used in different cultures for various purposes, including managing cardiovascular disorders, gastrointestinal problems, and nervous system disorders. [17, 18]. These traditional uses hint at the potential therapeutic effects of *R. damascena*.

Another area of research focuses on the chemical composition of *R. damascena* essential oil and its antimicrobial and antioxidant activities. The essential oil has shown promising antimicrobial properties, inhibiting the growth of microorganisms, and it exhibits antioxidant activity, which could have implications for health and well-being [3, 8, 24, 25]

Furthermore, there is scientific evidence supporting the pharmacological effects of *R. damascena*. In addition to aforesaid activities, studies have demonstrated that *R. damascena* could have broader applications in the field of pharmacology and medicine [5, 12, 13, 29, 33].

In recent years, efforts have been made to explore the waste generated during the extraction of rose oil from *R. damascena* flowers. Due to the relatively low oil content in the flowers, the industrial extraction process produces substantial amounts of waste, including solid residues and wastewater. In the past decade, several methods for valorisation of rose waste have been developed [30, 31]. Studies have revealed that the waste contains a significant amount of biologically active compounds, particularly polyphenols, flavonoids, and polysaccharides [2, 4, 6, 14, 11, 15, 20, 23]. These compounds hold potential for various applications in the pharmaceutical, nutraceutical, and cosmetic industries. By utilizing the waste, researchers aim to maximize the utilization of the *R. damascena* plant and minimize environmental impact.

Research on the polar and non-polar extracts obtained from *R. damascena* flower petals has provided valuable insights into their chemical composition, bioactive compounds, and potential applications. These extracts have attracted significant attention due to the presence of various bioactive components that contribute to their pharmacological properties and industrial significance. [27]. Non-polar extracts, such as essential oils obtained through processes like hydro distillation, have been a subject of interest due to their aromatic properties and commercial applications. *R. damascena* essential oil is highly valued in the perfume and cosmetic industries for its exquisite fragrance. The chemical composition of essential oils from *R. damascena* can vary significantly depending on factors such as climatic conditions, soil composition, and extraction techniques [10]. The major constituents of these oils typically include various terpenes and aromatic compounds. The unique composition of essential oils contributes to their pleasant scent and therapeutic effects [9]. Furthermore, the essential oil has been utilized in traditional

medicine for its medicinal properties, including its use as a base material in aromatherapy and for the treatment of certain diseases.

Recently we reported about biological potency of the aqueous extract of rose oil waste from the *R. damascena* Georgian cultivar [22] and the present study aimed to assess the pharmacological features of the potency of the different polarity fractions RDF-H₂O, RDF-50% and RDF-100% derived from the above-mentioned extract.

MATERIALS AND METHODS

Materials, chemicals and reagents

The plant material was collected from Kakheti floristical region of Georgia during the active flowering season in May, 2020. The identification of the plant was carried out in the Department of Botany at Ivel Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University. A voucher specimen (TBPH–21167) was deposited in the herbarium of the named institute. Aqueous extract of *R. damascena* petals (AERD) was obtained in accordance with the European Pharmacopoeia (Ph. Eur. 2008) guideline. All reagents, and solvents, and chemicals used in the experiments were of analytical grade and obtained from Sigma Aldrich (USA).

Fractionation of AERD

AERD was processed as following: a 20 g dry extract was chromatographed on a Diaion HP-20 column. H₂O-MeOH gradient (100:0, 50:50, and 0:100 v/v) was used as the mobile phase. The fractions were concentrated using rotary vacuum evaporator below 40°C and were frozen in a layer of 10 mm in the Petri dishes at -20 °C in freezer for 12 h, then vacuum dried at -90 °C under 3.33 Pa absolute pressure to constant weight. The dried material was powder-grinded and stored in vacuum desiccator until further use.

Biological assays

Animals:

Inbred white mice weighing 28 ± 2 g ($n = 40$) was housed under standard conditions: including a temperature of $20 \pm 2^\circ\text{C}$, humidity of 55-65%, a 12/12-hour light/darkness cycle, and provided with granulated food (4 g/animal/day) and water *ad libitum*. All experimental procedures adhered to the requirements of the EU Directive 2010/63 [7] and were approved by the Ethics Committee on Animal Research at Tbilisi State Medical University (registration number AP-61-2023).

Analgesic Activity (Hot plate assay)

The analgesic activity was evaluated using a setup comprising an open cylindrical space with transparent vertical walls and a metal floor heated to $52 \pm 2^\circ\text{C}$. The mice were administered the test compounds (50 mg/kg intraperitoneally), and the reaction time, either hind paw licking or jumping, was recorded at regular 30 min intervals over a period of one hour. The analgesic effect was calculated using the formula: $E\% = ((T_0 - T_n) / T_0) \times 100$, where T_0 represented the reaction time before the extract injection, and T_n represented the reaction time after the specified period (30 or 60 min) following injection [26].

Anti-inflammatory Activity (Carrageenan-induced edema assay)

The anti-inflammatory activity was assessed using the carrageenan-induced paw edema model [21]. A 50 μ l injection of 1% carrageenan solution in normal saline was administered into the aponeurosis of the right hind paw of each mouse. One hour prior to the carrageenan injection, the control group received 0.5 ml of normal saline, while the experimental groups received 0.5 ml of the test fractions at a dose of 50 mg/kg, both administered intraperitoneally. The thickness of the paw was measured using a digital micrometer before (baseline) and 2 hours after the carrageenan injection. The anti-inflammatory efficacy was calculated using the formula: $E\% = (1 - (\Delta T_{exp} / \Delta T_{con})) \times 100$, where ΔT_{con} and ΔT_{exp} represented the mean differences in paw thickness before and 2 hours after carrageenan administration in the control and experimental groups, respectively.

Gastroprotective Activity (Ethanol-induced ulcer model): The gastroprotective activity experiment followed the method described by Adinortey et al. [1]. A total of 24 outbred mice were randomly divided into four groups, with six mice in each group. Prior to the experiment, food intake was restricted for 24 hours, and to prevent coprophagy the mice were placed in cages with a raised wire mesh floor. To prevent excessive dehydration during fasting, all mice had free access to a nutritive solution of 8% sucrose in 0.2% NaCl. On the second day, the test group received test fractions (at a dose of 50 mg/kg) intraperitoneally, while the control group - normal saline (0.4 ml/animal). Subsequently, all animals were orally administered absolute ethanol (1 ml/100 g). After 1 hour, the mice were euthanized using CO₂ inhalation. The stomachs were promptly removed, opened along the greater curvature, and rinsed with water and a 10% formalin solution (containing approximately 4% formaldehyde w/v). The stomachs were then fixed on a white polystyrene board and digitally photographed. The macroscopic ulcer index (MUI) was calculated for each stomach based on the following scale, determined by three independent observers: 1 - no lesions, 2 - single petechial lesions, 2.5 - multiple petechial or short linear haemorrhagic lesions, 3 - long linear haemorrhagic lesions, 4 - continuous linear haemorrhagic lesions along the entire length of the glandular part of the stomach. The efficacy of test compounds was calculated using the following formula: $\% I = (MUI_{control} - MUI_{test}) / MUI_{control} \times 100$, where $MUI_{control}$ and MUI_{test} represent the macroscopic ulcer indexes in the control and test groups, respectively.

Statistical Analysis.

Statistical analysis of the experimental data was conducted using Student's t-test [19]. A significance level of $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Hot plate assay.

When evaluating the analgesic properties in "Hot plate" model in mice it was discovered that maximal analgesic effect for all tested fractions was observed 1 hour after the administration. RDF-50% and RDF-100% fractions exhibited nearly similar efficacy: 86.0% and 99.7%, respectively ($p < 0.05$). Fraction RDF-H₂O with 19.5% analgesic effect appeared 4.5-fold less active. (Fig. 1, Fig.4, A)

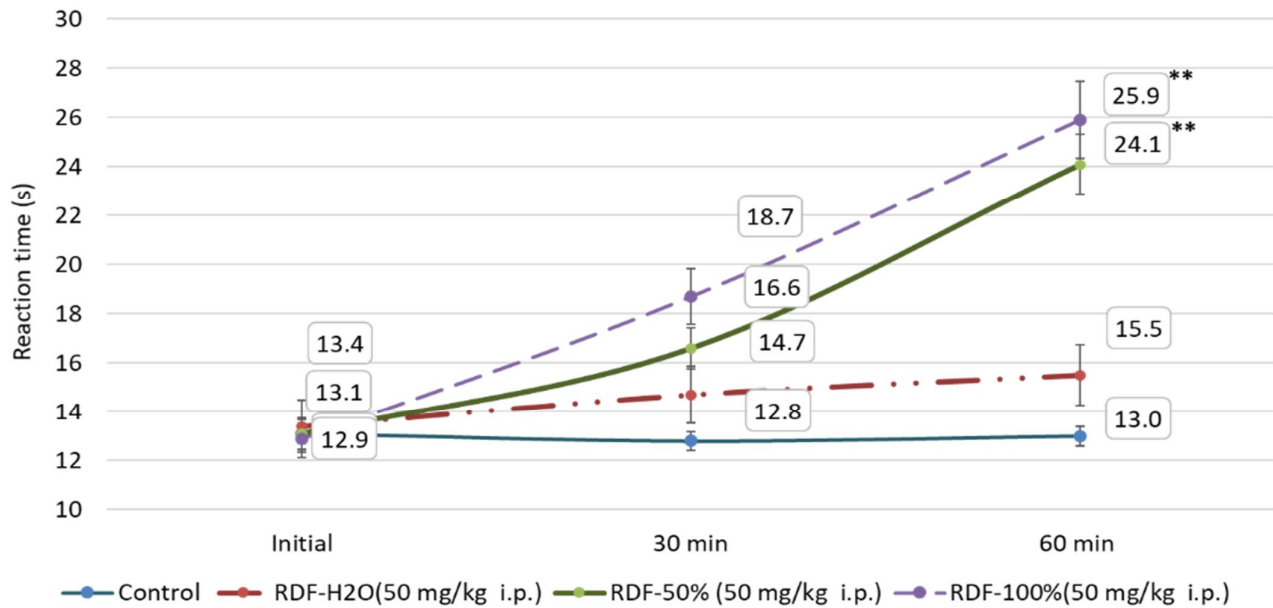


Figure 1. Analgesic efficacy of tested fractions in “Hot plate” model. Each value represents mean±SEM of 6 animals; * - $p < 0.05$; ** - $p < 0.01$ vs control.

Carrageenan-induced edema assay.

In the model of acute inflammation (carrageenan-induced swelling) in rodents, it was established that 2 hours after the carrageenan administration all three fractions - RDF-H₂O, RDF-50% and RDF-100% have an anti-inflammatory effect of higher-than-average strength. An increase in the thickness of the paw varied from 49 to 72 μm , whereas in control animals it was 131 μm (Fig. 2). Among fractions, RDF-50% appeared the most active one causing the 62.5% ($p < 0.01$) inhibition of the edema. (Fig.2, Fig. 4, B)

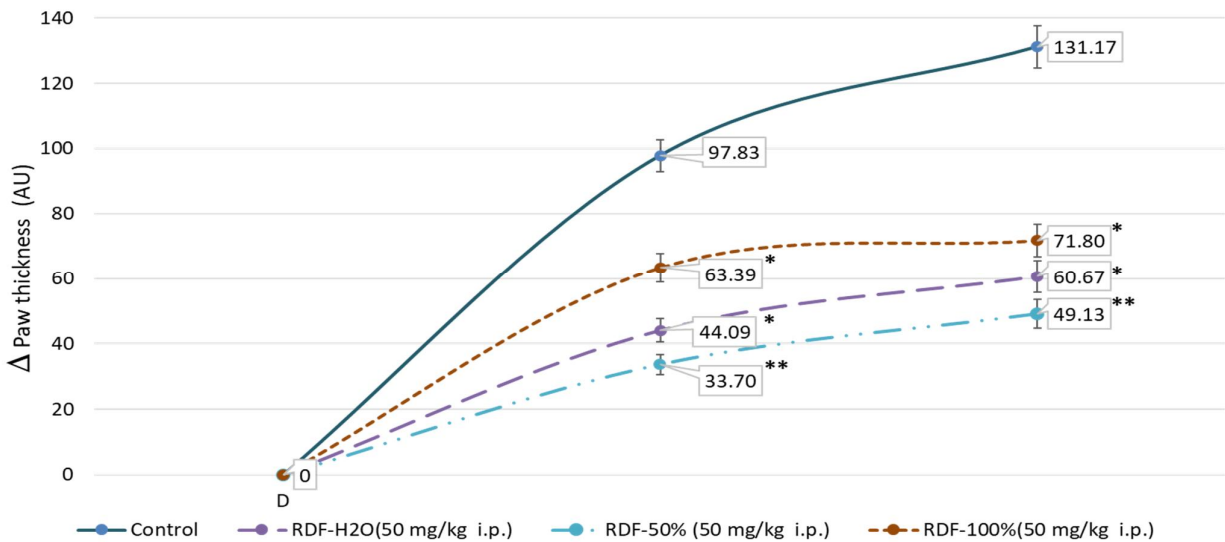


Figure 2. Dynamics of carrageenan-induced edema development in animals from control and experimental groups. Each value represents mean±SEM of 6 animals; * - $p < 0.05$; ** - $p < 0.01$ vs control.

Ethanol-induced ulceration assay

As a result of the experiment on the gastric ulcer model caused by absolute ethanol in rodents, it was revealed that the samples RDF-H2O and RDF-100% have a gastroprotective effect of medium strength (48 and 40%, respectively), whereas RDF-50% appeared less effective (Fig. 3, Fig 4, C). The findings of current study highlight the potential of further investigation of the above-mentioned fractions to identify individual constituents responsible for the observed pharmacological effects.

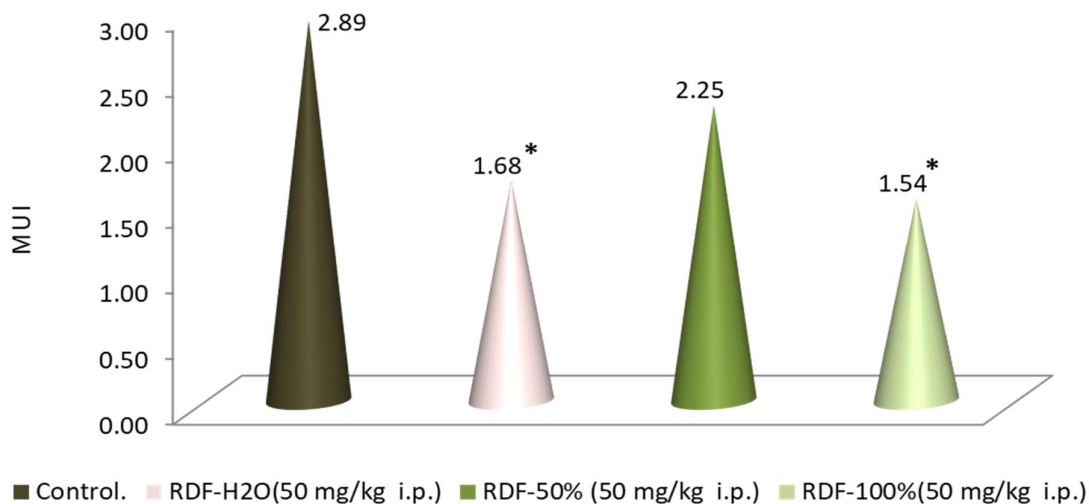


Figure 3. Gastroprotective activity of tested fractions in ethanol-induced gastric ulcer model in mice. MUI - Macroscopic ulcer index. Each value represents mean of 6 animals; * - $p < 0.05$ vs control

DISCUSSION

Polar extracts, such as hydroalcoholic and aqueous extracts, rich in biologically active compounds, including polyphenols, flavonoids, and polysaccharides [4, 15] have been extensively studied for their diverse pharmacological activities [2, 27]. Studies have shown that polar extracts of *R. damascena* exhibit antioxidant properties, which can help protect against oxidative stress-related diseases. The presence of polyphenols and flavonoids in these extracts contributes to their antioxidant effects by scavenging free radicals and inhibiting oxidative damage [3]. Additionally, the hydroalcoholic and aqueous extracts have demonstrated analgesic, anti-inflammatory [8, 12], hypnotic, memory-enhancing, and anticonvulsant activities [28, 29]

Thus, the results of our investigation well correlate with literary data. On the other hand, based on the results obtained (Fig. 4), it is obvious that further research is needed both to identify the compounds responsible for the revealed activities and to explore the full potential of these extracts in various industries and therapeutic applications.

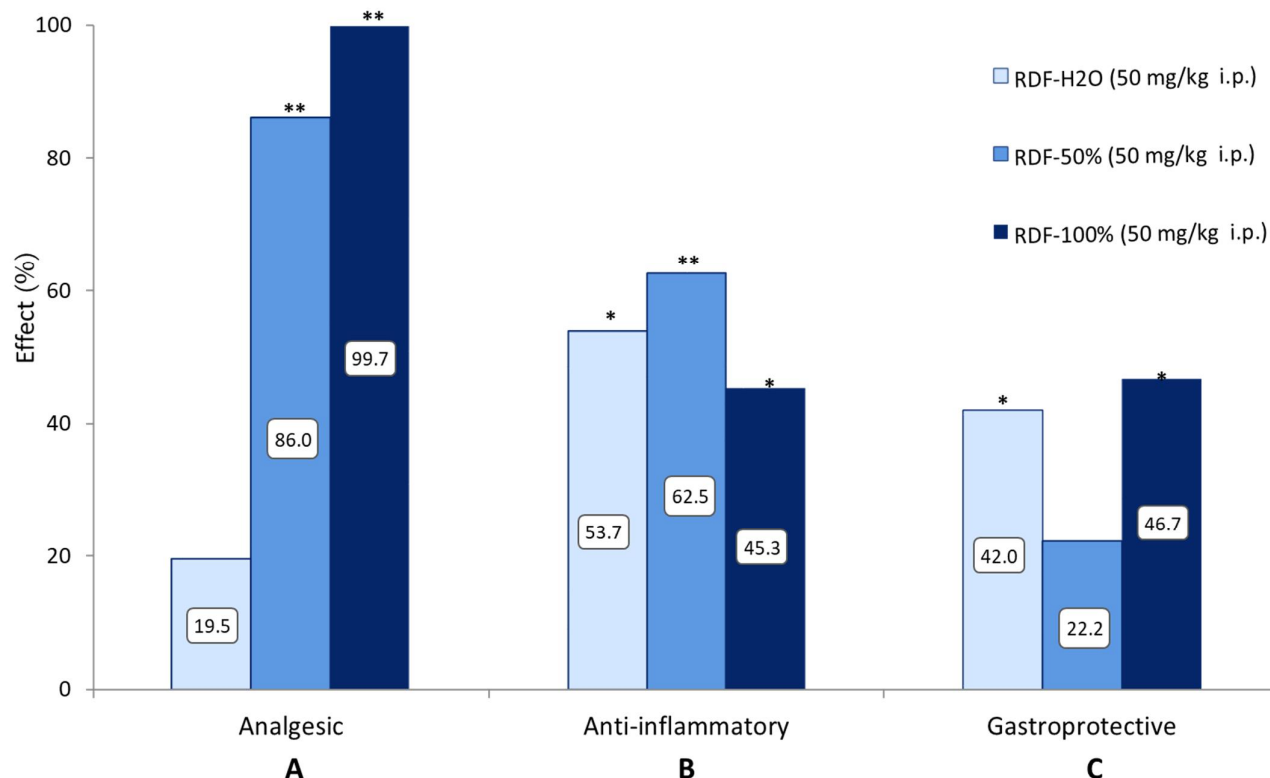


Figure 4. Pharmacological efficacy of tested fractions (consolidated data). * - $p < 0.05$; ** - $p < 0.01$ vs corresponding controls.

CONCLUSION

The present study provided additional data on biological potency of the rose oil waste products from the *R. gallica* Georgian cultivar. It can be concluded that these products contain valuable amounts of bioactive substances that can be used in the pharmaceutical and cosmetic industry as main or auxiliary components. The recovery of such substances additionally carries an ecological impact because it aids in the complete utilization and hence reduction the overall amount of waste produced during the production of rose oil.

COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

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Disclosure of conflict of interest

The authors declare no financial or any other conflicts of interest in this work.

Statement of ethical approval

The animal research protocol was approved by the Tbilisi State Medical University Ethics Committee on Animal Research (registration #AP-61-2023).

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საქართველოში კულტივრებული *Rosa x damascena*-ს ეთერზეთის წარმოებით მიღებული ნარჩენი წყლიანი ფრაქციების ფარმაკოლოგიური პოტენციალი კარენ მულკიჯანიანი¹, მარინე სულაქველიძე¹, ნათელა გოგიტიძე¹, ნადეჟდა მუშკიაშვილი¹, ჟანა ნოვიკოვა¹, ნინო ალექსიძე², სოფიო გოჭაძე^{2,3}, ლაშა მსხილაძე^{1,2*}

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რეზიუმე

დამასკოს ვარდი (*Rosa x damascena* Herrm) გვარი Rosa L.-ის ცნობილი სახეობაა, რომელიც ფართოდაა გავრცელებული მრავალ ქვეყანაში მისი დეკორატიული თვისებების და განსაკუთრებით ძვირფასი ეთერზეთის შემცველობის გამო. ეს უკანასკნელი საუკუნეების მანძილზე გამოიყენებოდა ხალხურ მედიცინაში, პარფიუმერიაში, კოსმეტოლოგიაში, არომათერაპიაში. დამასკოს ვარდზე ჩატარებული სამეცნიერო კვლევის შედეგები იძლევა პერსპექტიულ ინფორმაციას მის მრავალფეროვან ფარმაკოლოგიურ ეფექტებსა და სამკურნალო მიზნით გამოყენებაზე. თუმცა, უნდა აღინიშნოს, რომ ამ დასკვნების

დასადასტურებლად სიღმისეული კვლევებია საჭირო რათა დადგინდეს ბიოლოგიურად აქტიური ნაერთების მოქმედების მექანიზმები.

კვლევა მიზნად ისახავდა საქართველოში კულტივირებული *R. damascena*-დან ვარდის ეთერზეთის წარმოების დროს მიღებული ნარჩენი წყლიანი ექსტრაქტის სხვადასხვა პოლარობის ფრაქციების RDF-H₂O, RDF-50% და RDF-100% ფარმაცოლოგიური პოტენციალის შეფასებას *in vivo* ექსპერიმენტში.

კარაგენანით გამოწვეული მწვავე ანთების მოდელში მღრღნელებში დადგინდა, რომ სამივე ფრაქციას - RDF-H₂O, RDF-50% და RDF-100% აქვს საშუალოზე მაღალი სიძლიერის ანთების საწინააღმდეგო ეფექტი. მათ შორის, RDF-50% იყო მნიშვნელოვნად უფრო აქტიური და გამოიწვია შეშუპების 62.5% დათრგუნვა. თავგებში "ცხელი ფირფიტის" ტესტში ანალგეზიური თვისებების შეფასებისას, ფრაქციებმა RDF-50% და RDF-100% გამოავლინეს ყველაზე ძლიერი ტკივილგამაყუჩებელი ეფექტი, მაქსიმუმით (86.0 და 99.7%, შესაბამისად) მიღებიდან 1 საათის შემდეგ. მღრღნელებში აბსოლუტური ეთანოლით გამოწვეული კუჭის წყლულის მოდელზე გამოვლინდა, რომ ფრაქციებს RDF-H₂O და RDF-100% აქვთ საშუალო სიძლიერის გასტროპროტექტორული ეფექტი (48 და 40%, შესაბამისად), ხოლო RDF-50% ნაკლებად ეფექტური აღმოჩნდა. მიღებული შედეგები ხაზს უსვამს ფიტოქიმიური კვლევების სამომავლო პერსპექტიულობას, რათა დადგინდეს ზემოაღნიშნულ ფრაქციებში ფარმაცოლოგიურ ეფექტებზე პასუხისმგებელი ინდივიდუალური ნაერთები.

საკვანძო სიტყვები: *Rosa damascena*-ს ექსტრაქტი; ანთების საწინააღმდეგო; ტკივილგამაყუჩებელი; გასტროპროტექტორული

Aqueous Extract of *Rosa damascena* - AERD

