RESEARCH PAPER

Study of the Acute Toxicity and Local Anesthetic Properties of the Combined Flavonoids Isolated from *Salvia sclarea* L. and *Matricaria chamomilla* L.

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ABSTRACT

In this study, the acute toxicity (LD_{50}) and local anesthetic properties of the total flavonoid fraction isolated from *Salvia sclarea* L. (clary sage) and *Matricaria chamomilla* L. (chamomile) were investigated using experimental methods. The local anesthetic activity of the flavonoid complex was evaluated employing the Regnier, Bülbring, and Weid experimental models. The test substance was compared with the reference drug, lidocaine hydrochloride, to assess its anesthetic efficacy.

Keywords: *Salvia sclarea* L. (clary sage), *Matricaria chamomilla* L. (chamomile), flavonoids, local anesthesia, Regnier method, Bülbring and Weid method, acute toxicity, LD50

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INTRODUCTION

In recent years, there has been growing interest in modern pharmaceutical science and medicine in the development of medicinal products based on biologically active compounds of natural origin. Among these, flavonoids have attracted particular attention due to their broad spectrum of pharmacological activities. They exhibit antioxidant, anti-inflammatory, capillary-protective, antimicrobial, immunomodulatory, and analgesic effects [1,2]. Flavonoids exert their pharmacological activity in both animals and humans by scavenging free radicals, reducing lysosomal enzyme activity, and inhibiting prostaglandin biosynthesis [3].

Salvia sclarea L. (clary sage) and *Matricaria chamomilla* L. (chamomile) have long been used in traditional medicine as anti-inflammatory, antiseptic, and analgesic remedies. The combined flavonoid fraction isolated from these plants is believed to possess a synergistic pharmacological effect, making its toxicological safety and analgesic potential important subjects for further investigation [5,6].

The present study evaluated the acute toxicity and local anesthetic activity of the total flavonoid fraction isolated from *Salvia sclarea* L. and *Matricaria chamomilla* L. using established experimental models.

The primary objective of this research was to determine the LD_{50} value of the flavonoid complex and to compare its local anesthetic activity with that of the reference drug, lidocaine hydrochloride, using the Regnier, Bülbring, and Weid experimental models.

Materials and Methods

The study was conducted in accordance with the bioethical requirements of the Ministry of Health of the Republic of Uzbekistan and complied with both international and national regulatory standards, including GOST R 53434-2009 (“Safety Rules for Working with Laboratory Animals”) and GOST ISO/IEC 17025-2019 (“General Requirements for the Competence of Testing and Calibration Laboratories”) [7,8].

The object of investigation was the total flavonoid fraction isolated from *Salvia sclarea* L. and *Matricaria chamomilla* L. Acute toxicological

safety was assessed using healthy outbred white mice weighing 18–20 g. Throughout the experimental period, the animals were maintained under standard vivarium conditions, including a temperature of 22 ± 2°C, relative humidity of 60–70%, and a 12:12-hour light–dark cycle. The mice were provided with a standard laboratory pelleted diet and had unrestricted access to drinking water.

Acute Toxicity Assessment

To evaluate acute toxicity, the flavonoid preparation was administered orally to mice at doses ranging from 100.0 mg/kg to 14,000.0 mg/kg using an atraumatic metal gavage probe. Six animals were included in each dose group. The experimental observation period lasted for 14 days**.** During this time, the animals were monitored for their general clinical condition, locomotor activity, reflex responses, respiratory rate, heart rate, and changes in the appearance of the skin and mucous membranes. Signs of intoxication, mortality, and the time of death, when applicable, were also recorded. The median lethal dose (**LD_{50}**) was calculated using statistical analysis performed with IBM SPSS Statistics v27.0.1.0 software [9].

Evaluation of Local Anesthetic Activity

To investigate local anesthetic properties, 18 laboratory rabbits **weighing** 2.5–3.0 kg and 18 healthy guinea pigs weighing 300–400 g were selected. The test compounds were dissolved in 0.9% sodium chloride (NaCl) physiological saline at concentrations of 0.25%, 0.5%, and 1.0%**.** The control group received only physiological saline, whereas the reference group was treated with lidocaine hydrochloride solutions of corresponding concentrations.

Regnier Method

Surface anesthetic activity was assessed using the Regnier method**,** based on the corneal reflex in rabbits. Each rabbit received 0.2 mL of the test solution into the conjunctival sac, administered as two consecutive instillations of 0.1 mL separated by a 15-second interval**.** Corneal sensitivity was evaluated by mechanical pain stimulation, and the onset time, duration of complete anesthesia, and total duration of the anesthetic effect were recorded over a 30-minute observation period [10,11].

Bülbring and Weid Method

Infiltration anesthetic activity was evaluated according to the Bülbring and Weid method**.** Each guinea pig received a subcutaneous injection of 0.25 mL of the test solution. Sensory responsiveness was assessed using an entomological needle at 1–5, 5–10, 10–15, 15–20, 20–25, and 25–30 minutes after injection. The presence or absence of a pain response at each time interval was recorded using the symbols "+" (response present) or "–" (response absent). The obtained results were statistically compared with those observed for lidocaine hydrochloride [12–14].

Statistical Analysis

All experimental data were analyzed using IBM SPSS Statistics 27 software. Quantitative results were expressed as mean ± standard error (M ± m)**.** Differences between experimental groups were evaluated using Student's ***t***-test, and a P value of ≤ 0.05 was considered statistically significant [15].

Results and Discussion

To assess the acute toxicity level of the total flavonoid fraction isolated from *Salvia sclarea* L. and *Matricaria chamomilla* L., white mice weighing 18–20 g were administered the substance orally (*per os*) at doses ranging from **100.0 to 16,500.0 mg/kg**.

Clinical Manifestations of Acute Intoxication

Following administration of the total flavonoid fraction isolated from *Salvia sclarea* L. and *Matricaria chamomilla* L. at doses of **100.0–9,000.0 mg/kg**, the animals' motor activity and responses to sound and pain stimuli remained within normal limits. At doses of **10,000.0–14,000.0 mg/kg**, reduced motor activity and rapid, shallow breathing were observed within 5 minutes. Within 25 minutes, a decrease in response to external stimuli and unsteady movement were noted. At the 40th minute, relaxation of the motor muscles was observed.

No mortality was recorded among the experimental animals at the above-mentioned doses during the 14-day observation period (Table 1). When the substance was administered at a dose of **15,000.0 mg/kg**, rapid and shallow breathing, as well as decreased motor activity, were observed after 5 minutes. A reduced response to external stimuli was noted within 25 minutes, followed by relaxation of the motor muscles.

Table 1

Acute Toxicity Following Oral (*per os*) Administration of the Total Flavonoid Fraction Isolated from *Salvia sclarea* L. (Clary Sage) and *Matricaria chamomilla* L. (Chamomile)

№	**Name of the Substance**	**Dose (mg/kg)**	**Animal Mortality/ Survival Rate**	LD_{50}

1	**Total Flavonoid Fraction Isolated from *Salvia sclarea* L. (Clary Sage) and *Matricaria chamomilla* L. (Chamomile)**	100 500 1000 3000 6000 1000 0 1300 0 1400 0 1450 0 1500 0 1530 0 1550 0 1580 0 1600 0 1650 0	0/6 0/6 0/6 0/6 0/6 0/6 0/6 0/6 0/6 1/5 2/4 3/3 4/2 5/1 6/0	15183.4 (14481.0± 15729.6)

Confidence Limits

		95% Confidence Limits for VAR00001		95% Confidence Limits for log (VAR00001)		
	Вероятно					
PROBIT	,010	12660,178	9386,967	13670,662	4,102	3,973	4,136
	,020	12932,686	9902,333	13860,501	4,112	3,996	4,142
	,030	13108,617	10243,170	13983,297	4,118	4,010	4,146
	,040	13242,538	10506,792	14077,040	4,122	4,021	4,149
	,050	13352,482	10725,843	14154,259	4,126	4,030	4,151
	,060	13446,779	10915,561	14220,738	4,129	4,038	4,153
	,070	13530,008	11084,379	14279,653	4,131	4,045	4,155
	,080	13604,966	11237,488	14332,946	4,134	4,051	4,156
	,090	13673,498	11378,324	14381,898	4,136	4,056	4,158
	,100	13736,887	11509,289	14427,400	4,138	4,061	4,159
	,150	14002,476	12064,567	14621,186	4,146	4,082	4,165
	,200	14217,216	12519,567	14783,386	4,153	4,098	4,170
	,250	14404,066	12917,470	14931,017	4,158	4,111	4,174
	,300	14573,955	13278,075	15073,348	4,164	4,123	4,178
	,350	14733,171	13611,518	15217,278	4,168	4,134	4,182
	,400	14885,859	13922,685	15369,435	4,173	4,144	4,187
	,450	15035,092	14212,925	15537,300	4,177	4,153	4,191
	,500	15183,420	14481,074	15729,663	4,181	4,161	4,197
	,550	15333,211	14724,979	15956,100	4,186	4,168	4,203
	,600	15486,930	14943,876	16225,432	4,190	4,174	4,210
	,650	15647,429	15140,433	16544,689	4,194	4,180	4,219
	,700	15818,372	15320,786	16920,552	4,199	4,185	4,228
	,750	16004,942	15493,171	17363,340	4,204	4,190	4,240
	,800	16215,288	15667,223	17893,083	4,210	4,195	4,253
	,850	16463,962	15855,466	18550,989	4,217	4,200	4,268
	,900	16782,277	16079,620	19432,475	4,225	4,206	4,289
	,910	16860,078	16132,448	19653,760	4,227	4,208	4,293
	,920	16945,007	16189,436	19897,751	4,229	4,209	4,299
	,930	17038,885	16251,696	20170,316	4,231	4,211	4,305
	,940	17144,347	16320,827	20480,006	4,234	4,213	4,311
	,950	17265,423	16399,267	20839,987	4,237	4,215	4,319
	,960	17408,766	16491,018	21272,157	4,241	4,217	4,328
	,970	17586,619	16603,417	21817,200	4,245	4,220	4,339
	,980	17825,860	16752,495	22565,475	4,251	4,224	4,353
	,990	18209,557	16987,478	23801,082	4,260	4,230	4,377

Figure 1. Confidence Limits for the Toxic Dose

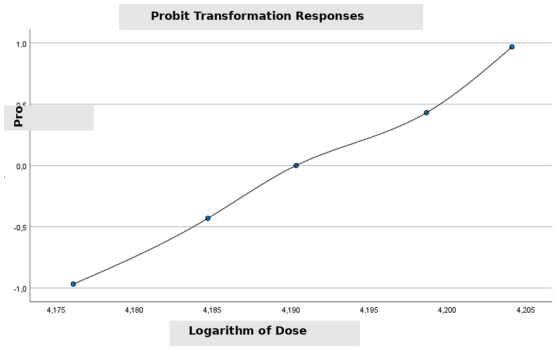


Figure 2. Increase in Toxicity.

Results of the Regnier Method (Rabbit Model):



Figure 3. Administration of the total flavonoid fraction isolated from *Salvia sclarea* L. (clary sage) and *Matricaria chamomilla* L. (chamomile) into the conjunctival sac of laboratory rabbits.

Local Anesthetic Activity

In studies conducted on rabbits using the Regnier method**, a** 1.0% lidocaine hydrochloride solution produced the onset of local anesthesia after 4.1 ± 0.5 minutes, with the anesthetic effect lasting for 17.3 ± 0.9 minutes**.** In comparison, a 1.0% solution of the total flavonoid fraction isolated from *Salvia sclarea* L. and *Matricaria chamomilla* L. initiated anesthesia after 4.5 ± 0.6 minutes**,** while the duration of action was 9.7 ± 0.4 minutes (Table 2).

Table 2. Evaluation of the local anesthetic properties of the total flavonoid fraction isolated from *Salvia sclarea* L. (clary sage) and *Matricaria chamomilla* L. (chamomile) in comparison with **lidocaine hydrochloride** using the **Regnier method** in laboratory rabbits.

№	Test Substance	Dose (%)	Anesthesia			
			Onset Time of Anesthesia (min)	End Time of Anesthesia (min)	Duration of Complete Anesthesia (min)	Total Duration of Anesthesia (min)

1	**Lidocaine Hydrochloride**	0.25	-	-	-	-
		0.5	5.2± 0.7	8.1± 0.4	2.9± 0.3	8.1± 0.4
		1.0	4.1± 0.5	17.3 ±0.9	13.2 ±0.8	17.3 ±0.9
2	Total Flavonoid Fraction Isolated from ***Salvia sclarea*** L. (Clary Sage) and ***Matricaria chamomilla*** L. (Chamomile)	0.25	-	-	-	-
		0.5	-	-	-	-
		1.0	4.5± 0.6	9.7± 0.4	5.2± 0.5	9.7± 0.4

Note: $P \leq 0.05$ compared with the control group.

The experimental findings demonstrate that the total flavonoid fraction possesses local anesthetic activity, although its duration of action is shorter than that of lidocaine**.** Nevertheless, its natural origin and low toxicity profile represent significant advantages that support its potential for clinical application.

In addition to their direct effects on pain receptors, flavonoids exhibit anti-inflammatory, membrane-stabilizing, and antioxidant properties, which are believed to enhance and potentiate their overall analgesic activity. These multifunctional pharmacological characteristics make the flavonoid complex a promising candidate for the development of safe and effective plant-derived local anesthetic agents.

Results of the Bülbring and Weid Method (Guinea Pig Model):



Figure 4. Subcutaneous administration of the total flavonoid fraction isolated from *Salvia sclarea* L. (clary sage) and *Matricaria chamomilla* L. (chamomile) into the subcutaneous adipose tissue of a guinea pig.

Local Anesthetic Activity

The studies conducted using the Bülbring and Weid method enabled the evaluation of the infiltration anesthetic properties of the test substances. Lidocaine hydrochloride at concentrations of 0.5% and 1.0% completely suppressed pain sensitivity during the 5–15 minute observation period. In comparison, the 1.0% flavonoid preparation exhibited a pronounced local anesthetic effect, with significant analgesic activity observed during the 15–30 minute interval (Table 3).

Table 3. Evaluation of the local anesthetic properties of the total flavonoid fraction isolated from *Salvia sclarea* L. (clary sage) and *Matricaria chamomilla* L. (chamomile) in comparison with lidocaine hydrochloride using the Bülbring and Weid method in laboratory rats.

№	**Test Substance**	**Dose %**	**Pain Sensitivity**					
			5 min	10 min	15 min	20 min	25 min	30 min
1	**Lidocaine Hydrochloride**	0.25	+	+	+	+	+	+
		0.5	-	-	+	+	+	+
		1.0	-	-	-	-	+	+
2	**Total Flavonoid Fraction Isolated from *Salvia sclarea* L. (Clary Sage) and**	0.25	+	+	+	+	+	+
		0.5	+	+	+	+	+	+
		1.0	-	-	-	+	+	+

	***Matricaria chamomilla* L. (Chamomile)**							

Note: $P \leq 0.05$ compared with the control group.

Although the 0.25% and 0.5% concentrations of the total flavonoid preparation also demonstrated analgesic activity, the highest efficacy was observed at the 1.0% concentration**.** The observed effects were found to be statistically significantly different from those of the control group ($P \leq 0.05$)**.**

Discussion

The experimental findings indicate that the total flavonoid fraction exhibits analgesic activity amounting to at least 50% of that produced by the conventional synthetic local anesthetic, lidocaine**.** At the same time, the flavonoid preparation is characterized by low toxicity, ease of preparation, and derivation from natural plant sources**,** making it a promising candidate for pharmaceutical development and future clinical evaluation.

In addition to its direct action on pain receptors, flavonoids possess anti-inflammatory, membrane-stabilizing, and antioxidant properties**,** all of which are considered to contribute synergistically to their analgesic effect. These multifunctional pharmacological characteristics may enhance their therapeutic potential and support their use as natural alternatives or adjuncts to synthetic local anesthetic agents.

Conclusion

The results of the experimental studies demonstrated that the total flavonoid fraction isolated from *Salvia sclarea* L. and *Matricaria chamomilla* L. possesses significant pharmacological properties and belongs to the category of compounds with a high level of toxicological safety, characterized by low toxicity and appreciable local anesthetic activity.

According to the acute toxicity assessment, the median lethal dose (**LD_{50}**) of the flavonoid complex was determined to be 15,183.4 mg/kg, indicating that the substance can be classified as low-toxic. This finding supports its favorable safety profile and suggests its potential suitability for pharmaceutical applications.

The Regnier test performed on rabbits demonstrated that a 1.0% solution of the flavonoid complex produced a local anesthetic effect with an onset time of 4.5 ± 0.6 minutes, maintaining analgesic activity for approximately 9.7 ± 0.4 minutes**.** Although its efficacy was compared with that of lidocaine hydrochloride, the flavonoid preparation exhibited promising anesthetic properties.

Furthermore, investigations using the Bülbring and Weid method confirmed the infiltration anesthetic activity of the flavonoid mixture. In particular, the 1.0% concentration produced a stable and sustained analgesic effect during the 15–30 minute observation period.

Overall, these findings indicate that the combined flavonoid fraction isolated from *Salvia sclarea* L. and *Matricaria chamomilla* L. may serve as a pharmacologically effective, safe, and promising natural alternative to conventional local anesthetics. Its low toxicity and the potential for fewer adverse effects compared with synthetic anesthetic agents enhance its clinical relevance and therapeutic value.

The present study provides a scientific basis for the further development of phytopharmaceutical products containing this flavonoid complex, optimization of dosage forms, and progression toward preclinical and clinical investigations. The results also support the evidence-based validation of medicinal plants traditionally used in folk medicine and highlight their potential for incorporation into modern pharmaceutical practice.

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