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Steroidal Glycosides from the Leaves of Caucasus Endemic Plant Solanum woronovii Pojark.: Pharmacological Insights

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ABSTRACT

Background: The global distribution encompasses approximately 2,000 species of Solanum L., with 12 species identified within the geographical boundaries of Georgia, among which Solanum woronowii Pojark. stands as an endemic species. Steroid glycosides extracted from Solanum L. species exhibit a spectrum of purported pharmacological properties, including antimicrobial, anti-ulcer, antiviral, antioxidant, analgesic, anti-inflammatory, cytotoxic, and fungicidal effects.

Objectives: Given the limited available data concerning the biological activities of chemical constituents derived from Solanum woronowii leaves, this investigation seeks to assess the pharmacological potential of a steroid glycosides-enriched fraction derived from this source.

Methods: Experiments were conducted in vivo to evaluate the abovementioned fraction's anti-inflammatory, analgesic, and gastroprotective activities. Established methodologies were employed, including carrageenan-induced acute inflammation, "hot plate" assays for analgesia, and ethanol-induced gastric ulceration models. Additionally, antioxidant activity was assessed using the ABTS assay.

Results: Pharmacological assessments revealed that the steroid glycosides-enriched fraction extracted from Solanum woronowii leaves demonstrated moderate anti-inflammatory (34%) and gastroprotective (32%) activities, along with mild short-term analgesia (23%). The fraction exhibited minor antioxidant activity.

Conclusions: While the pharmacological efficacy of the enriched fraction from Solanum woronowii leaves appears less potent than conventional drugs, its potential as an alternative therapy with reduced side effects warrants consideration. Further investigation must elucidate its safety profile and role in therapeutic strategies.

Keywords: Biological activity; Solanum woronovii Pojark.; steroid glycosides.

BACKGROUND

Solanum L. species, known as black nightshades, encompass many biologically active compounds. The genus comprises approximately 2,000 species globally, with 12 species identified in the region of Georgia, among which Solanum woronowii Pojark. stands as an endemic species of the Caucasus region.[1-3] Over the past three decades, up to 700 substances have been isolated and characterized from various Solanum L. species, including steroid compounds, glycoalkaloids, pregnane glycosides, terpenes, flavonoids, and other bioactive constituents. Steroid glycosides derived from black nightshade species are purported to possess various pharmacological effects, such as antimicrobial, anti-ulcer, antiviral, antioxidant, analgesic, anti-inflammatory, cytotoxic, and fungicidal activities.[4-9] Despite this, there remains to be more comprehensive data regarding the chemical composition and biological activity of Solanum woronowii leaves. It is important to note that the chemical profile of plants can vary significantly based on factors such as geographical location, environmental conditions, and the specific plant part analyzed.

Recent phytochemical investigations have focused on the leaves of S. woronovii, including an in vitro assessment of the antioxidant activity of a glycosides-enriched fraction. Given these considerations, the present study evaluates the pharmacological efficacy of fractions obtained from S. woronowii Pojark leaves, specifically enriched with steroid glycosides (referred to as SwSF).

METHODS

Materials and chemicals

ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) and gallic acid (2,4,6-trihydroxy benzoic acid) were procured from Sigma–Aldrich (Bornem, Belgium), while quercetin (3,3',4',5,7-pentahydroxy-2-phenylchromen-4-one) was obtained from ChromaDex (LGC Standard, France). All chemicals utilized were of analytical grade. The steroidal glycosides enriched fraction from S.woronowii Pojark leaves was obtained using previously established methods.[10]

ABTS assay

The antioxidant activity of the SwSF was evaluated using the ABTS assay.[11] In brief, antioxidants were introduced into a solution containing ABTS radical cation, resulting in decreased absorption due to ABTS termination. SwSF was prepared at 50, 25, 12.5, 6.25, and 3.125 µg/ml concentrations. Gallic acid and quercetin were utilized as reference compounds at final concentrations of 10, 5, 2.5, 1.25, 0.625, and 0.3125 µg/ml. Samples and standards were

dissolved in DMSO and distributed in 96-well microplates. The negative control comprised 0.02 mL DMSO in 1.98 mL of ABTS solution. Samples and standards were prepared and measured in triplicates using a Thermo Multiskan Ascent Plate reader at 734 nm.

Experimental animals

Inbred white mice weighing 28 ± 2 g (n = 36) were housed under standard conditions, including a temperature of 20 ± 2°C, humidity of 55-65%, and a 12/12-hour light/darkness cycle. Animals were provided granulated food (4 g/animal/day) and water ad libitum. All experimental procedures strictly adhered to the principles of the 3Rs outlined in the EU Directive 2010/6312 and internationally accepted guidelines.[12,13] Additionally, the study protocol received approval from the TSMU Ethics Committee on Animal Research (registration number AP-56-2022).

Analgesic activity (Hot Plate Assay)

The analgesic activity was assessed using an open cylindrical space with transparent vertical walls and a metal floor heated to 52 ± 2°C. Mice were administered the test compound (10 mg/kg intraperitoneally), and the reaction time, defined as hind paw licking or jumping, was recorded at regular 30-minute intervals over one hour. The analgesic effect was calculated using the formula E% = ((To-Tn) /To) × 100, where To represents the reaction time before the extract injection, and Tn represents the reaction time after the specified period (30 or 60 min) following injection. [14,15]

Anti-inflammatory activity (Carrageenan-induced Paw Edema Assay)

The carrageenan-induced paw edema model was employed to assess anti-inflammatory activity.[16] In brief, each mouse received a 50 µl injection of 1% carrageenan solution in normal saline into the aponeurosis of the right hind paw. One hour before carrageenan injection, mice in the control group received 0.5 ml of normal saline. In contrast, mice in the experimental group were administered 0.5 ml of the test fractions intraperitoneally at 100 mg/kg. Paw thickness was measured using a digital micrometer before carrageenan injection (baseline measurement) and 2 hours post-injection. Anti-inflammatory efficacy was calculated using the formula: E% = (1 - (ΔTexp/ΔTcon)) x 100, where ΔTcon represents the mean difference in paw thickness before and 2 hours after carrageenan administration in the control group, and ΔTexp represents the same difference in the experimental groups.

Ethanol-induced ulcer model

A total of 12 mice were randomly divided into three groups, each containing six mice. Before the experiment, food intake was restricted for 24 hours, and to prevent coprophagy, mice were placed in cages with a raised wire mesh floor. All animals had free access to 8% sucrose solution in 0.2% NaCl to prevent dehydration during fasting. On the second day, the test group received the SwSF fraction at 50 mg/kg intraperitoneally, while the control group received normal saline (0.4 ml/animal). After 1 hour, the mice were euthanized using CO2 inhalation. The stomachs were promptly removed, opened along the greater curvature, rinsed with water and a 10% formalin solution, fixed on a white polystyrene board, and digitally photographed. The macroscopic ulcer index (MUI) was calculated for each animal based on the following scale, determined by two independent observers as described in17:1 - no lesions, 2 - single petechial lesions, 2.5 - multiple petechial or short linear hemorrhagic lesions, 3 - long linear hemorrhagic lesions, 4 - continuous linear hemorrhagic lesions along the entire length of the glandular part of the stomach—scale, as described by two independent observers. The efficacy of the test compound (Curative Ratio or CR) was calculated using the formula: CR = (MUIc - MUIt) / MUIc × 100%, where MUIc and MUIt represent the macroscopic ulcer indexes in the control and test groups, respectively.

Statistical analysis

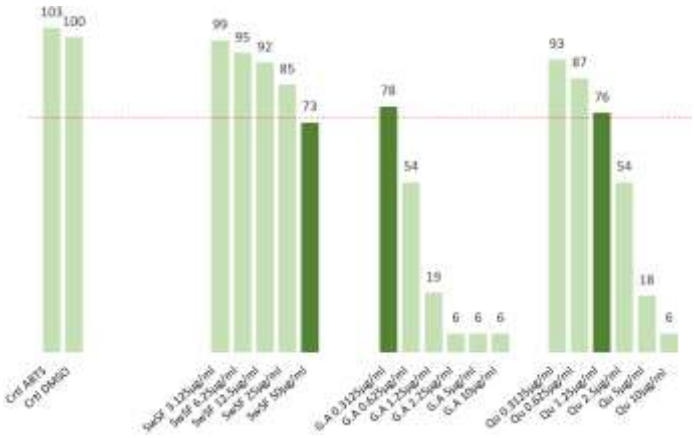
Statistical analysis of the experimental data was conducted using Student's t-test, with a significance level of $p \leq 0.05$ considered statistically significant.

RESULTS

ABTS assay

Figure 1 illustrates the results of the ABTS assay. The investigated fraction demonstrated comparatively low antioxidant activity compared to gallic acid and quercetin.

FIGURE 1. Antioxidant activity of the SwSF in ABTS assay



Abbreviations: ABTS, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid; SwSF, steroid glycosides.

"Hot plate "assay

In the "Hot Plate" assay conducted in mice, administering SwSF at 10 mg/kg intraperitoneally resulted in a mild analgesic effect. At the experiment's outset (baseline), the reaction times in the control and experimental groups were 16.7±1.9 and 16.3±2.3 seconds, respectively. After 60

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minutes, the corresponding values in animals receiving the tested compound exhibited a latency time of 20.8±3.7 seconds compared to 16.2±2.2 seconds in mice of the control group. The maximal analgesic effect of the SwSF extract was determined to be 23.2% (Tab.1).

TABLE 1. Analgesic effect of 10 mg/kg SwSF in "Hot plate" assay in mice

Number of animals	Reaction time (sec)			
	Control		SwSF 10 mg/kg i.p.	
	Baseline	60 min later	Baseline	60 min later
1	15.6	17.0	18.2	20.3
2	18.2	11.4	12.8	13.8
3	19.4	14.1	15.4	23.0
4	14.1	15.0	18.6	22.6
5	15.9	20.0	15.0	24.1
6	17.0	19.0	17.2	21.7
7	15.6	17.0	18.2	20.3
Mean ± SEM	16.7±1.9	16.1±3.2	16.2±2.2	20.±3.7

Abbreviations: SEM, standard error of mean; SwSF, steroid glycosides.

Carrageenan edema assay

In the model of acute inflammation induced by carrageenan swelling in rodents, it was observed that 2 hours after carrageenan administration, there was an increase in paw thickness in animals receiving 10 mg/kg SwSF (91.2±7.6 µm) compared to control animals (110.9±6.8 µm). Consequently, the fraction exhibited an anti-inflammatory effect of moderate strength (34%, $p<0.05$) (Tab.2).

TABLE 2. The anti-inflammatory action of 10 mg/kg SwSF in carrageenan-induced paw inflammation in mice

Number of animals	Thickness of the paw (µm)			
	Control		SwSF 10 mg/kg i.p.	
	Baseline	120 min later	Baseline	120 min later
1	198	300	198	296
2	192	281	209	314
3	188	322	208	280
4	186	293	198	308
5	191	311	202	296
6	195	326	208	283
Mean ± SEM	110±6.8		91.2±7.7	

Abbreviations: SEM, standard error of mean; SwSF, steroid glycosides.

Ethanol-induced ulcer model

In the ethanol-induced gastric ulcer model in mice, administration of absolute ethanol in control animals resulted in gross mucosal lesions characterized by full-length hemorrhagic streaks along the longitudinal axis of the glandular part of the stomach. However, mainly partial-length longitudinal hemorrhagic streaks were observed in mice pretreated with 10 mg/kg intraperitoneal SwSF. Correspondingly, the Macroscopic Ulcer Index (MUI) was reduced in animals pretreated with SwSF at 10 mg/kg (MUI=2.33±1.22) compared to untreated mice (MUI=3.50±0.45). The gastroprotective efficacy of SwSF reached 32% (Tab.3).

TABLE 3. Gastroprotective efficacy of 10 mg/kg SwSF in ethanol-induced gastric ulcer in mice

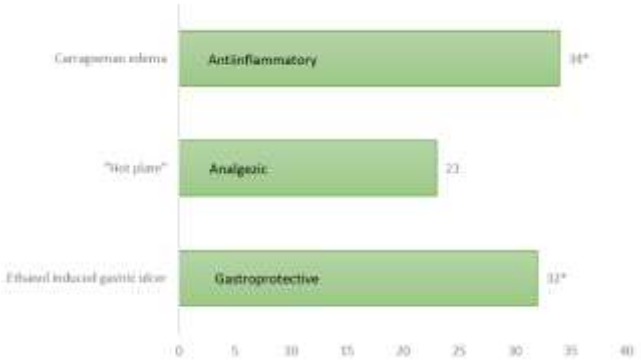
Number of animals	Macroscopic ulcer index			
	Control		SwSF 10 mg/kg i.p.	
	Observer 1	Observer 2	Observer 1	Observer 2
1	4	4	4	4
2	4	3	1	0
3	4	4	2	2
4	4	3	3	3
5	3	2	2	2.5
6	n/a	n/a	2.5	2
Mean ± SEM	3.5±0.45		2.33±1.18	

Abbreviations: n/a, not applicable; SEM, standard error of mean; SwSF, steroid glycosides.

CONCLUSIONS

In an in vivo experiment, the enriched fraction from the leaves of S. woronowii Pojark., enriched with steroid glycosides and administered at a dose of 10 mg/kg, demonstrated remarkable effects (Fig. 2). Specifically, when administered intraperitoneally, it exhibited 34% anti-inflammatory efficacy. In contrast, oral administration decreased activity with an efficacy of 14%. Additionally, the enriched fraction displayed moderate analgesic activity, providing 23.2% relief. Notably, it also demonstrated gastroprotective properties, offering a protective effect of 32.6% against ethanol-induced gastric damage.

FIGURE 2. Pharmacological efficacy of SwSF (10 mg/kg i.p.) in different animal models. * - $p<0.05$



Abbreviations: SwSF, steroid glycosides.

These findings align well with existing literature documenting the antiseptic, anti-inflammatory, expectorant, and diuretic effects of leaf extracts from S. villosum,[18-20] along with their ability to manage and prevent gastric ulcers.[21,22] When comparing these results to existing treatments, the enriched fraction appears less potent but could serve as an alternative with fewer side effects or as an adjunct therapy. Moreover, considering the widespread culinary use of black nightshades worldwide,[23,24] it seems

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reasonable to suggest the high potency of glycosides from the leaves of the Caucasus endemic S. woronowii Pojark. for use as biologically active additives. Further research is warranted to determine safety margins and elucidate their role in therapeutic strategies.

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REFERENCES

1. WFO (2024): Solanum woronowii Pojark. Published on the Internet; http://www.worldfloraonline.org/taxon/wfo-0001032533
2. Gagnidze R. Vascular plants of Georgia. A nomenclatural checklist. Tbilisi, 2005; 247p.
3. Pojarkova AI (Species novae generis Solanum L. ex URSS [in Russian]. Botanicheskie Materialy Gerbariya Botanicheskogo Instituta Imeni V. L. Komarova Akademii Nauk S S S R. Leningrad; 1955(a) 17: 328–340.
4. Kaund JS, Zhang VJ. The genus Solanum: an ethnopharmacological, phytochemical and biological properties: Review. Natural Products and Bioprospecting, 2019; 9: 77 -137
5. Zhou X, He X, Wang G, Gao H, Zhou G, Ye W, Yao X. Steroidal saponins from Solanum nigrum. J. Nat. Prod. 2006; 69: 1158-1163
6. Atanu F, Ebiloma H, Ajay E. A review of the pharmacological aspects of Solanum nigrum. Biotechnology and Molecular Biology Review. 2011; 6(1): 001 – 007
7. Wang Y, Xiang L, He X. Potential anti-inflammatory steroidal saponins from the berries of Solanum nigrum L. Journal of Agricultural and Food Chemistry. 2017; 65(21): 4262-4272
8. Jain R, Sharma A, Gupta S, Sarethy I, Gabran R. Solanum nigrum: current perspectives in therapeutic properties. Alternative Medical. Review, 2011; 16(1): 78-85
9. Zalaria ZA, Suleiman MR., Mursid NA, Aris A, Zainal H. Antinociceptive, anti-inflammatory and antipyretic effects of Solanum nigrum aqueous extract in animal models. Methods Find Exper. Clinic. Pharmacol. 2009; 31(2): 881-888.
10. Eremasvili M, Benidze M, Mchedlidze K, Mskhiladze L. Phytochemical study and evaluation of pharmacological activity of Solanum woronowii, common in Georgia. 2023 Collection of Scientific Works of the Tbilisi State Medical University (in press).
11. Liang N, Kitts DD. Antioxidant property of coffee components: assessment of methods that define mechanisms of action. Molecules. 2014; 19(11): 19180-208. doi: 10.3390/molecules191119180.
12. Directive 2010/63/EU on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276/33, 2010.
13. Guide for the Care and Use of Laboratory Animals, 8th Ed. USA: the National Academies Press: 2011.
14. Hunskaar S, Berge, O-G., Hole K. A modified hot-plate test sensitive to mild analgesics. Behavioral Brain Research, 1986; 21(2): 101–108.
15. Parle M, Yadav M. Laboratory models for screening analgesics. Int Res J Pharm. 2013; 4(1): 15-19.
16. Morris CJ. Carrageenan-Induced Paw Edema in the Rat and Mouse. In: Inflammation Protocols. Humana Press. 2003; 225: 115-122.
17. Adinortey MB, Ansah C, Galyuon I, Nyarko A. In Vivo Models Used for Evaluation of Potential Antigastroduodenal Ulcer Agents. Ulcers. 2013; 1-12.
18. Potawale SE, Sinha SD, Shroff KK, Dhalawat HJ, Boraste SS, Gandhi SP, Tondare AD (2008) Solanum nigrum Linn.: A phytopharmacological review. Pharacologyonline 3: 140–163.
19. Jagatheeswari D, Bharathi T, Sheik Jahabar Ali H. Black night shade (Solanum nigrum L.) – and updated review. International Journal of Pharmaceutical and Biological Archives. 2013; 4(2): 288–295.
20. Ignacimuthu S, Ayyanar M, Sankarasivaraman K. Ethnobotanical investigations among tribes in Madurai district of Tamil Nadu, India. Journal of Ethnobiology and Ethnomedicine 2006; 25. https://doi.org/10.1186/1746-4269-2-25
21. Lin CC, Lin WC, Yang SR, Shieh DE. Anti-inflammatory and hepatoprotective effects of Solanum alatum. The American Journal of Chinese Medicine. 1995; 23(01): 65–69. https://doi.org/10.1142/S0192415X95000092.
22. Heo KS, Lim KT. Glycoprotein isolated from Solanum nigrum L. modulates the apoptotic-related signals in 12-o-Tetradecanoylphorbol 13-Acetate-stimulated MCF-7 cells. Journal of Medicinal Food. 2005; 8(1): 69–77. https://doi.org/10.1089/jmf.2005.8.69.
23. Pojarkova AI. Solanaceae. In: Shishkin BK, Bobrov EG (Eds) Flora SSSR. Vol. 22. Academy of Science USSR, Leningrad [St. Petersburg], 1955(b); 1–105.
24. Yang R-Y, Ojiewo C (2013) African nightshades and African eggplants: Taxonomy, crop management, utilization and phytonutrients. ACS Symposium Series 1127: 137–165. https://doi.org/10.1021/bk-2013-1127.ch011.

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