



Subchronic Toxicity of *Sideritis Scardica*, Lamiaceae on Male Wistar Rats

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Abstract

Introduction: *Sideritis scardica*, Lamiaceae, is a plant with anti-inflammatory, antirheumatic, digestive, and antimicrobial properties that is widely used in folk medicine throughout the Balkan Peninsula. The name derives from the Greek word 'sideros', meaning iron, and it is believed that the plant was also used by soldiers to heal wounds caused by cutting weapons.

Aim: The study aimed to assess the subchronic toxicity of a dry hydromethanolic extract from *Sideritis scardica*, Lamiaceae.

Materials and methods: To investigate the subchronic toxicity, male Wistar rats were given orally a solution of dry hydromethanolic extract daily for 12-weeks at doses of 100, 200, and 400 mg/kg bw. Blood and blood serum were collected at the end of the experiment, and different organs were prepared for histopathological examination. Statistical analysis was performed with One-Way ANOVA test, using IBM SPSS 19.0.

Results: All hematological and biochemical results remained within the normal reference ranges described for the species. The histological examination showed no abnormalities in the morphology of the examined organs (brain, stomach, liver, and kidney).

Conclusions: The study contributes to a better understanding of the possible pharmacological effects, while documenting the absence of toxicity and safe use of the herb for future new indications.

Keywords

Lamiaceae, *Sideritis scardica*, toxicity, Wistar

INTRODUCTION

Sideritis scardica, Lamiaceae, also called Mursala or Pirin tea, is a plant endemic to the Balkan Peninsula, which has been known and used in ethnopharmacology for centuries. Many terpenes, flavonoids, organic acids, and polyphenolic biologically active substances are found in its composition.^[1,2] In recent years, there has been an increased interest in expanding the indications for this traditional

herb and efforts have been made to establish its safety in preclinical trials.

AIM

The aim of the study was to determine the toxicity of a *Sideritis scardica* plant preparation in Wistar rats, as well as to detect possible pathological changes in different tissues and biochemical and hematological parameters.

MATERIALS AND METHODS

Plant material was purchased from the NW Health Ltd, Bulgaria and a hydromethanolic extract (70% v/v) was produced using industrial spray dryer technology at Veselino Ltd, Kazanlak, Bulgaria.

Phytochemical Analysis

The amount of total phenols in the obtained extracts was determined by the Folin-Ciocalteu method. The results are presented as milligram equivalents of gallic acid per gram (mg GAE/g dry matter).^[3] The content of total flavonoids in the extracts was also determined spectrophotometrically using Al (NO₃).^[4] The results are presented as milligram equivalents of quercetin (mg QE/g dry matter). Determination of antioxidant activity was performed via the well-established methods - DPPH, ABTS, FRAP, and CUPRAC. The results of all four methods are presented as millimoles of Trolox equivalents (mM TE).^[5,6]

Subchronic toxicity

The subchronic toxicity experiment follows up an acute toxicity assessment in which no lethality was observed after a single oral administration of doses of 1000, 2000, 5000, and 10000 mg/kg bw.

Twenty-four male Wistar albino rats were obtained from the vivarium at the Medical University of Plovdiv. The animals were housed in a room providing ventilation with a temperature range of 25±2°C at a 12-h light-darkness cycle. Nutrition consisted of a standard feeding diet and use of tap water ad libitum. The animals were divided into four groups, each consisting of 6 rats. Group 1 was the control group which was treated with saline (2 ml). Groups 2, 3, and 4 were given orally water solution (2 ml) of a hydromethanolic dry extract from *Sideritis scardica* at doses of 100, 200, and 400 mg/kg bw for 12 weeks. The animals were fasted for 12 hours before serum and organ samples were taken.

Haematological analysis

Venous blood samples were obtained from the jugular vein under humane conditioned inhalation anesthesia. The tests were performed using RT-7600 Auto Hematology Analyzer and reagents provided by the producer.

Biochemical analysis

Venous blood samples were obtained from the jugular vein under humane conditioned inhalation anesthesia. The blood samples were collected in test tubes and centrifuged at 4000 rpm for 10 minutes. The serum was immediately frozen at -20 degrees Celsius for further analysis. An analysis of a series of biochemical parameters was performed using an automatic biochemistry analyzer Rayto Chemray-120 and kits from Biomaxima, Poland. The acquired data were analyzed using one-way ANOVA test with IBM

SPSS v. 19. Differences among the group means were evaluated by Tukey's test. Mean values were considered to be statistically significant at $p < 0.05$.

Histological analysis

Material from liver, kidney, brain, and stomach were collected from the animals of each experimental group and prepared for histological evaluation using the paraffin method.^[7] The samples were then fixated using 10% neutrally buffered formalin and placed in paraffin molds. The paraffin blocks were cut using a microtome into 5-µm slices and stained with hematoxylin-eosin, a widely used combination for many types of tissues, as it allows a clear and contrastive differentiation of the cell nucleus and cytoplasm. The nuclei tend to achieve blue-to-violet coloring, whereas the cytoplasm remain brighter and pink. This staining allows common understanding and estimation of the organic structure and pathological alteration. The acquired samples were observed using a light microscope Olympus, and microphotographic images were supplied via the microscope camera.

RESULTS

Phytochemical analysis (Tables 1-4; Fig. 1)

Table 1. Phytochemical properties

Method	Value
Saccharose, g/100 g	13.30
Glucose, g/100 g	4.61
Fructose, g/100 g	4.30
Total sugars, g/100 g	22.21
Total polyphenols, mg GAE/g	88.66±2.57
Total flavonoids, mg QE/g	22.01±1.23
Antioxidant activity, mM TE/g	
DPPH	693.89±3.61
ABTS	1009.60±13.82
FRAP	552.66±5.65
CUPRAC	1418.60±23.39

Subchronic toxicity

No lethality, macroscopic alterations, or pathological behavior was observed after 12 weeks of treatment with the preparation at doses of 100, 200, and 400 mg/kg bw. The weekly weight measurements showed that the treated groups gained about 10% more weight than the controls did.

Bodyweight

Table 2. Bodyweight

	Control	100 mg/kg	200 mg/kg	400 mg/kg
Weight gain, %	54.6	65.4	69.1	63.2

Hematology

Table 3. Hematology

	Control	100 mg/kg	200 mg/kg	400 mg/kg
WBC	4±0.70	4.01±1.2	3.74±0.84	4.11±1.26
RBC	7.93±0.46	7.44±0.71	7.33±0.6	7.57±0.52
HGB	147.67±8.87	143.83±8.86	140.5±8.85	140.33±7.87
HCT	0.42±0.02	0.41±0.02	0.34±0.14	0.4±0.02
MCV	52.53±1.04	54.63±2.35	53.48±1.65	52.6±1.17
MCH	18.63±0.33	19.37±0.83	18.32±1.3	18.53±0.48
MCHC	354.67±3.27	355±2.68	342.83±25.5	352.33±3.33
PLT	680.67±186.45	546.17±315.58	520.67±179.29	569.83±241.87

WBC: white blood cells; RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: MCH concentration; PLT: platelets. The obtained results show no statistically significant differences between the treated and control groups.

Biochemistry

Table 4. Biochemical analysis

	Control	100 mg/kg	200 mg/kg	400 mg/kg
Glu	65.5±11.43	81.17±19.05	80.33±14.36	83.83±17.94
ASAT	135.83±16,5	133±25.57	117.17±23.18	135.33±18.61
ALAT	5.5±1.05	7.33±2.73	5.67±1.37	6.17±1.94
Cho	69.79±13.11	78.33±6.76	72.82±15.94	66.54±10.31
Ua	1.73±0.73	1.82±0.67	1.85±1.50	1.92±0.44
Tgc	78.5±14.32	62.67±9.54	52.33±20.51*	47±10.26*
Ca	8.23±0.23	7.97±0.27	7.2±0.74*	7.7±0.30

Glu: glucose, ASAT: aspartate transaminase; ALAT: alanine transaminase; Cho: cholesterol; Ua: uric acid; Tgc: triglycerides; Ca: calcium; **p*<0.05

In comparison to the control group, the results revealed a statistically significant reduction in the triglyceride levels at doses of 200 mg/kg bw (*p*=0.023) and 400 mg/kg bw (*p*=0.006), as well as the concentration of calcium at a dose of 200 mg/kg (*p*=0.031). Despite this, according to scientific literature sources, these parameters seem to correlate with the normal reference levels of biochemical markers in albino rats.

Histological analysis

The organs of the animals of all examined groups demonstrated normal morphology.

Kidney

We observed no changes in the microstructure of the cortex and the medulla of the treated animals. This applies for the glomeruli and all systems of tubules.

Stomach

The images showed no differences in the gastric mucosae or glandulae of treated animals and the control group.

Liver

The liver lobules were well defined, with intact border and structure of v. centralis, triads, and cells. The hepatocytes did not display any type of alterations from normal morphology.

Brain

The sample images of the experimental animals showed normal structure and no remodeling of the cortex cerebri.

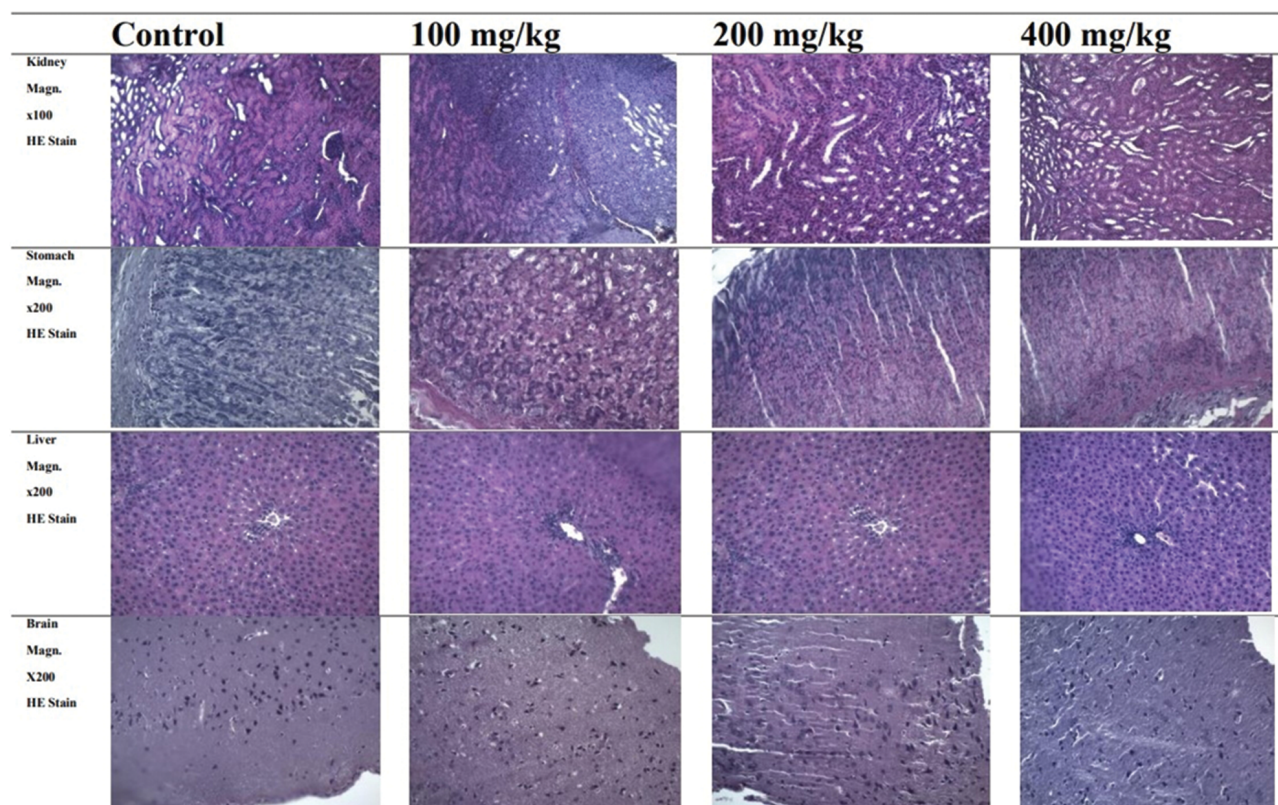


Figure 1. Kidney, stomach, liver, and brain of animals from the tested groups. HE staining.

DISCUSSION

Although plants of the genus *Sideritis* have been used in traditional medicine for centuries, little data has been acquired on their toxic properties. The European Medicines Agency's Committee on Herbal Medicinal Products published a monograph on *Sideritis scardica* in 2015, recommending its use to treat gastrointestinal problems and cold-related cough.^[8] Since then, numerous studies have been carried out by researchers in an effort to widen the indications for this traditional plant's use in the treatment of neurodegenerative diseases and to uncover its cytotoxic, antibacterial, and anti-inflammatory properties.^[9-11]

In an in vitro study by Tadic et al. involving B16 and HL-60 cells, a highly lipophilic diethyl ether extract of *S. scardica* has been demonstrated to exert moderate cytotoxic effects.^[9] An assessment of the toxicity of four *Sideritis scardica* extracts acquired via different solvents by Feistel et al. revealed no toxicity or concerns for mutagenic effects. In the same study, the authors performed an Ames test on bacterial cultures and observed no mutagenic effects, measured by the increase of revertant colony numbers as compared to control counts, for any of the extracts tested up to concentrations of 5000 µg/plate.^[12]

The 12-week dosage subchronic toxicity revealed no lethality or gross pathological occurrences in the present study. Altogether, significant toxicity of the plants from the family *Lamiaceae* has been associated more with the volatile components like terpenes found in the oils of different

plants, which do not accumulate in an alcoholic aqueous extract.^[13]

Contrary to Feistel et al., who documented an increase in calcium levels in the serum, the present study shows a statistically significant decrease in the calcium concentration (**Table 4**). Bearing in mind that the control group results were also below the reference values and did not illustrate a dose-effect tendency, this finding remains of minor relevance in respect to the direct toxic effects.^[12,14]

A dose-dependent reduction of triglycerides has been documented (**Table 4**). Despite this fact, all the values are within the reference range.^[15,16] Similar effects have been observed in numerous experiments involving plant preparations that, like *Sideritis scardica*, contain a high concentration of flavonoids and polyphenolic compounds and have high antioxidant activity (**Table 1**).^[17,18] Some of the flavonoids found in the composition of the Mursala tea, such as apigenin and luteolin, have been well documented to exert a decreasing effect on serum lipids.^[19,20]

The hematological results showed no statistically significant alterations (**Table 3**). A decrease in PLT levels is shown, yet it does not present a dosage effect tendency. The slight increase in bodyweight gain could be associated with the gastroprotective effects of the herb and the presence of different sugars in the extract (**Table 1, 2**).^[21]

Determining the outer morphology and histological structure of the main organs responsible for the elimination of xenobiotics and detoxication of the organism revealed no toxic effect or dysfunctionality (**Fig.1**).

CONCLUSIONS

The results of the present study suggest that using this *Sideritis scardica* preparation on Wistar rats causes no toxic effects. The decrease in the serum levels of triglycerides and calcium suggests a possible use of this medicinal herb in indications connected with metabolism and blood diseases. The documented results contribute to a better understanding and safety of *Sideritis scardica* and help introduce this herb further in the ever-evolving interest in plant derived nutrition supplements.

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Conflict of interests

The authors of this manuscript have declared no conflict of interests.

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Субхроническая токсичность *Sideritis Scardica*, Lamiaceae на самцах крыс Wistar

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Резюме

Введение: *Sideritis scardica*, Lamiaceae, представляет собой растение с противовоспалительными, противоревматическими, пищеварительными и противомикробными свойствами, которое широко используется в народной медицине на Балканском полуострове. Название происходит от греческого слова «сидерос», что означает железо, и считается, что это растение также использовалось солдатами для лечения ран, нанесённых режущим оружием.

Цель: Изучение субхронической токсичности сухого гидрометанольного экстракта из *Sideritis scardica*, Lamiaceae.

Материалы и методы: Для изучения субхронической токсичности самцам крыс линии Wistar ежедневно в течение 12 недель вводили перорально раствор сухого гидрометанольного экстракта в дозах 100, 200 и 400 mg/kg массы тела. В конце эксперимента собирали кровь и сыворотку крови, а различные органы готовили для гистопатологического исследования. Статистический анализ был выполнен с помощью теста One-Way ANOVA с использованием IBM SPSS 19.0.

Результаты: Все гематологические и биохимические результаты оставались в пределах нормальных референтных диапазонов, описанных для данного вида. Гистологическое исследование не выявило отклонений в морфологии исследуемых органов (головного мозга, желудка, печени и почек).

Заключение: Исследование способствует лучшему пониманию возможных фармакологических эффектов, документируя отсутствие токсичности и безопасное использование травы для будущих новых показаний.

Ключевые слова

Lamiaceae, *Sideritis scardica*, токсичность, Wistar
