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RESEARCH ARTICLE

Evaluation of Acute Oral Toxicity Induced by Tablets Based on Ethanolic Extract of *Salvia dumetorum* Leaves in Experimental Rats

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Abstract: The aim of this study was to assess the acute oral toxicity of a solid dosage form based on *Salvia dumetorum* Andrz. ex Besser leaf extract (SDT5) in experimental rats. Acute toxicity was evaluated over a 14-day observation period following a single oral administration at three dose levels (500 mg/kg, 1000 mg/kg, and 2000 mg/kg), prepared in 0.5 mL of distilled water. Animals were weighed during group formation, prior to administration, and every 24 h thereafter. Daily monitoring included food and water intake as well as behavioral observations. After euthanasia, liver, heart, and spleen tissues were collected for histopathological analysis, with a focus on vascular, inflammatory, and necrotic changes. Additional histological evaluation of vital organs was also performed. The results showed that no mortality occurred, and no statistically significant differences ($p > 0.05$) were observed between treated and control groups in body weight, organ histology, hematological parameters, or structural abnormalities. These findings suggest that SDT5 does not exhibit detectable acute oral toxicity under the tested conditions and may be considered a promising candidate for further preclinical and clinical investigations.

Keywords: Salvia dumetorum, Acute Oral Toxicity, Experimental Rats, SDT5 formulation, Histopathology, Ethanolic Extract, Dose-dependent Toxicity, Preclinical Safety, Organ Histology, Herbal Drug Development

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Introduction

Medicinal plants remain an essential component of healthcare systems worldwide, particularly in developing regions where access to conventional pharmaceuticals may be limited. Among these, species of the genus Salvia (family Lamiaceae) have attracted considerable attention due to their extensive therapeutic properties, such as antioxidant, anti-inflammatory, neuroprotective and antimicrobial effects [1].

Salvia dumetorum Andrz. ex Besser is a relatively understudied species within this genus, historically employed in folk medicine for treating gastrointestinal disorders, inflammation, and infections [2, 3]. Its traditional uses also include applications as a tonic and antimicrobial agent [4, 5]. Recent phytochemical investigations have confirmed the presence of bioactive compounds including flavonoids, phenolic acids, and essential oils in Salvia spp. [6-8], which likely contribute to its pharmacological activity.

Despite the growing interest in S. dumetorum, there is a noticeable gap in the scientific literature concerning its safety profile and formulation development. While other Salvia-based preparations such as topical gels, hydrogels, and microcapsules have been explored [9-12], no data are available regarding the acute toxicity or oral safety of S. dumetorum extract formulations.

In our preliminary research, a leaf extract of S. dumetorum demonstrated pronounced antibacterial effects on multiple Gram-positive and Gram-negative bacteria [13]. These findings supported the development of a novel phytopharmaceutical tablet formulation based on S. Dumetorum Leaf Extract (SDT5), intended for potential use in upper respiratory tract infections. This study represents, to our knowledge, the first evaluation of acute oral toxicity of a standardized S. dumetorum tablet formulation in vivo. Establishing its safety profile is a critical step toward further pharmacological investigation and potential clinical use.

Accordingly, this study was conducted to assess the acute oral toxicity of SDT5 in an animal model under OECD guidelines, providing foundational data for the formulation's continued development as a safe and effective phototherapeutic agent.

Materials and Methods

Chemicals and Drugs

Microcrystalline cellulose, α-lactose monohydrate, citric acid, calcium stearate, peppermint oil and eucalyptus oil used for tablet production were sourced from Sigma Aldrich Co. (St. Louis, MO, USA). Ethanol, diethyl ether, 10% neutral buffered formalin, xylenes histological grade, hematoxylin solution according to Mayer and eosin Y obtained from Merck (Darmstadt, Germany), highly purified water is prepared with purified water obtained from a water purification system Milli-Q (Millipore, France).

Salvia dumetorum Leaf Ethanolic Extract: Preparation Method

Leaves of Salvia dumetorum Andrz. ex Besser (syn. Salvia stepposa Des.-Schost) [14] were collected in July 2024 from the vicinity of Karaganda city (coordinates: N 49°88.898', E 73°15.569'). Taxonomic identification was confirmed by Dr. M.Yu. Ishmuratova, Botanist at the Department of Botany, Karaganda University named after Academician E.A. Buketov. A voucher specimen of Salvia dumetorum (voucher no. QAR00068) was previously collected on July 25, 1988, from the Karkaraly Mountains (Karkaraly district, Karaganda region) and is deposited in the university herbarium. Before extraction, the freshly harvested leaves and flowers were air-dried under shaded conditions at 25-30 °C for seven days to avoid direct sunlight. The dried material was ground, yielding a moisture content of 4.2%, and the resulting powder was sieved through a stainless-steel mesh with a 1.4 mm aperture.

Plant material was extracted by Microwave-Assisted Extraction (MAE) technique using ETHOS X microwave-assisted extraction system (Milestone, ETHOS X, Sorisole, Italy). One hundred grams of powdered S. dumetorum leaves were mixed with 1000 mL of 40% ethanol and loaded into the extraction chamber for 6 minutes, with the microwave power set at 600 W. MAE was carried out 3 times, following filtration, the extract was evaporated under reduced pressure using a rotary evaporator RV 8 (IKA, IKA-Werke GmbH & Co. KG, Germany). Subsequently, the extract was concentrated in a water bath at 55 °C, resulting in a yield of 22.01% relative to the dry weight.

The selection of 40% ethanol was based on its well-documented efficacy in extracting both polar and moderately non-polar secondary metabolites, such as flavonoids, phenolic acids, and terpenoids, which are characteristic constituents of Salvia species [15,16]. MAE was chosen due to its advantages over conventional methods, including reduced extraction time, solvent consumption, and thermal degradation [17]. The specific parameters (600 W, 6 min, 3 cycles) were adapted from prior optimization studies involving Lamiaceae plants [18, 19], and further validated in preliminary trials to achieve maximal extraction efficiency without compromising extract stability.

Design and Pharmaceutical Development of a Solid Dosage Form

Tablets based on the of *S. dumetorum* leaf extract were produced by the direct compression method using a 10 mm punch at 1400 psi on a hydraulic press [20]. All components, including 50 mg of the extract and excipients (microcrystalline cellulose 75 mg, α-lactose monohydrate 589.5 mg, citric acid 28 mg, calcium stearate 7.5 mg, peppermint oil 1.0 mL, and eucalyptus oil 0.5 mL), were accurately weighed, sieved, and thoroughly mixed. The powder blend was lubricated with calcium stearate

and mixed again to ensure uniform distribution. Moisture content was controlled and maintained within an acceptable range (5-6%) prior to compression. Tablet production was carried out to manufacture 400 pieces of SDT5. During tablet manufacturing, routine quality control was performed to assess weight uniformity, mechanical strength, friability, and dimensions.

Experimental Animals

The acute toxicity study was conducted using white albino female rats, weighing 210±10 g. The use of only female rats in the acute toxicity screening study is justified based on methodological guidelines, which allow for the use of a single sex at the initial stage, preferably females. This recommendation is based on the observation that females are generally more sensitive to the effects of toxic substances, thereby providing a more reliable assessment of the minimal toxic dose threshold. The rats were obtained from Karaganda Medical University vivarium, Kazakhstan, Karaganda. The rats were maintained under controlled environmental conditions, with a room temperature of 22-24 °C, relative humidity of 50 ± 5%, and a 12-hour light/dark cycle. They had free access to purified water and a standard pelleted diet. All experimental procedures were performed as dictated by the rules of “European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes” [21]. The study was approved by the Ethical Committee of Karaganda Medical University (No 12 from 11/07/2023).

Acute Toxicity Test

A single-dose acute toxicity study was conducted following the OECD Guideline 420 [22]. For this purpose, tablets with a total weight of 3500 mg were prepared. Prior to administration, the tablets were powdered using a sterile mortar and pestle, and subsequently suspended in 0.5 mL of purified water to form a homogeneous suspension, which was thoroughly mixed to ensure uniformity before each dose. The suspension was administered via oral gavage. Before dosing, the animals were subjected to a 12-hour fasting period with ad libitum access to water, in accordance with OECD 420 recommendations.

A total of 20 female rats with similar body weights (210 ± 10 g) rats were randomly divided into four groups, each containing five animals (n = 5) using simple random allocation, as no significant changes in body weight were observed between animals at baseline [23]. Group I was administered SDT5 at a dose of 500 mg/kg, Group II at 1000 mg/kg, Group III at 2000 mg/kg, while Group IV (control) received only purified water.

Following treatment, the animals had free access to food and water and were observed over a 24-hour period, with intensive monitoring during the first 4 hours and subsequent daily observation for at least 1 hour per day over the remaining 13 days for any signs of acute toxicity.

On the 15th day, the rats were anesthetized using diethyl ether. Blood samples were collected into EDTA-coated tubes for hematological analysis. The liver, heart, and spleen were excised, weighed, and preserved in 10% neutral buffered formalin for histopathological examination. The Relative Organ Weight (ROW) for each animal was subsequently calculated using Eq. 1:

$$\mathrm{ROW} = \frac{OW}{BW} \times 100 \tag{1}$$

Where:

ROW is relative organ weight (g).

OW is the organ weight (g).

BW is body weight of the animal on sacrifice day (g).

Analysis of Hematological and Biochemical Parameters

EDTA-coated tubes were used to collect whole blood for hematological analysis [24]. Hematological parameters were measured using an automated Mindray BC-3200 hematology analyzer (Mindray, China), including hemoglobin (Hb), total Red Blood Cells (RBCs), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), total White Blood Cells (WBCs), and Plateletcrit (PCT).

Histopathological Analysis

Tissue samples were fixed in 10% neutral buffered formalin for 24 hours, dehydrated, cleared with xylene, and embedded in paraffin. Sections of 5 µm thickness were prepared using a Leica SM 2000R microtome. (Leica, Germany) microtome and stained with Mayer's hematoxylin and eosin [25]. Microscopic analysis was performed using Zeiss AxioLab 4.0 (Carl Zeiss, Germany) microscope at x100, x200, and x400 magnification. Image acquisition was carried out using AxioVision 7.2 software for Windows.

Statistical Analysis

Data from all experiments are expressed as mean ± Standard Deviation (SD). Statistical comparisons were carried out using one-way ANOVA, with Tukey's post hoc test applied for multiple comparisons. Differences were considered statistically significant at $p < 0.05$. All analyses were performed using GraphPad Prism software, version 10 (GraphPad Software, San Diego, CA, USA).

Results and Discussion

Acute Toxicity

Throughout the experimemtal period, the animals remained healthy, active, and maintained normal appetite. They responded adequately to auditory and visual stimuli, and urination and defecation occurred without abnormalities. No signs of convulsions, respiratory distress, nor were any other signs of toxicity observed.

All animals showed a steady and continuous increase in body weight. (Table 1). Body weight was measured daily in four groups: Group I received 500 mg/kg of SDT5, Group II - 1000 mg/kg, Group III - 2000 mg/kg, and the control group received no SDT5. Statistical analysis using one-way ANOVA followed by Tukey's post hoc test showed no significant differences between treated and control groups on any of the recorded days ($p > 0.05$ for all comparisons).

Table 1: Daily Body Weight of Rats

Group	Doses, mg/kg	Weight (g)			
		Initial day	5th day	10th day	14th day
I	500	216.5 ± 2.06	217.0 ± 4.26	217.3 ± 2.06	217.5 ± 3.51
II	1000	199.0 ± 3.55	199.6 ± 3.48	199.8 ± 2.54	199.9 ± 1.89
III	2000	216.0 ± 1.25	216.4 ± 3.79	216.6 ± 2.45	216.7 ± 1.34
IV	Control	217.3 ± 1.48	217.5 ± 3.44	217.7 ± 3.62	217.9 ± 2.78

Values are expressed as the mean ± SD (n = 5; female rats); $p > 0.05$

Body weight dynamics indicated no adverse effects of SDT5 administration on growth. No statistically significant differences in body weight gain were observed among the groups; slight fluctuations were observed. For instance, animals in the 500 mg/kg group showed a very stable body weight throughout the study, while groups receiving 1000 and 2000 mg/kg doses exhibited a gradual but consistent increase in body weight.

These minor variations likely reflect normal biological variability and do not indicate any toxic effect of SDT5. The overall stable and slightly increasing body weight trend confirms the absence of adverse metabolic or systemic toxicity associated with the compound.

No signs of mortality were observed at any administered dose, which made it impossible to determine the LD_{50}. According to the widely accepted classification of chemical toxicity, SDT5 can be classified as a low-toxicity substance (Toxicity Class IV), suggesting a favorable acute safety profile under the tested conditions [26]. However, the minor histopathological findings observed warrant cautious interpretation and support the need for further toxicological evaluation.

Thus, the analysis of the obtained data demonstrated that SDT5, even at potentially toxic doses, had no negative impact on body weight in the experimental animals. No reduction in body weight commonly regarded as an indicator of systemic toxicity was observed in any group. Conversely, a gradual yet consistent increase in body weight was observed throughout the study period.

Relative Organ Weight

Relative organ weights are summarized in Table 2. Statistical analysis using one-way ANOVA (n = 5) showed no significant differences between the control and treated groups in the relative weights of the liver, heart, spleen, kidneys, or lungs ($p > 0.05$ for all comparisons).

Table 2: The relative organ weight of rats treated with different doses of SDT5

Organs	Control	I	II	III
Heart	0.46 ± 0.06	0.45 ± 0.01	0.43 ± 0.01	0.44 ± 0.02
Liver	3.05 ± 0.08	3.16 ± 0.10	3.14 ± 0.03	3.21 ± 0.04
Spleen	0.35 ± 0.01	0.33 ± 0.02	0.31 ± 0.01	0.32 ± 0.02
Kidneys	0.76 ± 0.01	0.78 ± 0.01	0.74 ± 0.01	0.73 ± 0.02
Lungs	0.84 ± 0.06	0.89 ± 0.04	0.86 ± 0.08	0.87 ± 0.01

Values are expressed as the mean ± SD (n = 5; female rats); $p > 0.05$

The results presented in Table 2 indicate no statistically significant differences in the relative weights of key internal organs (heart, liver, spleen, kidneys, and lungs), suggesting that the tested compound did not induce toxicity in these organs. Specifically, the relative mass of the heart remained stable across all groups and did not significantly differ from the control (0.46 ± 0.06 in control vs. 0.44 ± 0.02 at the highest dose of 2000 mg/kg). Similarly, the spleen and kidneys showed no significant changes in mass ratios, and the observed variations in liver weight (e.g., 3.05 ± 0.08 in control vs. 3.21 ± 0.04 in group III) remained within the expected physiological range and were not statistically significant ($p > 0.05$).

Hematological Parameters

The effects of acute SDT5 administration on hematological parameters are summarized in Table 3. Most parameters, including Hb, RBCs, PCV, MCV, MCH, MCHC, WBCs and PCT, did not differ significantly from the control. These findings indicate that the administered doses of SDT5 did not cause meaningful alterations in the hematological profile of rats, with all values remaining within the reference ranges. Other clinical hematological parameters also showed no significant differences compared to the control group.

Table 3: Impact of SDT5 on hematological parameters during acute toxicity assessment

Parameter	Unit	Control	I	II	III
Hb.	g/L	149.21 ± 0.11	151.23 ± 0.71	147.10 ± 0.61	149.97 ± 0.24
RBC's	10^{12}/L	7.96 ± 0.31	8.01 ± 0.10	7.38 ± 0.41	7.57 ± 0.17
PCV	L/L	0.48 ± 0.05	0.41 ± 0.07	0.44 ± 0.12	0.43 ± 0.01
MCV	fL	57.36 ± 0.17	54.35 ± 0.34	55.11 ± 0.36	55.06 ± 0.98
MCH	pg	17.31 ± 0.04	17.69 ± 0.07	17.54 ± 0.01	17.27 ± 0.05
MCHC	g/L	309.34 ± 0.01	310.78 ± 0.64	313.29 ± 0.09	312.18 ± 0.46
WBC's	10^9/L	7.04 ± 0.18	7.71 ± 0.22	7.67 ± 0.03	7.57 ± 0.47
PCT	**%**	**0.86 ± 0.03**	**0.70 ± 0.23**	**0.69 ± 0.11**	**0.77 ± 0.21**

Values are expressed as the mean ± SD (n = 5; female rats); $p > 0.05$

Hemoglobin levels remained stable across all groups, with only minor fluctuations observed (149.21 ± 0.11 g/L in control vs. 151.23 ± 0.71 g/L at 500 mg/kg and 149.97 ± 0.24 g/L at 2000 mg/kg), indicating preserved oxygen-carrying capacity. Similarly, the RBC showed no statistically significant differences among groups, although a slight decrease was noted at 1000 and 2000 mg/kg (7.38 ± 0.41 and 7.57 ± 0.17 × 10^{12}/L, respectively), which remained within physiological limits. PCV values varied slightly but showed no dose-dependent trend, with values ranging from 0.41 ± 0.07 to 0.48 ± 0.05 L/L. MCV and MCH values were also comparable across all groups, suggesting stability in erythrocyte size and hemoglobin content per cell. MCHC exhibited a slight increase in the treated groups, with the highest value at 1000 mg/kg (313.29 ± 0.09 g/L), though this variation was not statistically significant. WBC showed a slight elevation in treated groups compared to control (7.04 ± 0.18 × 10^9/L), with the highest at 500 mg/kg (7.71 ± 0.22 × 10^9/L), possibly may indicate a subclinical immune activation or a mild inflammatory response to constituents of the extract. While this finding remained within physiological limits, it suggests the

potential for immunomodulatory effects, which require further clarification. Despite minor fluctuations, PCT values remained within normal limits and did not differ significantly from the control group. Overall, the hematological profile of SDT5-treated animals was comparable to that of controls, with no signs of anemia, leukocytosis, or other toxicologically relevant alterations.

Histological Findings

Histopathological examinations of the liver, heart, and spleen were conducted to evaluate potential tissue-level toxicity of SDT5. Representative micrographs are shown in Figure 1.

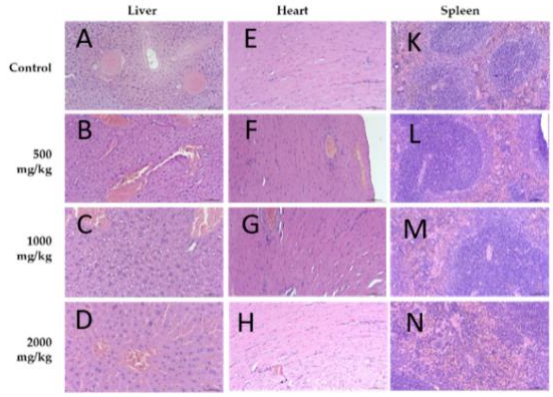

Fig. 1: Representative histological sections of the liver, heart, and spleen from female rats following 14-day oral administration of SDT5 (× 200). A-liver of the control group; B-liver of the group with a dose of 500 mg/kg, normal architecture; C- liver of the group with a dose of 1000 mg/kg, hepatocyte vacuolization; D- liver of the group with a dose of 2000 mg/kg, hemorrhagic and dilated sinusoids; E-heart of the control group; F-heart of the group with a dose of 500 mg/kg, mild venous congestion; G- heart of the group with a dose of 1000 mg/kg, mild venous congestion; H- heart of the group with a dose of 2000 mg/kg, mild venous congestion, focal cardiomyocyte hypertrophy; K-spleen of the control group; L-spleen of the group with a dose of 500 mg/kg, a clearly defined area of the spleen with a lymphoid follicle; M- spleen of the group with a dose of 1000 mg/kg, lymphoid follicle surrounded by giant macrophages; N- spleen of the group with a dose of 2000 mg/kg, vaguely defined structures with signs of slight stagnation

Liver sections from all treated groups (500, 1000, and 2000 mg/kg) exhibited normal hepatic architecture, with preserved hepatocyte morphology, intact central veins, and no signs of necrosis, inflammation, or fatty degeneration, comparable to the control group.

Heart tissues displayed intact myocardial fibers and no evidence of inflammatory infiltration, fibrosis, or degenerative changes in any group.

Spleen histology revealed preserved white and red pulp regions without lymphoid depletion, hemorrhage, or architectural disruption across all treatment groups. These findings indicate that SDT5 did not produce any histopathological changes in the examined organs, even at the highest administered dose. Additionally, macroscopic examination revealed no alterations in organ color or texture compared to the control group.

Staining with hematoxylin and eosin showed that SDT5 at the doses studied did not induce notable structural alterations in the liver, heart, or spleen relative to the control group.

Histological examination of liver sections from control female rats, which received purified water orally, revealed normal hepatic architecture. In all cases, normal, clearly well-defined histological structures were observed, with no evidence of vascular or inflammatory alterations. Liver sections showed a histologically The central vein and hepatic sinusoids exhibited a normal morphology, with intact endothelial and Kupffer cell lining. Hepatocytes were of normal size and shape, arranged radially, forming strands around the central vein, with no vacuoles observed in their cytoplasm.

To study toxicity, histopathological signs of histological changes in the liver, heart, and spleen were evaluated. The liver represents the primary target organ for toxic effects, so liver damage can disrupt the integrity of hepatocytes, leading to damage to the hepatobiliary system, potentially impairing the liver's biosynthetic and metabolic functions and leading to histopathological alterations, including micro- and macro vesicular vacuolization of the cytoplasm, hydropic dystrophy, hepatocyte necrosis, fibrosis and vascular alterations, including hyperemia of the central veins and hepatic sinusoids, which are commonly observed in hepatotoxicity.

In the toxicity study, histological changes in the liver included mild cytoplasmic vacuolization in some samples and vascular changes, including congested and dilated blood vessels. However, the observed vacuolization was mild, focal, and occurred in less than 25% of samples without a dose-dependent pattern, and was not accompanied by necrosis, inflammation, or other irreversible changes. Such vacuolization is likely a reversible adaptive response possibly related to transient metabolic or osmotic alterations induced by the phytochemical constituents of SDT5, consistent with reports that mild vacuolization can reflect hepatocyte adaptation rather than toxicity [27, 28]. This interpretation is supported by the absence of functional impairments, such as altered hematological parameters or organs weight changes. According to a number of authors, cytoplasmic vacuolization can be reversible, transient, or irreversible. The formation of vacuoles may simply be an adaptation to various changes in the environment [27]. Its transient form is in most cases caused by weakly basic amino-containing lipophilic compounds capable of passing through cell membranes, increasing osmotic pressure with water diffusion and the development of vacuolization. Irreversible vacuolization occurs as a result of a cytotoxic stimulus with cytopathological damage, resulting in cell death [28]. This pattern of vacuolization may be caused by caspase-independent mechanisms of cell death [29]. In our study, vacuolization in hepatocytes was observed in a small number of sections ($p>0.05$). Whether this was simply an adaptive response or the initiation of cell death needs to be clarified by further research. However, the observed changes appear to be adaptive and reversible, as no signs of necrosis were detected in hepatocytes even at high doses of the drug.

The mild level of histopathological changes observed in some cases needs to be clinically evaluated to assess the degree of potential liver failure; however, in the absence of other histopathological signs of liver damage, such as necrosis, severe dystrophy, etc., these mild changes appear to be of relatively minor significance.

In the study of the heart, moderate inflammatory and vascular changes in the heart muscle were observed in less than 10% of the material studied. There were no cases of acute inflammatory cell infiltration, hyalinosis of myocardial fibers, or pronounced muscle necrosis with the formation of contraction bands.

The study did not reveal any manifestations of SDT5 immunotoxicity leading to changes in the histopathological evaluation of lymphoid organs, including the spleen. Intragastric administration of SDT5 at doses of 500 mg/kg, 1000 mg/kg, 2000 mg/kg was not accompanied by any histopathological alterations in the spleen, since microscopic evaluation of spleen sections from rats receiving the drug at various concentrations was normal compared to that of rats in the control group.

The degree of histological changes observed with the administration of various doses of SDT5 was predominantly mild and focal in part of the samples (less than 25%) ($p>0.05$).

Consequently, according to the findings of the acute toxicity study of the tablets formulated from the leaf extract of *Salvia dumetorum*, oral administration at doses ranging from 500 to 2000 mg/kg had no adverse effects on hematological parameters or organs weights in rats. This study was conducted to a high scientific standard, as supported by data from contemporary literature on similar investigations [30-34]. Further subacute and chronic toxicity studies are needed to determine the long-term safety, identify potential target organs, and establish a no-observed-adverse-effect level (NOAEL) for SDT5.

Conclusion

As follows from the results obtained, single oral administration of SDT5 at doses of 500, 1000, and 2000 mg/kg exhibited low acute toxicity in rats. Histological examination revealed that hepatocytes in all treated groups remained largely intact, with no signs of moderate or severe histopathological damage. Similarly, cardiac muscle fibers and splenic tissues showed no evidence of significant injury in any of the examined samples.

The only accompanying findings were minor focal vascular and degenerative changes observed in the liver and heart in some cases; however, these alterations were not statistically significant ($p > 0.05$). Such minimal histological changes are considered within the range of normal biological variability and support the overall safety profile of SDT5.

These findings provide compelling evidence that SDT5, when administered as a single oral dose, can be classified as a low-toxicity substance (Toxicity Class IV), consistent with *in vivo* toxicological safety studies in animal models.

According to these findings, further investigations should include subacute and chronic toxicity studies to assess the effects of repeated administration over longer periods. In addition, efficacy studies, pharmacokinetic profiling, and dose-

ranging experiments will be essential to define the therapeutic potential, safety margins, and optimal dosing of SDT5 for future clinical application.

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Author's Contributions

Yana Levaya: Writing original draft, formal analysis, preparation and creation and/or preparation of the published work, specifically the visualization/data presentation. conceptualization, supervision and writing review and editing.
Karakoz Badekova: Methodology and formal analysis.
Mussa Zholdasbayev: Methodology and data curation.
Gayane Atazhanova: Conceptualization, formal analysis, funding acquisition, resources, investigation and supervision.
All authors have read and agreed to the published version of the manuscript.

Ethics

The animal study protocol was approved by the Ethics Committee of KARAGANDA MEDICAL UNIVERSITY (protocol code 12 from 07/11/2023).

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