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Acute Dermal Toxicity Study of *Aloe sinkatana*

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A B S T R A C T

Objective: Aloe sinkatana is a Sudanese medicinal plant that grows naturally in Eastern Sudan in the Red Sea Mountains, mainly in the Sinkat area, where it is popularly used extensively by residents of the region to treat skin diseases. The present study was designed to investigate the toxicity of ethanolic extracts of Aloe sinkatana for topical application via acute dermal toxicity analyses.

Methods: Acute dermal toxicity was studied Based on the Economic Co-operation and Development Guidelines for the Testing of Chemicals by Appling 2000 mg/kg body weight of plant extract on the shaved area of dorsal skin of rats, only once on the first day of the study. The study period was set at 14 days, and all rats were observed every day for behavioral (salivation, tremors, convulsions, diarrhea, and lethargy) and respiratory alterations, as well as for mortality and changes in their fur, eyes, and mucous membranes.

Results: Results showed, there were no poisonous symptoms or mortality throughout the trial period; there were also no changes in the eyes, mucous membranes, skin and fur, behavior patterns, salivation, lethargy, sleep, diarrhea, coma, or tremors. No significant alterations in behavior, skin impacts, respiration, inability to consume food or water, abnormal postural changes, or hair loss were observed in any of the rats.

Conclusion: This research presents the first report on the safety of topical application of Aloe sinkatana plant extract, supporting its application in traditional Sudanese medicine in treatment of multiple skin diseases especially for psoriasis. therefore, it has the potential to be developed into a suitable safe and effective pharmaceutical formula used to treat psoriasis.

Keywords: Aloe sinkatana, Dermatological studies, Extracts, Medicinal plant, Toxicity

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Introduction

Herbal medicines generally have fewer side effects compared to synthetic drugs. Nowadays, herbal resources are gaining more consistency and recognition due to their safety and efficacy, as supported by studies [1-3]. However, practically all herbal medicines are unlicensed and are not required to prove their quality, safety, or efficacy. Some medicinal plants have been shown to have previously unknown side effects [4].

Due to population growth and the increasing demand for medicinal plant resources, there is a growing need for medicinal plants in Africa. Pharmaceutical companies in industrialized nations are also becoming more interested in plant-derived medications [5]. On the other hand, medicinal plants are frequently used to treat various human diseases due to their minimal adverse effects, as noted in studies [6, 7].

Sudan, with its diverse meteorological, climatic, and topographical conditions, resulting in a wide variety of luxuriant flora, could supply many countries of the world with medicinal plants [8, 9]. Many drugs of established therapeutic value, used in the pharmacopeias of different countries, grow in various parts of Sudan [10-12]. Considering all of this, the main task of this study is to spotlight the safety of a very important Sudanese medicinal tropical plant known as *Aloe sinkatana*.

Aloe sinkatana grows naturally in the Eastern part of Sudan, primarily in the Sinkat area of the Red Sea Mountains, where it is extensively used by local residents to treat different type of skin diseases especially psoriasis. *Aloe sinkatana* leaves and their exudates are valued for treating a variety of

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ailments, including immune system disorders, skin diseases, constipation, fever, inflamed colon, and diabetes [13].

The antipsoriatic activity of *A sinkatana* was studied and confirm by studying the effect of 0.1% and 0.2% extracts of *A. sinkatana* on five groups of mice (imiquimod-induced psoriasis mice model) using dexamethasone as a control and the results show that the percent reduction of the psoriasis-like symptoms for the groups of 0.2% *A. sinkatana* extract, 0.1% *A. sinkatana* extract, and dexamethasone standard drug were 86%, 84%, and 68%, respectively. [13]

Asim *et al.* (2020), conducted an investigation into the antioxidant activity of Aloe sinkatana using the DPPH free radical scavenging assay. The findings revealed that the extracts were highly effective in scavenging free radicals, supporting the idea that Aloe sinkatana could be a valuable source of natural antioxidants or nutraceuticals, with potential applications in alleviating oxidative stress and promoting health benefits.[14]

Antibacterial and antifungal activity studies of the Aloe sinkatana plant were conducted by Ali et al. (2013)[15] .The results showed that the extracts of A. sinkatana leaves exhibited antimicrobial activities greater than the commercial standard drug (positive control), and these findings agree with Azza et al. (2022).[16]

A study conducted by Kamal et al. (2013) aimed to assess the impact of *Aloe sinkatana* on blood sugar levels and lipid profiles in patients with Type 2 diabetes. The study involved 110 randomly selected Type 2 diabetic patients from those regularly visiting Eldaba Chinese Hospital. Of these, ten patients served as the control group, while the remaining 100 were assigned to experimental groups, each consisting of ten individuals. Aloe sinkatana extract was administered to one of the experimental groups over a 30-day period, with all other factors kept consistent to facilitate statistical analysis. At the conclusion of the study, blood samples were taken, and various biochemical parameters were measured. The findings of the study suggest that Aloe sinkatana has anti-diabetic and hypolipidemic effects in Type 2 diabetic patients. It significantly reduced fasting blood glucose levels ($P < 0.05$) and led to a notable decrease in triglycerides (TG), total cholesterol (TC), and very low-density lipoproteins (VLDL) ($P < 0.04$). Furthermore, there was a significant increase in high-density lipoproteins (HDL) ($P = 0.03$). [17]

Ten active compounds from Aloe sinkatana extracts were separated by Gihan *et al.* (2012), and the chemical structures of these compounds were identified. These compounds are: Compound 2, 8-dihydroxy-6-(hydroxymethyl)-1-methoxyanthracene-9,10-dione, Aloe-emodin, feralolide, 1-hydroxy-5-methoxy-3-methyl-9,10-dihydroanthracene-9,10-dione, β-sitosterol, microdontin, homoaloins A, homoaloins B, aloins A, and aloins B. [18]. Furthermore, Gihan *et al.* (2012), reported that compound 2,8-dihydroxy-6-(hydroxymethyl)-1-methoxyanthracene-9,10-dione exhibit an inhibitory effect on early-stage protein glycation and significantly inhibit the effects against glucose-induced advanced glycation end-products.[18].

According to certain research, individuals who are allergic to aloe may occasionally have skin irritation, rashes, cramps, and diarrhea while using other aloe species, such as the aloe vera plant [19-21].Since A. sinkatana is a common medicinal plant that has been used traditionally to treat a wide range of diseases and no previous research has been done to investigate its toxicity, it was crucial to analyze and look into its toxicity. As far as we are aware, this is the first report on the plant A. sinkatan's acute dermal toxicity.

Acute dermal toxicity is defined as side effects that appear shortly after a single dosage of a test chemical is applied topically [22]. Any toxicology study for new consumer products must include an assessment of the toxicity of a single dermal dose in order to protect people from any potential negative consequences [23,24].

Material and Methods

Materials

Plant Collection and Authentication:

The *Aloe sinkatana* plants were collected in August 2022, from the Sinkat area, Red Sea state, Sudan, the identification of plant was done accurately according to the organoleptic properties, and morphological features such as appearance, shape, and size of the plant [25]. They were authenticated by taxonomist in the herbarium unit of the Department of Chemistry, Medicinal & Aromatic Plants Research Institute and Traditional Medicine, National Research Center, Khartoum, Sudan.

Experimental Animals

Adult albino rats (200-250g) were obtained from the University of Khartoum, Faculty Pharmacy Animal House. Albino rats were kept in stainless steel cages at the animal house, Department of Pharmacology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute, the pathogen free animals were placed in stainless steel, open-mesh cages in a room maintained under standard conditions (12 h light and dark cycle at an ambient temperature of 25 ±1°C and humidity 55±10%). The

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animals were fed with standard laboratory animal food pellets with water ad libitum.

The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments.

Methods

Extraction of *Aloe sinkatana*:

Mature, healthy, and fresh leaves of *Aloe sinkatana* having a length of approximately 75 to 90 cm were washed with fresh water.

The inner gel is scrapped and cut into pieces. A traditional hand filleting method of processing Aloe leaves was used. In this method, the lower leaf base, the tapering point at the leaf top, and the short spines located along the leaf margins were removed by sharp blades. The blade was then introduced into the mucilage layer below the green rind avoiding the vascular bundles, and the top rind was removed. The epidermis of the leaves was peeled off, and the colorless, solid mucilaginous gel was cut into pieces. Then 250 gm of gel was loaded into a 1000ml flask and 500 ml solvent (Ethanol) was added. Ultrasound-assisted extraction was performed at a frequency of 35 kHz with a maximum input power of 240 W, for 60 mins, at 60°C. After that the solution was filtered and the solvent was removed under reduced pressure in a Rotary evaporator until it became completely dry [26].

Experimental design of Acute dermal toxicity Test

Acute dermal toxicity test was made in accordance with the guidelines number 402 provided by the OECD for the chemical testing [27]. The test animals' dorsal trunks were clipped to eliminate fur 24 hours before to the test. For the test material to be applied, roughly 10% of the body surface area was free. In order to assess the toxic effects of the plant extract, 2000 mg/kg body weight (Approximately 0.2 to 0.5 mg of plant extract) was administered once on the first day of the study to the shaved area of the rats' dorsal skin. The study length was set at 14 days. Twenty albino rats were chosen at random and placed into two groups (n=10), the treatment and control groups. In the control group, sterile water was applied topically. On the first day of the trial, rats underwent a single clinical examination. Rats were observed every day for 24 hours, but the initial 6 hours and every day for the next 14 days received particular attention. Rats were weighed, and their mortality was visually observed.

The time of death must be recorded as precisely as possible. As accurately as feasible, the hour of death must be noted. Behavior patterns including salivation, tremors, convulsions, diarrhea, lethargy, sleep, and coma should be observed with special attention. Throughout the study period, physical appearance, injury, pain, and disease indicators were assessed once a day. Additionally, any alterations to the skin, eyes, or mucous membranes, as well as changes in behavior, breathing rate, circulation, autonomic nervous system, central nervous system. At the end of the experiment, the rats were humanely sacrificed under general anesthesia.

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Table 1: Experimental design for Acute dermal toxicity Test

Groups	Drug administered (mg/kg b. wt.)	Rats number
Group 1	Control (No treatment)	10
Group 2	2000 mg/kg of ethanolic extract of *A. sinkatana*	10

Histological studies

The rats were humanely sacrificed in accordance with ethical protocols. Skin fragments were fixed in 10% buffered neutral formalin and then embedded in paraffin. For the purpose of histopathological evaluation, sections measuring 4.0 µm were prepared and stained with hematoxylin and eosin (H&E), following standard staining procedures to highlight cellular structures. [28]

Histological examinations were conducted using an Olympus CX-21LED optical microscope (Japan), fitted with 10X and 20X objectives, and accompanied by an Olympus digital camera (Japan) for capturing images.

Statistical analysis

The data obtained were statistically analyzed using the Statistical Package for Social Science (SPSS) software version 20. The values were expressed as mean ± standard deviation (SD) for different parameters. Repeated measures analysis of variance (ANOVA) tests was conducted to compare the differences between and within the groups. [29,30]. Before performing the ANOVA, the assumptions of the analysis were examined. The normality of the distribution was tested using the Shapiro-Wilk test, which indicated that the data followed a normal distribution. Additionally, homogeneity of variance was assessed using Levene's test, which showed no significant differences in variances between groups. Post hoc analysis was conducted using Duncan's test to determine the level of statistical significance, which was set at $P < 0.05$. This effort is critical to ensure the reliability of the results derived from the analysis, thereby enhancing the accuracy of the study conclusions.

Results of Toxicity Study

Mortality and general assessment on signs and behavioral changes

During the 14-day study period, no mortality was observed following the dermal application of 2000 mg/kg body weight of *Aloe sinkatana* extract (Table 2). The behavior of study animals is a key indicator of toxicity, as changes in typical behaviors can reflect the effects of a toxic substance.[31] The finding indicating that, there were no clinical alterations in the skin, fur, eyes, mucous membranes, diarrhea, respiratory rate, no significant changes or impairments in behavior, sleep, coma, or tremors, autonomic and central nervous systems, tremors, seizures, salivation, sedation, drowsiness and food intake or water consumption were observed. (Table) .

Table 2: Mortality of dermal toxicity

Groups	Dosing phase			Final mortality
	1 day	≤ 1 weeks	≤ 2 weeks	
Group 1	0	0	0	0/10
Group 2	0	0	0	0/10

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Table 3: Summary of general assessment on signs and behavioral changes

Parameter	Group 1	Group 2
Skin and Fur	Normal	Normal
Mucous Membrane	Normal	Normal
Eyes	Normal	Normal
Behavioral pattern	No change	No change
Sleep	No change	No change
Salivation	No change	No change
Diarrhea	Not Found	Not Found
Tremors	Not Found	Not Found
Coma	Not Found	Not Found

Body Weight Changes

Because it can directly reflect the physiological state of animals in toxicity cases and is a crucial record of physiological and obsessive status in creatures, body weight evaluation is regarded as an important factor in determining acute dermal toxicity. This is because it is crucial in determining whether or not the organ was exposed to damage [32].

Generally speaking, variations in body weight indicate lethality following exposure to harmful compounds [33]. Changes in body weight are indicators of adverse effects from drugs and chemicals, and it is significant if the decrease in body weight is greater than 10% from the initial weight [34, 35]. The findings indicate that there was no significant change in body weight of the animals of the two groups during the experimental period.

Macroscopic and Microscopic Skin Changes

Allergy is a condition characterized by hypersensitivity of the skin or an excessive immune response to an antigen, typically presenting as erythema and edema [36]. Erythema refers to redness of the skin or mucous membranes caused by hyperemia of superficial capillaries, while edema is the accumulation of excess serous fluid between tissue cells [37]. In this study, the skin morphology in both control and treated groups appeared normal, with no signs of irritation, erythema, eschar, edema, or any other adverse reactions. Furthermore, microscopic examination of the skin of rats treated with the extract revealed no changes in the layers of the skin, including the epidermis, dermis, and hypodermis, compared to the control group.

Discussion

In vivo experimental studies are required to provide verified scientific evidence, especially on skin toxicity studies, as people's interest in dermal care products is growing these days. In addition, toxicity studies provide information on the range of dosages or concentrations needed to create safe products [38, 39]. In this study the extract of Aloe sinkatana was tested for acute dermal toxicity

The findings of this study, indicate that no toxic signs or mortality were observed in any animals, all of which survived up to 14 days after a single topical application of the extract at a dose level of 2000 mg/kg body weight. There were no significant changes in the body weight of animals in either group during the experimental period.

All animals appeared normal and did not display significant changes in behavior, breathing, food intake, water

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consumption, posture, or hair condition. This provides strong evidence that the extract is safe for topical application.

Throughout the entire test period, no adverse effects or deaths occurred in either the control group or the treated group. Furthermore, microscopic examination of the skin of rats treated with the extract revealed no changes in the epidermis, dermis, or hypodermis layers compared to the control rats.

Conclusions

In conclusion, to the best of our knowledge, this toxicity study represents the first investigation into the safety of the topical application of 2000 mg/kg Aloe sinkatana extract in rats as a single dose over a 14-day period. However, further toxicity studies focusing on chronic toxicity are needed to examine the effects of varying doses and prolonged exposure. Additionally, human clinical trials are necessary to confirm the safety of Aloe sinkatana extract and to investigate its potential effects on other vital organs (e.g., liver, kidneys, etc.).

Statements and Declarations

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Competing interests

The authors declare there is no Competing interests

Ethics approval

This study was performed in line with the principle of the declaration of Helsinki. Approved by the Pharmacology Department, Medicinal and Aromatic Plants & Traditional Medicine Research Institute, National Center for Research, Sudan (2022–0002).

Authors' contributions

The investigation, data collection Writing an original draft, Writing review, & editing: Azza Dawoud; editing, and data analysis: Sali Dawoud Hussien & Mohammed abdalbagi, Supervision: Mohamed El Hassan Shayoub

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