Toxicological safety assessment of a *Sceletium tortuosum* extract

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Abstract

A standardized hydroethanolic extract of *Sceletium tortuosum*, a South African plant with a long history of traditional use, is marketed under the trade name Zembrin® as an ingredient for use in functional foods and dietary supplements. It is standardized to contain 0.35–0.45% total alkaloids (mesembrenone and mesembrenol ≥60%, and mesembrine <20%). A 14-day repeated oral toxicity study was conducted at 0, 250, 750, 2500, and 5000 mg/kg bw/d. A 90-day subchronic repeated oral toxicity study was conducted at 0, 100, 300, 450, and 600 mg/kg bw/d. Because *Sceletium tortuosum* has a long history of human use for relaxation, a functional observation battery (FOB), including spontaneous locomotor activity measured using LabMaster ActiMot light-beam frames system, was employed. Several parameters, such as locomotion (time resting, moving, and hyperactive in seconds), rearing behavior (time in rearing, number of rearings), spatial parameters, and turning behavior, were investigated in the final week of the study. No deaths or treatment-related adverse effects were observed in male or female Crl:(WI)BR Wistar rats in the 14- or 90-day studies. In the 14-day study, the NOAEL was concluded as 5000 mg/kg bw/d. The NOAEL from the 90-day study was determined to be 600 mg/kg bw/d, the highest dose tested.

Introduction

- *S. tortuosum* (*Mesembryanthemum tortuosum* L.) is a decumbent succulent in the Mesembryanthemoideae subfamily of the Aizoaceae family (5 subfamilies, 135 genera, ~1900 species).[1,2]
- *S. tortuosum* is one of 8 *Sceletium* species endogenous to South Africa and has an affinity for arid environments.[1]
- The genus name means skeleton, which refers to the venation pattern of the dried leaves.[1]
- Common names include kanna (Khoi) and kougoed (Afrikaans), the latter referring to use by chewing.[1]
- Present research on *S. tortuosum* focuses on its alkaloid constituents and their central nervous system effects.[3-6]

Test Article, Zembrin®

- Hydroethanolic extract of dried above ground parts.
- Manufactured by Polifenoles Naturales, SL (Spain).
- Standardized to 0.35–0.45% total alkaloids.
  - Mesembrenone + mesembrenol ≥60%; mesembrine <20%.
- 100% water solubility.
- Formulated in water and administered by gavage.

14-Day Oral Toxicity Study

Wistar rats (aged 47–50 days) and OECD (407) and GLP compliant.

Group (mg/kg bw/d)	Treatment									
	0		250		750		2500		5000	
10 mL/kg bw	M	F	M	F	M	F	M	F	M	F
Animals (no.)	5	5	5	5	5	5	5	5	5	5
Deaths (no.)	0	0	0	0	0	0	0	0	0	0

- Evaluated: BW, food consumption, behavior and clinical observations, ophthalmologic examination, hematological and clinical chemistry analysis, gross and histopathology and organ weights (absolute and relative).
- Outcomes:
- The test article did not alter food consumption or BW compared to control.
- No signs of treatment related toxicological abnormalities. Slight to moderate transient salivation occurring in the majority of high-dose group animals and one 2500 mg/kg dose group male was considered incidental.
- Tested parameters, organ weights, and pathology were similar between groups and/or remained within normal/historical findings with 3 exceptions: (1) Increased liver to body weight ratio of males in the 5000 mg group (3.308 ± 0.270% compared to 3.025 ± 0.097% in control). (2) Increased thymus to brain weight ratio in females of the 250 mg group (25.09 ± 6.35% compared to 15.83 ± 3.93% in control). These changes were of small magnitude, unrelated to pathological lesions, and without dose-response. (3) Inflammation of the renal pelvis, prostate, and urinary bladder in a single male of the 5000 mg that was considered an individual disorder.

90-Day Subchronic Oral Toxicity Study

Wistar rats (aged 55–60 days) and OECD (408) and GLP compliant.

Group (mg/kg bw/d)	Treatment									
	0		100		300		450		600	
10 mL/kg bw	M	F	M	F	M	F			M	F
Animals (no.)	10	10	10	10	10	10	10	10	10	10
Deaths (no.)	0	0	0	0	0	0	0	0	0	0

- Evaluated: BW, food consumption, behavior and clinical observations, functional observation battery (including spontaneous locomotor activity measured using LabMaster ActiMot light-beam frames system), ophthalmologic examination, hematological and clinical chemistry analysis, gross and histopathology and organ weights (absolute and relative).
- Outcomes:
- BW did not differ between groups throughout the study; a few transient fluctuations observed in mean BW gain were of low degree and were not dose dependent.
- Food consumption compared to control was slightly lower in all male treatment groups during the first week of the study, but did not affect BW and remained within historical ranges.
- No signs of treatment related toxicological abnormalities. A single male in the 100 mg group exhibited slight to moderate decreased activity, dyspnea, sanguineous nasal orifices, and piloerection during the final two weeks.
- No differences in behaviour or reactions to stimuli were observed on the functional observation battery.
- Tested parameters and organ weights compared to that of controls or remained within or marginal to historical findings with the following exceptions and were attributable to an individual lesion or were slight and considered to be without biological significance: (1) Significant leukocytosis and blood filled thoracic formation in a single 100 mg group male. (2) Decreased liver to BW ratio of males in the 600 mg group (2.339 ± 0.135% compared to 2.543 ± 0.210% in control). (3) Decreased absolute adrenal weight (0.081 ± 0.011 g compared to 0.096 ± 0.017 g in control), adrenal to BW (0.0300 ± 0.0031% compared to 0.037 ± 0.006% in control), and heart to BW (0.326 ± 0.029% compared to 0.359 ± 0.023% in control) in females of the 600 mg group.

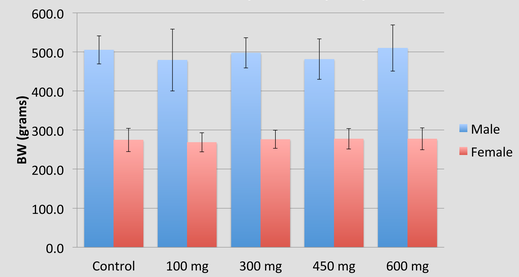


IMAGE: ©Nigel Gericke

Group (mg/kg bw/d at 10 mL/kg bw)		0		300	450	600	
Organs:	*Observations*	M	F	M	M	M	F
Cervical lymph nodes:	Hemorrhages	0	0	—	1	0	0
Kidneys:	Pyelectasia—unilateral	0	0	1	—	0	0
Lungs:	Alveolar emphysema	2	1	—	—	2	2
	Alveolar histiocytosis	1	1	—	—	1	1
	Hyperplasia of BALT	2	1	—	—	2	2
Testes:	Decreased intensity of spermatogenesis	1		—	—	0	
Epididymides:	Lack of mature spermatoza	1		—	—	0	
Uterus:	Dilatation		4				2

- Sporadic histopathological lesions occurred with similar frequencies in the control and 600 mg groups and were attributable to exsanguination, physiological phenomenon, age, and sexual cycle. Sporadic lesions observed in single males of the 300 mg, 450 mg, and control groups were considered individual lesions unrelated to the test article.

Conclusion

- No deaths occurred in either study, and no target organs or treatment-related toxicological effects were identified.
- The NOAEL of the 14- and 90-day repeated dose oral toxicity studies, respectively, are 5000 and 600 mg/kg bw/d—the highest doses tested.
- These animal studies can be used to estimate a level of safe human consumption of 420 mg daily based on a 100 fold safety factor and 70 kg person (600 mg/kg ÷ 100 x 70 kg = 420 mg).

References

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