Supplementary information

Acute effects of *Sceletium tortuosum* (Zembrin®), a dual 5-HT reuptake and PDE4 inhibitor, in the human amygdala and its connection to the hypothalamus

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Investigational product

Extract *Sceletium tortuosum* (Zembrin®) was manufactured by the company PoliNat, Las Palmas, Spain, according to European Union Good Manufacturing Practice (GMP). The extract, lot number SCE0411-1605, was in the form of a fine dry powder with the dry plant material: extract ratio of 2:1, standardized to a total alkaloid content of not less than 0.38% by dry weight for the total of the four alkaloids: mesembrenone, mesembrenol, mesembranol and mesembrine. Harvey and colleagues (2011) characterized the alkaloid composition by the relative amounts of three key alkaloids, whereby mesembrenone + mesembrenol > 70% of the total weight of the four alkaloids, mesembrine < 20%, and the minor compound mesembranol is present. In order to establish the most likely active components of Zembrin®, the activities of the isolated three main alkaloids present in the extract (mesembrenol, mesembrenone and mesembrine) were further studied on the 5-HT transporter, and on PDE4. All three were potently active in the 5-HT transporter binding assay (Ki's 1–60 nM) and against PDE4B activity (IC_{50}'s 0.5–16 μM). Of the three alkaloids mesembrenone is the pure compound closest to being a dual-acting 5-HT uptake and PDE4 inhibitor as the difference between concentrations for 50% effect on the two assays was 17 times, whereas it was 258 times for mesembrenol and 5500 for Mesembrine. Mesembrine was the compound showing most selectivity for the 5-HT transporter over PDE4B. Furthermore, the plant extract Zembrin® was shown *in-vitro* to have an IC_{50} of 4.3 μg/ml in the 5-HT transporter binding assay and an IC_{50} of 8.5 μg/ml on phosphodiesterase 4 (Harvey *et al*, 2011). The extract can thus be considered to be almost twice as potent on 5-HT reuptake than on PDE4.

No pharmacokinetic studies have yet been done on the extract, but in-vitro mucosal permeation studies (Shikanga *et al*, 2012) indicated that the main alkaloids found in Zembrin®; mesembrine, mesembrenone, mesembrenol and mesembranol, both as isolated pure compounds and in extract form, do cross intestinal mucosal surfaces. The permeability of mesembrine across intestinal mucosa was shown to be more rapid than that of caffeine used as a reference compound. Moreover, during ethnobotanical field work in Namaqualand in 1995 local pastoralists who chew *Sceletium* informed one of the authors (NG) that the calming effect of *Sceletium* could be felt within some twenty minutes of chewing the plant and swallowing the resulting saliva mixed with plant sap.

Thus, reports by indigenous people of rapid onset of activity, and supporting in-vitro permeation studies suggested that effects of the investigational product should be apparent after two hours after ingestion.

Table S1. Participants' gender, age, race, guess on administration order, and subjective side-effects for each session.

Gender	Age	Race	Guessed correct?	Zembrin	Placebo
Female	18	White	Yes	Nauseus and calm	-
Female	18	Coloured	No	-	Tasks felt easier
Female	19	Black	Yes	A bit woozy	-
Female	18	Coloured	No	Drowsy and calm	-
Female*	18	Black	Yes	Tired and as if she had taken something	-
Female	18	Indian	Yes	More alert and relaxed	-
Female	18	White	Yes	A bit sleepy	-
Female	19	Coloured	Yes	-	-
Male	20	White	Yes	More alert	Less alert
Male	19	White	No	More calm and different	Anxious
Male	21	White	Yes	Relaxed	Tired and slow
Male	19	Coloured	Yes	Calm and confident	Anxious
Male	21	Black	No	-	More relaxed
Male	19	White	Yes	Clearer thinking and relaxed	Less focused
Male	21	Black	No	-	Better concentration, more comfortable
Male	21	White	No	More tired	More awake, mentally sharp

* Participant excluded from analyses based on task performance, which was more than 3*SD* below average

References

Harvey AL, Young LC, Viljoen AM, Gericke NP (2011). Pharmacological actions of the South African medicinal and functional food plant Sceletium tortuosum and its principal alkaloids. *J Ethnopharmacol* **137**(3): 1124-1129.

Shikanga EA, Hamman JH, Chen W, Combrinck S, Gericke N, Viljoen AM (2012). In vitro permeation of mesembrine alkaloids from Sceletium tortuosum across porcine buccal, sublingual, and intestinal mucosa. *Planta Med* **78**(3): 260-268.