BEHAVIOURAL EFFECTS OF ALKALOID FRACTION FROM NARCISSUS CV. “HAWERA” ON RATS


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Abstract

Sceletium-type alkaloids containing plants have a long history of traditional use, but recent studies emphasize their use as supplements in the treatment of anxiety, stress, and depression disorder. This study aimed to investigate the effects of Sceletium-type alkaloid fraction from Narcissus cv. “Hawera” (MZM) on the anxiety and depressive-like behaviour in healthy female Wistar rats and rats with an experimental model of diabetes mellitus type 1 (T1DM), induced by streptozotocin injection. “Forced swimming”, “Open field”, “Elevated plus-maze” and “Paw-pressure” tests were used for the study of anxiety, depressive-like behaviour, habituation, and nociception, respectively. T1DM caused a significant decrease in overall motor activity, increased depressive-like behaviour, and impaired habituation to a new environment without altering the anxiety behaviour. MZM (20 mg/kg, 20 days) significantly attenuated depressive-like behaviour, reduced anxiety behaviour, and improved habituation in healthy rats without altering their overall motor activity. MZM treatment exerted weak influence on the DM-induced metabolic changes and did not change DM-induced behavioural abnormalities.

Key words: Sceletium-type alkaloid, anxiety, depression

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**Introduction.** *Sceletium*-type alkaloids containing herbal products have a long history of traditional use to maintain and improve the sense of well-being, and recently the attention of scientists has been focused on their possible use as a tool for the treatment of people with anxiety disorder, stress and depression. These alkaloids are found in high concentrations in species of the genus *Sceletium* (Aizoaceae, subfamily Mesembryanthemoideae), some species of the genus *Narcissus* (Amaryllidaceae) as *Narcissus palidulus*, *Crinum oliganthum*, *Hymenocallis arenicola* and cultivated *Narcissus* cv. “Hawera” [1–3].

Substantial data for the pharmacodynamics of the *Sceletium* alkaloids and mesembrine and mesembrenone in particular, showed a strong inhibitory effect on the 5-HT transporter, GABA, opioid, cholecystokinin-1, EP4 prostaglandin, and melatonin-receptors that suggested a putative inhibitory effect of the alkaloids on the neuronal transmission [4].

Depression and anxiety are some of the most common mental illnesses, defined as a condition characterized by significant behaviour changes and disturbance in the psychological feedback [5–9]. Studies had identified that a large proportion of adults with diabetes, presented with complications related to anxiety and depression associated with moderate encephalopathy [10,11]. We have previously demonstrated that this model of T1DM induces a decrease in exploratory behaviour both in male and female rats and provokes anxiety and depressive-like behaviour only in female rats with T1DM [12].

The aim of the present study is focused on the effects of alkaloid fraction extracted from *Narcissus* cv. “Hawera” on the behavioural parameters in healthy and T1DM female rats.

**Materials and methods.** The aerial parts of *Narcissus* cv. “Hawera” (Holland Biodiversity BV, Lisse, the Netherlands) were dried at 60°C and extracted with methanol. The experiments were approved by BFSA (No 155/15.11.2016) and carried out on female Wistar rats (250–300 g) housed in individual metabolic cages. The alkaloid fraction (MZM) was dissolved in sterile saline and injected intraperitoneally (IP) at a daily dose of 20 mg/ml/kg of body weight, 10 days before and 10 days after the injection of STZ/saline [13]. The experimental model of diabetes mellitus type 1 (T1DM) was induced by streptozotocin (STZ; Sigma-Aldrich), IP at a dose of 65 mg/kg, in citrate buffer (pH = 4.5). Diabetes was confirmed 48 h later by blood glucose level above 16 mmol/L (Accu-Chek® test strips) [14]. All behavioural tests were carried out during the last week of drug treatment. The “Open field” apparatus, [12], and Elevated plus maze [15], were equipped with a camera connected to a video tracking system (SMART PanLab software). The paw pressure pain threshold was determined with an analgesimeter (Ugo Basile, Italy) [16]. Forced swimming test was carried out and the immobility time was recorded [17]. The experimental data were analyzed statistically by one-way ANOVA and Bonferroni post hoc test and represented as means ± standard error means. The results with \( p < 0.05 \) are accepted for statistically significant.

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Results. Chronic treatment of healthy controls with MZM significantly decreased the immobility time in FST (F 1.15 = 7.291, p = 0.017). Experimental T1DM caused depression-like behaviour expressed by increased immobility time in FST (F 1.18 = 95.357, p < 0.001), which was not affected by treatment with MZM (Fig. 1A).

Fig. 1. Immobility time in “Forced swimming” test (A), and habituation to a new environment (decreasing in motor activity) in “Open field” test in rats injected with saline (Controls and T1DM), or MZM (Controls + MZM and T1DM + MZM)

Healthy rats were normally characterized by habituation to a new environment evidenced by a decrease in spontaneous exploration with time (F 1.36 = 14.419, p < 0.001; Fig. 1B). T1DM was accompanied by a considerable decrease in total ambulation (F 1.75 = 31.859, p < 0.001) and habituation (F 1.24 = 6.866, p < 0.001) in OF test (Fig. 1B). Chronic treatment with MZM improved the habituation in both controls (F 1.80 = 27.908, p < 0.001) and T1DM (H = 9.016, p = 0.003) (Fig. 1B).

T1DM decreased significantly the motor activity (F 1.19 = 41.385, p < 0.001) and this was not prevented by the treatment with MZM (Fig. 2A). Controls

Fig. 2. Total motor activity (A) and the ratio of distance travelled in the open arms vs. total distance (B) in the “Elevated plus-maze” test in rats injected with saline (Controls and T1DM), or MZM (Controls + MZM and T1DM + MZM)
treated with MZM passed a longer trajectory in the aversive open arms of EPM (H = 4.725, p = 0.030) vs. controls (Fig. 2B). T1DM did not alter the anxiety-like behaviour (Fig. 2B).

The pain threshold was significantly decreased only in controls treated with MZM (F 1.12 = 6.231, p = 0.030) (Fig. 3).

**Discussion.** In our study, we showed that the main effect of the MZM was suppression of anxiety-like behaviour and improvement of habituation in a new environment, supporting the suggested anxiolytic effect. These effects were demonstrated in healthy rats, which also demonstrated a decreased pain threshold. This result can be explained by the reported inhibitory action of *Sceletium* alkaloids on the δ2- and μ-opioid receptors [4].

**Conclusions.** These results suggest that MZM fraction from *Narcissus* cv. “Hawera” have a beneficial effect on emotional behaviour in healthy individuals, but does not affect the diabetes-induced complications associated with hypoactivity and depressive-like behaviour, probably due to different mechanisms affected by the metabolic disease.

**REFERENCES**


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