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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.

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).

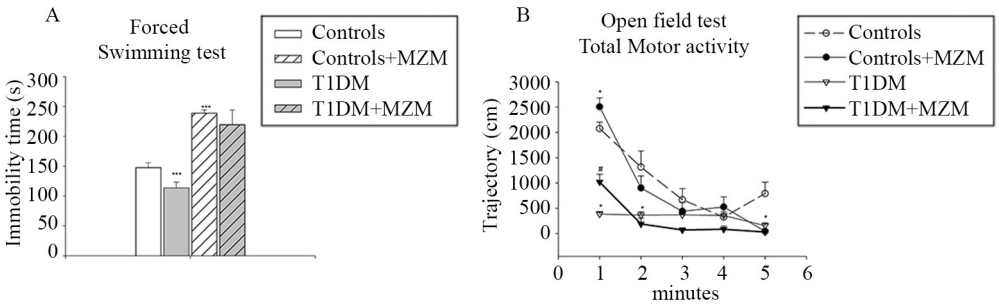

Fig. 1. Immobility time in "Forced swimming" test (A), and habituation to a new environment (decreasing in motor activity) in "Open field" test in rats injected with saline (Controls and T1DM), or MZM (Controls + MZM and T1DM + MZM)

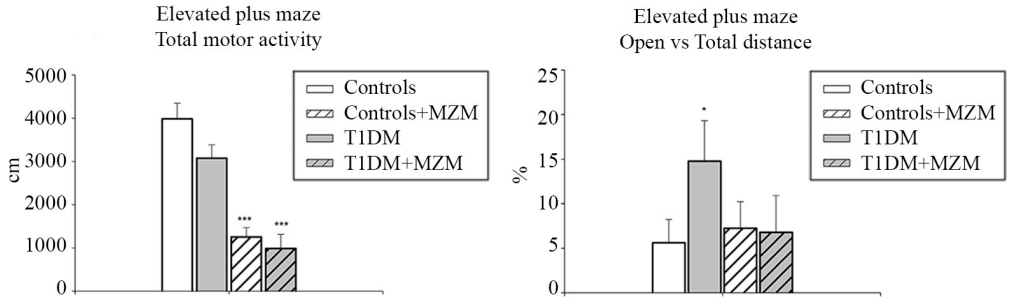

Fig. 2. Total motor activity (A) and the ratio of distance travelled in the open arms vs. total distance (B) in the "Elevated plus-maze" test in rats injected with saline (Controls and T1DM), or MZM (Controls + MZM and T1DM + MZM)

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

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).

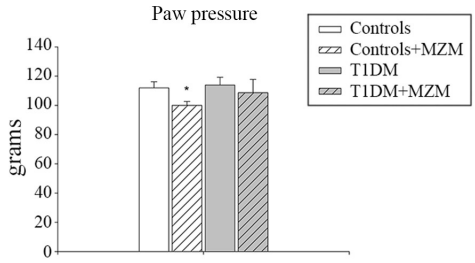

Fig. 3. Pain threshold (in grams) in rats injected with saline (Controls and T1DM), or MZM (Controls + MZM and T1DM + MZM)

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.

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