Supporting Information

Phytochemical and Antinociceptive Investigations of *Anemone coronaria*

Active Part Ameliorating Diabetic Neuropathy Pain

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Chemicals, solvents and standards

All chemicals, solvents and standards (purity > 98%) used in the study were commercially obtained from Sigma-Aldrich and of analytical grade without further purification. The positive standards, including glibenclamide (purity ≥ 99%) and tramadol hydrochloride (purity ≥ 99%) were also obtained from Sigma-Aldrich. The purity of the tested compound 3 was ca. 95%, HPLC.

Animals

Swiss-albino male healthy mice (25.0-30.0 g) of Wistar-strain were obtained from the University animal house (BAU, Lebanon). The mice were housed for one week before experimentation under standard controlled conditions of temperature (25.0 ± 1.0°C) with a twelve hour dark/light cycle, and free access to water and commercially obtained mice-feed [1]. Mice were deprived of food sixteen hours before the experimentation, but still had free access to water.

Study design and assessment of oxidative stress markers

Freshly prepared alloxan was dissolved in 0.9% cold sterile saline (vehicle) and 180 mg/kg were administered every 48 hours to the mice to provoke T1DM. Three days after the administration of the last dose of alloxan, HbA1c and blood glucose levels (BGL) were recorded for each mouse utilizing a HbA1c micro column method (Analyticon) and Roche Accu-chek Active Test Meter and Accu-chek Active glucose strips, respectively. The blood samples were collected by snipping the mouse tail-vein [2]. The mice with HbA1c > 8% and BGL ≥ 200 mg/dl were considered diabetic and were included in the experiments. Moreover, BGL was measured for each tested mouse acutely (6 h) and subchronically (8 days) as described in (Table 1).
addition, HbA$_{1c}$ levels were tested at pre-dose and 8 weeks post-administration [3, 4]. As a positive control, glibenclamide (GB; 5 mg/kg) was used (Table. 1). On the experiment day, all test solutions were freshly prepared [5] and UV bacterially-sterilized utilizing optimal in-line bacterial filtration (ZHENFU) [1]. All doses were chosen equivalently to the amount of substance in *A. coronaria*. In addition, markers of oxidative stress (catalase, lipid peroxidation, and reduced glutathione) were assessed to evaluate alloxan induced oxidative stress, and to assess *in vivo* antioxidant potentials of the test compounds [6]. Serum catalase (CAT) levels (kU/l) were subchronically measured using the modified method elucidated before in the literature [7] (Table 1B). Moreover, lipid peroxidation was measured by the thiobarbituric acid test that was modified from a previously described method [8]. Reduced glutathione (GSH) was also assessed by a modified method illustrated before [9].

**Assessment of diabetic neuropathy in mice**

The diabetic mice provoked peripheral neuropathy after 8 weeks of induction of T1DM with alloxan [1], which was manifested from a significant hyperalgesia and tactile-allodynia accompanied by decrease in pain and withdrawal threshold response in tail flick, hot plate and Von Frey filaments. Only diabetic mice with persistent hyperglycemia (HbA$_{1c}$ > 8%) showed clear thermal hyperalgesia (significant sensory loss of heat sensitivity below 10S) after 8 weeks of alloxan administration [1, 10-12]. Tail withdrawal, hot plate and Von Frey filament were performed to monitor the extent of the DN within control, positive control, utilizing 10 mg/kg tramadol (TRA), and test groups of mice. Thermal hyperalgesia was assessed by hot plate and tail withdrawal assays. The mechanical responses were evaluated by Von Frey filaments. These
tests were repeated for 8 weeks post-administration in control, positive control, and test groups of mice.

**Assessment of thermal hyperalgesia**

After a basal recording of nociceptive thresholds (pain thresholds) in week 8 post last alloxan administration, the changes in pain thresholds values were monitored in control, negative, positive control, and test groups of mice. Thermal hyperalgesia of the hind paw was evaluated by using a Ugo-Basile hot plate apparatus, for assessing the reactivity against nociceptive thermal stimuli. Additionally, spinal thermal sensitivity was monitored using a Hugo Sachs Elektronik tail withdrawal apparatus according to a previously described method [10-12].

**Assessment of mechano-tactile allodynia**

Non-noxious tactile stimuli responses were monitored in mice by assessing the hind-paw withdrawal-threshold in response to OptiHair, Marstock Nervtest flexible von Frey filaments stimulation [13]. The principle for the mechano-tactile thresholds was equivalent to the von Frey filament provoking paw withdrawal or flinching, measured in grams. The mechano-tactile threshold responses in diabetic mice for recording of basal nociceptive reaction were measured 8 weeks post control, positive control or test administration. The mechano-tactile threshold responses values (g) were compared with the control group (**Table 1B**).
Table S1: $^1$H NMR data for isolated compounds catalin (1); thaliporphine (2); glaucine (3).

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<td>δH+ m (J in Hz)</td>
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<td>6.678</td>
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<td>7.001</td>
<td>ddd (8.2, 4.2, 1.6)</td>
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<td>7.226</td>
<td>ddd (7.9, 7.5, 1.6)</td>
<td>ddd (6.8, 5.1, 1.5)</td>
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<td>7.736</td>
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<td>6.907</td>
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<td>6.896 d (2.8)</td>
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Fig. S1 HPLC chromatogram of *A. coronaria* extract (AC). Major peaks are as follows: I corrurine (12.6%); II catalin (9.3%); III thaliporphine (5.2%) IV glaucine (19.9%) V protopine (12.4%), using mobile phase of triethylamine (0.1%) and acetonitrile (60:40) at a flow rate of 1 mL/min at 280 nm using UV detector.

References


5. **Breitinger U, Raafat KM, Breitinger HG.** Glucose is a positive modulator for the activation of human recombinant glycine receptors. J Neurochem 2015:


