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Proceeding Paper

Nepeta nuda L. Plant Extract Preserves the Morphology of Red Blood Cells Subjected to Oxidative Stress †

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Abstract: We show that an extract from catmint (*Nepeta nuda* L.) flowers is not hemotoxic and does not alter erythrocytes' morphology. H_2O_2-induced oxidative stress leads to an increase in lipid peroxidation, accompanied by a reduction in the number of biconcave cells and an increase in the number of echinocytes. Pre-treatment of erythrocytes with this extract does not reduce the lipid peroxidation level; however, it results in partial restoration of the relative abundance of biconcave cells and a respective reduction in the echinocytes' quantity. Our data reveal the concentrations at which the examined extract exhibits a protective effect on erythrocytes' morphology under the condition of H_2O_2-induced oxidative stress.

Keywords: plant extract; red blood cells; oxidative stress

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1. Introduction

Medicinal plant extracts are widely explored for their beneficial effects on human health. A number of previous reports highlight the protective effect of plant extracts against oxidative damage, including on erythrocytes [1]. The catmint *Nepeta nuda* L. (Lamiaceae) has been reported to exhibit antioxidant effects due to its phenolic compounds and iridoids [2]; however, to the best of our knowledge, no studies have been performed so far on the effect of catmint extract on red blood cells (RBCs).

2. Materials and Methods

The plant extract (PE) from catmint flowers was prepared at 60 °C using water solvent and maceration [2]. After lyophilization, the dry substance was stored at −20 °C until further use. For all experiments, aqueous solutions of 0.01–1 mg/mL PE and 0.1 mM Trolox (TX) antioxidant (Acros Organics Fisher Scientific (Geel, Belgium), water-soluble analogue of the vitamin E) were utilized.

Donor blood was used for the preparation of RBCs as described by Langari et al. [3]. The experiments were performed in three replicates. Samples with a concentration of 1 mg Hb/mL were incubated for 1 h at 37 °C with the PE with concentrations of 1, 0.1 and 0.01 mg/mL and 0.1 mM TX. Untreated RBCs were used as the control. Hemolysis was determined spectrophotometrically based on hemoglobin absorption at 543 nm corrected for non-specific absorption at 650 nm. Full (100%) hemolysis was achieved via 100 times dilution of the RBCs in distilled water.

In vitro lipid peroxidation was induced by incubation of the control and the PE/TX treated samples with 1 mg Hb/mL and 0.8 mM H_2O_2 for 2 h. The lipid peroxidation reaction was evaluated via thiobarbituric acid reactive substances (TBARS) according to Gilbert et al. [4], and the absorption at 532 nm was corrected for non-specific absorption at 650 nm. The reaction was stopped with 150 µL of 10 mM ethylenediaminetetraacetic acid before the absorption readings.

RBC morphologic evaluation on air-dried smears was performed by means of optical microscopy (3D Optical profiler, Zeta-20, Zeta Instruments, Milpitas, CA, USA).

The data are represented as the mean values and standard deviation (SD).

3. Results and Discussion

As a first step in our study, we probed the hemolytic effect of the studied PE and TX on the RBCs. The evaluation of the hemolysis level after 1 h of PE incubation in the selected concentration range resulted in similar cell lysis for the control and PE/TX-treated cells of ca. 6–11% (Figure 1a). The morphology of the treated cells was similar to the one of the control RBCs—the dominant cell species was the biconcave one accounting for 74–85% followed by echinocytes (11–26%) and spherocytes (0–3%) (Figure 1b).

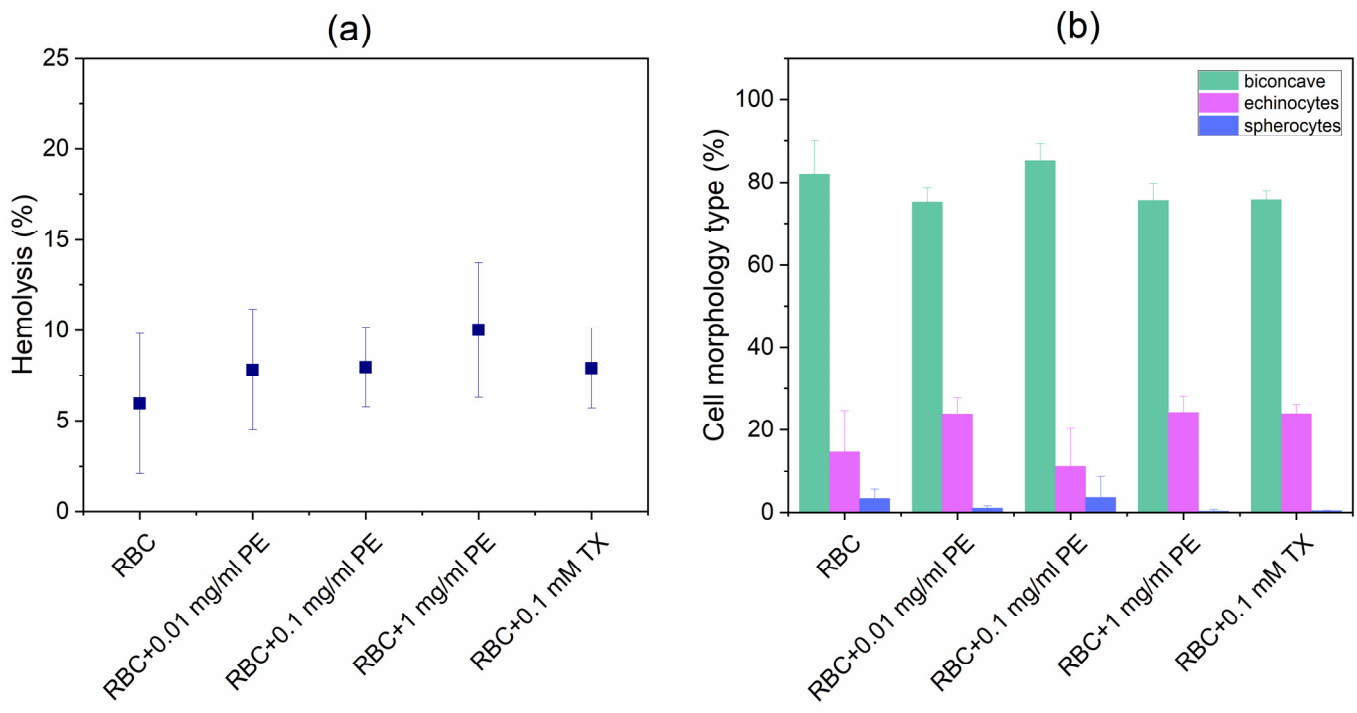

Figure 1. Hemolysis (**a**) and relative abundance of different morphological types (**b**) of RBCs subjected to 1 h treatment with different concentrations of catmint plant extract (PE) and 0.1 mM Trolox (TX). Mean ± SD.

Next, we evaluated the PE effect under the condition of H_2O_2-induced oxidative stress. The applied incubation with H_2O_2 resulted in a five-fold increase in TBARS levels in the RBCs (designated RBCoxi). Incubation with 0.01 and 0.1 mg/mL PE and 0.1 mM TX had no additional effect, while 1 mg/mL PE induced a further increase in TBARS values (Figure 2a). Lipid peroxidation also led to a four-fold decrease in the relative abundance of biconcave RBCs and a five-fold increase in the echinocyte population, while the number of spherocytes was not substantially affected, as compared to the RBCoxi sample. All three applied concentrations of PE, as well as TX, led to an increase in the number of biconcave cells by 1.5–2.1 times and a reduction in echinocytes by 0.6–0.8 times under the conditions of oxidative stress (Figure 2b).

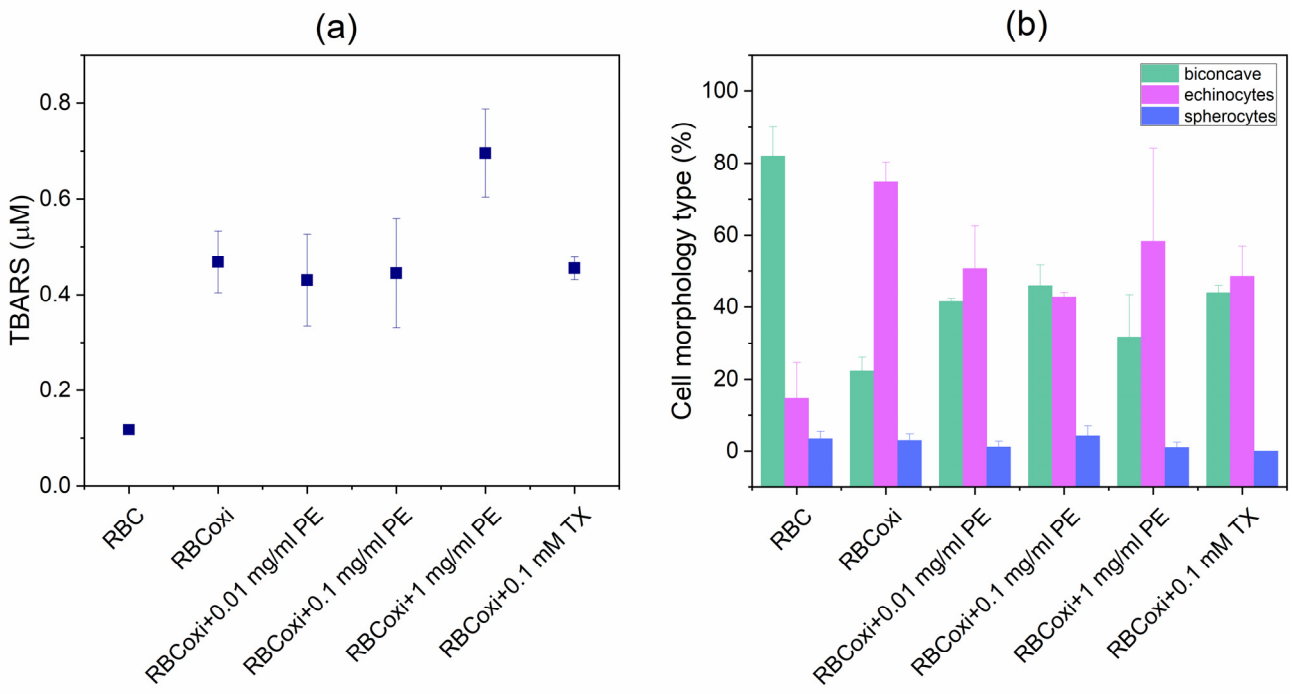

Figure 2. TBARS amount per mg Hb (**a**) and relative abundance of different morphological types (**b**) of RBCs subjected to 1 h treatment with different concentrations of catmint plant extract (PE) and 0.1 mM Trolox (TX) followed by 2 h *in vitro* oxidation by H_2O_2. The control RBCs were not exposed to either PE, TX, or H_2O_2 treatment, while RBCoxi is cells subjected to *in vitro* H_2O_2 oxidation. Mean ± SD.

The presented data clearly show that under our experimental conditions, the PE itself has no detrimental effect on RBC features. However, under the conditions of *in vitro* H_2O_2 oxidation, the PE does not express any protective effect towards the formation of TBARS and even induces a further increase in their values at the highest tested concentration. Surprisingly TX, a compound with well-established antioxidant properties, also did not reduce TBARS, which might be due to the short incubation period and/or the utilized concentration in the applied experimental protocol. Indeed, Antosik et al. [5] demonstrated a dose- and time-dependent effect of TX in long-term stored RBCs on membrane integrity, lipid peroxidation, and cellular morphology, with clear protective function only after 20 days of storage in TX supplemented medium.

A clear protective effect of the PE and TX was observed in the morphological features of the oxidized RBCs, with a similar extent for the 0.01–1 mg/mL PE range and 0.1 mM TX. This important finding strongly suggests that the PE stabilizes RBCs' shape, which is essential for their functioning and resistance to different pathologies and ageing [6,7]. The mechanism of this process will be the subject of our future studies.

4. Conclusions

Our data showed that incubation of RBCs with 0.01–1 mg/mL aqueous catmint extract exhibits a protective effect on cell morphology under the conditions of H_2O_2-induced oxidative stress.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available upon request.

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Conflicts of Interest: The authors declare no conflict of interest.

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