Neurochemistry International 128 (2019) 206–214

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/neuint

Monoaminergic system is implicated in the antidepressant-like effect of hyperoside and protocatechuic acid isolated from *Impatiens glandulifera* Royle in mice

Jolanta Orzelska-Górka[a,*], Katarzyna Szewczyk[b], Monika Gawrońska-Grzywacz[c], Ewa Kędzierska[a], Ewelina Głowacka[a], Mariola Herbet[c], Jarosław Dudka[c], Grażyna Biała[a]

[a] Chair and Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Chodźki 4A, 20-093, Lublin, Poland
[b] Chair and Department of Pharmaceutical Botany, Medical University of Lublin, Chodźki 1, 20-093, Lublin, Poland
[c] Chair and Department of Toxicology, Faculty of Pharmacy, Medical University of Lublin, Jaczewskiego 8b, 20-090, Lublin, Poland

ARTICLE INFO

Keywords:
Antidepressant
Monoaminergic system
BDNF
Hyperoside
Protocatechuic acid
Mice

ABSTRACT

We have recently demonstrated that the hydroethanolic extracts of *Impatiens glandulifera* Royle (*Balsaminaceae*) have antianxiety effect in mice. The present study was aimed to investigate an antidepressant activity of hyperoside (HYP) and protocatechuic acid (PCA), two polyphenols isolated from the aerial parts of this plant, using the forced swimming test (FST) and tail suspension test (TST) in mice. The implication of the monoaminergic system in this effect was assessed and *brain-derived neurotrophic factor* (BDNF) expression was measured. At doses 1.875, 3.75 and 7.5 mg/kg, HYP and PCA significantly reduced immobility in the FST and TST, without affecting locomotor activity of mice. Pretreatment with p-chlorophenylalanine (PCPA 100 mg/kg, a serotonin synthesis inhibitor) or α-methyl-DL-tyrosine (AMPT 100 mg/kg, a catecholamine synthesis inhibitor) was able to prevent antidepressant-like effect of HYP and PCA (3.75 mg/kg). Sub-effective doses of fluoxetine (5 mg/kg) or reboxetine (2 mg/kg) were capable of potentiating the effect of a sub-effective dose of HYP (0.94 mg/kg) in the FST. Co-administration of sub-effective dose of PCA (0.94 mg/kg) and reboxetine (2 mg/kg) resulted in reducing immobility in the FST. The antidepressant-like effect of HYP and PCA was also prevented by the administration of sulpiride (50 mg/kg), a D2 antagonist. In addition, HYP (3.75 and 7.5 mg/kg) and PCA (7.5 mg/kg) improved the expression of hippocampal BDNF of mice subjected to TST. Altogether, our findings suggest that HYP and PCA exert antidepressant-like effects in mice, which was possibly mediated by monoaminergic system and the upregulation of BDNF level.

1. Introduction

Depression is estimated to affect around 322 million people across the world making it a major prevalent neuropsychiatric problem. The current therapies have many limitations. Approximately half of all patients with major depressive disorder are non-responders to first-line therapy, and more than 65% do not achieve complete remission despite intensive management (Hamon and Blier, 2013). As a consequence, there is a clear need to develop more efficient and safer drugs as alternative and/or complimentary therapy for depression (Knight and Baune, 2017). In this context, herbal products seem to be a crucial source of new antidepressant agents. Over the past two decades, a lot of natural plants and their constituents that improve depression symptoms have been extensively explored. For example, *Hypericum perforatum*, *Hypericum androsaemum*, *Nelumbo nucifera*, *Rosmarinus officinalis*, *Ginkgo biloba* and *Aniba riparia* have been suggested to have antidepressant activity (de Sousa et al., 2014; Kwon et al., 2010; Nabavi et al., 2018). Due to the fact that numerous preclinical and clinical studies have revealed the therapeutic potential of extracts and isolated compounds of medicinal plants against depression (Lee and Bae, 2017; Nabavi et al., 2015), it is important to search and identify new phytochemicals for treatment of psychiatric disorders, including depression, as an alternative for conventional drugs.

Impatiens glandulifera Royle (Himalayan Balsam), belonging to the *Balsaminaceae*, is an annual invasive species in Europe (Beerling and Perrins, 1993; Hejda and Pyšek, 2006; Pyšek and Prach, 1995), North America and New Zealand (Weber, 2003). Decoction of the leaves of this plant is commonly used in stress and mental tension in India

* Corresponding author.
E-mail address: jolanta.orzelska@umlub.pl (J. Orzelska-Górka).

https://doi.org/10.1016/j.neuint.2019.05.006
Received 23 February 2019; Received in revised form 23 April 2019; Accepted 8 May 2019
Available online 09 May 2019
0197-0186/

(Kumar et al., 2009). *I. glandulifera* is also one of the Bach flower remedies that is recommended as a treatment for acute anxiety and which is supposed to be protective in stressful situations (Thaler et al., 2009). Furthermore, recent studies, carried out with the different extracts and fractions of this species, show that *I. glandulifera* exerts several biological effects, such as antimicrobial (Szewczyk et al., 2016), antioxidant antimicrobial (Szewczyk et al., 2016; Szewczyk and Olech, 2017), cytostatic (Cimmino et al., 2016), and cytotoxic activities (Szewczyk et al., 2018a). We have recently demonstrated that the hydroethanolic extracts of *I. glandulifera* have antianxiety effect in the elevated plus-maze test (Szewczyk et al., 2018c). Phytochemical studies have showed presence of the biologically active compounds in this species, such as flavonoids (Szewczyk et al., 2016, 2018b; Vieira et al., 2016), phenolic acids (Szewczyk and Olech, 2017; Szewczyk et al., 2018b), coumarins (Tříska et al., 2013), fatty acids (Szewczyk et al., 2018a), quinones (Cimmino et al., 2016), and glucosylated steroids (Cimmino et al., 2016), and some of these compounds have proven antidepressant effect (Machado et al., 2013; Szafrański, 2014).

Two polyphenols – hyperoside (HYP) and protocatechuic acid (PCA) – also found in *I. glandulifera* were previously shown to exert antidepressant activity in rodents (Butterweck and Schmidt, 2007; Haas et al., 2011; Thakare et al., 2017). Antidepressant effect of HYP was reported to be mediated, at least in part, by the dopaminergic system through the activation of dopamine D2 receptors (Haas et al., 2011).

Depression is a complex emotional disorder with symptoms not only manifested at the behavioral, but also at the physiological level. Insufficient amounts of monoamine neurotransmitters serotonin (5-HT), noradrenaline (NE), and/or dopamine (DA) have been long related to pathogenesis of depression. This hypothesis grew out of observations that antidepressant therapies raise neurotransmission tone depending on one or more of these neurotransmitters (Hamon and Blier, 2013). In addition, many studies have suggested that there is a reduction in brain-derived neurotrophic factor (BDNF) levels in depressive individuals and that BDNF is required for the normal action of antidepressant drugs (Kozisek et al., 2008).

Based on the above presented data, the aim of the study was to clarify the mechanisms underlying antidepressants-like effects of HYP and PCA. The implication of the monoaminergic system in this action was assessed and BDNF expression was measured.

2. Materials and methods

2.1. Isolation of hyperoside and protocatechuic acid

Hyperoside (HYP) and protocatechuic acid (PCA) were extracted and purified from the aerial parts of *Impatiens glandulifera* as previously described with slight modifications (Szewczyk et al., 2018b). Air-dried and powdered aerial parts (3.0 kg) of *I. glandulifera* were extracted with 50% ethanol (3 x 3L, reflux, 3h), and then were sonicated with mixture of ethanol-acetone-water (3:1:1, v/v/v) (3 x 3L) at a controlled temperature (40 ± 2 °C) for 30 min. Extractants were evaporated under reduced pressure to afford a residue (493.8 g), which was re-dissolved in hot distilled water (1000 mL) and partitioned between chloroform (2 × 300 mL, 8 × 200 mL), ethyl acetate (1 × 300 mL, 8 × 250 mL) and n-buthanol (6 × 300 mL), yielded 61.3, 123.5, and 176.0 g of each dried fraction, respectively.

A dried ethyl acetate fraction (123.5 g) was successively subjected to polyamide column with MeOH–H_2O (0:100 to 100:0, v/v, in 6 steps) as eluent to afford each component. The methanol-water (40:60, v/v) fraction (5.79 g) was re-chromatographed on Sephadex LH-20 column eluting with mixtures of MeOH–H_2O (60:40 to 100:0, v/v in 4 steps). The methanol-water (50:50, v/v) fraction (3.08 g) was subjected to the preparative HPLC (A: 0.1% HCOOH in H_2O, B: 0.1% HCOOH in MeCN, flow 20 ml/min, column temperature 25 °C, detection 280 nm, gradient 0 min–6%B, 50 min – 23%B) to yield protocatechuic acid (127.2 mg, t_r = 7.0–7.6) and hyperoside (519.4 mg, t_r = 32.2–32.6). PCA (3,4-dihydroxybenzoic acid): ESI-MS (negative) *m/z*: 153 $[M-H]^-$, MS/MS *m/z*: 109 $[M-H-CO_2]^-$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 7.45 (1H, *d*, *J* = 1.5 Hz, H-2), 7.33 (1H, *dd*, *J* = 8.0, 1.5 Hz, H-6), 6.78 (1H, *d*, *J* = 8.0 Hz, H-5); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 167.34 (C-7), 150.13 (C-4), 141.97 (C-3), 120.67 (C-6), 122.67 (C-1), 116.15 (C-2), 114.58 (C-5); HYP: ESI-MS (negative) *m/z*: 463 $[M-H]^-$; ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 12.63 (*s*, 1H, 5-OH), 10.53 (*s*, 1H, 7-OH), 9.40 (*s*,1H, 4′-OH), 7.68–7.65 (*dd*, 1H, *J* = 5.9 Hz, 6′-H), 7.53 (*s*, 1H, 2′-H), 6.84 (*d*, 1H, *J* = 8.5 Hz, 5′-H), 6.20 (*s*, 1H, 6-H), 5.38 (*d*, 1H, *J* = 7.6 Hz, H-1″), 5.13 (br s, 1H, galactosyl OH), 4.86 (br s, 1H, galactosyl OH), 4.39 (br s, 2H, galactosyl OH), 3.82 (*s*, 1H, galactosyl H), 3.86–3.26 (*m*, 6H, H-2″-6″); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 177.60 (C-4), 164.01 (C-7), 161.46 (C-5), 156.17 (C-2, C-9), 148.56 (C-4′), 144.78 (C-3′), 132.08 (C-3), 121.92 (C-6′), 119.96 (C-1′), 115.90 (C-5′), 114.98 (C-2′), 103.22 (C-10), 101.84 (C-1″), 98.19 (C-6), 93.39 (C-8), 75.53 (C-5″), 73.18 (C-3″), 70.63 (C-2″), 67.57 (C-4″), 59.67 (C-6″).

2.2. Animals

The experiments were performed on male Albino Swiss mice (20–25 g), where 4 animals were kept in a cage, at room temperature of 22 ± 1 °C, with free access to food and water. All manipulations were conducted in the light phase, between 9:00 a.m. and 5:00 p.m. Each experimental group consisted of 8 animals. All behavioral experiments were carried out according to the European Community Council Directive for Care and Use of Laboratory Animals (2010/63/EU), and approved by the Local Ethics Committee for Animal Experimentation.

2.3. Drugs

All substances were administered intraperitoneally (i.p.) in a constant volume of 10 ml/kg body weight. HYP and PCA dissolved in DMSO (its final concentration of 0.1%), suspended in 0.5% Tween-80 (1–2 drops) and then diluted with saline solution (0.9% NaCl). HYP and PCA were administered 60 min before tests. Bupropion (BUP), p-chlorophenylalanine methyl ester (PCPA) and α-methyl-DL-tyrosine (AMPT) were purchased from Sigma-Aldrich Co. (MO, USA). Fluoxetine (Flx; Lilly, USA), reboxetine (Rbx; Tocris, USA), sulpiride (SUL; RBI, USA), PCPA and BUP were dissolved in 0.9% NaCl, and AMPT was suspended in saline with 10% (v/v) Tween-80 (polyoxyethylenesorbitanmonooleate, Sigma-Aldrich Co.). The control animals received the vehicle (0.9% NaCl).

2.4. Experimental design

In order to investigate a possible contribution of the 5-HT system to the effect of HYP or PCA in reducing the immobility time in the FST, animals were pretreated with PCPA (100 mg/kg, i.p., an inhibitor of serotonin synthesis) or vehicle, once a day, for four consecutive days. Then, 24 h after the last PCPA or saline injection, animals were treated with PCA (3.75 mg/kg, i.p.), HYP (3.75 mg/kg, i.p.) or Flx (15 mg/kg, i.p., a serotonin reuptake inhibitor, a positive control) or vehicle and were tested in the FST and in an actometers 60 min later (Colla et al., 2014).

In the experiments designed to study a possible role of the noradrenergic/dopaminergic system in the antidepressant-like effect of HYP or PCA, mice were pretreated with AMPT (100 mg/kg, i.p., an inhibitor of tyrosine hydroxylase, a critical enzyme for the synthesis of noradrenaline (NE) and DA), or vehicle 4 h before HYP or PCA administration (3.75 mg/kg, i.p.). After 60 min, the behavioral tests were carried out (FST and the mobility measurement).

In another two sets of experiments, the effect of the administration of a sub-effective dose of HYP or PCA (0.94 mg/kg, i.p.), followed by the administration of a sub-effective dose of Flx (5 mg/kg; ip.) or Rbx (2 mg/kg, i.p., a selective noradrenaline reuptake inhibitor), was

investigated after 60 min in the behavioral tests.

To assess the possible involvement of the dopaminergic system in the antidepressant activity of HYP or PCA in the FST, animals were treated with SUL (50 mg/kg, i.p., a DA receptor antagonist), and after 30 min they received HYP or PCA (3.75 mg/kg, i.p.), BUP (a DA reuptake inhibitor, 30 mg/kg, i.p.) or saline. Subsequently, animals were evaluated in the FST and actometer 1 h later (de Sousa et al., 2014).

2.5. Behavioral tests

2.5.1. Spontaneous locomotor activity

Spontaneous locomotor activity was measured using an animal activity meter Opto-Varimex-4 Auto-Track (Columbus Instruments, OH, USA), as was described in details previously. A distance travelled (in cm) by a tested mouse was measured after 6 min (corresponded with the time duration of the FST). The cages were cleaned up with 70% ethanol after each mouse (Fiorino et al., 2017).

2.5.2. Forced swimming test (FST)

The experiment was carried out according to the method of Porsolt et al. (1977). Mice were individually placed in a glass cylinder (25 cm high; 10 cm in diameter) containing 10 cm of water maintained at 23–25 °C, and were left there for 6 min. A mouse was regarded as immobile when it remained floating on the water, making only small movements to keep its head above it. The total duration of immobility was recorded during the last 4 min of a 6-min test session.

2.5.3. Tail suspension test (TST)

Mice were individually suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded during the final 4 min interval of the 6-min test. Mice were considered immobile only when they hung passively and completely motionless. The immobility time was recorded by observers blind to the drug treatment (Steru et al., 1985).

2.5.4. Determination of BDNF concentration

The non-TST group (a group of mice with saline treatment only), the TST group (a group of mice exposed to the TST procedure with saline treatment only) and mice administered with Flx (15 mg/kg), HYP or PCA (1.875, 3.75 and 7.5 mg/kg) exposed to TST were sacrificed immediately after the test by decapitation. The hippocampus was dissected from each animal, and then stored at −80 °C for later analysis of BDNF level. The concentration of above factor was measured by a ready-to-use sandwich enzyme immunoassay (ELISA) diagnostic kit dedicated to mouse fluids and tissues (Enzyme-linked Immunosorbent Assay Kit For BDNF). All the procedures were performed according to the instructions supplied by the manufacturer (Cloud-Clone Corp., Katy, USA). The tissue homogenate was prepared from each mouse hippocampus by sonication in fresh lysis buffer (w:v = 1:50, 1 ml buffer/50 mg tissue sample) (Cloud-Clone Corp., Katy, USA) with addition of 2 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich Co., MO, USA) to avoid protein degradation. Then, the homogenates were centrifuged for 5 min at 10,000 × *g* and the supernatants were collected to assay.

2.6. Statistical analysis

The results were calculated by the one- or two-way analysis of variance (ANOVA), followed by Bonferroni's *post hoc* test as appropriate. The results are presented as means ± standard errors of means (S.E.M). The level of $p < 0.05$ was considered statistically significant. All figures were prepared by the GraphPad Prism version 5.00 for Windows, GraphPad Software (San Diego, California, USA), www.graphpad.com.

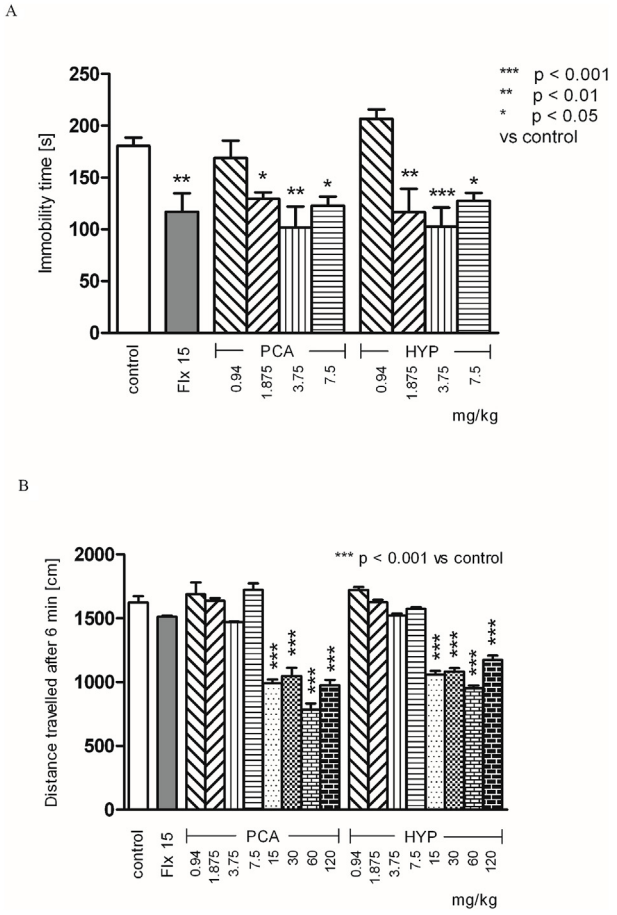

Fig. 1. Effects of administration of PCA and HYP at doses 0.94, 1.875, 3.75 and 7.5 mg/kg on the immobility time in the FST (A). Effects of administration of PCA and HYP at doses 0.94, 1.875, 3.75, 7.5, 15, 30, 60 and 120 mg/kg on the distance travelled (B). Each column represents the mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001 *vs*. control group (one-way ANOVA followed by Bonferroni's test).

3. Results

3.1. Effects of HYP and PCA on the immobility time in the FST

One-way ANOVA revealed that the acute i.p. doses of PCA (1.875, 3.75 and 7.5 mg/kg), HYP (1.875, 3.75 and 7.5 mg/kg) and Flx (15 mg/kg) exerted a statistically significant effect on immobility time values [$F_{(9,81)} = 6{,}036$; $p < 0.0001$; Fig. 1A]. The applied *post hoc* Bonferroni's test showed PCA (1.875, 3.75 and 7.5 mg/kg), HYP (1.875, 3.75 and 7.5 mg/kg) and Flx (15 mg/kg) to have significantly decreased immobility time, as compared with the saline-treated control group [$p < 0.05$, $p < 0.01$ and $p < 0.05$; $p < 0.01$, $p < 0.001$ and $p < 0.05$; $p < 0.01$ respectively], indicating that these constituents and Flx exert an antidepressant-like effect. The lowest dose of PCA and HYP (0.94 mg/kg) did not affect the immobility time.

3.2. Effects of treatments on the locomotor activity of mice

One-way ANOVA revealed that a treatment with PCA or HYP affected the distance travelled [$F_{(17,126)} = 68{,}32$; $p < 0.0001$; Fig. 1B]. The *post hoc* Bonferroni's test showed that PCA and HYP at doses 15, 30, 60 and 120 mg/kg significantly shortened the distance travelled compared to saline group ($p < 0.001$). There were no substantial alterations in the locomotor activity of mice after PCA and HYP administration at dose: 0.94, 1.875, 3.75 and 7.5 mg/kg ($p > 0.05$).

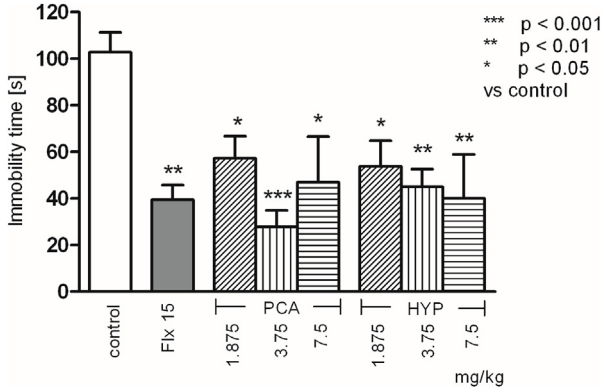

Fig. 2. Effects of administration of PCA and HYP at doses 1.875, 3.75 and 7.5 mg/kg on the immobility time in the TST. Each column represents the mean ± SEM. *p < 0.05, **p < 0.01 and ***p < 0.001 *vs.* control group (one-way ANOVA followed by Bonferroni's test).

3.3. Effects of PCA and HYP on the immobility time in the TST

One-way ANOVA revealed that the acute i.p. doses of PCA (1.875, 3.75 and 7.5 mg/kg), HYP (1.875, 3.75 and 7.5 mg/kg) and Flx (15 mg/kg) exerted a statistically significant effect on immobility time values [$F_{(7,45)}$ = 3,999; p = 0.0018; Fig. 2]. The applied *post hoc* Bonferroni's test showed PCA (1.875, 3.75 and 7.5 mg/kg), HYP (1.875, 3.75 and 7.5 mg/kg) and Flx (15 mg/kg) to have significantly decreased immobility time, as compared with the saline-treated control group [p < 0.05, p < 0.001 and p < 0.05; p < 0.05, p < 0.01 and p < 0.01; p < 0.01 respectively], indicating that these constituents and Flx exert an antidepressant-like effect.

3.4. Involvement of the serotonergic system in the antidepressant-like effect of HYP and PCA in the FST

Two-way ANOVA revealed that there was a statistically significant effect caused by PCPA (100 mg/kg) pretreatment [$F_{(1,62)}$ = 15.93; p = 0.0002] and interaction between PCPA (100 mg/kg) and PCA (3.75 mg/kg), HYP (3.75 mg/kg) or Flx (15 mg/kg) treatment [$F_{(3,62)}$ = 9.81; p < 0.0002].

The acute injection of PCA (3.75 mg/kg), HYP (3.75 mg/kg) and Flx (15 mg/kg) caused antidepressant-like effect, reducing the immobility time in the FST (p < 0.001, p < 0.001 and p < 0.01, respectively; the post hoc Bonferroni's test; Fig. 3A). Pretreatment with PCPA (100 mg/kg) significantly prevented anti-immobility actions induced by Flx (15 mg/kg) and HYP (3.75 mg/kg) (p < 0.05 and p < 0.001, respectively, the *post hoc* Bonferroni's test; Fig. 3A).

Two-way ANOVA revealed that there was a statistically significant effect caused by Flx (5 mg/kg) pretreatment [$F_{(1,41)}$ = 5.84; p = 0.0202] and interaction between Flx (5 mg/kg) and HYP (0.94 mg/kg) treatment [$F_{(2,41)}$ = 3.97; p = 0.0266]. Co-administration of sub-effective doses of Flx (5 mg/kg) and HYP (0.94 mg/kg) significantly reduced the immobility time in the FST in comparison with the control group (p < 0.05, the post hoc Bonferroni's test; Fig. 4A).

None of the treatments caused significant changes on locomotor activity (Figs. 3B and 4B).

3.5. Involvement of the noradrenergic/dopaminergic system in the antidepressant-like effect of HYP and PCA in the FST

Two-way ANOVA revealed that there was a statistically significant effect caused by AMPT (100 mg/kg) pretreatment [$F_{(1,53)}$ = 20.90; p < 0.0001], treatment with PCA (3.75 mg/kg) or HYP (3.75 mg/kg) [$F_{(2,53)}$ = 87.20; p < 0.0001] and interaction between AMPT (100 mg/kg) pretreatment and PCA (3.75 mg/kg) or HYP (3.75 mg/kg)

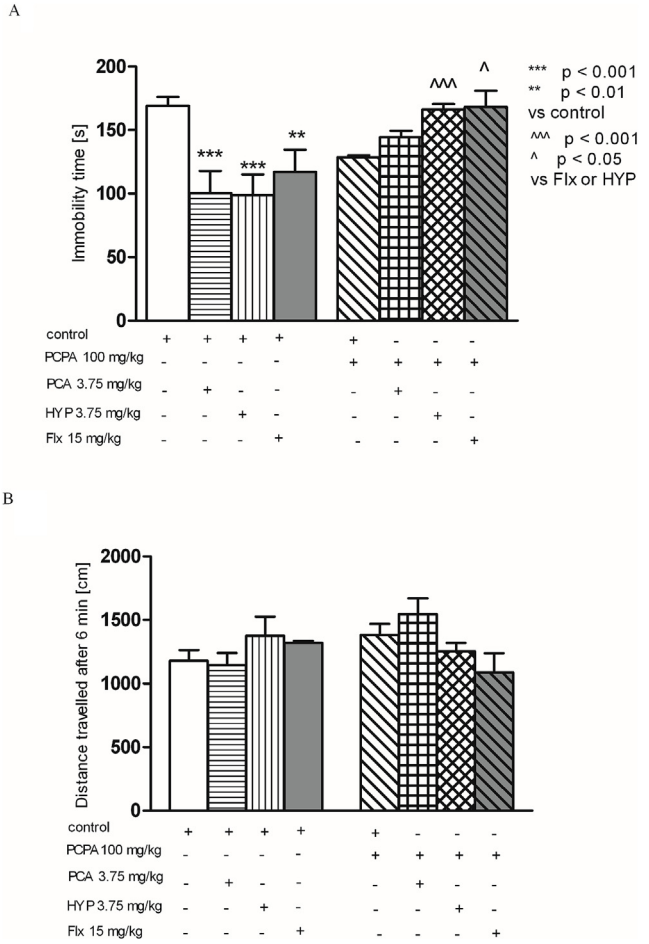

Fig. 3. Effect of pretreatment of mice with PCPA (100 mg/kg, i.p.) and treatment with PCA (3.75 mg/kg, i.p.) or HYP (3.75 mg/kg, i.p.) on the immobility time in the FST (A) and on the distance travelled (B). Each column represents the mean ± SEM. **p < 0.01, ***p < 0.001 *vs.* control group; ^ p < 0.05, ^^^ p < 0.001 *vs.* the same group pretreated with vehicle (two-way ANOVA followed by Bonferroni's test).

treatment [$F_{(2,53)}$ = 31.13; p < 0.0001].

The acute injection of HYP (3.75 mg/kg) and PCA (3.75 mg/kg) caused antidepressant-like effect, reducing the immobility time in the FST (p < 0.001 and p < 0.01, respectively; the *post hoc* Bonferroni's test; Fig. 5A). Pretreatment with AMPT (100 mg/kg) significantly prevented anti-immobility actions induced by HYP (3.75 mg/kg) or PCA (3.75 mg/kg) (p < 0.05, the *post hoc* Bonferroni's test; Fig. 5A).

Two-way ANOVA revealed that there was a statistically significant effect caused by Rbx (2 mg/kg) pretreatment [$F_{(1,31)}$ = 87.62; p < 0.0001], treatment with PCA (0.94 mg/kg) or HYP (0.94 mg/kg) [$F_{(2,31)}$ = 6.78; p = 0.0036] and interaction between Rbx (2 mg/kg) pretreatment and HYP (0.94 mg/kg) or PCA (0.94 mg/kg) treatment [$F_{(2,31)}$ = 14.25; p < 0.0001]. Co-administration of sub-effective doses of Rbx (2 mg/kg) and HYP (0.94 mg/kg) or PCA (0.94 mg/kg) significantly reduced the immobility time in the FST in comparison with control group (p < 0.05, the post hoc Bonferroni's test; Fig. 6A).

Two-way ANOVA revealed that there was a statistically significant effect caused by SUL (50 mg/kg) pretreatment [$F_{(1,57)}$ = 197.97; p < 0.0001], treatment with HYP (3.75 mg/kg), PCA (3.75 mg/kg) or BUP (30 mg/kg) [$F_{(3,57)}$ = 65.84; p < 0.0001] and interaction between SUL (50 mg/kg) pretreatment and HYP (3.75 mg/kg), PCA (3.75 mg/kg) or BUP (30 mg/kg) [$F_{(3,57)}$ = 14.63; p < 0.0001].

The acute injection of HYP (3.75 mg/kg), PCA (3.75 mg/kg) and BUP (30 mg/kg) caused antidepressant-like effect, reducing the immobility time in the FST (p < 0.01, p < 0.01 and p < 0.001,

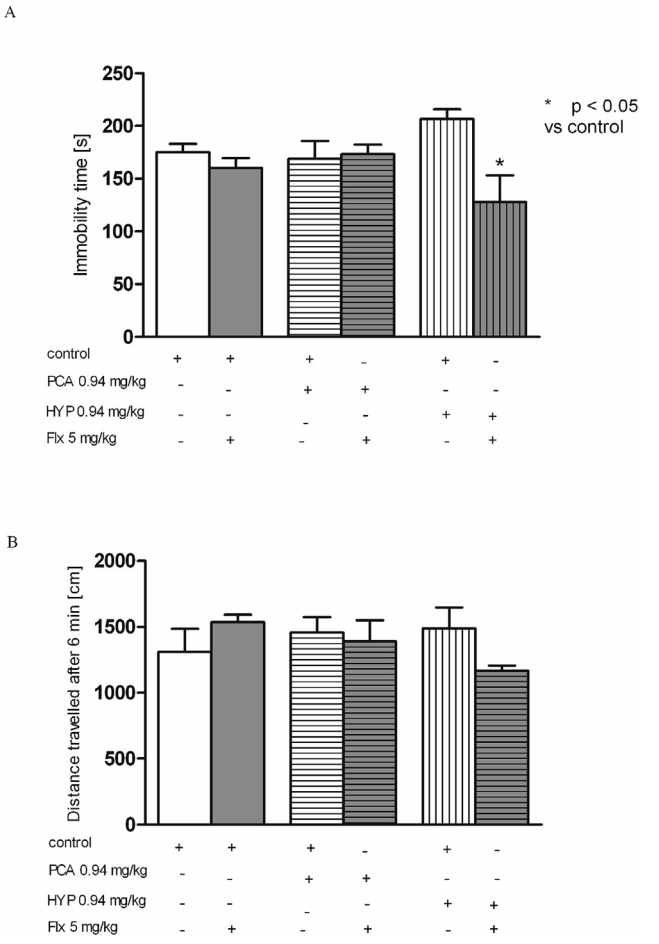

Fig. 4. Effect of the treatment with sub-effective doses of PCA (0.94 mg/kg, i.p.) and HYP (0.94 mg/kg, i.p.) in combination with sub-effective dose of fluoxetine (Flx, 5 mg/kg, i.p.) on the immobility time in the FST (A) and on the distance travelled (B). Each column represents the mean ± SEM. *p < 0.05 *vs.* control group (two-way ANOVA followed by Bonferroni's test).

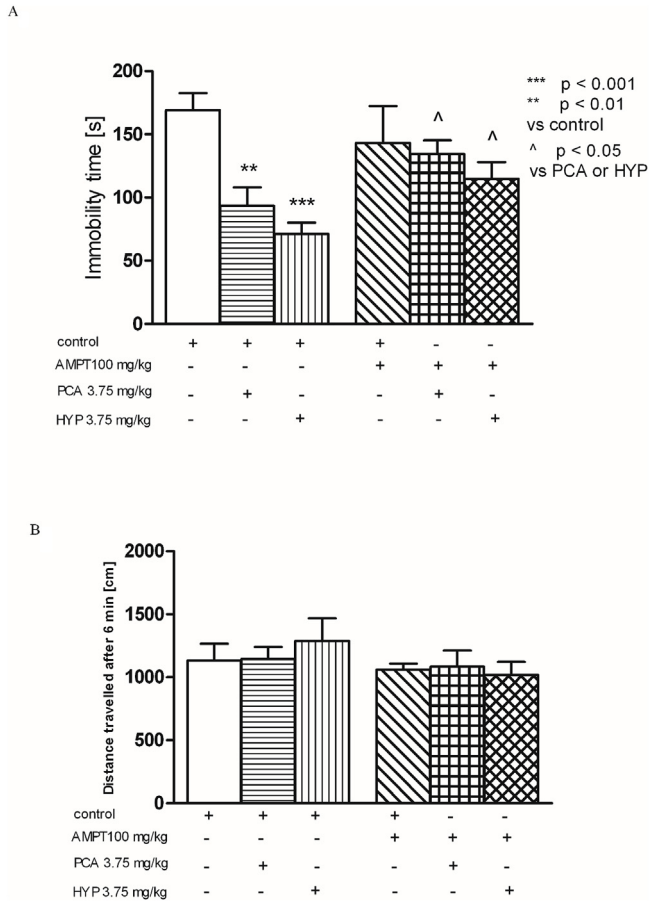

Fig. 5. Effect of pretreatment of mice with AMPT (100 mg/kg, i.p.) and treatment with PCA (3.75 mg/kg, i.p.) or HYP (3.75 mg/kg, i.p.) on the immobility time in the FST (A) and on the distance travelled (B). Each column represents the mean ± SEM. **p < 0.01, ***p < 0.001 *vs.* control group; ^ p < 0.05 *vs.* the same group pretreated with vehicle (two-way ANOVA followed by Bonferroni's test).

respectively; the *post hoc* Bonferroni's test; Fig. 7A). Pretreatment with SUL (50 mg/kg) significantly prevented anti-immobility actions induced by BUP (30 mg/kg), PCA (3.75 mg/kg) and HYP (3.75 mg/kg) ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively, the *post hoc* Bonferroni's test; Fig. 7A).

None of the treatments caused significant changes on locomotor activity (Figs. 5B, 6B and 7B).

3.6. Effects of PCA and HYP on BDNF concentration in the hippocampus

A significant decrease in concentration of BDNF ($F_{[2.18]} = 4.061$, $p < 0.01$; Fig. 8) in the hippocampus of mice exposed to TST (TST group) compared to the non-TST group was recorded. In the group of TST rodents receiving Flx (15 mg/kg, i.p.), the significant rise of BDNF level ($F_{[2.18]} = 4.061$, $p < 0.01$; Fig. 8) was noted in comparison to TST group. PCA (7.5 mg/kg i.p.) administered to mice exposed to TST significantly increased the BDNF concentration ($F_{[2.18]} = 4.061$, $p < 0.01$; Fig. 8) when compared with the TST group. BDNF levels were also recovered in a dose-depend manner by administration of HYP at a dose of 3.75 mg/kg i.p. ($F_{[2.18]} = 4.061$, $p < 0.01$; Figs. 8) and 7.5 mg/kg i.p. ($F_{[2.18]} = 4.061$, $p < 0.001$; Fig. 8) in comparison to the TST group.

4. Discussion

In the present study, we aimed to investigate the potential antidepressant-like effect of two polyphenols, isolated from *I. glandulifera*, in FST and TST, frequently used as screening models for new antidepressant agents. Both tests are sensitive to all major classes of antidepressants and are leading tools to assess the neurobiological mechanisms involved in the antidepressant response. In these tests, mice are placed in an inescapable situation which leads to the development of immobile posture and substances that have antidepressant-like effect promote the occurrence of escape-related behavior decreasing the immobility time duration (Kara et al., 2018; Porsolt et al., 1977; Steru et al., 1985).

To verify that the changes in immobility time in the FST and TST were not attributed to non-specific effects, locomotor activity of mice was measured. The first stage of experiment consisted of assessing the impact of different doses of HYP and PCA on the mobility of animals. In the further treatments, HYP and PCA were used at doses (0.94, 1.875, 3.75 and 7.5 mg/kg) which did not affect spontaneous locomotion of mice.

It was demonstrated that an acute treatment with HYP and PCA, administered i.p., produced a significant antidepressant-like response, evidenced by a decrease in the immobility duration in the FST and TST. These effects were observed in three used doses of HYP and PCA – 1.875, 3.75 and 7.5 mg/kg. Antidepressant activity of HYP and PCA at a dose of 3.75 mg/kg was comparable with that of Flx (15 mg/kg).

Extensive studies showed that monoaminergic neurotransmission that involves 5-HT, NE and DA exerts major influence on brain circuits concerned by the regulation of mood, reactivity to psychological stress,

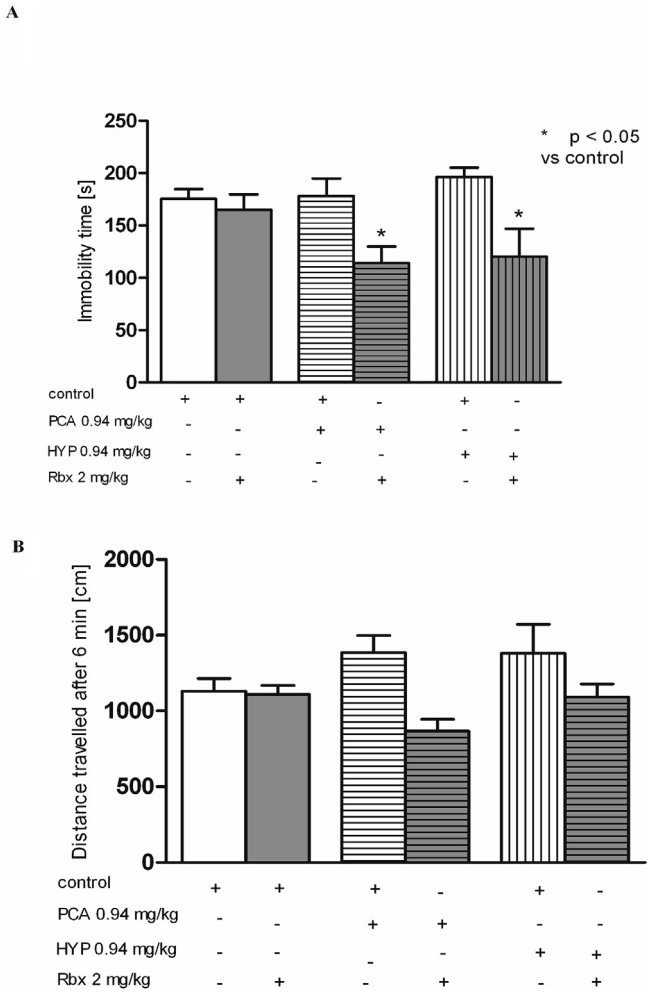

Fig. 6. Effect of the treatment with sub-effective doses of PCA (0.94 mg/kg, i.p.) and HYP (0.94 mg/kg, i.p.) in combination with sub-effective dose of reboxetine (Rbx, 2 mg/kg, i.p.) on the immobility time in the FST (A) and on the distance travelled (B). Each column represents the mean ± SEM. *p < 0.05 *vs.* control group (two-way ANOVA followed by Bonferroni's test).

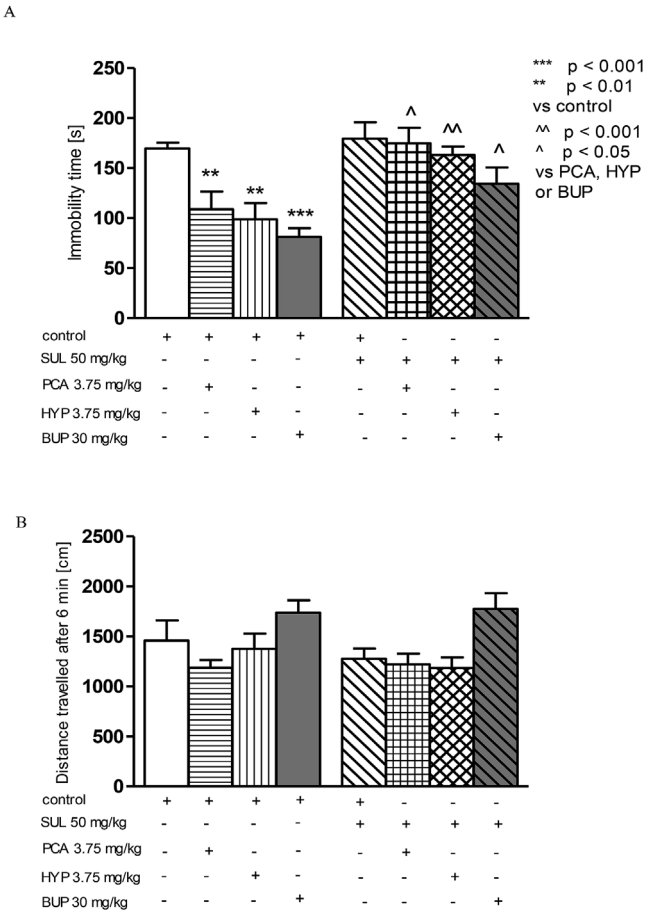

Fig. 7. Effect of pretreatment of mice with SUL (50 mg/kg, i.p.) on PCA (3.75 mg/kg)-, HYP (3.75 mg/kg)- or BUP (30 mg/kg)-induced reduction in immobility time in the FST (A). Effects of administration of SUL (50 mg/kg, i.p.), PCA (3.75 mg/kg) and HYP (3.75 mg/kg) or BUP (30 mg/kg) on the distance travelled (B). Each column represents the mean ± SEM. **p < 0.01, ***p < 0.001 *vs.* control group; ^ p < 0.05 or ^^ p < 0.01 *vs.* the same group pretreated with vehicle (two-way ANOVA followed by Bonferroni's test).

motivation and drive. Indeed, the therapeutic efficacy of available antidepressants is dependent on the increase in the level of monoamines in the brain caused by the therapy with these drugs (Hamon and Blier, 2013).

In order to verify the involvement of serotonergic system in the antidepressant-like effect of HYP and PCA, serotonergic lesion was performed. PCPA, an inhibitor of the enzyme tryptophan hydroxylase (an enzyme that plays a pivotal role in biosynthesis of 5-HT in CNS neurons), was reported to produce a significant depletion of cortical 5-HT content in mice (O'Leary et al., 2007). In our experiment, PCPA (100 mg/kg) was able to abolish antidepressant-like effect of HYP (3.75 mg/kg) and Flx (15 mg/kg), a positive control in the FST. This effect suggests that anti-immobility action of HYP is dependent on the bioavailability of 5-HT in the synaptic cleft, similar to Flx. Reinforcing this hypothesis, the co-administration of sub-effective dose of HYP (0.875 mg/kg) in combination with Flx (5 mg/kg) produced a synergistic antidepressant-like response in the FST. Flx belongs to SSRIs which mechanism of antidepressant action is associated with selective activity at the rise of the serotonergic system in the CNS by inhibiting the reuptake of 5-HT (Hamon and Blier, 2013). PCPA, when given alone, had no impact on the FST behavior. The administration of PCPA at the same dose did not prevent the antidepressant-like effect of PCA in the FST. The combination of subthreshold doses of PCA (0.94 mg/kg) an Flx (5 mg/kg) does not imply a convincing interaction.

It should be underlined that antidepressants acting selectively on only one monoamine, such as selective inhibitors of 5-HT or NE reuptake, alleviate depression symptoms in a limited percentage of patients, and are poorly effective to prevent recurrence. Thorough investigations for the last 30 years allowed the demonstration of the existence of functional interactions between 5-HT, NE and DA systems (Hamon and Blier, 2013). Therefore, in the subsequent stages of research, the effect of AMPT – a selective inhibitor of tyrosine hydroxylase – on the antidepressant-like activity of HYP and PCA was explored. Tyrosine hydroxylase is a rate-limiting enzyme in the synthesis of NE and DA and the administration of AMPT reduced NE and DA levels in mice, whereas it does not affect 5-HT levels (Mayorga et al., 2001). In the present study, it was found that a pre-treatment with AMPT caused a significant inhibition of the antidepressant effect in the FST when co-administered with HYP or PCA at a dose of 3.75 mg/kg. To better address this issue, we also evaluated the effect of combined administration of subeffective doses of HYP and PCA (0.94 mg/kg) with Rbx, a selective NE reuptake inhibitor. Its mechanism of action leads to an increased availability of NE around synaptic cleft (Eyding et al., 2010). The synergism of the antidepressant action, observed in the experimental scheme, suggests that the NE system can be implicated in the antidepressant-like response of HYP and PCA. It was also shown that the anti-immobility effect of HYP and PCA (3.75 mg/kg) was prevented by SUL (50 mg/kg) – a dopamine D2 receptor antagonist. The result was comparable to the antagonist interaction between SUL

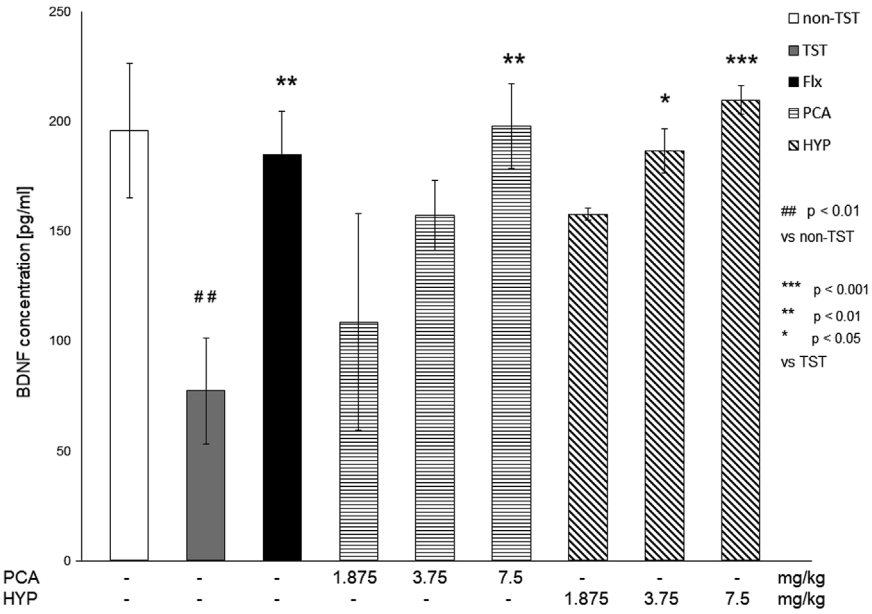

Fig. 8. The impact of administration of PCA and HYP at doses of 1.875, 3.75 and 7.5 mg/kg on the concentration of BDNF in hippocampus of mice. Data is displayed as mean ± SEM. Significance: $\#\#p < 0.01$ compared to non-TST group; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to TST group (one-way ANOVA with a *post-hoc* Bonferroni multiple comparison test).

and BUP (30 mg/kg), used as a positive control. However, we cannot exclude that the interaction between SUL and BUP in the FST was affected by increased locomotion. Locomotor activity seems to be enhanced in the SUL + BUP group, however, statistical comparisons are not significant. BUP is suggested to selectively inhibit the neuronal reuptake of DA and is significantly more potent than either imipramine or amitriptyline in this regard. Obtained result seems to support that DA system could be implicated in the antidepressant effects of studied polyphenols (de Sousa et al., 2014).

Our results regarding to antidepressant activity of HYP are consistent with previous research. It was shown that HYP (0.6 and 1.3 mg/kg) exerted antidepressant activity in rats assessed in the FST. The duration of immobility was not changed in animals which received HYP at higher doses – 2.61, 5.22 and 7.61 mg/kg. HYP was administered orally 24h, 5h and 1 h before the test (Butterweck and Schmidt, 2007). Haas et al. (2011) presented that HYP (10 and 20 mg/kg) reduced the immobility time in rats measured in the FST. At our experiments, we did not use so high doses of HYP because of the mobility impairment caused by these doses. In addition, these authors reported that the activation of D2-like receptors accounted for the anti-immobility effect of HYP which is in agreement with our results. HYP was also able to reverse depressive symptoms in the FST and sucrose preference test in rats exposed to chronic mild stress (CMS). Simultaneously, HYP increased the expression of BDNF in hippocampus of CMS rats (Gong et al., 2017).

PCA - the other phenolic compound isolated from *Impatiens* tested in the present study – previously have been demonstrated to show antidepressant activity in FST in mice (Thakare et al., 2017). Treatment with PCA, at much higher doses than in our experiment – 100 and 200 mg/kg, was able to prevent stress induced immobility time in the FST without altering locomotor activity.

The results reported herein suggest that antidepressant-like effect of PCA and HYP in the FST in mice is due to the up-regulation of monoaminergic neurotransmission. It can be concluded that brain monoamines should be maintain at a certain level for PCA (DA and NE concentration) and HYP (5-HT, DA and NE concentration) antidepressant response. Further studies are needed to make these mechanisms clearer. Based on the presented results and known mechanisms of monoaminergic antidepressants we can only speculate that polyphenols isolated from *I. glandulifera* modulate the monoamines levels in brain, maybe through the inhibition of neuronal reuptake, the stimulation of monoamine-synthesizing enzymes – tryptophan hydroxylase or/and tyrosine hydroxylase or the inhibition of monoamine oxidase (MAO). It can be also suggested that the anti-immobility effects of PCA and HYP is mediated by D2 receptor activation, based on the results showing that the anti-immobility effects of PCA and HYP were prevented by sulpiride (D2 antagonist). In addition, it was found through in vitro screening test that PCA exhibited significant inhibition on MAO-B and dopamine-β hydroxylase (DBH), revealing a weak MAO-A inhibition. PCA is expected to elevate the level of released DA effectively, by preventing DBH from converting DA to NE (Kim et al., 2012).

Depression is a disease that is too intricate to be explained by the monoamine hypothesis alone. Thus, other hypotheses have been suggested. According to the neurotrophin theory of depression, there is a reduction in BDNF levels in depressive individuals and the reversal of this situation could be involved with the production of antidepressant action (Kozisek et al., 2008). BDNF is an endogenous neurotrophic factor that modulates neuronal plasticity and inhibits cell death cascades. It has been extensively investigated in recent years as one of the targets of antidepressants. BDNF has one effect relevant to the therapeutic actions of antidepressants: the regulation of 5-HT neurons; local infusion of BDNF into the cerebral cortex or midbrain elevates 5-HT levels (Siuciak et al., 1994) and protects 5-HT neurons from neurotoxin-induced damage (Mamounas et al., 1995). It was also shown that chronic (21 d), but not acute (1 d), administration of several different antidepressant drugs, including tranylcypromine, sertraline, desipramine, or mianserin, significantly increased BDNF mRNA in hippocampus (Nibuya et al., 1995). Numerous studies have demonstrated that monoaminergic antidepressants produced antidepressant-like effect through BDNF-dependent neurotrophic/neuroplastic mechanisms (Paizanis et al., 2007). Our findings demonstrated that the exposure to TST was accompanied by lower BDNF level, while PCA (7.5 mg/kg) and HYP (3.75 and 7.5 mg/kg) normalized BDNF level. In addition, it should be marked that BDNF concentration in groups administered with PCA at lower dose - 3.75 mg/kg and HYP - 1.8 mg/kg also seems to indicate a visible increase in BDNF concentration in comparison with the group subjected to TST (the results are not statistically significant). This effect correlated with the antidepressant action assessed in the FST and TST. However, our behavioral approach does not allow us concluding about the mechanism by which PCA and HYP may affect BDNF level. Data mentioned above underlying that monoaminergic antidepressants influence BDNF concentration but after chronic, not acute treatment. Further studies dealing with the effects of PCA and HYP on

BDNF level after prolonged administration are necessary to clarify this issue.

The data on monoamine neurocircuitry provided a rationale for the design of new, multimodal, therapeutic strategies involving drugs affecting three monoamines - 5-HT, NE, and DA to improve efficacy and tolerability antidepressant therapy (Hamon and Blier, 2013). Considering this assumption, polyphenols isolated from *I. glandulifera* –PCA and HYP seem to be useful candidates for new antidepressants. Additionally, the obtained results suggest that the use of PCA or HYP together with currently available antidepressant e.g. Rbx (synergism with PCA and HYP) or Flx (synergism with HYP) agents can allow lowering the doses of these drugs and contributing to a more effective and safer pharmacotherapy of depression.

Collectively, PCA and HYP displayed an antidepressant-like effect in mice, and this effect may be partially mediated by regulating the central monoamine neurotransmitter system and increasing the levels of BDNF, which is similar to the effect of Flx. However, the results of this study would benefit from further investigation into the antidepressant mechanism of this potential treatment option.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The study was supported by statutory table funds provided by the Polish Ministry of Science and Higher Education for Medical University of Lublin, Poland (G. Biała).

References

Beerling, D.J., Perrins, J.M., 1993. *Impatiens glandulifera* Royle (*Impatiens roylei* Walp). J. Ecol. 81, 367–382. https://doi.org/10.2307/2261507.

Butterweck, V., Schmidt, M., 2007. St. John's wort: role of active compounds for its mechanism of action and efficacy. Wien Med. Wochenschr. 157, 356–361. https://doi.org/10.1007/s10354-007-0440-8.

Cimmino, A., Mathieu, V., Evidente, M., Ferderin, M., Banuls, L.M.Y., Masi, M., De Carvalho, A., Kiss, R., Evidente, A., 2016. Glanduliferins A and B, two new glucosylated steroids from *Impatiens glandulifera*, with *in vitro* growth inhibitory activity in human cancer cells. Fitoterapia 109, 138–145. https://doi.org/10.1016/j.fitote.2015.12.016.

Colla, A.R., Oliveira, A., Pazini, F.L., Rosa, J.M., Manosso, L.M., Cunha, M.P., Rodrigues, A.L., 2014. Serotonergic and noradrenergic systems are implicated in the antidepressant-like effect of ursolic acid in mice. Pharmacol. Biochem. Behav. 124, 108–116. https://doi.org/10.1016/j.pbb.2014.05.015.

de Sousa, F.C., Oliveira, I.C., Silva, M.I., de Melo, C.T., Santiago, V.R., de Castro Chaves, R., Fernandes, M.L., Gutierrez, S.J., Vasconcelos, S.M., Macêdo, D.S., Barbosa Filho, J.M., 2014. Involvement of monoaminergic system in the antidepressant-like effect of riparin I from *Aniba riparia* (Nees) Mez (*Lauraceae*) in mice. Fundam. Clin. Pharmacol. 28, 95–103. https://doi.org/10.1111/j.1472-8206.2012.01069.x.

Directive 2010/63/EU of the European parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, Official Journal of the European Union, L 276/33, document 32010L0063.

Eyding, D., Lelgemann, M., Grouven, U., Harter, M., Kromp, M., Kaiser, T., Kerekes, M.F., Gerken, M., Wieseler, B., 2010. Reboxetine for acute treatment of major depression: systematic review and meta-analysis of published and unpublished placebo and selective serotonin reuptake inhibitor controlled trials. BMJ 341, c4737. https://doi.org/10.1136/bmj.c4737.

Fiorino, F., Magli, E., Kędzierska, E., Ciano, A., Corvino, A., Severino, B., Perissutti, E., Frecentese, F., Di Vaio, P., Saccone, I., Izzo, A.A., Capasso, R., Massarelli, P., Rossi, I., Orzelska-Górka, J., Kotlińska, J.H., Santagada, V., Caliendo, G., 2017. New 5-HT1A, 5HT2A and 5HT2C receptor ligands containing a picolinic nucleus: synthesis, *in vitro* and *in vivo* pharmacological evaluation. Bioorg. Med. Chem. 25, 5820–5837. https://doi.org/10.1016/j.bmc.2017.09.018.

Gong, Y., Yang, Y., Chen, X., Yang, M., Huang, D., Yang, R., Zhou, L., Li, C., Xiong, Q., Xiong, Z., 2017. Hyperoside protects against chronic mild stress-induced learning and memory deficits. Biomed. Pharmacother. 91, 831–840. https://doi.org/10.1016/j.biopha.2017.05.019.

Hamon, M., Blier, P., 2013. Monoamine neurocircuitry in depression and strategies for new treatments. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 45, 54–63. https://doi.org/10.1016/j.pnpbp.2013.04.009.

Haas, J.S., Stolz, E.D., Betti, A.H., Stein, A.C., Schripsema, J., Poser, G.L., Rates, S.M., 2011. The anti-immobility effect of hyperoside on the forced swimming test in rats is mediated by the D2-like receptors activation. Planta Med. 77, 334–339. https://doi.org/10.1055/s-0030-1250386.

Hejda, M., Pyšek, P., 2006. What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? Biol. Conserv. 132, 143–152. https://doi.org/10.1016/j.biocon.2006.03.025.

Kara, N.Z., Stukalin, Y., Einat, H., 2018. Revisiting the validity of the mouse forced swim test: systematic review and meta-analysis of the effects of prototypic antidepressants. Neurosci. Biobehav. Rev. 84, 1–11. https://doi.org/10.1016/j.neubiorev.2017.11.003.

Kim, J.H., Kim, G.H., Hwang, K.H., 2012. Monoamine oxidase and dopamine β-hydroxylase inhibitors from the fruits of *Gardenia jasminoides*. Biomol. Ther. 20, 214–219.

Knight, M.J., Baune, B.T., 2017. Psychosocial dysfunction in major depressive disorder-rationale, design, and characteristics of the cognitive and emotional recovery training program for depression (CERT-D). Front. Psychiatry 8, 280. https://doi.org/10.3389/fpsyt.2017.00280.

Kozisek, M.E., Middlemas, D., Bylund, D.B., 2008. Brain-derived neurotrophic factor and its receptor tropomyosin-related kinase B in the mechanism of action of antidepressant therapies. Pharmacol. Ther. 117, 30–51. https://doi.org/10.1016/j.pharmthera.2007.07.001.

Kumar, M., Paul, Y., Anand, V.K., 2009. An ethnobotanical study of medicinal plants used by the locals in Kishtwar, Jammu and Kashmir, India. Ethnobot. Leaf. 13, 1240–1256. https://opensiuc.lib.siu.edu/ebl/vol2009/iss10/5.

Kwon, S., Lee, B., Kim, M., Lee, H., Park, H.J., Hahm, D.H., 2010. Antidepressant-like effect of the methanolic extract from Bupleurum falcatum in the tail suspension test. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34, 265–270. https://doi.org/10.1016/j.pnpbp.2009.11.015.

Lee, G., Bae, H., 2017. Therapeutic effects of phytochemicals and medicinal herbs on depression. BioMed Res. Int. 2017, 6596241. https://doi.org/10.1155/2017/6596241.

Machado, D.G., Cunha, M.P., Neis, V.B., Balen, G.O., Colla, A., Bettio, L.E.B., Oliveira, Á., Pazini, F.L., Dalmarco, J.B., Simionatto, E.L., Pizzolatti, M.G., Rodrigues, A.L.S., 2013. Antidepressant-like effects of fractions, essential oil, carnosol and betulinic acid isolated from *Rosmarinus officinalis* L. Food Chem. 136, 999–1005. https://doi.org/10.1016/j.foodchem.2012.09.028.

Mamounas, L.A., Blue, M.E., Siuciak, J.A., Altar, C.A., 1995. Brain-derived neuro-trophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J. Neurosci. 15, 7929–7939.

Mayorga, A.J., Dalvi, A., Page, M.E., Zimov-Levinson, S., Hen, R., Lucki, I., 2001. Antidepressant-like behavioral effects in 5-hydroxytryptamine (1A) and 5-hydroxytryptamine(1B) receptor mutant mice. J. Pharmacol. Exp. Ther. 298, 1101–1107. http://jpet.aspetjournals.org.

Nabavi, S.M., Nabavi, S.F., Sureda, A., Caprioli, G., Iannarelli, R., Sokeng, A.J.T., Braidy, N., Khanjani, S., Moghaddam, A.H., Atanasov, A.G., Daglia, M., Maggi, F., 2018. The water extract of tutsan (*Hypericum androsaemum* L.) red berries exerts antidepressive-like effects and in vivo antioxidant activity in a mouse model of post-stroke depression. Biomed. Pharmacother. 99, 290–298. https://doi.org/10.1016/j.biopha.2018.01.073.

Nabavi, S.F., Braidy, N., Hebtemariam, S., Orhan, I.E., Daglia, M., Manayi, A., Gortzi, O., Nabavi, S.M., 2015. Neuroprotective effects of chrysin: from chemistry to medicine. Neurochem. Int. 90, 224–231. https://doi.org/10.1016/j.neuint.2015.09.006.

Nibuya, M., Morinobu, S., Duman, R.S., 1995. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J. Neurosci. 15, 7539–7547.

O'Leary, O.F., Bechtholt, A.J., Crowley, J.J., Hill, T.E., Page, M.E., Lucki, I., 2007. Depletion of serotonin and catecholamines block the acute behavioral response to different classes of antidepressant drugs in the mouse tail suspension test. Psychopharmacology (Berlin) 192, 357–371. https://doi.org/10.1007/s00213-007-0728-9.

Paizanis, E., Kelai, S., Renoir, T., Hamon, M., Lanfumey, L., 2007. Life-long hippocampal neurogenesis: environmental, pharmacological and neurochemical modulations. Neurochem. Res. 32, 1762–1771.

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.

Pyšek, P., Prach, K., 1995. Invasion dynamics of *Impatiens glandulifera* - a century of spreading reconstructed. Biol. Conserv. 74, 41–48. https://doi.org/10.1016/0006-3207(95)00013-T.

Siuciak, J.A., Altar, C.A., Wiegand, S.J., Lindsay, R.M., 1994. Antinociceptive effect of brain-derived neurotrophic factor and neurotrophin-3. Brain Res. 633, 326–330.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology (Berlin) 85, 367–370. https://doi.org/10.1007/BF00428203.

Szafrański, T., 2014. Herbal remedies in depression – state of the art. Psychiatr. Pol. 48, 59–73.

Szewczyk, K., Bonikowski, R., Maciąg-Krajewska, A., Abramek, J., Bogucka-Kocka, A., 2018a. Lipophilic components and evaluation of the cytotoxic and antioxidant activities of *Impatiens glandulifera* Royle and *Impatiens noli-tangere* L. (Balsaminaceae). Grasas Aceites 69 (3), e270. https://doi.org/10.3989/gya.023418.

Szewczyk, K., Cicek, S.S., Zidorn, C., Granica, S., 2018b. Phenolic constituents of the aerial parts of *Impatiens glandulifera* Royle (Balsaminaceae) and their antioxidant activities. Nat. Prod. Res. 3, 1–5. https://doi.org/10.1080/14786419.2018.1499644.

Szewczyk, K., Olech, M., 2017. Optimization of extraction method for LC-MS based determination of phenolic acid profiles in different *Impatiens* species. Phytochem. Lett. 20, 322–330. https://doi.org/10.1016/j.phytol.2017.02.005.

Szewczyk, K., Orzelska-Górka, J., Polakowska, M., Biała, G., 2018c. Antinociceptive and antianxiety activity of hydroethanolic extracts of three *Impatiens* species in mice. Acta Pol. Pharm. 75, 989–1001.

Szewczyk, K., Zidorn, C., Biernasiuk, A., Komsta, Ł., Granica, S., 2016. Polyphenols from *Impatiens* (Balsaminaceae) and their antioxidant and antimicrobial activities. Ind.

Crops Prod. 86, 262–272. https://doi.org/10.1016/j.indcrop.2016.03.053.
Thakare, V.N., Dhakane, V.D., Patel, B.M., 2017. Attenuation of acute restraint stress-induced depressive like behavior and hippocampal alterations with protocatechuic acid treatment in mice. Metab. Brain Dis. 32, 401–413. https://doi.org/10.1007/s11011-016-9922-y.
Thaler, K., Kaminski, A., Chapman, A., Langley, T., Gartlehner, G., 2009. Bach Flower Remedies for psychological problems and pain: a systematic review. BMC Complement Altern. Med. 9, 16. https://doi.org/10.1186/1472-6882-9-16.
Tříska, J., Vrchotová, N., Sýkora, J., Moos, M., 2013. Separation and identification of 1,2,4-trihydroxynaphthalene1-O-glucoside in *Impatiens glandulifera* Royle. Molecules 18, 8429–8439. https://doi.org/10.3390/molecules18078429.
Vieira, M.N., Winterhalter, P., Jerz, G., 2016. Flavonoids from the flowers of *Impatiens glandulifera* Royle isolated by high performance countercurrent chromatography. Phytochem. Anal. 27, 116–125. https://doi.org/10.1002/pca.2606.
Weber, E., 2003. Invasive Plant Species of the World: A Reference Guide to Environmental Weeds. CAB International Publishing, Wallingford, USA, pp. 548.