International Journal of Phytopharmacy
Vol. 6 (1), pp.14-26, Jan-Feb 2016
ISSN: 2277-2928 (Online)
Journal DOI:10.7439/ijpp

www.ssjournals.com

Research Article

Antidepressant-like effect of hydroalcoholic extract of *Valeriana prionophylla* Standl. from Guatemala: Evidence for the involvement of the monoaminergic systems

Iandra Holzmann*, Valdir Cechinel Filho, Armando Caceres, José Vicente Martinez, Sully M Cruz, Márcia Maria de Souza

Universidade do Vale do Itajaí, Galeria - Rodovia Sc 401, 5025B - Saco Grande, Florianópolis - SC, 88032-005, Brazil

*Correspondence Info:

Iandra Holzmann
Universidade do Vale do Itajaí,
Galeria - Rodovia Sc 401, 5025B - Saco Grande,
Florianópolis - SC, 88032-005, Brazil
E-mail: iandrah@hotmail.com

Abstract

Background: Previous studies carried out in our laboratories have shown that *Valeriana prionophylla* displays central effects in mice. The antidepressant effect of the *Valeriana prionophylla* was the most prominent effect. The goal of this study was to evaluate the mechanism of action of the antidepressant property of HEVp, and its effect when used chronically.

Methods: Experiments were performed using the forced swimming test and the open field test. Female Swiss mice (25-30 g, 3 months) were divided into groups (N=8-10): negative control (saline), antagonist systems evaluated, HEVp and antagonist/HEVp. The treatments with NAN-190 (0.5 mg/kg, i.p.), ketanserin (5 mg/kg, i.p.), prazosin (1 mg/kg, i.p.), yohimbine (1 mg/kg, i.p.), haloperidol (0.2 mg/kg, i.p.), pimozide (0.2 mg/kg, i.p.), SCH-23390 (0.05 mg/kg, i.p.), L-arginine (750 mg/kg, i.p.), bicuculline (1 mg/kg, i.p.) and phaclofen (1 mg/kg, i.p.) were administered 30 min before HEVp. The treatments with reserpine (2 mg/kg, i.p.) and PCPA (100 mg/kg, i.p.) were administered 4 hours and 4 days before the test, respectively. Sub-chronic treatment was administered for 15 days, and the test was performed on days 1, 7 and 15 of the treatment.

Results: The anti-immobility effect caused by HEVp was significantly attenuated by treatment of the reserpine, PCPA, NAN-190, ketanserin, prazosin, yohimbine, L-arginine, bicuculline and phaclofen, but was not affected by haloperidol, pimozide and SCH-23390.

Conclusion: The mechanisms involved in their effects indicate that HEVp produces antidepressant effects through pathways that involve interaction with L-arginine-nitric oxide, serotonergic and GABAergic systems, as well as interaction with the α-adrenoceptors.

Keywords: *Valeriana prianophylla*, antidepressant effect, forced swimming, mechanism of action.

1. Introduction

The World Health Organization global burden of disease study places depression disorders among the ten leading medical causes of disability worldwide, and second only to ischemic heart disease [1]. Depression is a highly prevalent psychiatric disorder with a life time risk close to 20%, and is associated with high levels of morbidity and mortality [2]. Depressed patients are at higher risk of serious physical health problems such as coronary artery disease and diabetes, and worsening prognosis of other medical conditions [3]. In addition, a series

of evidence has also suggested that changes occur in the perception and response to pain processes, which are more intense in depressed patients [4]. Besides causing peripheral and central neuroplastic changes by altering the physiology of important processes such as hunger, sleep, and pain, depression is a disease that is still poorly understood in social terms, and is often viewed with prejudice by many.

Although the underlying pathophysiological mechanisms of depression are not completely established, novel targets have been identified for the development of new pharmacological treatments [5]. Conventional treatment of depression involves the use of monoamine oxidase inhibitors, tricyclics antidepressants, selective serotonin reuptake inhibitors or selective noradrenaline reuptake inhibitors. These drugs provide complete remission in only 50% of the patients, and produce side effects that can lower patient adhesion to the treatment [1,5]. Alternative therapies are highly sought after by patients with depression, especially the use of herbal medicines [6]. Thus, developing safe and effective agents from traditional herbs may provide a good way to lessen the side effects, as well as to improve the efficacy of conventional treatment [7]. In this context, *Valeriana prionophylla* (Vp) may be a treatment option.

Previous studies have shown that Vp displays hypnotic, anxiolytic and antidepressant effects, validating, in part, its use in folk medicine [8]. The antidepressant effect of hydroethanolic extract of *Valeriana prionophylla* (HEVp) was similar to the reference drugs such as fluoxetine and imipramine. Antidepressant activity of plants from the Valeriana genus are already known in the literature, and many of them are used in folk medicine [9-11]. The biological underpinnings of depression and the precise mechanism of effectiveness of antidepressants have not yet been clarified. Current therapy based on depression monoamine theory, which attributes the cause of the disease to a deficit in transmission of neurotransmitters such as dopamine, norepinephrine and serotonin [12]. However, it is known that other neurotransmitter systems, such as glutamatergic and oxidonitrergic, are also involved in the genesis of depression [13,14]. That said, the search for new therapeutic agents that can serve as alternatives to the current agents is required, not only to minimize the adverse effects of the medicines in this class but also decrease the latency period for obtaining therapeutic effects. The results obtained earlier with HEVp [8] have prompted the continuation of studies with this plant, evaluating the mechanism of action by which the antidepressant effect occurs.

2. Materials and Methods

2.1 Collection and preparation of extracts

Vp was collected from cultivation in Tierra Blanca, Concepción Tutuapa, San Marcos (15°14.808'N, 91°55.430'W), Guatemala. Botanical samples were determined by Mario Veliz at the Herbarium BIGU, School of Biology, USAC, and a voucher sample was deposited (No. 49183). Dry extract, HEVp, was prepared by percolation in 50% ethanol, concentrated in a rotavapor and desiccated in a vacuum dryer with silica. The average yield of the extractable solids was 28.5%.

2.2 Animals

For the experiments, female Swiss mice were used (25 to 30g). The animals were obtained from pharmacology department of UNIVALI, and were kept in a controlled-temperature environment (22 ± 3°C), illuminated by daylight and supplemented with electric light from 7:00 a.m. to 7:00 p.m., with free access to food and water. The experiments were performed after approval of the protocol by the Institutional Ethics Committee of UNIVALI 3008/2013, and were carried out in accordance with the current guidelines for the care of laboratory animals, and the ethical guidelines for investigations in Brazil, COBEA (Brazilian College of Animal Experimentation). The number of animals and intensity of the noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.3 Drugs and treatments

The following drugs were used: Reserpine, 4-Chloro-L-phenylalanine (PCPA), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl] piperazine-hydrobromide (NAN-190), ketanserin tartarate, prazosin hydrochloride, yohimbine hydrochloride, (R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390), 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone, 4-[4-(4-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone, 4-[4-(p-hlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone (haloperidol), 1-[1-[4,4-bis (4-Fluorophenyl)butyl]-4-piperidinyl]-1,3-dihydro-2H-benzimidazol-2-one (pimozide), (S)-(+)-2-Amino-5-[(aminoiminomethyl) amino] pentanoic acid

monohydrochloride (L-Arginine) and imipramine (Sigma, USA). All compounds were dissolved in saline 0.9% at the time of testing. All drugs (except HEVp) were administered by the intraperitoneal (i.p.) route, at a constant volume of 10 mL/kg body weight.

3. Behavioural procedures

3.1 Forced swimming test (FST)

The FST was conducted using the method described by Porsolt *et al* [15] with minor modifications. Mice were individually forced to swim in open plexiglass cylinders (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1°C. The total time of immobility during a 6 min test was scored live. This test procedure was carried out according to the previously standardized and validated experiments performed in our and other laboratories. Animals exposed to a situation of forced swimming became passive and immobile after a period of vigorous activity (struggling) and produced just enough movements to keep their heads above the water. The absence of hind leg movement was recorded as immobility using a stopwatch, by a single observer during the exposures [16].

3.2 Open field test (OFT)

The open field test (OFT) is a model that evaluates motor coordination in mice [17]. The apparatus consists of a wooden box measuring 40 x 60 x 50 cm (length/width/height), the surface of which is divided into 12 quadrants. The main objective is rule out the possibility that the possible antidepressant-like effect that occurs is due to a change in locomotor activity caused by the substances tested. Animals were subjected to OFT one hour after administration of the HEPV; the number of quadrants crossed by animals was recorded over a 6-minute period.

3.3 Mechanism of action

Considering that HEVp (150 mg/kg, p.o.) was the most effective dose in reducing the immobility time in the FST in previous studies performed in our laboratories [8], this dose was chosen for all subsequent experiments to investigate the mechanisms involved in the antidepressant-like effect of HEVp. The doses of the drugs and protocols were selected on the basis of literature data for in vivo treatments [18-21].

3.4 Influence of the monoaminergic system on the antidepressant-like effect of HEVp

In order to investigate the possible contribution of monoaminergic system to the anti-immobility effect of HEVp in the FST, animals were pre-treated with reserpine (2 mg/kg, i.p., catecholamine depletor of the synaptic vesicles) and after 4 hours of treatment, the animals received HEVp (150 mg/kg, p.o.) and/or vehicle. One hour after treatment, mice were analyzed in the FST and OFT.

3.5 Influence of serotonergic system in the antidepressant-like effect of HEVp

To assess the possible involvement of the serotonergic system on the mechanism of action of antidepressant-like effect of HEVp, animals were pretreated for 4 consecutive days with PCPA (100 mg/kg, i.p., tryptophan hydroxylase inhibitor responsible for synthesis of 5-HT and depletor stocks 5-HT in the CNS). After 24 h the last injection of PCPA and/or vehicle, animals received the HEVp (150 mg/kg, p.o.) and were subjected to the FST and OFT. In order to investigate the possible involvement of serotonergic receptor subtypes (5-HT1A and 5-HT2A receptor) on the antidepressant-like effect of HEVp, the mice were pretreated with NAN-190 (0,5 mg/kg, i.p.) and ketanserin (5 mg/kg, i.p.), or vehicle, respectively. After 30 minutes of treatment with antagonists, the animals received the HEVp (150 mg/kg, p.o.) and after 60 min, were subjected to the FST and OFT.

3.6 Influence of the noradrenergic system on the antidepressant-like effect of HEVp

To investigate the possible involvement of the noradrenergic system in the antidepressant-like effect of HEVp, the animals were pretreated with prazosin (1 mg/kg, i.p., α1 antagonist), yohimbine (1 mg/kg, i.p., α2 antagonist), 30 min before receiving the HEVp and/or vehicle. After 60 min, the animals were analyzed in the FST and OFT.

3.7 Influence of the dopaminergic system on the antidepressant-like effect of HEVp

To test the possible involvement of the dopaminergic system in the antidepressant-like effect of HEVp, the animals were pretreated with haloperidol (0.2 mg/kg, i.p., non- selective dopamine receptor antagonist), pimozide (0.2 mg/kg, i.p., a dopamine D_2 receptor antagonist), SCH23390 (0.05 mg/kg, i.p., dopamine D1 receptor antagonist) and/or vehicle, 30 min before receiving the HEVp. After 60 min of treatment, the animals were evaluated in the FST and OFT.

3.8 Influence of the nitric oxide system on the antidepressant-like effect of HEVp

To evaluate the possible involvement of the nitric oxide system on the antidepressant-like effect of HEVp, L-arginine (750 mg/kg, i.p., precursor of nitric oxide) was administered 30 min before the animals received treatment with HEVp and/or vehicle. After 60 min, the animals were evaluated in the FST and OFT.

3.9 Influence of the GABAergic system on the antidepressant-like effect of HEVp

In order to check the influence of the GABAergic system on the mechanism of action of HEVp, animals were pretreated with GABAA (bicuculline, 1 mg/kg, i.p.) or GABAB (phaclofen, 1 mg/kg, i.p.) receptor antagonists and/or vehicle. After 30 minutes, the mice received HEVp (150 mg/kg, p.o.) and 60 minutes later, were evaluated in FST and OFT.

3.10 Antidepressant-like effect of sub-chronic treatment with HEVp

To investigate the antidepressant-like effect of chronic administration of HEVp, animals received for 15 consecutive days, daily treatments of vehicle (negative control), HEVp (150 mg/kg, p.o.) and imipramine (10 mg/kg, p.o., positive control). The experiments were conducted on the 1st, 7th and 15th days of treatment, when the mice were submitted to the FST and OFT

3.11 Statistical analysis

The results were analyzed using one- or two-way analysis of variance (ANOVA), depending on the experimental protocol, followed by post-hoc Tukey test where appropriate. Differences between groups were considered significant when $P<0.05$.

4. Results

4.1 Monoaminergic system

The results shown in Fig. 1A indicate that the pretreatment of mice with reserpine (2 mg/kg, i.p.) prevented the antidepressant-like effect elicited by HEVp (150 mg/kg, p.o.) in the FST. This effect was observed when the reserpine+HEPv group was compared with the antidepressant-like effect of HEPv shown in the vehicle+HEPv group. Two-way ANOVA revealed significant differences for reserpine [$F(1,36)=25.92$, $P<0.001$], HEPv [$F(1,36)=35.48$, $P<0.001$] and reserpine×HEPv interaction [$F(1,36)=4.53$, $P<0.01$]. Treatment with reserpine alone or in combination with HEVp (Fig. 1B) was not able to alter the locomotor activity of mice in the OFT ($P>0.05$).

4.2 Serotonergic system

The pre-treatment of animals with PCPA (100 mg/kg, i.p.), reverted the antidepressant-like effect of HEVp (150 mg/kg, p.o.) since it significantly increased the immobility of the animals, when compared to the group that received only HEVp (Fig. 1C). The two-way ANOVA revealed significant differences of PCPA [$F(1,30)=5.14$, $P<0.05$] and PCPA×HEPv interaction [$F(1,30)=5.41$, $P<0.05$]. The treatment of mice with PCPA, alone or in combination with HEVp (Fig. 1D), did not alter the locomotor activity in the OFT ($P>0.05$). It was also noted that the NAN-190 promoted the reversal of the antidepressant-like effect of HEVp (Fig. 2A), suggesting that this receptor may be involved in the antidepressant-like effect of HEVp. Two-way ANOVA revealed significant differences in NAN-190 [$F(1,36)=8.29$, $P<0.01$], HEPv [$F(1,36)=4.28$, $P<0.05$] and NAN-190×HEPv interaction [$F(1,36)=21.56$, $P<0.001$]. The treatment with NAN-190 alone or in combination with HEVp (Fig. 2B) was not able to alter the locomotor activity of mice in the OFT ($P>0.05$). The serotonergic (5HT2A) receptor subtype was also assessed using ketanserin as the antagonist, where it was noted that it promoted a reversal of the antidepressant-like effect of HEVp (Fig. 2C). The two-way ANOVA revealed significant differences for the ketanserin [$F(1,36)=43.73$, $P<0.001$], HEPv [$F(1,36)=50.05$, $P<0.001$] and ketanserin×HEPv interaction [$F(1,36)=7.49$, $P<0.01$]. Treatment with ketanserin alone and/or associated with HEVp (Fig. 2D) did not change the locomotor coordination of mice in the OFT ($P>0.05$).

4.3 Noradrenergic system

The results shown in Fig. 3A indicate that pretreatment of mice with prazosin (1 mg/kg, i.p.) prevented the antidepressant-like effect caused by HEPv (150 mg/kg, p.o.) in the FST. Two-way ANOVA revealed significant differences for prazosin [$F(1,36)=14.38$, $P<0.001$], HEPv [$F(1,36)=29.43$, $P<0.001$] and prazosin×HEPv interaction [$F(1,36)=8.08$, $P<0.01$]. Treatment of mice with prazosin, alone or in combination with HEVp (Fig. 3B), did not alter locomotor activity in OFT ($P>0.05$). The results illustrated in Fig. 3C show that administration of yohimbine (1 mg/kg, i.p.), reverted the antidepressant-like effect of HEVp (150 mg/kg, p.o.) in the FST. Two-way ANOVA revealed significant differences for yohimbine [$F(1,36)=17.73$, $P<0.001$], HEPv [$F(1,36)=18.16$, $P<0.001$] and

yohimbine×HEPv interaction [F(1,36)=8.40, P<0.01]. Treatment with yohimbine alone and/or in association with HEVp (Fig. 3D) did not change the locomotor coordination of mice in the OFT (P>0.05).

4.4 Dopaminergic system

Fig. 4A shows that pretreatment of animals with haloperidol (0.2 mg/kg, i.p.) did not revert the antidepressant-like effect of HEVp, since it did not interfere significantly in immobility. Two-way ANOVA did not show significant differences for haloperidol [F(1,36)=2.31, P=0.13] and for haloperidol×HEPv interaction [F(1,36)=2.69, P=0.10]. Treatment with haloperidol alone or in combination with HEVp (Fig. 4B) was not able to alter the locomotor activity of mice in the OFT (P>0.05). Treatment with pimozide also did not promote the antidepressant-like effect rollback so statistically significant of HEVp (Fig. 4C). The two-way ANOVA did not revealed significant differences for pimozide [F(1,36)=0.07, P=0.79] and for pimozide×HEPv interaction [F(1,36)=0.01, P=0.92]. Treatment with pimozide, alone and/or in association with HEVp (Fig. 4D), did not change the locomotor coordination of mice in the OFT (P>0.05). Selective receptors D1 were also evaluated. Fig. 5A shows that pretreatment of animals with SCH23390 (0.05 mg/kg, i.p.) did not alter the antidepressant-like effect of HEVp. Two-way ANOVA did not show any significant differences for SCH23390 [F(1,36)=0.34, P=0.56] and for SCH23390×HEPv interaction [F(1,36)=0.03, P=0.86]. Treatment with SCH23390, alone or in combination with HEVp (Fig. 5B), was not able to alter the locomotor activity of mice in the OFT (P>0.05).

4.5 Nitric oxide system

Fig.5C shows that pretreatment of animals with L-arginine (750 mg/kg, i.p.) reverted the antidepressant-like effect of HEVp, as demonstrated by the increased immobility time of the animals when compared to the group that received only HEVp. Two-way ANOVA revealed significant differences for L-arginine×HEPv interaction [F(1,36)=28.67, P<0.001]. Treatment with L-arginine, alone and/or in association with HEVp (Fig. 5D), did not change the locomotor coordination of mice in the OFT (P>0.05).

4.6 Evaluation of GABAergic system

Fig. 6A shows that pretreatment of mice with bicuculline (GABAA antagonist receptors, 1 mg/kg, i.p.) significantly reverted the antidepressant-like effect caused by HEPv. Two-way ANOVA revealed significant differences for bicuculline [F(1,36)=5.38, P<0.05], HEVp [F(1,36)=14.22, P<0.001] and bicuculline×HEPv interaction [F(1,36)=13.53, P<0.001]. Treatment with bicuculline alone, or in combination with HEVp (Fig. 6B), was not able to alter the locomotor activity of mice in the OFT (P>0.05). The administration of phaclofen (GABAB receptor antagonist, 1 mg/kg, i.p.) was also able to prevent the antidepressant-like effect caused by HEVp in the FST (Fig. 6C). Two-way ANOVA shows significant differences for phaclofen [F(1,36)=7.67, P<0.01], HEVp [F(1,36)=21.44, P<0.001] and phaclofen×HEPv interaction [F(1,36)=11.43, P<0.01]. Treatment with phaclofen, alone or in combination with HEVp (Fig. 6D), was not able to change the locomotor activity of mice in the OFT (P>0.05).

4.7 Antidepressant effect of sub-chronic treatment with HEVp

The sub-chronic effects of oral administration of HEVp (150 mg/kg. p.o.) on immobility time in the FST are shown in Fig. 7. HEVp decreased immobility time in the FST compared to the control group, when evaluated on the 1st, 7th and 15th days of treatment. One-way ANOVA revealed a significant effect of HEVp in the FST on the 1st day [F(3,31)=17.26, P<0.001], 7th day [F(3,31)=20.34, P<0.001] and 15th day [F(3,31)=18.82, P<0.001]. Additionally, imipramine (a tricyclic antidepressant, 10 mg/kg p.o.) was also effective in reducing the immobility time of mice in the FST. Treatment with HEVp and/or imipramine (data not showed) were not able to change the locomotor activity of mice in the OFT (P>0.05).

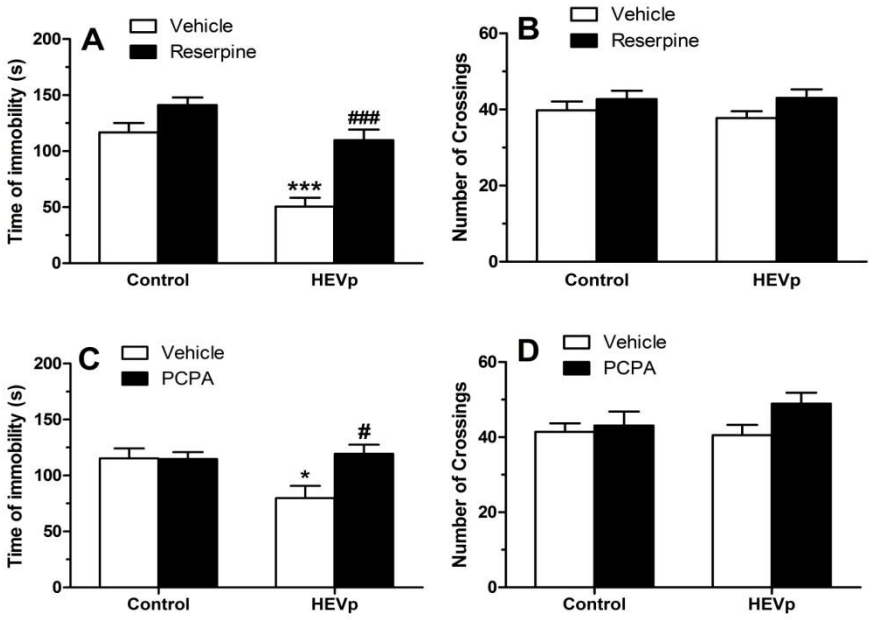

Fig1: Effect of pretreatment of mice with reserpine (2 mg/kg, i.p.) 4 hours prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 1A) and the number of crossings in OFT (figure 1B), and effect of pretreatment of mice with PCPA (100 mg/kg, i.p.) during 4 days consecutive prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 1C) and the number of crossings in the OFT (figure 1D). Values were expressed as mean + standard error (n=8-10). * P<0,05, *** P<0.001 compared with the control group treated with saline; # P<0.05, ### P<0.001 compared with the same group pretreated with saline (two-way ANOVA followed by Tukey's test).

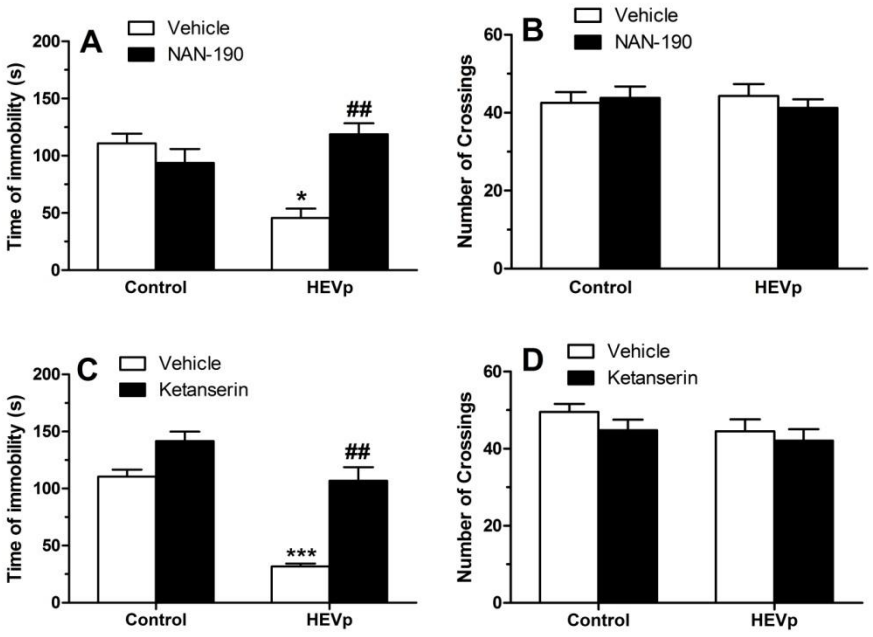

Fig 2: Effect of pretreatment of mice with NAN-190 (0.5 mg/kg, i.p.), 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 2A) and the number of crossings in OFT (figure 2B) and effect of pretreatment of mice with ketanserin (5 mg/kg, i.p.) 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 2C) and the number of crossings in the OFT (figure 2D). Values were expressed as mean + standard error (n=8-10). * P<0.05, *** P<0.001 compared with the control group treated with saline; ## P<0.01 compared with the same group pretreated with saline (two-way ANOVA followed by Tukey's test).

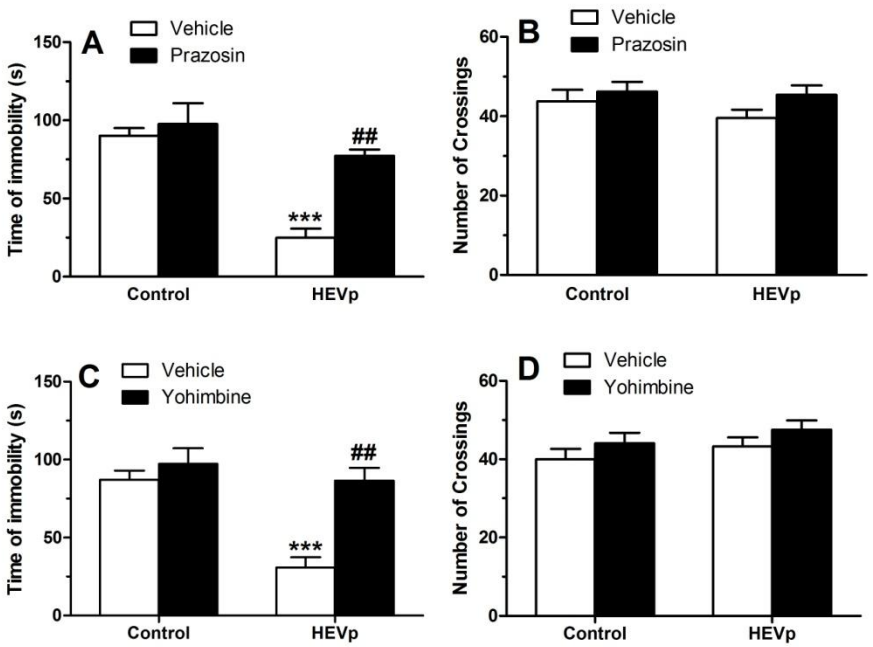

Fig 3: Effect of pretreatment of mice with prazosin (1 mg/kg, i.p.), 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 3A) and the number of crossings in OFT (figure 3B) and effect of pretreatment of mice with yohimbine (1 mg/kg, i.p.) 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 3C) and the number of crossings in the OFT (figure 3D). Values were expressed as mean + standard error (n=8-10). *** P<0.001 compared with the control group treated with saline; ## P<0.01 compared with the same group pretreated with saline (two-way ANOVA followed by Tukey's test).

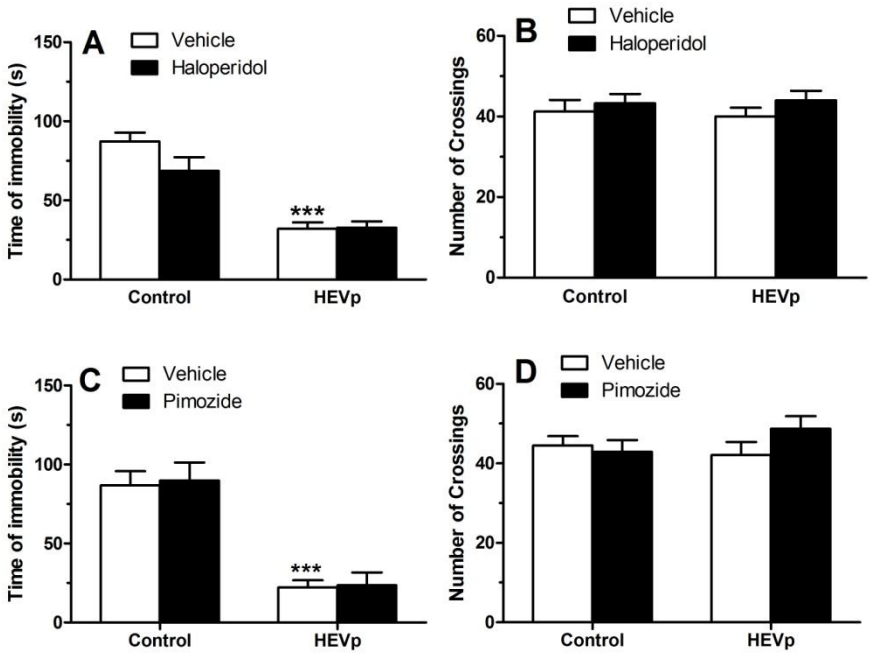

Fig 4: Effect of pretreatment of mice with haloperidol (0.2 mg/kg, i.p.), 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 4A) and the number of crossings in OFT (figure 4B) and effect of pretreatment of mice with pimozide (0.2 mg/kg, i.p.) 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 4C) and the number of crossings in the OFT (figure 4D). Values were expressed as mean + standard error (n=8-10). *** P<0.001 compared with the control group treated with saline (ANOVA two-way followed by Tukey's test).

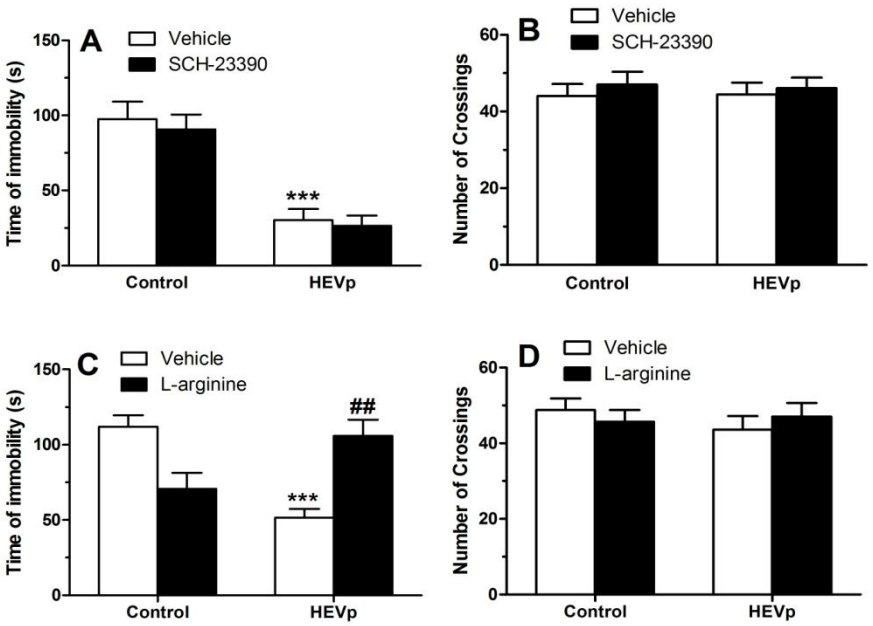

Fig 5: Effect of pretreatment of mice with SCH-23390 (0.05 mg/kg, i.p.), 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 5A) and the number of crossings in the OFT (figure 5B) and effect of pretreatment of mice with L-arginine (750 mg/kg, i.p.) 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in FST (figure 5C) and the number of crossings in the OFT (figure 5D). Values were expressed as mean + standard error (n=8-10). *** P<0.001 compared with the control group treated with saline; ## P<0.01 compared with the same group pretreated with saline (ANOVA two-way followed by Tukey's test).

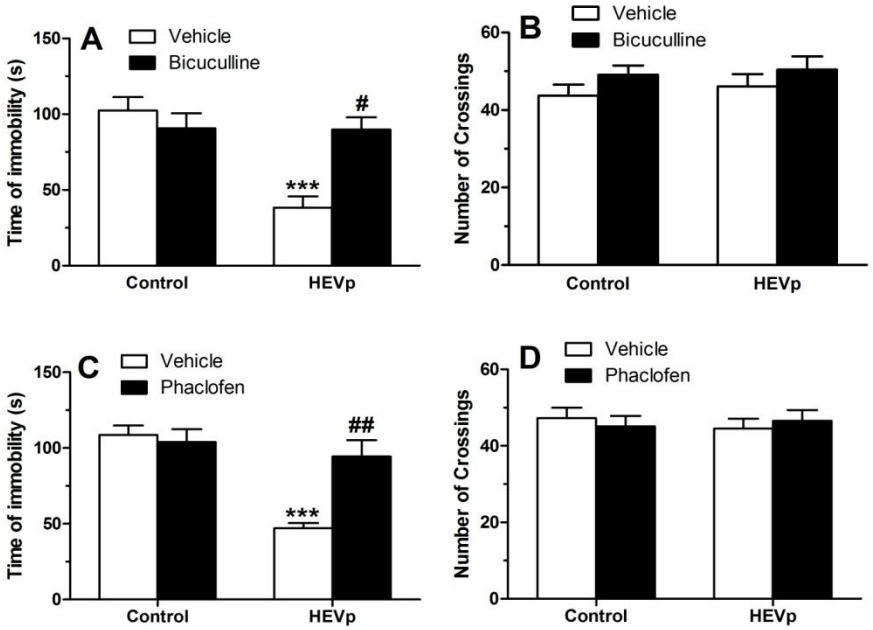

Fig 6: Effect of pretreatment of mice with bicuculline (1 mg/kg, i.p.), 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in the FST (figure 6A) and the number of crossings in OFT (figure 6B) and effect of pretreatment of mice with phaclopen (1 mg/kg, i.p.) 30 min prior to administration of HEVp (150 mg/kg, p.o.) on the anti-immobility effect in FST (figure 6C) and the number of crossings in the OFT (figure 6D). Values were expressed as mean + standard error (n=8-10). *** P<0.001 compared with the control group treated with saline; # P<0.05, ## P<0.01 compared with the same group pretreated with saline (two-way ANOVA followed by Tukey's test).

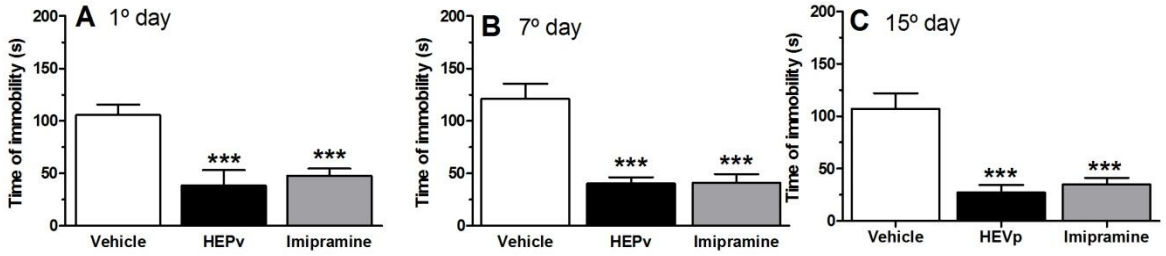

Fig 7: Effect of the sub-chronic treatment of mice with HEVp (150 mg/kg, p.o.), 1º day (figure 7A), 7º day (figure 7B) and 15º day (figure 7C) on the immobility time in the FST. Each column represents the mean+S.E.M. of 8–10 animals. ***P<0.001, compared with the vehicle-treated control.

5. Discussion

The increased use of medicinal plants in clinical practice reflects the increase in alternative therapies for depression. Phytochemicals from medicinal plants play a vital role in maintaining the brain's chemical balance, by influencing the function of the receptors for the major inhibitory neurotransmitters [22]. Recently, we reported that Vp displays central effects in mice, validating, in part, its popular use in Guatemala and Brazil for the treatment of psychological illnesses such as depression, insomnia and anxiety [8]. Among all the pharmacological properties tested, the antidepressant effect of the HEVp was the most prominent, showing superior results to antidepressants such as imipramine and fluoxetine, when administered to mice subjected to the FST. Therefore, in this study, we investigated the pharmacological mechanism of the antidepressant-like effect of HEVp in the FST in mice. This test is an experimental model for testing the efficacy of antidepressant drugs; the animals develop a learned helplessness syndrome characterized by a lowered motivation to escape, as evidenced by increased periods of immobility. Several authors have proposed that immobility during the test could be an efficient adaptive response to this stress [15,16]. The OFT was the model used to evaluate a possible interference in the locomotor system of mice. We found that a single administration of the HEVp was able to promote a significant antidepressant-like effect in the FST, without altering the locomotor activity of mice.

The monoaminergic system is one of the most important, or perhaps, the main target for the treatment of depression, in which the deficiency of biogenic amines, such as serotonin, norepinephrine and dopamine, is one of the factors responsible for the outbreak of the disease [23]. Drugs such as reserpine, used for a long time as a antihypertensive agent and currently only used experimentally, is able to deplete stocks of these neurotransmitters, by blocking vesicular monoamine [4] In the present study, reserpine reversed the antidepressant-like effect of HEVp, which suggests the involvement of monoamines in the mechanism of action.

The serotonergic system is more pronounced in relation to the antidepressant drugs [24]. In this study, the involvement of this system has been investigated primarily with the administration of PCPA serotonin synthesis inhibitor, and with selective serotonergic receptor antagonists, such as NAN-190 (a 5-$HT1_A$ selective receptor antagonist) and ketanserin (a 5-$HT2_A$ receptor antagonist). PCPA was previously reported to result in extensive (>90%) depletion of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the same strain of animals used in our current study [20]. However, there was no effect on noradrenaline or dopamine levels [25-27]. In our experiments the antidepressant-like effect of HEVp, characterized by a decrease in immobility of the animals, was reverted by pre-treatment with PCPA, indicating that an decrease in 5-HT levels in the synapse plays a critical role in the pharmacological effect of HEVp. These results confirm previously reported data in the literature that show that the PCPA reverses the effect of antidepressants selective serotonin reuptake inhibitors such as fluoxetine, and can also partially revert the effect of tricyclic antidepressants such as imipramine, which inhibits the reuptake of 5HT and noradrenaline [28].

Among the various subtypes of serotonergic receptors as $5HT1_A$ and $5HT2_A$ has received attention as a therapeutic of the depression clinical data record low levels of these receptors in pathogenesis [29]. In the present study, their involvement was evidenced by the reversal of the antidepressant-like effect of HEVp in FST by selective antagonists of receptors $5HT1_A$, $5HT2_A$, NAN-190 and ketanserine, respectively. Norepinephrine also deserves mention in the pathophysiology of depression, as clinically used drugs increase the synaptic concentration of NOR

and also can act on noradrenergic receptors [30]. Many antidepressants, such as doxepin, amitriptyline and nefazodone, have significant α_1-adrenoceptor affinity [31]. Moreover, α_1 and α_2-adrenoceptors have been shown to underlie some of the antidepressant-like responses of drugs in behavioral models of antidepressant activity [32]. Our experiments have shown that both the α1-adrenergic antagonists (prazosin) and α2-adrenergic antagonist (yohimbine) reverted the antidepressant-like effect of HEVp in the FST. Thus, the results obtained indicate that the HEVp may be involved with the noradrenergic system, and possibly exert their effect by interaction with the adrenergic receptors.

The dopaminergic system has also been investigated, since biochemical studies have shown that metabolites of dopamine are reduced in depressive patients [33] In addition; major depression is characterized by diverse debilitating symptoms that include hopelessness and anhedonia. Dopamine neurons involved in reward and motivation are among many neural populations that have been hypothesized to be relevant, and certain antidepressant treatments, including medications and brain stimulation therapies, can influence the complex dopamine system [34]. Studies have shown that the anti-immobility effect of imipramine was reverted using D2 antagonists, and agonists D1 increased the effects of SSRI antidepressants in the FST [20]. The results of our study show that the dopaminergic system is not involved in the antidepressant-like effect of Vp, since pre-treatment of animals with haloperidol (a nonselective dopaminergic receptor antagonist), SCH23390 (a dopamine D_1 receptor antagonist) and pimozide (a dopamine D_2 receptor antagonist) did not prevent the antidepressant-like effect caused by administration of HEVp in the FST.

We also investigated the involvement of nitric oxide in the antidepressant-like effect of HEVp. These studies found that the modulation of depression depends on NOS (nitric oxide synthetase) inhibition to exert antidepressant effects in pharmacological depression models [19,35]. In addition, other studies suggest that NOS inhibition can be used as a strategy to enhance the effectiveness of antidepressants that affect other systems, such as the serotonergic system [36]. In this study, we showed that pre-treatment of mice with L-arginine (NOS substrate) reversed the decrease in immobility time in the FST elicited by HEVp. These results indicate that the antidepressant-like effect of HEVp in the FST also may be related to the inhibition of nitric oxide synthesis in the CNS.

Although the etiology of depression is mainly related to monoaminergic system dysfunction, there is evidence between the dysfunction of the GABAergic system with the depressive disorders [37]. Previous studies have found a reduction in density of the GABAergic neurons in the prefrontal and cingulate cortex, in autopsies of patients with major depression and bipolar disorder, respectively [38]. In this study we investigated the involvement of the GABAergic system on the antidepressant-like effect of HEVp using GABAA and GABAB receptor antagonists. The pretreatment of bicuculline (GABAA antagonist) and phaclofen (GABAB receptor antagonist) were effective in abolishing the anti-immobility effect of HEVp in the FST, suggesting a possible involvement of this system in the antidepressant-like effect of HEVp. Corroborating with these results, several studies have demonstrated a link between the pharmacological effects of valerenic acid with the GABAergic system. In pre-clinical experiments, valerenic acid and/or valerian extracts showed a tranquilizing role and/or sedative activity [39,40] . Valerenic acid also was able to modulate, at high concentrations, the GABAA receptors, as shown for recombinant receptors expressed in *Xenopus oocytes* [41] (or neonatal brain stem neurons [42]. Benke *et al* [43], described the existence of a specific binding site on the GABAA receptors with affinity for valerenic acid and valerenol, common constituents of valerian species. Both agents enhanced the response to GABA in multiple types of recombinant GABAA receptors.

In this study, after detecting the possible mechanisms by which HEVp exerts its antidepressant effect, we sought to investigate whether the sub-chronic treatment of animals, dates back to the data on the clinical therapeutic effects of conventional antidepressants only occurs after at least 14 days of constant use [44]. In the treatment of depression, pharmacological therapy promotes long-term processes of neuronal plasticity and adaptability, and this explains the effect antidepressant of most therapeutic agents used, not only pharmacodynamic [45] Sub-chronic administration of HEVp for 15 days showed a significant reduction in the duration of the forecast total immobility, which corroborates the findings of Kitamura *et al* [46], showing that compounds with properties in mice administered antidepressant qualities for at least 15 days of course, reduced the length of time immobility time. Concerning the chemical constituents of HEVp used in our experiments, classical phytochemical methods were used to determine the main constituents. The gas chromatography technique showed metabolites of interest, such as isovaleric, 3-methylvaleric, isovalerate allyl valeric, and 3-methyl-2-oxo valerenic acids, glucuronide, and morphine

3-hydroxybromazepan [8]. Generally, two strands of research have attempted to indicate the chemical constituents responsible for the central effects of the valerian species. The first proposes that valerenic acids are responsible for the central effects, while the second proposes that flavonoids are responsible for these effects.

In relation to flavonoids, several species of valerian (including Vp) present these compounds in their composition. Wasowski *et al*, [47] through a "ligand searching approach" using purified extracts as far as possible, were able to report the presence of 6-methylapigenin in *Valeriana wallichii* DC and *V. officinalis.* They also reported the presence of 2S (−) hesperidin in *V. wallichii* and in *V. officinalis.* Fernandez *et al*. [48]reported the identification of the flavone glycoside linarin in *V. officinalis* and the discovery that it has, like hesperidin, sedative and sleep-enhancing properties that are potentiated by simultaneous administration of valerenic acid. 6-methylapigenin, another flavonoid was also identified in extracts of *V. officinalis.* The antidepressant effect of these compounds in animals [49] and in humans [50] (has also been reported in the literature.

Concluding, at present, considering that treatment of depression with conventional antidepressant produces various side effects that can reduce patient compliance to treatment, there is a need to develop strategies for antidepressant treatment with fewer side effects, but with better results in the management of depression. Current evidence for the efficacy of a variety of readily available herbal extracts and chemicals that may improve brain function has attracted research in this area. In this study, we deepened the research on the antidepressant-like effect of HEVp, and showed that this plant may have potential therapeutic value for the treatment of depressive disorders.

Conflict of interest

The Authors declare that there is no conflict of interest.

Acknowledgments

This study was supported by grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa do Estado de Santa Catarina (FAPESC). This work represents part of the Dissertation of Iandra Holzmann, for the Master of Science of the Postgraduate program in Pharmaceutical Sciences of the University of Vale do Itajaí– UNIVALI (Brazil). We are grateful to Facultad de Agronomía, Universidad de San Carlos, Guatemala for identifying the plant materials.

References

[1] Palazidou E. The neurobiology of depression. *Br Med Bull.* 2012;101:127-45.

[2] Kessler RC, Berglund P, Demler O. Lifetime prevalence and age of onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005; 62:593-602.

[3] Knol MJ, Twisk JW, Beekman AT. Depression as a risk factor for the onset of type 2 diabetes mellitus A meta analysis. *Diabetologia* 2006; 49:837-45.

[4] Lewandowski HA, Law EF, Zhou C, Murphy L, Clarke G, Palermo TM. Reciprocal longitudinal associations between pain and depressive symptoms in adolescent. *Eur J Pain* 2013; 17:1058-1067.

[5] Lee HR, Hwang IS, Kim JE, Choi SI, Lee YJ, Goo JS, Lee EP, Choi HW, Kim HS, Lee JH, Jung YJ, Hwang DY.Altered expression of γ-secretase components in animal model of major depressive disorder induced by reserpine administration. *Lab Anim Res* 2012; 2:109-114.

[6] Nahas R, Sheikh O. Complementary and alternative medicine for the treatment of major depressive disorder. *Can Fam Physician* 2011; 57:659-63.

[7] El-Alfy AT, Abourashed EA, Matsumoto RR. Nature against depression. Curr Med Chem 2012; 19:2229-41.

[8] Holzmann I, Filho VC, Mora TC, Cáceres A, Martínez JV, Cruz SM, Souza MM. Evaluation of Behavioral and Pharmacological Effects of Hydroalcoholic Extract of *Valeriana prionophylla* Standl. From Guatemala. *Evidence-Based Complementary and Alternative Medicine*. 2011;doi:10.1155/2011/312320.

[9] Müller LG, Salles LA, Stein AC, Betti AH, Sakamoto S, Cassel E, Vargas RF, Von Poser GL, Rates SM. Antidepressant-like effect of *Valeriana glechomifolia* Meyer (Valerianaceae) in mice. *Prog Neuropsychopharmacol Biol Psychiatry* 2012; 10:101-109.

[10] Liu XG, Gao PY, Wang GS, Song SJ, Li LZ, Li X, Yao XS, Zhang ZX. *In vivo* antidepressant activity of sesquiterpenes from the roots of *Valeriana fauriei* Briq. *Fitoterapia* 2012; 83:599-603.

[11] Sah SP, Mathela CS, Chopra K. Antidepressant effect of *Valeriana wallichii* patchouli alcohol chemotype in mice: Behavioural and biochemical evidence. *J Ethnopharmacol* 2011; 135:197-200.

[12] Mulinari S. Monoamine theories of depression: historical impact on biomedical research. *J Hist Neurosci* 2012; 21:366-92.

[13] Harkin AJ, Bruce KH, Craft B, Paul IA. Nitric oxide synthase inhibitors have antidepressant-like properties in mice. Acute treatments are active in the forced swim test. *Eur J Pharmacol* 1999; 372:207-213.

[14] Pedrazza EL, Rico EP, Senger MR, Pedrazza L, Zimmermann FF, Sarkis JJF, Bogo MR, Bonan CD. Ecto-nucleotidase pathway is alternad by different treatments with fluoxetine and nortriptyline. *Eur J Pharmacol* 2008; 583:18-25.

[15] Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn* 1977; 36:229-327.

[16] Tolardo R, Zetterman L, Bittencourtt DR, Mora TC, de Oliveira FL, Biavatti MW, Amoah SK, Bürger C, de Souza MM. Evaluation of behavioral and pharmacological effects of Hedyosmum brasiliense and isolated sesquiterpene lactones in rodents. *Journal of Ethnopharmacology* 2010; 128:63-70.

[17] Cunha MP, Machado DG, Capra JC, Jacinto J, Bettio LE, Rodrigues ALS. Antidepressant-like effect of creatine in mice involves dopaminergic activation. *J Psychopharmacol* 2012; 26:1489–501.

[18] Aragão GF, Carneiro LMV, Junior APF, Vieira LC, Bandeira PN, Lemos TLG, Viana BGS. A possible mechanism for anxioliytic and antidepressant effects of alpha and beta-amyrin from *Protium heptaphyllum* (Aubl) March. *Pharmacology Biochemical Behaviour* 2006; 85:827-834.

[19] Brocardo PS, Budni J, Kaster MP, Santos ARS, Rodrigues ALS. Folic acid administration produces an antidepressant-like effect in mice: evidence for the involvement of the serotonergic and noradrenergic systems. *Neuropharmacol* 2008; 54:464-473.

[20] Gonçalves AE, Burger C, Solomon KS, Tolardo R, Biavatti MW, De-Souza MM. (2012). Antidepressant-like effect of *Hedyosmum brasiliense* and its sesquiterpene lactone, podoandin in mice: Evidence for the involvement of adrenergic, dopaminergic and serotonergic systems. *Eur J Pharmacol* 2012; 674:307-314.

[21] Kulkarni SK, Dhir A. On the mechanism of antidepressant-like action of berberine chloride. *European Journal of Pharmacology* 2008; 589:163-172.

[22] Kumar GP, Khanum F. Neuroprotective potential of phytochemicals. *Pharmacogn Rev* 2012; 12:81-90.

[23] Bao AM, Ruhé HG, Gao SF, Swaab DF. Neurotransmitters and neuropeptides in depression. *Handb Clin Neurol* 2012; 106:107-36.

[24] Domínguez-López S, Howell R, Gobbi G. Characterization of serotonin neurotransmission in knockout mice: implications for major depression. *Rev Neurosci* 2012; 23:429-443.

[25] Koe BK, Weissman A. p-Chlorophenylalanine: a specific depletor of brain serotonin. *J Pharmacol Exp Ther* 1966;154:499-516.

[26] Can OD, Demir Özkay U, Uçel UI. Anti-depressant-like effect of vitexin in BALB/c mice and evidence for the involvement of monoaminergic mechanisms. *Eur J Pharmacol* 2012; 699:250-257.

[27] Rodrigues-Landa JF, Contreras CM. A review of clinical and experiment observations about antidepressant actions and side effects producet by *Hypericum perforatum* extracts. *Phytomedicine* 2003; 10:688-699.

[28] Eckeli AL, Dach F, Rodrigues ALS. Acute treatments with GMP produce antidepressant-like effect in mice. *Neuro Report* 2000; 11:839-843.

[29] Noro M, Antonijevic I, Forray C, Kasper S, Kocabas NA, Lecrubier Y, Linotte S, Mendlewicz J, Montgomery S, Snyder L, Souery D, Verbanck P, Zohar J, Massat I. 5HT1A and 5HT2A receptor genes in treatment response phenotypes in major depressive disorder. *Int Clin Psychopharmacol* 2010; 25:228-231.

[30] Elhwuegi AS. Central monoamines and their role in major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2004:28; 435-451.

[31] Souza LC, Gomes MG, Goes AT, Del Fabbro L, Filho CB, Boeira SP, Jesse CR. Evidence for the involvement of the serotonergic 5-HT(1A) receptors in the antidepressant-like effect caused by hesperidin in mice. *Prog Neuropsychopharmacol Biol Psychiatry* 2013; 40:103-109.

[32] Kaster I, Raupp RW, Binfaré R, Andreatini ALS. Antidepressant-like effect of lamotrigine in the mouse forced swimming test: evidence for the involvement of the noradrenergic system. *Eur J Pharmacol* 2007; 565:119-124.

[33] Sher L, Mann J, Traskman-Bendz L, Winchel R, Huang YY, Fertuck E, Stanley BH. Lower cerebrospinal fluid homovanillic acid levels in depressed suicide attempters. *Journal of Affective Disorders* 2006; 90:83-89.

[34] Tye KM, Mirzabekov JJ, Warden MR, Ferenczi EA, Tsai HC, Finkelstein J, Kim SY, Adhikari A, Thompson KR, Andalman AS, Gunaydin LA, Witten IB, Deisseroth K. Dopamine neurons modulate neural encoding and expression of depression-related behaviour. *Nature* 2013; 493:537-541.

[35] Wegener G, Volke V, Harvey BH, Rosenberg R. Local, but not systemic, administration of serotonergic antidepressants decreases hippocampal nitric oxide synthase activity. *Brain Research* 2003; 959:128-134.

[36] Harkin A, Connor TJ, Burns MP, Kelly JP. Nitric oxide synthase inhibitors augment the effects of serotonin reuptake inhibitors in the forced swimming test. *European Neuropsychopharmacology* 2004; 14:274-281.

[37] Sullivan G, Cooper TB, Xie S, Currier D. Anxiety in major depression and cerebrospinal fluid free gamma-aminobutyric acid. *Depress Anxiety* 2014; 31:814-821.

[38] Gos T, Günther K, Bielau H, Dobrowolny H, Mawrin C, Trübner K, Brisch R, Steiner J, Bernstein HG, Jankowski Z, Bogerts B. Suicide and depression in the quantitative analysis of glutamic acid decarboxylase-Immunoreactive neuropil 2009;113:45-55.

[39] De-Feo V, Faro C. Pharmacological effects of extracts from *Valeriana adscendens*: effects on GABA uptake and amino acids. *Phytotherapy Research* 2003; 17:661-664.

[40] Felgentreff F, Becker A, Meier B, Brattström A. Valerian extract characterized by high valerenic acid and low acetoxy valerenic acid contents demonstrates anxiolytic activity. *Phytomedicine* 2012; 19:1216-1222.

[41] Khom S, Strommer B, Ramharter J. Valerenic acid derivatives as novel subunit-selective GABAA receptor ligands- in vitro and in vivo characterization. *British Journal of Pharmacology* 2010; 161:65-78.

[42] Jiang X, Zhang JC, Liu YW, Fang Y. Studies on chemical constituents of *Valeriana officinalis*. *Zhong Yao Cai* 2007; 30:391-393.

[43] Benke D, Barberis A, Kopp S. GABAA receptors as in vivo substrate for the anxiolytic action of valerenic acid, a major constituent of valerian root extracts. *Neuropharmacology* 2009; 56:174-181.

[44] Miguelez C, Berrocoso E, Mico JA, Ugedo L. L-DOPA modifies the antidepressant-like effects of reboxetine and fluoxetine in rats. *Neuropharmacology* 2013; 67:349-358.

[45] Pittenger C, Duman RS. Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* 2008; 33:88-109.

[46] Kitamura Y, Araki H, Gomita Y. Influence of ACTH on the effects of imipramine, desipramine and lithium on duration of immobility of rats in the forced swim test. *Pharmacology Biochemical Behaviour* 2002; 71:63-69.

[47] Wasowski C, Marder M, Viola H, Medina JH, Paladini AC. Isolation and identification of 6-methylapigenin, a competitive ligand for the brain GABAA receptors, from *Valeriana wallichii. Planta Medica* 2002; 68:934-936.

[48] Fernandez S, Wasowski C, Paladini AC, Marder M. Sedative and sleep-enhancing properties of linarin, a flavonoid-isolated from *Valeriana officinalis*. *Pharmacology Biochemistry and Behavior* 2004; 77:399-404.

[49] Donato F, de Gomes MG, Goes AT, Filho CB, Del Fabbro L, Antunes MS, Souza LC, Boeira SP, and Jesse CR. Hesperidin exerts antidepressant-like effects in acute and chronic treatments in mice: possible role of L-arginine-NO-cGMP pathway and BDNF levels. *Brain Res Bull* 2014; 104:19-26.

[50] Dias GP, Cavegn N, Nix A, Nascimento BMC, Stangl D, Zainuddin MS, Nardi AE, Gardino PF, Thuret S. The role of dietary polyphenols on adult hippocampal neurogenesis: molecular mechanisms and behavioural effects on depression and anxiety. *Oxid Med Cell Longev* 2012;doi:10.1155/2012/541971.