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Contribución original

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La administración sub-crónica de hierba de San Juan revierte las conductas de ansiedad y depresión inducidas por dos protocolos distintos de estrés crónico.

Sub-chronic administration of St. John's wort reverses anxiety- and depressive-like behaviors induced by two different protocols of chronic stress.

Keywords

herbal medicine; stress; anxiety; depression; benzodiazepines.

Abstract

Introduction. St. John's wort (Hypericum perforatum, HP) is one of the most used herbal medicines. Here we investigated the putative antidepressive- and anxiolytic-like effects of HP, by using a well-validated rat model of anxiety and depression based on chronic stress.

Methods. To this purpose, subjects were either immobilized (INM) or randomly exposed to different stressors (chronic unpredictable stress: CUS) during 30 days. Forty-eight hours after the last stress session, subjects of each stress condition were randomly assigned to the HP (100 mg/kg), diazepam (5 mg/kg) or saline groups. Immediately after a baseline measure and during 10 consecutive days, drugs were intragastrically administrated. During this period, four open-field and elevated plus-maze tests were carried out every other day.

Results. CUS and INM stress were found to induce an anxiety- and depressive-like phenotype in both tests, whereas HP and diazepam progressively restored this stress-dependent phenotype. HP potency was almost equivalent to that of the diazepam. However, diazepam peaked slightly sooner and remained unaltered throughout the testing days, whereas HP peaked gradually and required more administrations to reach diazepam levels.

Conclusion. HP seems to be a promising alternative treatment for anxiety and mood disorders that may have wider safe-dosing ranges and fewer side-effects than benzodiazepines.

Resumen

Introducción. La hierba de San Juan (Hypericum perforatum, HP) es una de las plantas medicinales más utilizadas. Por ello, se ha investigado su efecto antidepresivo y ansiolítico a través de un modelo ampliamente validado de depresión y ansiedad en ratas basado en el estrés crónico.

Métodos. Con ese fin, los sujetos fueron inmovilizados (INM), o bien, expuestos de forma aleatoria a diferentes estresores (estrés crónico impredecible: CUS) durante 30 días. Cuarenta y ocho horas después de la última sesión de estrés, los sujetos de cada condición fueron aleatoriamente asignados a los grupos de HP (100 mg/kg), diazepam (5 mg/kg) o salina. Las drogas fueron administradas de forma intragástrica inmediatamente después de una medición de línea base y durante 10 días consecutivos. Durante este periodo, se ejecutaron cuatro sesiones de evaluación de la prueba del campo abierto y del laberinto elevado en cruz con 24 hrs. de separación entre cada sesión.

Resultados. Se encontró que el INM y CUS indujo un fenotipo depresivo y ansioso en ambas pruebas, en donde el HP y el diazepam redujeron este fenotipo inducido por el estrés. La potencia del HP fue casi equivalente a la del diazepam. Sin embargo, los efectos del diazepam se observaron más temprano y se mantuvieron sin cambios a lo largo de las evaluaciones, mientras que los efectos del HP aparecieron progresivamente y requirieron de más administraciones para igualar los niveles del diazepam.

Conclusiones. El HP muestra ser una alternativa promisoria para el tratamiento de la ansiedad y los desórdenes del estado de ánimo, ya que posee rangos más amplios de dosis seguras y menores efectos secundarios en comparación con las benzodiacepinas.

Palabras clave

hierbas medicinales; estrés; ansiedad; depresión; benzodiacepinas.

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Introduction

Anxiety disorders (AD) have a global prevalence of 7.3%[1], and constitute the most common mental health illness in the U.S.[2] Although several therapeutic approaches have proved effectiveness to treat AD, an important percentage of patients will not respond to traditional pharmacological therapies.[3] Since the neural basis of anxiety and depression are linked to different neurotransmitter systems, the development of experimental drugs with different brain targets is highly encouraged.[4] In the field of drug development research, is also desirable to find affordable, clinically effective molecules with minor side effects. To accomplish such endeavor, the investigation of herbal extracts have become a promising avenue for alternative treatments.[4] One of the most used herbal medicines is the St. John's wort (Hypericum perforatum, HP). HP is a multi-target extract that is thought to exert antidepressant- and anxiolytic-like actions by blocking the mono-amine oxidase and stimulating the type-A gama-aminobutyric acid (GABA) receptor, respectively.[5] Even though some evidence about the mechanism of action of HP has recently accumulated, more preclinical research is needed to clearly address the behavioral effects of this extract in well-validated animal models of anxiety and depression.

With the aim to explore the anti-depressive and anxiolytic effects of HP, we used two rat models of chronic predictable[6-7] (restrain by immobilization, INM) and unpredictable stress (CUS)[8] in order to induce anxiety- and depressive-like behaviors. Then, we treated the chronic stressed animals with HP or the gold-standard anxiolytic, diazepam (DZ), and tested them in the open-field test (OF) and the elevated plus maze (EPM). Rodents display complex behavioral patterns when confronted to uncertain threats as the OF and EPM. This repertoire is referred to as risk-assessment and plays a major role in defensiveness.[9] Increases in risk-assessment responses are considered to be as anxiety-like behavior.[9] Accordingly, stress normally potentiated such behaviors, whereas anxiolytic drugs reduce them.[10] In contrast, when stress is maintained over long periods of time,[11] the motivation to engage in exploratory activities drastically decreases. Such an effect is defined as a sort of depressive-like behavior.

It has been found that CUS-induced behavioral deficits in the EPM can be restored by chronic administrations of some antidepressants.[13] Interestingly, DZ reverses not only anxiety- but also depressive-like behaviors induced by CUS.[14] This effect can be attributed, in part, to the ability of DZ to modulate the monoaminergic transmission,[15] in addition to its recognized activity as agonist of GABAA receptors. In our study, therefore, DZ was used as a positive control to compare the effects of HP. Since INM7, [16] and CUS[8, 13] stress are well known to alter OF and EPM behavioral parameters, we expected that HP reverses stress-induced anxiety- and depressive-like phenotypes with similar potency as compared to DZ.

Materials and methods

Stress protocols. Forty two male Sprague-Dawley rats (200–220 g) were randomly assigned to three groups: non-stress (NS, n=6), CUS (n=18), and INM (n=18). CUS animals were daily exposed to one of the following stressors in an unpredictable, randomized order: cold chamber (4° C during 1 h), food deprivation (during 24 h), wet bedding (350 mL of water during 18 h), tilt cage (45° during 18 h), and inverted light cycle (during 24 h). INM animals were pressed down by means of a custom-designed press plate during 1h every other day (16 sessions). Rats remained in these conditions for 30 days and were kept undisturbed thereafter. All animals were group housed (3 per cage, 37.5 x 22 x 18 cm) and bedding was changed three times per week, with food and water provided ad libitum, except otherwise specified. Animals were maintained under a 12h light/dark cycle (lights on at 7 h) with a room temperature of 22–25°C and a relative

humidity of 40–60%. All experimental procedures were done in accordance with the guidelines of the Costa Rican Ministry of Science and Technology for the Care and Use of Laboratory Animals and were approved by the Institutional Committee for Animal Care and Use of the University of Costa Rica.

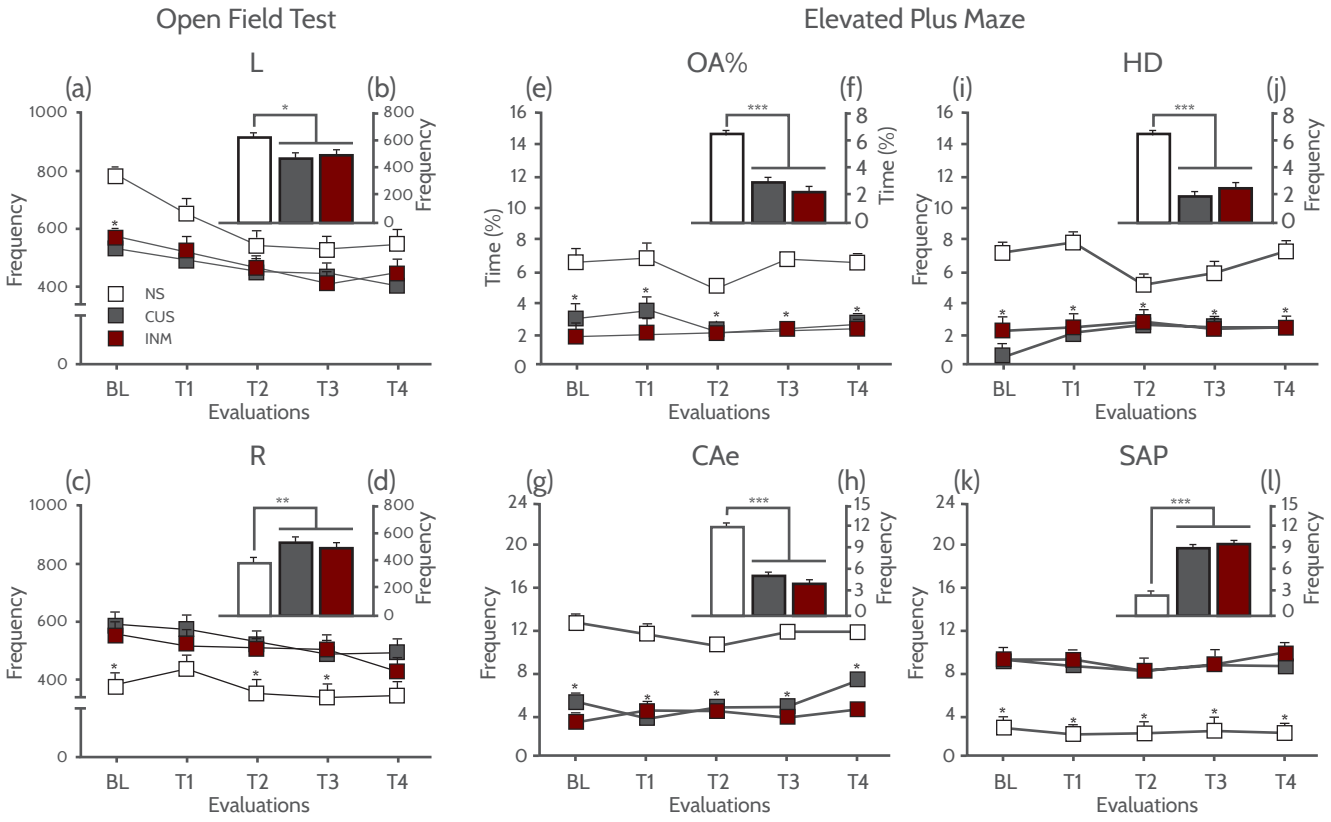
Experimental design, drug treatments, and data analysis. Forty eight hours after the last stress session, six animals of each stress condition were randomly assigned to one of the following treatments: HP (100 mg/kg, Felicicaps, Total Natural®, San José, Costa Rica), DZ (5 mg/kg, Raven®, San José, Costa Rica) or saline (SS, 0.9%, 3.5 ml). OF behaviors were automatically measured during 10 min with an actophotometer (41.5 x 41.5 x 23.6 cm, Columbia Instruments, Ilinois, USA). Interruptions of low and high 16-photobeam frames allowed the detection of horizontal (locomotion) and vertical (rearing behavior) activity, respectively. The activity in the EPM was manually scored from videotapes during 5 min (OA: 50 x 10 x 1 cm; CA: 50 x 10 x 40 cm; 63 cm from the floor), and the following parameters were measured: permanence on the OA (OA%: percentage of time spent on the OA relative to the total time), entries to the CA (CAe: frequency of four-paws arm entries) head-dipping (HD: frequency of head-dips over the edges of the CA), and stretch-attend posture (SAP: frequency of body elongations, and head and forepaws extensions outside the CA). As a baseline (BL), all animals were tested -prior to drug administration- in an OF and several hours later in an EPM. Immediately after BL and during 15 consecutive days, drugs were intragastrically administrated. During this period, four OF and EPM tests (T) were carried out every 48 h.

To identify the effect of stress protocols, non-drug treated groups (i.e. NS, CUS and INM) were compared to each other and over the testing days with a mixed ANOVA model. Likewise, DZ, HP, and SS rats were also compared using a mixed ANOVA test to identify the effect of treatments on both stress protocols. Pairwise between-groups comparisons were adjusted with the Bonferroni correction. Sphericity was corrected with the Greenhouse-Geisser method when appropriate. OF and EPM parameters were correlated (Pearson), both within and between tests, using the overall average of the four days evaluated. Data were expressed as means +SEM. Significance was defined as $p<.05$.

Results

Stress effects. In the OF, locomotion ($F_{(4,60)}=12.16$, $p<.001$, $\eta^2=.45$), but not rearing ($F_{(4,60)}=2.38$, $p=.06$, $\eta^2=.14$), decreased progressively over testing days in all groups. However, both CUS and INM rats showed less locomotor activity ($F_{(2,15)}=15.49$, $p<.001$, $\eta^2=.67$) and more rearing behavior ($F_{(2,15)}=6.67$, $p<.01$, $\eta^2=.47$) than NS counterparts throughout all testing points ($p<.05$). It is worth noting that both stress protocols seemed to be equally effective in inducing chronic stress, and therefore, no differences between them on any OF behavior were observed (Figure 1.a–d). In the EPM, no differences over days were detected. When comparing groups, we found that CUS and INM rats showed significantly less OA% ($F_{(2,15)}=44.43$, $p<.001$, $\eta^2=.47$), CAe ($F_{(2,15)}=101.58$, $p<.001$, $\eta^2=.93$), and HD ($F_{(2,15)}=93.35$, $p<.001$, $\eta^2=.93$) levels as compared to NS conspecifics (all p-values <.001). The Pearson correlation analysis computed with all stressed rats revealed a positive association among locomotion, CAe, and HD parameters (L*HD: $r=.61$, $p<.01$; L*CAe: $r=.61$, $p<.01$; CAe*HD: $r=.87$, $p<.01$), indicating that the reduction in exploratory activity was similar between both tests. In contrast, SAP ($F_{(2,15)}=54t.76$, $p<.001$, $\eta^2=.88$) appeared significantly augmented in both stressed groups in relation to the NS group (all p-values: <.001) (Figure 1.b–c). Again, stress groups did not differ from each other on any EPM parameter (Figure 1.c). The correlation analysis indicated that rearing and SAP parameters were positively correlated ($r=.73$, $p<.001$), whereas exploratory and risk-assessment behaviors were inversely associated, both within and between tests (L*SAP: $r=-.62$, $p<.001$; OA%*R: $r=-.71$, $p<.001$; OA%*SAP: $r=-.88$, $p<.001$).

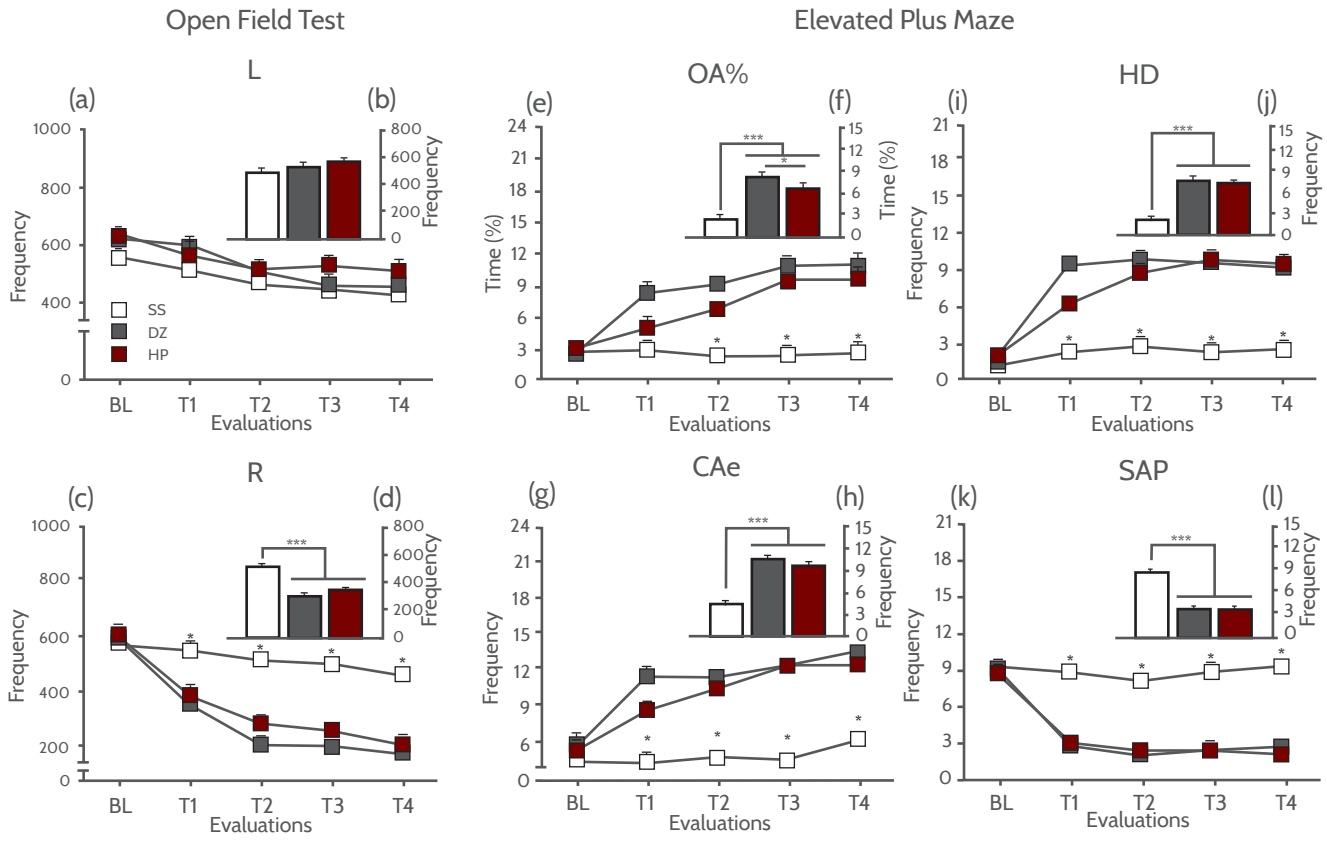
Figure 1. Effects of chronic stress on open field (a–d) and elevated plus maze (e–l) tests. Notes: Groups – Unpredictable stress (CUS, n=18), immobilization stress (INM n=18), and non-stress (SS, n=6). Between-groups significance: *p<.05; **p<.01; ***p<.001. Abbreviations: L: locomotion; R: rearing; OA%: open-arm time; CAe: closed-arm entries; HD: head-dipping; SAP: stretch-attend posture; BL: baseline.



Drug effects. Since stressed rats from both CUS and IM groups were similarly affected by the drug treatments, such groups were pooled for further analyses. In the OF, HP and DZ treatments equal and consistently restored stress-induced reductions in locomotor activity on all testing days, so that groups no longer differed among each other (p>.05). As compared to both, BL levels and SS counterparts, HP and DZ treatments significant and gradually reduced rearing behavior ($F_{(8,132)}$=64.12, p<.001, η2=.66; $F_{(2,33)}$=59.27, p<.001, η^2=.78). This reduction appeared as soon as in test 1, and continued to decrease on test 2. On the following testing points no further reductions were observed, however, DZ rats reached the lowest rearing levels slightly sooner than HP conspecifics (Figure 2.a–d). In the EPM, HP and DZ treatments significant and progressively restored stress-induced alterations on OA% ($F_{(2,33)}$=61.67, p<.001, η^2=.79; $F_{(6.45,106.51)}$=6.20, p<.001, η^2=.27), CAe ($F_{(2,33)}$=64.12, p<.001, η^2=.66; $F_{(8,132)}$=6.37, p<.001, η^2=.28), HD ($F_{(2,33)}$=103.09, p<.001, η^2=.86; $F_{(6.14,101.34)}$=8.64, p<.001, η^2=.34), and SAP ($F_{(2,33)}$=88.32, p<.001, η^2=.84; F(6.4,105.60)=7.05, p<.001, η^2=.30) as compared to SS conspecifics (all p-values <.001). Except for SAP, where both drugs exhibited the same trend, the effects of DZ peaked sooner (Test 1) and remained unaffected thereafter, whereas the action of HP peaked gradually and needed more administrations to reach the DZ levels (Figure 2.e–l). In addition, the correlation analysis including only drug-treated groups showed that exploratory and locomotor parameters increased within the EPM (OA%*CAe: r=.80, p<.001), and between the EPM and the OF tests (OA%*L: r=.46, p<.01). Also, risk-assessment

behaviors reduced to a similar extent in both tests (R*SAP: *r*=.80, p<.001). Consistently, exploratory activity and risk-assessment behaviors followed opposite directions, as indicated by the negative correlations observed between OA% and rearing behavior (*r*=-.78, p<.001), and SAP and OA% parameters (*r*=-.76, p<.001).

Figure 2. Effects of Hypericum perforatum (HP), diazepam (DZ), and saline (SS) on open field (a–d) and elevated plus maze (e–l) tests. Notes: All subjects were exposed to chronic stress; see text for details. Groups – 12 rats each. Between-groups significance: *p<.05; **p<.01; ***p<.001. Abbreviations: L: locomotion; R: rearing; OA%: open-arm time; CAe: closed-arm entries; HD: head-dipping; SAP: stretch-attend posture; BL: baseline.



Discussion

The research on herbal extracts[4] has become a favorable option for developing alternative medical treatments, which can be both clinically effective and affordable. The present study aimed to extent the evidence about the putative antidepressive- and anxiolytic-like effects of the herbal medicine HP, by using well-validated animal models of chronic stress in rats.

In our experiment, subjects were either immobilized (predictable stress, INM) or randomly exposed to different stressors (unpredictable stress, CUS) during 30 days. We found that INM and CUS stress inhibited exploratory behavior in the OF (locomotion) and EPM (CAe and HD) to a similar extent, as indicated by the positive associations found among behaviors of these tests. This effect

on exploratory behaviors has been traditionally interpreted as a depressive-like phenotype induced by chronic stress.[8,10,11]
Anxiogenic stimuli (e.g., wide, bright, and open environments) use to trigger risk-assessment behaviors, such as vertical exploration (rearing) and horizontal elongation of the body (SAP), which are displayed for monitoring areas where unspecific threats are likely to appear.[9] In this regard, CUS and INM stress were found to increase risk-assessment behaviors in the OF and EPM, with rearing and SAP parameters being closely associated between them. In addition, anxiety- and depressive-like behaviors were consistently affected by chronic stress, with rats showing less exploratory activity also displaying more risk-assessment responses, in agreement with previous reports.[7,8,13,16]

Based on previous evidence[12,16] we initially anticipated that the severity and predictability of INM would have potentiated depressive-like behaviors (i.e. inhibition of locomotor activity), whereas the mildness and unpredictability of CUS would have rather increased anxiety-like behaviors (i.e. increasing rearing and SAP).[6,13] However, EPM and OF parameters appeared to be equally sensitive to chronic stress. CUS and INM groups, whose effects were almost identical, already differed from the control group at the first test and remained unaltered thereafter. Thus, it is very likely that an early ceiling effect prevented stress-induced behavioral changes to be further detected. Also, as CUS stress was daily administered, it could become predictable irrespective of the randomized order of the stressors, and therefore, CUS and INM effects eventually became alike.

Once established that both stress protocols increased anxiety and depressive-like behaviors, we then evaluated whether sub-chronic, oral administration of HP was able to reverse such behavioral effects. As a positive control, the anxiolytic drug DZ was used. We found that HP administration progressively restored stress-induced depressive- and anxiety-like behaviors, similarly as DZ did. However, DZ showed a slightly higher potency as indicated by the left-shifted curves obtained for almost all behavioral parameters. Interestingly, both drug treatments restored the effects of chronic stress irrespective of the protocols used. Low doses of DZ have been found to restore depressive- and anxiety-like behaviors in a mouse model of severe stress.[17] However, a previous study using chronic INM (2 h for days) reported no effects of HP (350 mg/kg) on anxiety and depressive-like behaviors[18], even though a dose 3.5-fold higher than the one we used, was administered. One factor that can account for these contradictory results is that stressed animals did not differ from controls, and therefore, there were no behavioral deficits to be further reversed by HP. Since only a trend on EMP and no effects on OF were found there,[18] longer periods of administration, instead of higher doses, seemed to be required. In this regard, extremely high doses of HP (from 9.3- to 27.8-fold higher than the one we used) administered to non-stressed rats led again to inconsistent results.[19] There, HP increased overall OF activity, but reduced anxiogenic responses in the dark-light box test.19 In contrast, a low dose of HP (the same one we used) but given to stressed subjects (INM), was enough to reverse stress-induced hypolocomotion and anxiety-like behaviors in the OF and the mirror-chamber test, respectively.[20] The fact that this effect was observed even though mice were only acutely stressed (6 h), highlights the importance of employing stress pretreatments and low HP doses to achieve more valid results.

In human studies HP has been found to be as effective as classical anxiolytics and antidepressants,[21-23] but without the typical side effects of these drugs (e.g. memory problem, drowsiness, dizziness, irritability).[24] Most of the preclinical studies here discussed lacked of positive controls (e.g. groups treated with DZ) to estimate the relative potency of HP.[18-20] Contrary to our current investigation, many studies do not include well-validated animal models of depression and anxiety in which construct and predictive validity of experimental treatments can be properly judged.[18- 20]

Conclusion

Based on our preclinical findings, HP seems to be a promising alternative treatment for anxiety and mood disorders that may have wider safe-dosing ranges, without the side effects associated with benzodiazepines. The results here presented, however, must be taken cautiously, as more studies including different doses and biobehavioral parameters should be carried out before translational interpretations could be made. Considering the clinical implications of developing new alternative treatments for anxiety and affective disorders, further research on this topic is warranted.

Conflict of interest
There is no relevant conflict of interest to declare for the purposes of this work.

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