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RESEARCH ARTICLE

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Double-blind placebo controlled trial of the anxiolytic effects of a standardized Echinacea extract

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Earlier studies suggested that specific Echinacea preparations might decrease anxiety. To further study the issue, we performed a double blind, placebo controlled trial with a standardized Echinacea angustifolia root extract. Participants were volunteers scoring above 45 points on the state or on the trait subscale of the State Trait Anxiety Inventory (STAI). They were treated with 40 mg Echinacea or with placebo tablets twice daily for 7 days followed by a 3 week-long washout period. Participants were also administered the Beck Depression Inventory (BDI) and the Perceived Stress Scale (PSS). In the Echinacea group, state anxiety scores decreased by approximately 11 points by the end of the treatment period, whereas the decrease was around 3-points in the placebo group ($p < 0.01$). The effect maintained over the washout period. The difference from placebo was significant from the 7th day of treatment throughout. Changes were less robust with trait anxiety scores, but the preparation performed better than placebo in patients with high baseline anxiety. Neither BDI nor PSS scores were affected by the treatments. Adverse effects were rare and mild, and all were observed in the placebo group. These findings suggest that particular Echinacea preparations have significant beneficial effects on anxiety in humans.

KEYWORDS
anxiety, Echinacea, clinical trial, STAI

1 | INTRODUCTION

Echinacea preparations present a series of potential health benefits, including anti-inflammatory, antibiotic and anxiolytic activities (Manayi, Vazirian, & Saeidnia, 2015; Parsons, Cameron, Harris, & Smith, 2018), and were considered promising, albeit not sufficiently studied new targets for the herbal treatment of anxiety (Sarris, McIntyre, & Camfield, 2013; Sarris, 2018).

Anxiolytic activities of Echinacea preparations were evidenced in both laboratory and clinical studies. The uniqely standardized Echinacea angustifolia root extract, specifically the one studied here (Anxiofit-1), significantly decreased anxiety in four preclinical tests e.g. the elevated plus-maze, social interaction, conditioned fear and social avoidance tests (Haller, Hohmann, & Freund, 2010, 2013). In addition, the same preparation rapidly decreased anxiety in human subjects, an effect that was maintained for at least 7 days after stopping the one week-long treatment (Haller et al., 2013). The anxiolytic effects of the preparation followed a bell shaped dose-response curve in rats, and was present at doses considerably lower than those that stimulate immune responses, a more common indication of the herb (Rehman et al., 1999; Zhai et al., 2007).

Anxiolytic effects were attributed to lipophilic constituents of Echinacea preparations called alkamides, which occur in relatively large amounts especially in the roots of Echinacea purpurea and angustifolia (Bauer & Remiger, 1989). These bind to the cannabinoid CB1 receptor, and inhibit the enzyme fatty acid amide hydrolase (FAAH), which degrades the endocannabinoid anandamide, and the inhibition of which increases anandamide levels in the brain (Woelkart et al., 2005), both of which were implicated in anxiety and are targets of anxiolytic drug development (Haller, Bakos, Szirmay, Ledent, & Freund, 2002; Pertwee, 2012; Piomelli, Tarzia, Duranti, et al., 2006). Receptor binding and effects on FAAH may be due to the structural similarity between Echinacea alkamides and the endocannabinoid anandamide (Fig. 1).

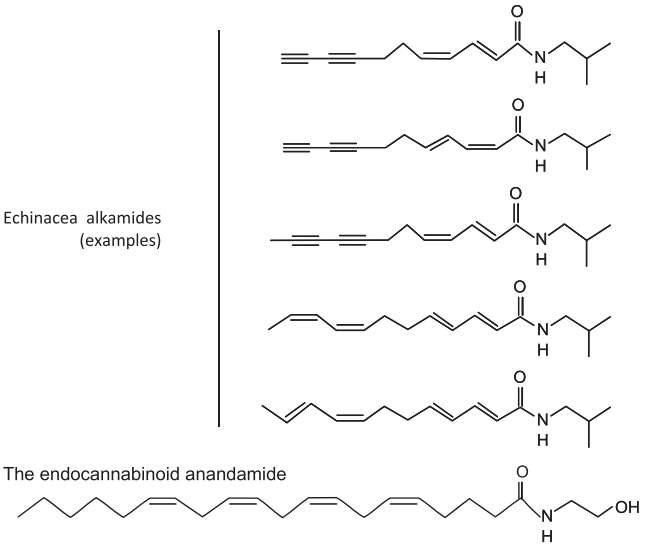

FIGURE 1 The chemical structures of anandamide (lower panel) and several Echinacea alkamides (upper panel)

Subsequent [35S]GTPcS-binding studies with purified Echinacea alkamides showed that their effects on the CB1 receptor is complex. Alkamides may be neutral with respect to CB1 activation (e.g. dodeca-2Z,4E,10Z-trien-8-ynoic acid isobutylamide), but may also exert inverse agonist (e.g. dodeca-2E,4Ediene-8,10-diynoic acid isobutylamide) partial agonist (e.g. dodeca-2E,4E,8Z,10E-tetraenoic acid methylbutylamide) or antagonist effects (e.g. dodeca-2E,4E,10Z-trien-8-ynoic acid isobutylamide) (Hohmann et al., 2011). The inhibition of FAAH was also characteristic to a few Echinacea alkamides only, e.g. to tetradeca-2E-ene-10,12-diynoic acid isobutylamide (Woelkart et al., 2005). Assuming that alkamides are indeed responsible for the anxiolytic effects of Echinacea preparations, these findings suggest that anxiolysis depends largely on the alkamide composition of the preparation, which is highly dependent on various factors, including the species, plant part, habitat and climate (Kraus, Bae, Wu, & Wurtele, 2006; Wu et al., 2009). This assumption was experimentally supported by our earlier findings showing that the HPLC fingerprint of alkamides differentiated those extracts that did from those that did not affect anxiety (Haller et al., 2010).

The effects of Echinacea preparations on human anxiety were investigated in a single trial so far, where an Echinacea angustifolia root extract standardized for alkamide fingerprint rapidly decreased anxiety (Haller et al., 2013). In this earlier human trial, however, no placebo control was employed, and sample size was small. To correct these deficiencies, here we performed a double-blind, placebo-controlled trial on a larger sample by using the preparation that proved effective in earlier studies 2010.

2 | METHODS

2.1 | Participants

62 participants were enrolled via advertisements placed in local media. The demographic characteristics of participants and their baseline psychometric features were summarized in Table 1. Overall, the trial population consisted of middle-aged, physically healthy, highly educated people, who had their residence or studied in Budapest the Capital of Hungary. Gender ratio was balanced. The trial was approved by Regulatory Authority, the Public Health Administration of the Budapest Capital Government Office (approval No. IF-2372-12/2017), based on the expert advice of the Scientific and Research Ethics Committee of the Medical Research Council (ETT-TUKEB, Budapest) acting as Central Ethics Committee in review of this type of research. The trial was conducted in compliance with the ethical and medical data privacy principles set by local regulations, ICH Good Clinical Practice (ICH GCP) guidelines and the Declaration of Helsinki. Prior to the inclusion in the trial voluntary consent has

TABLE 1 The characteristics of subjects according their prospective experimental treatment

Characteristic	Placebo	Echinacea
Sample size	32	32
Gender ratio		
male/female	16/16	14/18
Age	37.1±2.5	37.5±2.3
Marital status		
single	17	13
divorced	6	5
married/spouse	8	13
widow	1	1
Education		
university	28	25
high school	4	7
Residence		
capital (Budapest)	24	22
city	80	91
village		
Somatic disease last 6 month		
no	30	30
yes	2*	2†
Mental disorder,last 3 month		
no mental disorder	31	32
mental disorder	1#	0
Inventory scores at recruitment		
State anxiety	60.8±1.1	58.9±1.5
Trait anxiety	48.9±1.7	47.9±1.6
Beck depression	10.5±1.0	11.6±1.5
Perceived stress	34.4±1.1	33.1±1.1

*hypertonia (treated) and hyperthyroidism (clinically not significant, not treated);
†hypertonia (clinically not significant, not treated) and kidney stone (clinically not significant, not treated);
#incipient anxiety and depression (treated earlier with Mirtazapine, but treatment stopped more than 3 month prior to the trial). None of the group differences were significant.

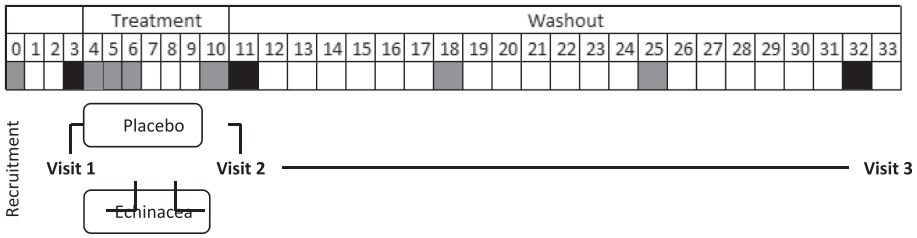

FIGURE 2 *The schematic of the study design*. The numbers indicate the study days. Gray-filled squares show the days when the STAI was filled in. Black-filled squares indicate the days when the BDI and PSS inventories were filled in

been obtained from each participant. Potential participant received a patient information sheet, which provided an overall description of the trial, the available information on the Echinacea extract, including drug incompatibilities, possible side effects, and the procedures, and potential risks and benefits associated with the participation. They had the opportunity to ask for more information verbally. After having their questions answered, those intending to participate signed an informed consent form.

2.2 | Procedure

We performed a double-blind placebo-controlled trial, which started by the Recruitment Visit. Recruitment was performed by a trained psychiatrist. Patients received information and signed the informed consent form as described above, after which they filled in the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970). We used the validated Hungarian version of the inventory (Sipos & Sipos, 1978). After checking for inclusion and exclusion criteria, patients were asked to return within 3 days for Visit 1 (day 1 of the trial). At Visit 1, patients filled in the STAI again to check for the reliability of their scores. In addition, they filled in the Beck Depression Inventory (BDI), and the Perceived Stress Scale (PSS). Patients were randomly assigned to either Echinacea or placebo treatment, received the assigned medications, and pre-printed trial diaries. Randomization was based on a randomization list created by a trained statistician of the manufacturer of the tablets, by using the randomcodegenerator.com platform. Based on this list, a unique identification number of each tablet container was established and attached to the container. The identification number was five character in length, and consisted of upper case letters and digits. The statistician in a sealed envelope retained the list, which was opened after the completion of the study. Once a participant was enrolled into the study, s/he was provided with one of the tablet containers and the identification number of the container was noted on the first page of the trial diary by the principal investigator.

Over the following 7 days, each patient self-administered the treatment (see below). Visit 2 was performed on the 11th day of the trial. Remaining tablets were returned to the experimenter, and adherence to the treatment protocol was checked via the trial diaries. Patients filled in the three inventories (see above), and trial diaries were returned to them. Visit 2 was followed by a three weeks washout period, after which subjects returned to the psychiatrist for Visit 3. Trial diaries were checked and collected; patients filled in the three inventories and were discarded from the trial.

The investigational products (Echinacea tablets and placebo tablets) were provided to the study subjects free of charge as per the legislation in force and the above mentioned guidelines, and they were provided with monthly tickets for public transportation to ensure free transport to the trial site. They received no other compensation for participation. The trial design was summarized in Fig. 2.

2.3 | Inclusion and exclusion criteria

Patients were included in the trial if they were over 18 years, were in good health and in their legal capacity, and showed signs of elevated anxiety. Elevated anxiety was defined as STAI scores more than one standard deviation above the average of the general population on either the State or the Trait Anxiety subscale of STAI (i.e. the cut-off score was 46 at either subscale). Participants were excluded from the trial if they suffered from major depression, bipolar disorder, anorexia, bulimia, alcohol or drug abuse or dependence, schizophrenia, schizoaffective disorder, psychosis, delirium, dementia, or any other major cognitive disorder. Furthermore, we excluded those patients, who showed signs of any personality disorder, those who were considered by the psychiatrist to be unable to complete the trial, or who showed risk for suicidality. We also excluded those who were treated with Echinacea, received psychotropic medication or underwent psychotherapy over the three month that preceded the trial. Allergy towards plants of the family Asteraceae (e.g. Chamomile), pregnancy, as well as AIDS and cancer were also exclusion criteria.

2.4 | Psychometric instruments

Anxiety was assessed by means of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970) which is a self-reporting questionnaire designed to evaluate the severity of anxiety symptoms. It consists of 20 questions for measuring state anxiety and 20 questions regarding trait anxiety. We used the validated Hungarian version of the inventory (Sipos & Sipos, 1978). Albeit no clear cut-off scores were established with this instrument, scores larger than the population mean (~35 point on each subscale) plus its standard deviation (~ 10 points on each subscale) are considered to indicate increased anxiety.

Signs of depression were investigated by means of the Beck Depression Inventory (BDI; Beck, Ward, Mendelsohn, & Mock, 1961) a self-reporting instrument used to evaluate the severity of depression symptoms mainly in research. We used the validated Hungarian version of the scale (Ágoston and Szili, 2001). The cut-off scores of

this instrument are as follows: 0-9, no depression; 10-18, mild depression; 19-25, moderate depression; 25+, severe depression.

General feelings of distress were investigated by means of the Perceived Stress Scale (PSS), which assesses the degree to which the participants appraised their lives as being stressful during the past month (Cohen, Kamarck, & Mermelstein, 1983). This widely used psychological instrument was designed to determine how unpredictable, uncontrollable and overloaded respondents find their lives. We used the 10-item version, which was previously validated in Hungary (Stauder and Konkoly Thege, 2006). Although the inventory has no well-established cut-off scores, 0-13 points are considered to indicate low levels of perceived distress; 14-26 points indicate moderate stress, whereas 27-40 points would be considered high perceived stress.

Except for visits, data were collected by a structured self-assessment diary technique. This procedure was employed earlier in a variety of disorders including anxiety (Bakker, van Dyck, Spinhoven, & van Balkom, 1999; Siddall et al., 2006; Thewissen et al., 2011). The preprinted diaries included an overall presentation of the trial, detailed instructions for each trial day, and six STAI forms, which were filled in by patients on days 4, 5, 6, 10, 18, and 25. Note that the rest of the STAI inventories were filled in during the Visits. The time when the forms were filled in as well as any deviation from the protocol were also noted. On treatment days, patients noted the time when the tablets were taken, and any side effects or discomfort noticed on that particular day. The latter information served to identify side effects.

2.5 | Treatments

We used an Echinacea angustifolia extract Anxiofit-1 in a tablet form (marketed as AnxioCalm in the US) that was characterized earlier in preclinical studies (Haller et al., 2010; Hájos et al., 2011; Hohmann et al., 2011) and used previously in a human trial (Haller et al., 2013). Ratio of herbal drug to native extract was 1: 8. Regarding the alkamide content of the extract, it should be noted that absolute values cannot be given because of the lack of specific alkamides to be used as standards. As a standard, 4-hydroxybenzoic acid butyl ester is being used; however, estimates of absolute alkamide contents are very approximate based on its own HPLC peak. Based on the standard (4-hydroxybenzoic acid butyl ester), the alkamide content of the extract was approximately 1-1.5%. The extract was administered in the form of film-coated tablets manufactured by the pharmaceutical company ExtractumPharma Zrt (Budapest, Hungary). Each tablet contained 40 mg of the Echinacea extract used in laboratory studies and suitable inactive ingredients. The product was registered by the National Institute for Food and Nutrition Science (file No. 2249-4/2010 OÉTI). Placebo and Echinacea tablets were indistinguishable; their size, weight, color, shape, and odor were similar, and neither had any taste. Neither the psychiatrist nor the patients were aware of the content of the tablets. Codes were broken by the end of the trial.

2.6 | Treatment compliance

Compliance with intervention was checked by the following two measures: (1) Subjects were provided with pre-printed trial diaries, having a separate page for each of the days of the trial, where the tasks of the day were described in detail. For treatment days, the diary had two specific entries for noting the taking of the tablets and its timing. For the days of psychological measurements, the page contained the inventory, and an entry for the date and the timing of the filling in. (2) At Visit 2, when the treatment period ended, subjects presented the trial diary and the tablet container to the principal investigator who checked both. Discrepancies between diaries and the number of tablets remaining in the container were discussed, and reasons were recorded, similar to any other deviations from the protocol. The procedure was repeated at Visit 3, when the trial ended. All deviations from the protocol were documented and recorded on a dispensing log.

2.7 | Sample size calculation

Sample size was first calculated by the "Power Analysis/Sample Size Calculation" module of the STATISTICA software for a 2-factor ANOVA model, where the number of levels coincided with that planned for our study. According to the module, the minimum sample size was 22 per group and 43 as for the total N to reach the level of interaction at $p < 0.05$. Sample size calculation made by the STATISTICA software was checked based on the empirical study performed earlier (Haller et al., 2013) by using the formula $n = 16\sigma 2 / W2$, where n denotes the minimum number of samples required, σ the standard deviation, and W the magnitude of the confidence interval (Kenny, 1987). The calculated sample size (n) was 26.6 per group, which was in a good agreement with the results of the first calculus. Based on the above two approaches, the required sample size was determined at 60 (30/ group). To account for dropouts, this sample size was increased to 64 (32/group).

2.8 | Statistics

Data were presented as mean ± the standard error of the mean. Findings were evaluated by two-factor repeated measures ANOVA, where the repeated measures factor was time (the days of the trial as levels), and factor 2 was treatment (levels: Echinacea and Placebo). Data were square root transformed to fulfill ANOVA requirements. P values lower than 0.05 were considered significant. Multiple comparisons, specifically those related to the repeated measure underwent Bonferoni correction. Differences between the share of high and low responders were evaluated by cross tabulation. Analyses adopted the intention to treat approach. Note however, that in the case of the primary objective (changes in STAI scores), deviations from the protocol were very mild; consequently the intention to treat approach approach was very close to the per protocol approach. We used the STATISTICA software (Tulsa, USA) for statistical calculi.

3 | RESULTS

3.1 | Treatment compliance and side effects

Two subjects were eliminated from the trial for non-adherence to the protocol. Both were from the placebo group. The rest of the sample

TABLE 2 Side effects observed over the treatment period

		Treatment day							
Treatment	**Side effect**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Placebo	Dry eye	1	0	0	0	0	0	0	0
	Insomnia	0	1	0	1	0	0	0	0
	Stomach complaint	0	0	0	0	1	1	0	1
	Headache	1	0	1	0	0	0	0	0
	Total	*8 complaints in 4 patients*							
Echinacea	Dry eye	0	0	0	0	0	0	0	0
	Insomnia	0	0	0	0	0	0	0	0
	Stomach complaint	0	0	0	0	0	0	0	0
	Headache	0	0	0	0	0	0	0	0
	Total	*No complaints*							

Data were shown as the number of patients reporting a particular complaint on specific treatment days.

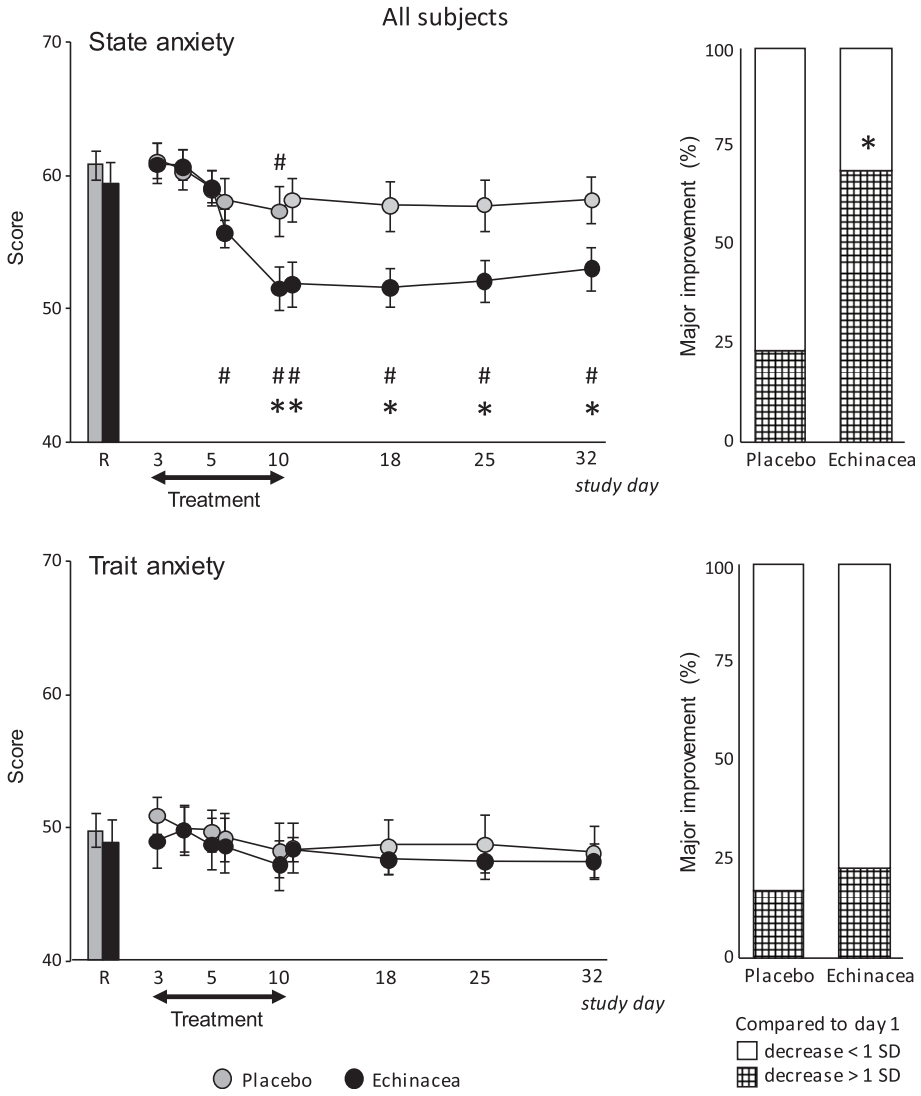

FIGURE 3 *The effects of Echinacea in the whole study sample*. Note that there was a significant interaction between factors in the case of state anxiety (F time*treatment (9,540) = 4.23; $p < 0.0001$) whereas TAI scores did not show significant changes. R, recruitment visit. #, significant difference from day 3 of the study, when patients were not yet treated (Visit 1). *, significant Placebo – Echinacea difference. In all cases, $p < 0.05$ at least after Bonferroni correction. For other explanations see text

completed the trial, and adhered to the protocol with minor deviations; e.g. the timing of tablet administration slightly deviated from that prescribed, one out of the 40 items of the STAI were omitted, etc.

Side effects were observed during the treatment phase only. In total, four patients reported 8 side effects (Table 2).

3.2 | Effects on anxiety

Baseline state anxiety (SAI) and trait anxiety (TAI) scores recorded during recruitment were similar in the Echinacea and Placebo groups (Fig. 2). Scores recorded 3 days later during Visit 1 were highly similar with those recoded on the recruitment visit. By contrast, baseline SAI and TAI scores differed irrespective to the treatment the participants subsequently received, the former being approximately 10 points higher than the latter.

Changes in state anxiety (SAI) significantly depended on the interaction between treatment and time (F *time*treatment* (9,540) = 4.23; p < 0.0001). Differences between the Echinacea and Placebo groups became significant beginning with the 10th day of the trial (7th day of the treatment) and remained significant until the end of the trial. By the end of the treatment phase, a SAI scores decreased by 3.3±0.9 and 11.7±1.7 points in the placebo and Echinacea groups, respectively. In addition to group differences, both groups showed decreases from baseline values. Placebo treatment decreased SAI scores significantly on the 10th day of the trial i.e. on the last day of the 7-day treatment period compared to scores recorded in Visit 1 (Fig. 3 upper left-hand panel). Scores remained low thereafter but did not differ significantly from Visit 1 due to a somewhat larger variation than on day 10. Echinacea decreased anxiety as compared to Visit 1 beginning with the 6th day of the trial (3rd day of treatment) and scores remained low throughout the rest of the trial.

The share of those patients who showed a high responsiveness to the treatment was significantly larger in the Echinacea as compared to the Placebo group (Fig. 3, upper right-hand panel) (Chi square= 12.14; p < 0.001). High responsiveness was defined here as a SAI score that was smaller than the mean recorded at Visit 1 minus its standard deviation. Less than one quarter of Placebo patients showed such large decreases, whereas the share of high responders was almost three quarters in the Echinacea group.

Changes in TAI scores were not significant (Fig. 3 lower left-hand panel) (F *treatment* (1,60) = 0.07; p< 0.8; F *time* (9,540) = 1.72; p< 0.1; F *time*treatment* (9,540) = 0.31; p < 0.9). The share of high responders was also similar (Fig. 3 lower right-hand panel) (Chi square= 0.27; p < 0.6.)

To investigate whether the lack of effect on TAI scores was due to the relatively low level of baseline trait anxiety (see above), those patients who showed high TAI scores at Visit 1 (N = 11) were studied in a second analysis. High TAI score was defined as two standard deviations above the population mean i.e. 55 points. Albeit differences

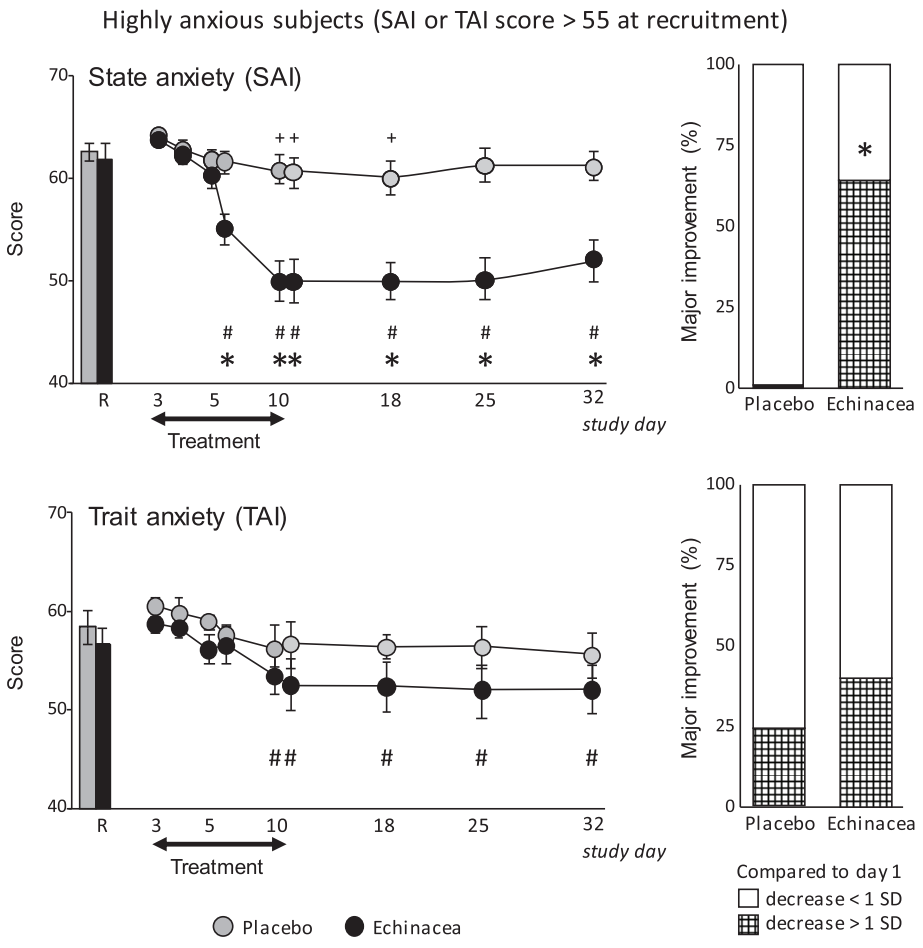

FIGURE 4 *The effects of Echinacea in patients showing high baseline anxiety.* Again, there was significant interaction between factors in the case of state anxiety (F time*treatment (9,423) = 11.49; p < 0.0001). In the case of trait anxiety, only the main effect of time was significant (F time (9, 230) = 2.72; p< 0.05). R, recruitment visit. #, significant difference from day 3 of the study, when patients were not yet treated (Visit 1); *, significant Placebo – Echinacea difference. In all cases, p < 0.05 at least after Bonferroni correction. For other explanations see text

TABLE 3 The impact of Echinacea treatment on depression and perceived stress

Treatment	Visit 1 (day 1)	Visit 2 (day 11)	Visit 3 (day 32)
Beck depression inventory (scores)			
Placebo	10.5±1.0	10.0±0.9	5.6±0.9#
Echinacea	11.6±1.5	11.3±1.3	4.9±0.6#
Statistics	F *treatment* (1, 114) = 0.05; p > 0.8		
	F *timing* (2, 114) = 12.97; p < 0.0001		
	F *interaction* (2, 114) = 0.17; p > 0.8		
Perceived Stress Scale (scores)			
Placebo	34.4±1.1	31.7±1.2	31.2±1.2#
Echinacea	33.1±1.1	31.9±1.4	28.1±0.9#
Statistics	F *treatment* (1, 114) = 2.11; p > 0.1		
	F *timing* (2, 114) = 5.59; p < 0.01		
	F *interaction* (2, 114) = 0. 74; p > 0.4		

Values were shown as mean ± standard error of the mean.
#significant difference compared to Visit 1.

between treatments were not significant in this analysis either, Echinacea patients showed a significant decrease from Visit 1 scores beginning with the 10th day of the trial (7th day of the treatment) and values remained low until the end of the trial (Fig. 4 lower left-hand panel). No significant decrease was observed in the placebo group. By the end of the treatment phase, TAI scores decreased by 0.2±1.3 and 5.3±2.4 points in the placebo and Echinacea groups, respectively. The share of high responders did not differ between the two groups (Fig. 4 lower right-hand panel) (Chi square= 0.19; p < 0.6).

A similar analysis was performed on SAI scores (Fig. 4 upper panel). As with TAI, here we evaluated those subjects who had SAI scores higher than 55 at Visit 1 (N = 23). The two groups remained significantly different (SAI scores: F *time*treatment* (9,423) = 11.49; p < 0.0001; share of high responders: Chi square= 21.02; p < 0.0001). By the end of the treatment phase, SAI scores decreased by 2.9±1.1 and 12.0±1.8 points in the placebo and Echinacea groups, respectively. The significant difference between Placebo and Echinacea treatment occurred 4 days earlier (on the 3rd day of treatment vs. the 7th day as with the whole population). In addition, a significant decrease compared to baseline levels was not observed in the Placebo group (all differences were marginally significant), and in this group high responders were lacking (Fig. 4 upper right-hand panel). The share of such patients remained high in the Echinacea group.

3.3 | Depression (BDI) and Perceived Stress (PSS)

On average, Beck scores indicated a low level of depression symptoms in both groups, as averages were slightly above the cut-off score (i.e. 9 points; Table 3). By contrast, the scores of the PSS indicate high levels of perceived stress. Both scores decreased over the trial period, but this decrease became significant after the washout period only. Scores slightly above the cut-off for mild depression lowered into the no-depression range of the scale. Although PSS scores also decreased significantly, they remained in the high perceived stress range. Echinacea treatment affected neither of these changes.

4 | DISCUSSION

4.1 | Main findings

The Anxiofit-1a uniquely standardized root extract of Echinacea angustifolia rapidly decreased state anxiety in subjects. Compared with placebo, scores were significantly decreased on the sixth day of treatment (day 10 of the trial). The share of patients showing large SAI decreases was significantly higher in the Echinacea as compared with the placebo group. Effects were stronger in patients scoring high on the recruitment day (above 55 SAI points) i.e. in those who had larger anxiety problems than the rest of the sample. In this subpopulation, scores decreased significantly compared to placebo on the fourth day of treatment. In addition, two thirds of Echinacea-treated subjects showed large SAI decreases, whereas no placebo-treated subject showed large decreases in this subpopulation. Effects on TAI were less evident. The likely reason was that the average baseline TAI scores were within the normal range i.e. were not indicative of trait anxiety. When subjects with high baseline TAI scores were considered separately, a mild decrease in TAI was observed. Patients belonging to this subgroup did not differ significantly from placebo patients who had similar baseline characteristics, but TAI scores decreased compared to the baseline, an effect that was not observed in the placebo group. Taken together, these findings show that the standardized root extract of Echinacea angustifolia performed considerably better than placebo as it regards state anxiety and moderately better than placebo as it regards trait anxiety, likely because a trait is less responsive to treatments than a state. The effect developed rapidly (within a few days) and was persistent as it did not vanish over the three weeks of washout. The extract did not affect Beck and PSS scores.

4.2 | Adverse effects

In this trial, no adverse effects were observed with the Echinacea preparation; all the adverse effects were observed in the placebo group. This finding is consistent with those obtained in laboratory studies performed earlier as well as with earlier human studies. In laboratory experiments, the acute toxicity of the standardized Echinacea angustifolia preparation was well above 3,000 mg/kg. No dose of the preparation affected locomotor activity, learning or memory and none of the employed doses showed addictive potential as shown by the conditioned place preference test (Haller et al., 2013). Note that the doses employed in safety experiments were several times larger than those showing anxiolytic effects in laboratory studies. In human studies, Echinacea preparations proved to be safe, except for allergy for Asteraceae plants (e.g. Chamomile, sunflower, Echinacea), and rare skin rushes, which resolved quickly when treatment were discontinued (Barnes, Anderson, Gibbons, & Phillipson, 2005; Birt, Widrlechner, Lalone, et al., 2008; Borchers, Keen, Stern, & Gershwin, 2000; Izzo & Ernst, 2001; Tesch, 2003). As such, the anxiety-related benefits of Echinacea pose minimal risks.

4.3 | Limitations

The two main limitations of the study are related to sample size and the duration of treatment. The necessary sample size was calculated by two methods (see subsection Sample size calculation), which provided consistent estimates, and in conformity to the expectations the sample size was sufficient to evidence significant changes over the trial. However, the sample size was well below those generally employed by studies on the efficacy of anxiolytic medications, and additional trials in larger study populations are required to support the validity of the findings reported here. The duration of the treatment was considerably shorter than that usually employed in anxiolytic studies. The choice of the length of the treatment period was motivated by the rapidity of the anxiolytic effect observed in our previous trial, which raised the possibility that subjects having anxiety problems may benefit from the Echinacea preparation studied here in an intermittent, "as required" basis, especially so as it occurs that the short-term treatment had long-term benefits. Yet, the effects of longer treatment regimens still need to be established.

4.4 | Beneficiaries of putative treatments

There are several well-established treatments for anxiety disorders, all of which are effective and reasonably well tolerated by patients. Herbal preparations are unlikely to replace such medications, but are still required in some special cases, particularly in sub-threshold and mild forms of anxiety.

Sub-threshold, or subliminal cases differentiate from anxiety disorders either by their intermittent nature (no anxiety phase reaching the temporal requirements for a diagnosis) and/or by incomplete or weaker symptoms. Despite their "incompleteness", sub-threshold anxiety symptoms induce significant suffering and lower the quality of life (Unick, Snowden, & Hastings, 2009). Importantly for the present trial, sub-threshold symptoms are not only persistent (Karsten, Penninx, Verboom, Nolen, & Hartman, 2013), but have the tendency to progress into clinical-level anxiety i.e. anxiety disorders (Bystritsky et al., 2010; Van't Veer-Tazelaar et al., 2010). Residual symptoms also increase the risk of relapse in successfully treated anxiety patients. These studies demonstrate that the risks for anxiety-disorders and depression are increased by sub-threshold symptoms of anxiety, which often remain untreated because both physicians and subjects are reluctant to administer and receive, respectively, anxiolytics in lack of a formal diagnosis. The same often holds true for mild anxiety disorders: a small fraction of people presenting mild anxiety disorders ask and receive medical help (Burgess et al., 2009).

For the reasons outlined above, the alleviation of sub-threshold and mild anxiety requires non-conventional (alternative) interventions (Batelaan et al., 2010; Karsten et al., 2013; Nauta et al., 2012;). A variety of interventions were proposed to this end, and these proved successful in alleviating sub-threshold symptoms. We claim that the standardized Echinacea angustifolia root extract studied here may be effective and useful in patients showing sub-threshold and/or mild forms of anxiety.

CONFLICT OF INTEREST STATEMENT

JH is one of the authors of a US patent on the anxiolytic effects Echinacea preparations.

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