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RESEARCH ARTICLE

Bacopa monnieri as augmentation therapy in the treatment of anhedonia, preclinical and clinical evaluation

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Bacopa monnieri (L.) is widely used in Ayurvedic medicine as a neural tonic for improving intelligence and memory. Several studies highlighted its efficacy in neuropsychiatric diseases but there is no evidence regarding anhedonia. Aim of the present work was to preclinically and clinically test against anhedonia a standardized *B. monnieri* extract (20% bacosides). In a mouse model of a depressive-like syndrome induced by lipopolysaccharide (LPS), the daily administration of the extract (50–200 mg kg^{-1}, p.o.) for 1 week, dose-dependently counteracted the immobility time in Porsolt and Tail suspension tests ($p < .01$). At the sucrose preference test (directly related to the ability for feeling pleasure) the extract treatment (100 and 200 mg kg^{-1}) counteracted the reduction of sucrose intake induced by LPS ($p < .01$). Moreover, *B. monnieri* significantly reduced cytokines, cortisol, and artemin LPS-dependent alterations in plasma while increased the brain-derived neurotrophic factor levels ($p < .05$). The efficacy of the same extract was tested in a clinical study in which 42 patients with significant degree of anhedonia (evaluated as Snaith-Hamilton Pleasure Scale [SHAPS] score ≥ 3) were enrolled. Patients were divided into two groups and treated with citalopram or citalopram associated with *B. monnieri* (300 mg bid) for 4 weeks. The Pears Sample *T*-test showed a significant improvement ($p < .05$) in relevant scales (Hamilton depression rating scale, SHAPS, and strength and difficulties questionnaire) in the extract-treated group in comparison to citalopram alone was recorded. These data suggest that *B. monnieri* extract may be effective for the management of anhedonia and therefore should be considered for future controlled trials.

KEYWORDS
artemin, BDNF, HAM-D, LPS, SDQ, SHAPS

1 | INTRODUCTION

Bacopa monnieri, a plant native to the wetlands of Southern and Eastern India, is known in India under the name of Brahmi and is traditionally used in Ayurvedic medicine as neural tonic, sedative, anti-epileptic, memory, and learning enhancer (Pham et al., 2019). Several preclinical studies have shown numerous pharmacological effects of this plant, such as improved memory in Alzheimer's disease and schizophrenia, potential anti-stress, anticonvulsant and anti-Parkinson's actions and recent clinical trials have

Abbreviations: ARTN, artemin; BDNF, brain-derived neurotrophic factor; CEQ, credibility/expectancy questionnaire; HAM-D, Hamilton depression rating scale; LPS, bacterial lipopolysaccharide; RDoC, Research Domain Criteria; SDQ, strength and difficulties questionnaire; SHAPS, Snaith–Hamilton Pleasure Scale; TAU, conventional therapy; TBARS, thiobarbituric acid reactive substances.

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confirmed the efficacy of *B. monnieri* on learning and cognitive performance (Negi, Singh, & Kushwaha, 2000). Furthermore, numerous preclinical studies have demonstrated the antidepressant effects of a *B. monnieri* standardized extracts in paradigms commonly used to evaluate depression (Banerjee, Hazra, Ghosh, & Mondal, 2014; Sheikh et al., 2007). Moreover, *B. monnieri* can inhibit the release of IL-6 and TNF-α from activated microglia (Nemetchek, Stierle, Stierle, & Lurie, 2017) and increase concentration of the brain-derived neurotrophic factor (BDNF) in rat hippocampus (Banerjee et al., 2014; Hazra, Kumar, Saha, & Mondal, 2017; Kumar & Mondal, 2016). Lesser evidence exist about the clinical efficacy of *B. monnieri*, a double-blind RCT study on mood and stress (Benson et al., 2014) was carried out on 17 healthy volunteers treated with 320 and 640 mg of extract after being exposed to stress induced by multitasking: the results showed a cortisol levels reduction and mood improvement (Benson et al., 2014).

Depression may be intended as the expression of different endophenotypes (Hasler, Drevets, Manji, & Charney, 2004). The association between psychopathological (impaired reward and cognitive functions, neurovegetative signs, increased stress sensitivity) and biological (functional and structural brain abnormalities, dysfunctions in neurotransmitters levels, and intracellular signaling) endophenotypes generates multiplex behavioral manifestations related to different neural circuits according to the Research Domain Criteria (RDoC) model (Insel, 2014). Beyond heterogeneity, anhedonia, the reduced ability to experience pleasure, is a recurrent, transnosographic dimension. It is a hallmark symptom of major depression, schizophrenia, and other neuropsychiatric disorders that represents a negative prognostic factor and leads to adverse mental health outcomes across the lifespan and predicts poor psychosocial functioning. The neural bases of the construct of anhedonia reflects deficits in hedonic capacity and are closely linked to the constructs of reward valuation, decision-making, anticipation, and motivation (Treadway & Zald, 2011). Since anhedonia is a difficult-to-treat target, several therapeutic approaches have been proposed, including psychosocial interventions, antipsychotics (for schizophrenia conditions) (Aleman et al., 2017), antidepressants (in mood disorders) (Craske, Meuret, Ritz, Treanor, & Dour, 2016), and neuromodulation interventions (in addictive use disorder; Pettorruso et al., 2018; Pettorruso, Di Giannantonio, De Risio, Martinotti, & Koob, 2019).

On this base, the possibility to intervene on the anhedonic dimension with a natural complementary approach becomes an intriguing therapeutic possibility. *B. monnieri* is a theoretically optimal candidate to be evaluated. Aim of the present work was to study the efficacy of a characterized *B. monnieri* extract in a mouse model of LPS-induced anhedonia and, in the subsequent clinical phase of the study, to test the effect of the extract in addition to conventional therapy (TAU) in patients with this symptom.

2 | MATERIALS AND METHODS

2.1 | Extract and pure Bacoside II

A commercial *B. monnieri* powder extract (Galeno, Carmignano, Italy) was used for the tests, batch n. C17070624. Bacoside II was an international reference standard (purity 98%) from Abacipharm Corp (Maryland, USA).

2.2 | HPLC-DAD and ESI-TQ-MS/MS apparatus and conditions

The HPLC analysis was performed on a HPLC 2695 Waters instrument coupled with TQ-Four micro Waters using an ESI source. The chromatographic separation was performed using a Gemini C18 analytical column 150 × 2.0 mm (5 μm, particle size, 110 Å) Phenomenex. The flow rate was 0.35 ml/min and the injection volume was 20 μl. Mobile phase (water/acetonitrile) was a gradient water starting with 20% of acetonitrile until 80% in 23 min. Identification of peaks was based on comparisons of ESI-MS/MS spectra with those reported in the literature and co-injection with standard Bacoside II. Samples were dissolved in MeOH (1 mg/ml) and filtered in a 0.45 μm membrane filter. Acquisition in full scan MS, positive ion mode from m/z 430 to m/z 1,200. The quantification of the contents of the *B. monnieri* saponins was performed using HPLC-MS with external standard method, using Bacoside II (IS, 99.7% pure by HPLC) as standard compound. The HPLC method was validated in terms of linearity, limit of detection, limit of quantification, precision, accuracy, and robustness according to the International Conference on Harmonization (ICH) guidelines (ICH, 2005).

2.3 | Preclinical investigation

2.3.1 | Animals

Male CD-1 albino mice (Envigo, Varese, Italy) weighing approximately 22–25 g at the beginning of the experimental procedure, were used. Animals were housed in CeSAL (Centro Stabulazione Animali da Laboratorio, University of Florence) and used at least 1 week after their arrival. A maximum of 12 mice were housed per cage (size 26 × 41 cm); animals were fed a standard laboratory diet and tap water ad libitum, and kept at 23 ± 1°C with a 12 h light/dark cycle, light at 7 a.m. All animal manipulations were carried out according to the Directive 2010/63/EU of the European parliament and of the European Union council (September 22, 2010) on the protection of animals used for scientific purposes. The ethical policy of the University of Florence complies with the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 85-23, revised 1996; University of Florence assurance number: A5278-01). Formal approval to conduct the experiments described was obtained from the Italian Ministry of Health (No. 54/2014-B) and from the Animal Subjects Review Board of the University of Florence. Experiments involving animals have been reported according to ARRIVE guidelines (McGrath & Lilley, 2015). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.3.2 | *B. monnieri* treatment

The extract of *B. monnieri* (L.) Pennell (dry extract, 20% bacosides, Galeno Srl, Italy) was suspended in 1% carboxymethylcellulose sodium salt (CMC) and orally (p.o.) administered every day for 1 week in a range dose of 50–200 mg kg^{-1}. Control group was treated with vehicle.

2.3.3 | LPS-induced anhedonia

Lipopolysaccharide (LPS) from *Escherichia coli* was purchased from Sigma-Aldrich, freshly dissolved in sterile saline and injected intraperitoneally (i.p.) at the dose of 1.25 mg kg^{-1} (Biesmans et al., 2013) after 1 week of *B. monnieri* treatment, 1 hr after the last administration of the extract. Behavioral tests were performed before 6 and 24 hr after LPS administration. Control group was treated with vehicle.

2.3.4 | Forced swimming test

The forced swimming test used was the same described by Porsolt, Le Pichon, and Jalfre (1977). More information are reported in Supporting Information.

2.3.5 | Tail suspension test

A piece of tape was adhered to the upper middle of the tail of each animal, creating a flap with the overlap of tape. Mice were suspended from a plastic rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape. The duration of the test was 6 min and the immobility time was measured in the first 2 min, when animals react to the inescapable stress, and in the last 4 min of the test, when the behavioral despair is established. Immobility was defined as the absence of any limb or body movements, except those caused by respiration.

2.3.6 | Sucrose preference

The preference for 2% sucrose solution was examined as a measured for anhedonia. More information are reported in Supporting Information.

2.3.7 | Tissue collection and measurements of soluble factors

At the end of the behavioral test session, 24 hr after LPS administration, animals were sacrificed by decapitation, the prefrontal cortex was collected and frozen using liquid nitrogen. IL-6, IL-1β, cortisol, BDNF, and ART were detected using ELISA kit according to the manufacturer instructions.

2.3.8 | Determination of thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) were quantified in brain tissue homogenates as described previously, with some minor modifications (Pan et al., 2009).

About 100 μg of prefrontal cortex tissue homogenate were added to 4 ml reaction mixture consisting of 36 mM thiobarbituric acid (Sigma-Aldrich, Milan, Italy) solubilized in 10% CH_3COOH, 0.2% SDS, pH was adjusted to 4.0 with NaOH. The mixture was heated for 60 min at 100°C and the reaction was stopped by placing the vials in ice bath for 10 min. After centrifugation (at 1,600*g* at 4°C for 10 min) the absorbance of the supernatant was measured at 532 nm (Perkin-Elmer spectrometer, Monza, Italy) and TBARS were quantified in μmoles/milligram of total protein using 1,1,3,3-tetramethoxypropane as standard. Protein homogenate concentration was measured by bicinchoninic acid (BCA; Sigma-Aldrich) assay.

2.3.9 | Catalase activity

Catalase activity was measured in the supernatant of brain tissue homogenate by Amplex Red Catalase Assay Kit (Invitrogen, Monza, Italy) following the manufacturer's instructions as previously described (Zanardelli et al., 2014). Protein concentration was quantified by bicinchoninic acid assay (Sigma-Aldrich). Catalase activity for each sample was normalized to protein concentration. Control conditions in the absence of treatment were set as 100%.

2.4 | Clinical investigation

2.4.1 | Sample

Our study was conducted on a sample of patients described in Table 1, dealing with outpatients and Day Hospital of the Psychiatric unit in the University Hospital of Florence and Institute of Neuroscience (INS), which had a clinically significant degree of anhedonia (evaluated as SHAPS score ≥ 3) being diagnosed for major depression (DSM 5.0) and after adequate treatment with citalopram and stable for 4 weeks with an unsatisfactory response.

2.4.2 | Assessment

To measure the severity of anhedonia, every participant was administered the Snaith–Hamilton Pleasure Scale (SHAPS; Snaith et al., 1995) and the Temporal Experience of Pleasure Scales (TEPS) scale immediately before and after the 4-week treatment period. Moreover, the following scales were administered and evaluated: strengths and difficulties questionnaire (SDQ), patient health questionnaire, Hamilton

TABLE 1 Study of the sample

Sample	42 Patients, 19 cases (BM), and 23 controls (TAU)
Sex	F > M (64.3% *N* = 27 vs. 35.7% *N* = 15)
Age	In cases of average age 47.22 years (DS = 12.891), between checks 48.53 years (DS = 13.443) For the years of illness, the two groups show no statistically significant differences ($p < .05$): The cases have average disease duration of 15.55 years (DS = 10.828) and the controls of 8.76 years (DS = 11.098)
Average school age	12.47 years (DS 3.31)
Smoke	21% smokers, 21.4% *N* = 9 smokers
Job	Employees 59.5% *N* = 25, freelancers 9% *N* = 5, retired 16.7% *N* = 7, unemployed 11.9% *N* = 5

depression rating scale (HAM-D; Hamilton et al., 1960), credibility-expectation questionnaire (CEQ), and clinical global impression (CGI).

2.4.3 | Treatment

Patients were randomly assigned to two groups. The first group (n = 23) was treated only with (40 mg) citalopram for the follow 4 weeks (TAU); the second group (n = 19) with conventional therapy associated with a nutraceutical supplement consisting of *B. monnieri* extract titrated at 20% in bacosides (one capsule 300 mg twice daily). The treatment lasted 4 weeks.

2.4.4 | Statistical analysis

For preclinical study, behavioral measurements were performed on 16 mice for each treatment carried out in two different experimental sets. Results were expressed as means ± *SEM* and the analysis of variance was performed by ANOVA. A Bonferroni's significant difference procedure was used as post-hoc comparison. p Values of less than .05 and .01 were considered significant. Data were analyzed using the "Origin 8.1" software. For clinical study, in order to characterize the study sample, a descriptive statistical analysis was performed. In particular for the categorical variables study the chi-square test was used, while the t-test was used for the continuous variables. For some variables, in addition to the paired t-test, a nonparametric test; a multivariate linear model was therefore used to study the association between the use of the bacopa and the outcome of the tests and to correct for the baseline CIB-3 imbalance.

Pearson's r coefficient was calculated to evaluate the correlation of clinical variables.

Statistical tests were two-tailed. Level of significance was set at $p = .05$.

All analyses were performed on study completers and were carried out using the SPSS statistical software version 15.0 (SPSS, Inc., Chicago, 2007).

3 | RESULTS

3.1 | Characterization of the commercial extract

B. monnieri commercial extract was characterized by HPLC-PDA-MS analysis. The chromatogram of *B. monnieri* commercial extract solubilized in MeOH (100 ng/μl) displayed four principal peaks related to Bacopa saponins with retention times of 16.5, 16.8, 17.2, and 17.5 min. The identification of Bacopaside II with the peak having a retention time of 16.8 min was based on the co-elution with the reference standard (20 ng/μl in methanol) and confirmed by the m/z 951.8 of the $[M + Na]^+$.

The other three peaks of the chromatogram of *B. monnieri* commercial extract at 16.5, 17.2, and 17.5 min were unambiguously attributed to Bacoside A3, Bacopasaponin C, and Bacopaside X, respectively. Peak at 16.5 was assigned to Bacoside A3, with the same MS data of Bacopaside II, which represents its isomer. Bacopasaponin C and Bacopaside X were assigned to the peaks at 17.2 and 17.5 min because the characteristic MS data (m/z 921.8 or 921.9 of the [M + Na]+), as reported in Figure 1.

The repeatability was determined by HPLC analysis of three samples, and the RSD of the contents was calculated for the four major constituents. RSD values ranged between 0.55 and 3.80. Lastly, the external standard method was applied to quantify each compound, using a regression curve, with each point determined in triplicate. The resulting percentage of total saponins expressed as Bacopaside II was ca. 20%.

3.2 | Preclinical results

3.2.1 | Forced swimming test

We evaluated the protective effect of *B. monnieri* extract on LPS-induced anhedonia in the mouse. In the forced swimming test, behavioral despair was evaluated by measuring the time during which the animal remains immobile after being placed in a water filled cylinder from which it cannot escape (Figure 2). LPS (1.25 mg kg^{-1}) acute administration was able to significantly increase the immobility time of the animals in this test both at 6 and 24 hr after injection with respect to the control group (168.0 ± 13.7 s vs. 63.5 ± 9.2 s at 6 hr and 104.8 ± 14.9 s vs. 40.0 ± 7.9 s at 24 hr, respectively). Daily administration of *B. monnieri* extract for 1 week (50–200 mg kg^{-1}) was able to counteract the immobility time induced by LPS in a dose-dependent manner (Figure 2). The higher dose (200 mg kg^{-1}) significantly diminished the reduction of movements induced by LPS systemic injection at 6 and 24 hr (30.6 ± 17.4 s and 31.2 ± 7.2 s, respectively). The dose of 100 mg kg^{-1} was still active at both time points but with a lower efficacy (70.3 ± 21.0 s and 61.0 ± 7.6 s, respectively) while the dose of 50 mg kg^{-1} was inactive both at 6 hr than 24 hr (156.3 ± 36.4 s and 95.7 ± 8.7 s, respectively).

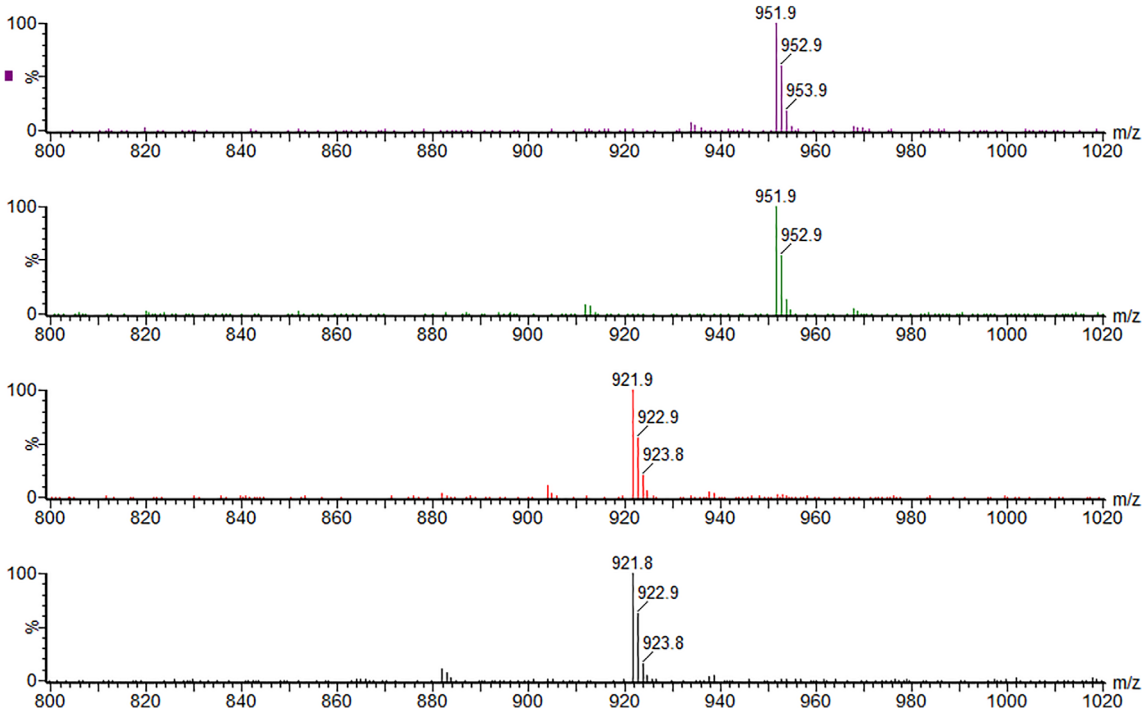

FIGURE 1 MS spectra of the positive ions of the major four peaks corresponding to the four detected saponins [Colour figure can be viewed at wileyonlinelibrary.com]

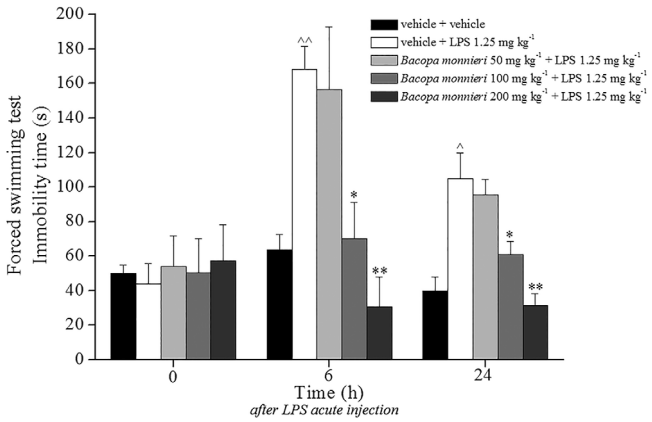

FIGURE 2 Forced swimming test. Effect of *B. monnieri* extract on LPS-induced anhedonic-like behavior. *B. monnieri* (50–200 mg kg^{-1}) was p.o. daily administered for 1 week before the acute i.p. injection of LPS (1.25 mg kg^{-1}). The immobility time was evaluated before and after LPS administration (6 and 24 hr). Each value represents the mean of 16 mice performed in two different experimental sets. Data are shown as mean ± *SEM*; ^p < .05 and ^^p < .01 versus vehicle + vehicle-treated mice; *p < .05 and **p < .01 versus vehicle + LPS-treated mice. LPS, lipopolysaccharide

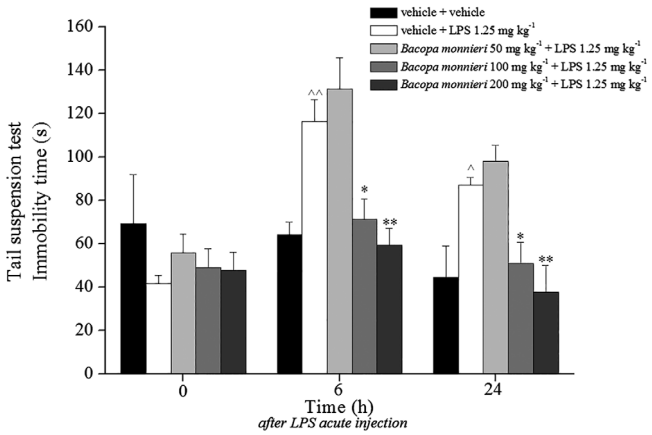

FIGURE 3 Tail suspension test. Effect of *B. monnieri* extract on LPS-induced anhedonic-like behavior. *B. monnieri* (50–200 mg kg^{-1}) was p.o. daily administered for 1 week before the acute i.p. injection of LPS (1.25 mg kg^{-1}). The immobility time was evaluated before and after LPS administration (6 and 24 hr). Each value represents the mean of 16 mice performed in two different experimental sets. Data are shown as mean ± *SEM*; ^p < .05 and ^^p < .01 versus vehicle + vehicle-treated mice; *p < .05 and **p < .01 versus vehicle + LPS-treated mice. LPS, lipopolysaccharide

3.2.2 | Tail suspension test

Similar results were obtained in the tail suspension test (Figure 3). In this test, behavioral despair was evaluated by measuring the time during which the animal remains immobile after being suspended by the tail. Repeated treatment with *B. monnieri* extract counteracted the increased of immobility time induced by LPS acute injection in a dose-dependent manner. The group treated with the higher dose (200 mg kg^{-1}) reached the value of 59.4 ± 7.5 s at 6 hr and 37.8 ± 12.2 s at 24 hr after LPS injection in comparison to the values of

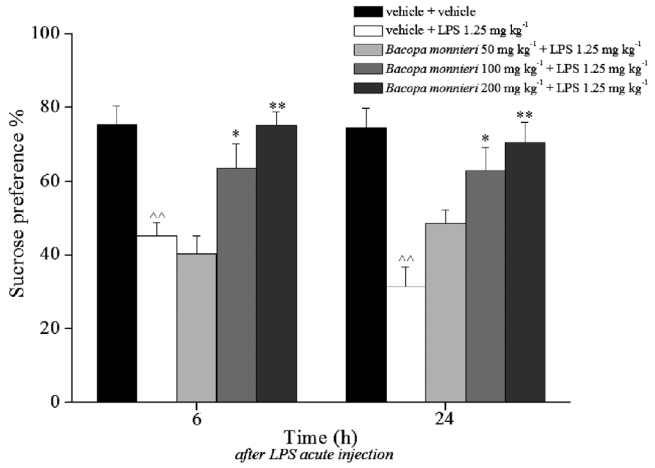

FIGURE 4 Sucrose preference test. Effect of *B. monnieri* extract on LPS-induced anhedonic-like behavior. *B. monnieri* (50–200 mg kg^{-1}) was p.o. daily administered for 1 week before the acute i.p. injection of LPS (1.25 mg kg^{-1}). The sucrose preference was evaluated after 6 and 24 hr LPS administration. Each value represents the mean of 16 mice performed in two different experimental sets. Data are shown as mean ± *SEM*; $^{\wedge\wedge}p < .01$ versus vehicle + vehicle-treated mice; $^{*}p < .05$ and $^{**}p < .01$ versus vehicle + LPS-treated mice. LPS, lipopolysaccharide

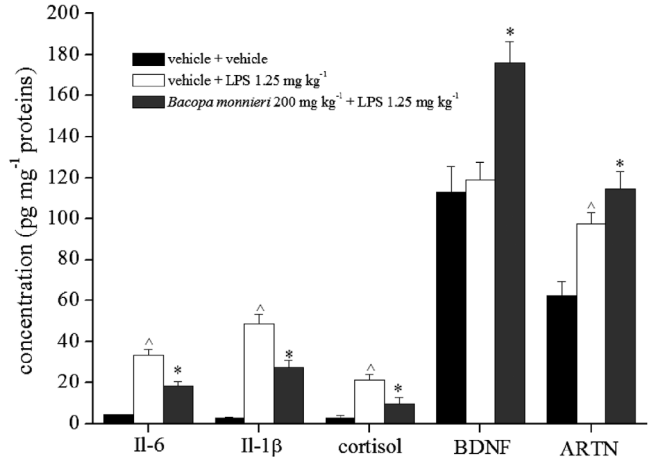

FIGURE 5 Measurements of soluble factors in the prefrontal cortex. Effect of *B. monnieri* extract on LPS-induced Il-6, Il-1β, cortisol, BDNF, and ARTN alterations. *B. monnieri* (200 mg kg^{-1}) was p.o. daily administered for 1 week before the acute i.p. injection of LPS (1.25 mg kg^{-1}). The measurements of Il-6, Il-1β, cortisol, BDNF, and ARTN were performed in the prefrontal cortex 24 hr after LPS administration. Each value represents the mean of 16 mice performed in two different experimental sets. Data are shown as mean ± *SEM*; $^{\wedge}p < .05$ versus vehicle + vehicle-treated mice; $^{*}p < .05$ versus vehicle + LPS-treated mice. ARTN, artemin; BDNF, brain-derived neurotrophic factor; LPS, lipopolysaccharide

116.6 ± 9.8 s and 87.3 ± 3.4 s reached by LPS-treated group (Figure 3). The dose 100 mg kg^{-1} was less effective while the lower dosage was inactive.

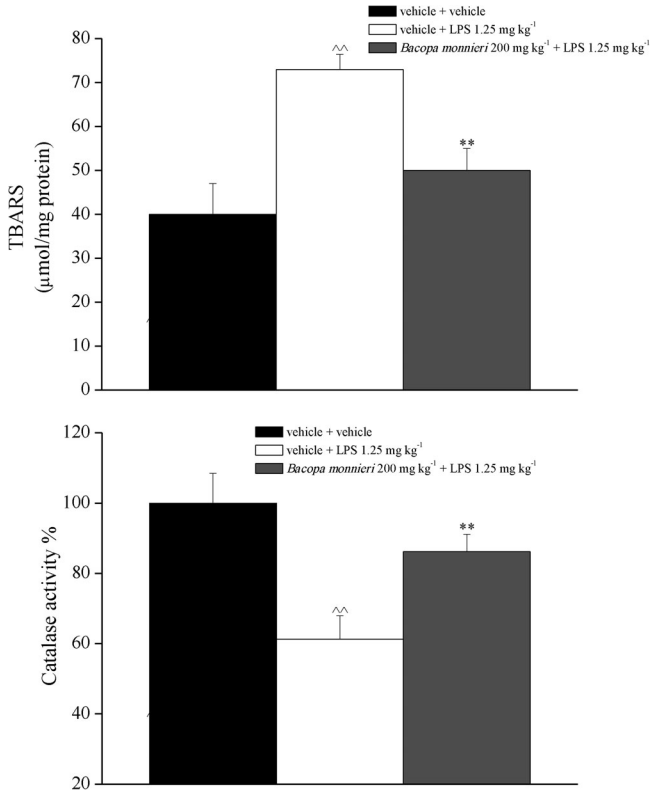

FIGURE 6 Evaluation of TBARS and catalase activity in the prefrontal cortex. Effect of *B. monnieri* extract on LPS-induced oxidative stress and enzymatic alterations. *B. monnieri* (200 mg kg^{-1}) was p.o. daily administered for 1 week before the acute i.p. injection of LPS (1.25 mg kg^{-1}). The measurements of (a) TBARS and (b) catalase activity were performed in the prefrontal cortex 24 hr after LPS administration. Each value represents the mean of 16 mice performed in two different experimental sets. Data are shown as mean ± *SEM*; $^{\wedge\wedge}p < .01$ versus vehicle + vehicle-treated mice; $^{**}p < .01$ versus vehicle + LPS-treated mice. LPS, lipopolysaccharide; TBARS, thiobarbituric acid reactive substances

3.2.3 | Sucrose preference test

Another anhedonic-like behavior was the reduction of sucrose intake. Six and twenty-four hours after LPS injection, mice in the LPS group showed significantly less preference for sucrose than animals in the control group (45.2% vs 75.4% at 6 hr and 31.5% vs 74.7% at 24 hr; Figure 4). *B. monnieri* extract counteracted this phenomenon at both time points when was administered at the dose of 100 and 200 mg kg^{-1}. The lower dosage was ineffective.

3.2.4 | Measurement of soluble factors

Moreover, LPS injection significantly increased the prefrontal cortex concentration of IL-6, IL-1β, cortisol, and ARTN with respect to the control group 24 hr after injection. No alteration was recorded for the neurotrophic factor BDNF (Figure 5). *B. monnieri* (200 mg kg^{-1})

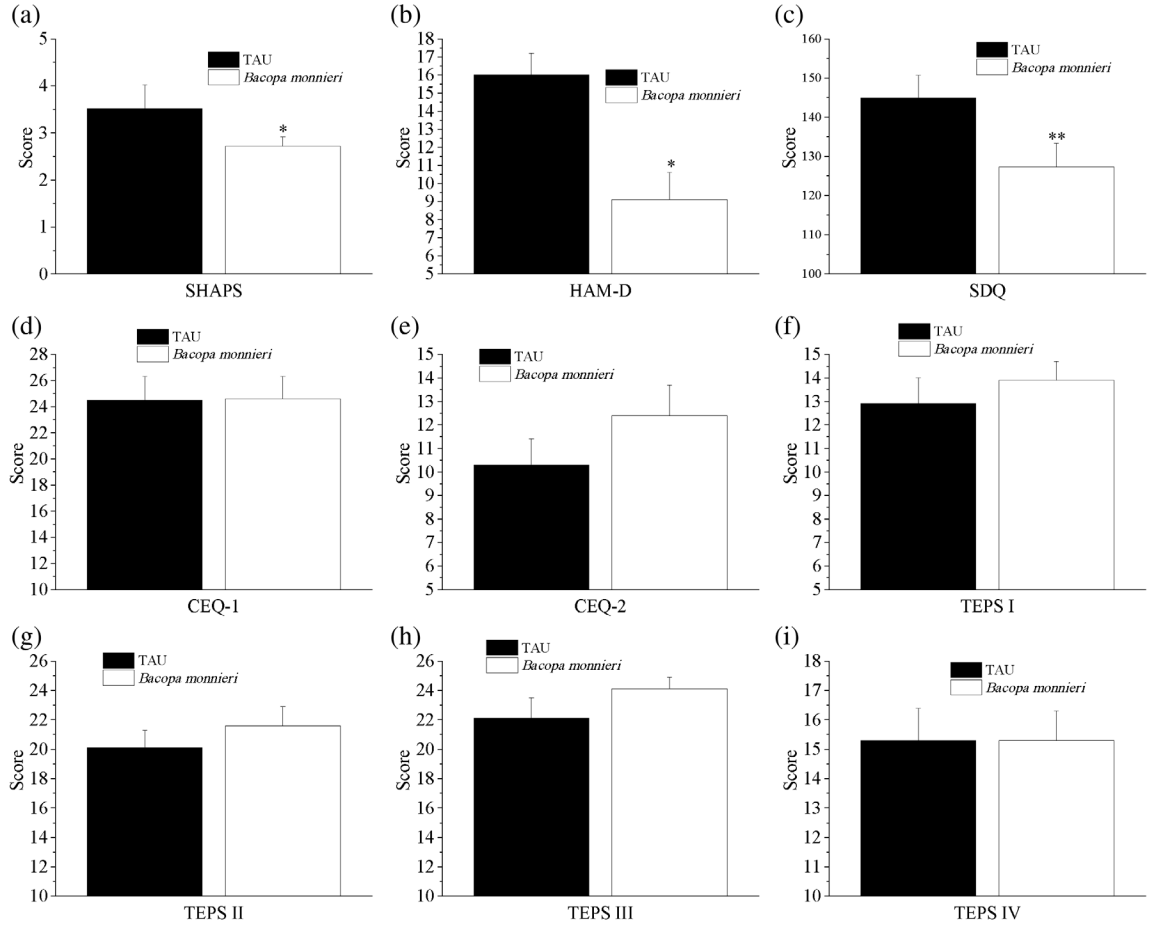

FIGURE 7 Clinical outcomes post-treatment in the group of patients treated with *B. monnieri* compared to the group treated with citalopram 40 mg (TAU). The first group (n = 21) was treated only with (40 mg) citalopram for the follow 4 weeks (TAU); the second group (n = 19) with conventional therapy associated with a nutraceutical supplement consisting of *B. monnieri* extract titrated at 20% in bacosides (one capsule 300 mg twice daily). The treatment lasted 4 weeks. Significant differences were measured for HAM-D, SHAPS, and SDQ. $^{*}p < .05$ and $^{**}p < .01$ versus TAU. HAM-D, Hamilton depression rating scale; SHAPS, Snaith–Hamilton pleasure scale; SDQ, strength and difficulties questionnaire

significantly reduced the LPS-alterations recorded for IL-6, IL-1β, cortisol, and ARTN while increased the BDNF value with respect to control and LPS group (Figure 5).

3.2.5 | Effect of *B. monnieri* on oxidative stress and catalase activity

Aimed to evaluate the protective effect of *B. monnieri* treatment against LPS-induced oxidative damage, we analyzed the lipid peroxidation measuring TBARS levels in the prefrontal cortex. Acute injection of LPS induced an alteration of oxidative stress parameter evaluated as a significantly increased of TBARS levels in comparison to the control groups, *B. monnieri* treatment restored the unbalance as shown in Figure 6a. Moreover, the lipopolysaccharide administration promoted a decrease in the catalase activity that was fully counteracted by *B. monnieri* repeated treatment (Figure 6b).

3.3 | Clinical results

Forty-two patients (Table 1) with anhedonia (SHAPS ≥ 3) and unsatisfactory response to drug therapy (CGI-S ≥ 3 and CGI-I between 3 and 7) were consequently enrolled, a first group (cases) of 19 patients have been prescribed *B. monnieri* extract in 300 mg tablets to be taken twice a day, for 4 weeks of treatment, while the second of 23 subjects (controls) with the same characteristics of the cases, which were treated with citalopram 40 mg (TAU).

In Figure 7, the differences in clinical outcomes post-treatment in the group of patients treated with *B. monnieri* compared to the group treated with citalopram 40 mg (TAU) are reported. Significant differences were measured for HAM-D, SHAPS, and SDQ. Raw data of each group pre- and post-treatment are reported in the Table S1. In the group treated with *B. monnieri*, there was no statistically significant correlation between the scores of both of the CEQ subscales and the T1 outcomes, excluding a possible autosuggestion effect by the patient. In the light of these data, the expectation and credibility in

the treatment of augmentation would not seem to be relevant for the final answer.

4 | DISCUSSION

Considering our initial hypothesis, this study indicates that *B. monnieri* extract can reduce anhedonia-like behavior in animals and anhedonia-related symptoms in humans. In mice, the repeated administration of 100 and 200 mg kg^{-1} extract, dose-dependently, reverted the pathological alterations induced by LPS. Efficacy was highlighted by different paradigms. The forced swimming test induces a "state of despair" that immobilizes the animal in swimming (Carbajal, Ravelo, Molina, Mas, & Arruzazabala Mde, 2009). This test is considered to have proven reliability and validity (Porsolt et al., 1977) for the analysis of antidepressants. The efficacy of Bacopa in this test was confirmed in the similar tail suspension analysis but also in the sucrose preference test that represents the most indicative analysis for anhedonia evaluation (Liu et al., 2018).

Based on the clinical evidence about the involvement of inflammatory processes in the pathogenesis of depression (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008), LPS is one of the most used models of inflammation-associated depression. Behavioral studies in rodents have shown that systemic LPS injection, by a potent activation of the immune response, induces a sickness response, characterized by social withdrawal, fatigue, anorexia, and alterations in sleep patterns and cognition, followed by a behavior similar to clinically relevant symptoms of depression in humans, including anhedonia (Biesmans et al., 2016; Henry et al., 2008; Kang et al., 2011; O'Connor et al., 2009).

The inflammatory response induced by LPS parallels with the production of pro-inflammatory cytokines and the cortisol-dependent depressive-like behavior promoted by chronic stress in laboratory animal (Knapp et al., 2011). Interestingly, treatment with antidepressants, besides normalizing depressive behavior induced by bacterial endotoxin, decreases pro-inflammatory cytokines' level (Castanon, Leonard, Neveu, & Yirmiya, 2002).

In the present results, we showed enhanced brain levels of the pro-inflammatory cytokines IL-1β and IL6 after LPS treatment. Bacopa extract significantly prevented these alterations suggesting anti-inflammatory effects of this product in the CNS. Accordingly, Nemetchek et al. (2017) showed that *B. monnieri*, as well as Bacoside A, significantly inhibited the release of TNF-α and IL-6 from LPS-activated microglial cells.

Moreover, it is known that activation of the immune system causes disruption of the HPA axis, a physiological recurrence often observed in depression (Capuron & Miller, 2011). According to previous results (Goble et al., 2001; Silva & Madeira, 2012), we found that systemic injection of LPS significantly increased brain cortisol levels. In this condition, the treatment with *B. monnieri* extract significantly prevented the HPA axis dysregulation.

In close relationship with the inflammatory state of the CNS, the altered redox unbalance plays a critical role in the pathophysiology of several neuropsychiatric disorders (Floyd, 1999). Brain tissue is particularly exposed to reactive oxygen species since 20% of total body oxygen is normally metabolized by brain in the presence of a limited amount of antioxidant strategies (Floyd & Carney, 1992). In situations where the generation of free radicals exceeds the capacity of antioxidant defense, oxidative stress may lead to membrane degradation, cellular dysfunction, and apoptosis (Ott, Gogvadze, Orrenius, & Zhivotovsky, 2007). Recent studies reported enhanced concentrations of lipid peroxidation products and alterations of the major antioxidant enzymes in patients with affective disorders, in particular with depression (Floyd, 1999; Ozcan, Gulec, Ozerol, Polat, & Akyol, 2004; Sarandol et al., 2007); these features were sensitive to antidepressant therapies (Machado-Vieira, Salvadore, Luckenbaugh, Manji, & Zarate Jr., 2008). *B. monnieri* treatment was able to restore the oxidative damage occurring in the brain of LPS-treated animals, preventing lipoperoxidation and enhancing the activity of the peroxisome-characteristic detoxifying enzyme catalase.

Interestingly, LPS did not alter the concentration of the neuronal factor BDNF but increased levels of ARTN, a factor of glial origin involved in depression signaling (Di Cesare Mannelli et al., 2011). *B. monnieri* treatment increased BDNF (considered as a marker of brain functionality increased after antidepressants treatment; Tsai, Hong, & Liou, 2010) without alter the LPS-induced increase of ARTN suggesting a possible role of these factors, and the consequent cellular plasticity, in the anti-anhedonic effect of the extract. On this base, the extract of *B. monnieri* was clinically tested. Subjects were evaluated by the MINI (Sheehan et al., 1998) to have a diagnosis standardized according to the DSM 5.0 criteria. After treatment, the group treated with *B. monnieri* extract showed significant lower value of SHAPS and HAM-D in comparison to TAU group.

Despite the exploratory characteristics of this study (it is an open study with a low number of samples), various corrective measures have been done to validate it. In order to characterize the study sample, a descriptive statistical analysis was performed, in particular for the categorical variables study the chi-square test was used, while the *t*-test was used for the continuous variables. From the first analysis, the two groups were found to be homogeneous for almost all the clinical variables considered, with the exception of CGI3 ($p < .01$).

To compensate for the lack of a placebo-controlled study, considering the probability of autosuggestion of the patients treated with "natural medicine," it was decided to provide a scale to measure that variable to the base line in order to limit its potential confusing effect. For this purpose, the credibility/expectancy questionnaire (CEQ), developed by Devilly and Borkovec (2000). The credibility of the treatment refers to how credible, convincing and logical the treatment seems to the patient, while the expectation of treatment refers to the actual improvement that the patient feels will be achieved by him/her personally. The CEQ showed a high internal consistency and good test–retest reliability.

It has been postulated that credibility with regard to treatment and expectation may be modifiable during treatment. It is also likely that expectation is a stronger predictor of the treatment outcome than its credibility (Goossens, Vlaeyen, Hidding, Kole-Snijders, &

Evers, 2005). A critical issue of the studies in literature is that they have only evaluated the pre-treatment phase immediately prior to the onset of therapy, which means that patients may have difficulty in judging what outcome they might achieve. It is to be specified, therefore, that in our study the CEQ was completed immediately after the patient had obtained the necessary treatment explanations, so that the patient was able to judge the credibility of the treatment and mature one's expectation.

Devilly and Borkovec argued that even if a patient thinks a new treatment is credible, this may differ from what he really feels. The authors hypothesized that expectation may be more related to an emotional aspect than the credibility that is most based on cognitive elements, and therefore may have a higher level of association with the final outcome of treatment, at least in Psychotherapy (Devilly & Borkovec, 2000). The authors argue that the sub-scale of credibility is more closely related to the logical thinking processes of patients, while the subscale of expectation ("how much is really expected to improve?") is functionally more linked to affective processes similar to those involved in hope or faith. Our study, based on the difference in the magnitude of R2's change for expectation and credibility, did not find clear indications for their hypothesis.

In conclusion, we evaluated a possible effect of a standardized extract of *B. monnieri* on positive symptoms, especially anhedonia, regardless of categorical diagnosis. The hypothesis of a possible therapeutic application of the botanical on the specific size was initially tested in an animal model that reflected the theoretical construct, targeting specific neurocircuits. The results of the preclinical phase were the basis on which to build the next clinical trial. The significant positive impact of *B. monnieri* treatment in patients was demonstrated. These results reflect the opportunities in applying the RDoC concept to clinical research, and it would of interest to investigate the clinical effect of *B. monnieri* on the same dimension, anhedonia, in clinical condition different from depression such as negative symptoms in schizophrenia. A harmonization of the models is still needed to effectively isolate the symptoms of "negative valence" in depression and other mental illnesses, and advances their translation to treatment. In particular in the field of Complementary Therapy (CAM), an increasingly evolving field in terms of clinical use but poorly of valid scientific evidence and studies conducted with rigorous scientific criteria according with current neuroscience research. Our work, although it presents all the limits of an exploratory study, may serve as a procedural model for other neurobiological domains, creating the bases for producing a more rigorous and translator clinical research in the field of integrative medicine.

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CONFLICT OF INTEREST

The authors declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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