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Neuroprotective and anxiolytic effects of *Matricaria chamomilla* ethanolic extract against harmine-induced anxiety and oxidative stress in rats exposed to forced swimming stress

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Abstract: The global rise in anxiety and depression disorders has drawn attention to compounds like harmine, a *Peganum harmala* alkaloid, which induces a pronounced anxiety response. Herbal treatments exhibit the potential to relieve such symptoms with minimal or no side effects. This study aimed to investigate the behavioral, neurobiological, and protective effects of the ethanolic extract of *Matricaria chamomilla* (EEC) in rats previously exposed to harmine and subjected to a forced swimming stress protocol. Six groups (n=8) of *Wistar* rats were formed as follows: group 1 (control); group 2 that received a single injection of harmine (40 mg/kg); groups 3 and 4 received 200 mg/kg or 500 mg/kg EEC, respectively; groups 5 and 6 received an injection of harmine (40 mg/kg) with administration of 200 mg/kg or 500 mg/kg EEC). Rats underwent behavioral testing alongside biochemical and histological analyses. Oxidative-stress markers were quantified, including malondialdehyde (MDA), glutathione peroxidase (GPx), glutathione S-transferase (GST), reduced glutathione (GSH), and acetylcholinesterase (AChE) activity. The EEC extract ameliorated depression-like behavior by counteracting harmine-induced reductions in locomotor activity, environmental exploration, and memory performance. It also lowered circulating adrenocorticotropic hormone (ACTH) levels and restored antioxidant enzyme activity, reflected by reductions in brain oxidative-stress markers. The findings indicate that oral EEC supplementation may enhance exploratory behavior, suggesting a potential neural mechanism for mitigating depressive states. Its modulation of brain oxidative-stress responses points to a possible prophylactic effect, supporting further investigation of EEC as an adjunctive therapeutic candidate.

Keywords: harmine, ethanolic extract, anxiety disorders, adrenocorticotropic hormone (ACTH), *Matricaria chamomilla*

INTRODUCTION

Psychiatric diseases are prevalent mental illnesses typified by extreme future-focused anxiety, intense sadness, and a loss of interest in routine activities [1]. Among these disorders, anxiety represents a stress condition that disturbs homeostatic balance and elicits physiological and psychological reactions [2]. Anxiety disorders (AD) affect 4.4% of people worldwide according to the World Health Organization [3]. Physiological responses to anxiety involve the activation of two main stress pathways: the sympatho-adrenomedullary (SAM) axis, responsible for the rapid release of epinephrine and norepinephrine from the adrenal medulla and sympathetic nerves, and the hypothalamic-pituitary-adrenal (HPA) axis, leading to a slower response through corticotropin-releasing hormone (CRH) release from the hypothalamus, stimulating adrenocorticotropic hormone (ACTH) secretion from the anterior pituitary and glucocorticoid production from the adrenal cortex [4]. These substances exert an inhibitory effect on serotonergic receptors in the amygdala and hippocampus, thereby increasing vulnerability to anxiety reactions. Data in AD suggest that oxidative stress, characterized as an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defence system [5], plays a major role in the development and progression of the disorder. Prolonged exposure to stress further increases ROS production, leading to neuronal damage and neurochemical disturbances [6]. Such alterations in oxidative and neurotransmitter balance represent the underlying

mechanism of anxiety, which is primarily associated with dysregulation of γ-aminobutyric acid (GABA), serotonin, noradrenaline, and dopamine systems. Among the bioactive compounds known to influence these pathways, Harmine, the main β-carboline alkaloid in *Peganum harmala*, has attracted attention due to its interactions with several types of receptors, including 5-hydroxytryptamine 2A (5-HT_2A) and 2C (5-HT_2C) receptors, as well as the induction of serotonergic side effects such as hypothermia, hallucinations, and tremors [7].

The herbal extract field has gained popularity for enabling the implementation of multicomponent approaches in preventing and treating various diseases [8]. *Matricaria chamomilla* (German chamomile) is a widely recognized medicinal plant from the Asteraceae family, native to southern and eastern Europe. According to earlier research, *M. chamomilla* demonstrates significant neuroprotective and anxiolytic properties through multiple mechanisms. The hydroalcoholic extract exhibits anxiolytic and antidepressant effects in scopolamine-induced rat models, with doses of 25-75 mg/kg effectively reducing anxiety and depressive-like behaviors [9]. These effects are mediated through modulation of cholinergic activity, neuroinflammation reduction, and enhanced antioxidant action in the hippocampus [10]. The extract contains bioactive compounds, including chlorogenic acid, apigenin-7-glucoside, rutin, and luteolin, which contribute to memory enhancement and restoration of brain-derived neurotrophic factor (BDNF) expression while reducing IL1β levels [10]. Chamomile's antioxidant properties are further demonstrated through protection against oxidative stress [11-12].

This study investigated the potential preventive role of *M. chamomilla* extract against harmine-induced oxidative and anxiogenic effects in rats. Using behavioral, biochemical, and histological analyses, the study evaluated whether chamomile could counteract harmine-induced disturbances in oxidative balance, neuroendocrine function, and anxiety-related behavior. The results confirm this objective, demonstrating the therapeutic potential of chamomile for neurological and psychiatric disorders.

MATERIALS AND METHODS

Ethics statement

All procedures involving animals were conducted in strict accordance with the guidelines for the care and use of laboratory animals of the Algerian Institutional Ethical Committee for Animal Research under agreement number 45/DGLPAG/DVA/SDA/14, issued by the General Directorate of Agricultural and Genetic Production Laboratories, Directorate of Veterinary Services, and Sub-Directorate of Animal Health on December 18, 2014.

Chemicals and extract

The harmine (7-methoxy-1-methyl-β-carboline) was purchased from Sigma-Aldrich, which is based in St. Louis, MO, USA (purity>98%; Cat. No. 286044; CAS No. 442-51-3; Aldrich, USA; Lot No. 101583680). The product is dissolved in a solution that contains 2% acetic acid (AcCOOH) and 0.9% sodium chloride (NaCl). Selected experimental groups received an intraperitoneal (i.p.) injection of 1 mL harmine solution. The control group received a 4 mL/kg injection of distilled water.

Plant identification and extraction

Matricaria chamomilla is an aromatic annual plant that grows to a height of 60 cm. It belongs to the Asteraceae family and has fragrant white flowers and feathery leaves [13]. The whole plant of *M. chamomilla* was obtained in March from a local herbalist in Annaba, Algeria (No. YO733DB0IG3N0NAFAMZ). Prof. Rebbas Khellaf, a taxonomist in the Department of Biology, University of M'sila, Algeria, identified the plant. The laboratory archived a voucher specimen under (No. 012022). In a flask, 100 g of *M. chamomilla* flower powder was macerated in 1 L of 70% ethanol for 24 h, with shaking at room temperature. The extracted solvent was filtered and removed using a rotary evaporator under vacuum at 45°C. The solvent was dried in an oven at 46°C, and the total yield of the ethanolic extract of *M. chamomilla* (EEC) was 18.11%. The extract was then stored at 4°C and reconstituted with distilled water to obtain different doses for treating the animals.

Animals and experimental design

The study included 48 male Wistar rats, weighing 220-260 g, obtained from the animal house of the Pasteur Institute of Algiers. The animals were maintained in a controlled environment with a 12-h light-dark cycle at constant temperature (20-25°C) and humidity (45-55%) at the UBMA University animal facility. They had unlimited access to tap water and standard rat food for the two-week adjustment period. Six groups were created after the animals had been acclimated for 14 days (n=8 per group). The control group was injected with 1 mL of distilled water intraperitoneally on day 1 and orally from day 11 to day 21. The harmine-treated group received a single intraperitoneal (i.p.) dose of harmine (40 mg/kg) on day one. Two additional groups were given oral doses of *M. chamomilla* ethanolic extract at 200 mg/kg or 500 mg/kg daily from day 11 to day 21 and were designated as EEC200 and EEC500, respectively. Finally, two combined-treatment groups received harmine (40 mg/kg, i.p.) on day 1, followed by oral administration of *M. chamomilla* extract (200 mg/kg or 500 mg/kg) during days 11-21, identified as H+EEC200 and H+EEC500, respectively. The selected harmine dose (40 mg/kg) is equivalent to 1/5 of its reported LD_{50}, as established by [14]. The ethanolic extract dosages of *M. chamomilla* were determined based on the experimental design suggested in[15].

Protocol on swimming-related stress

This test was performed between days 5 and 10 of the study. Rats were forced to swim for 20 min in a cylindrical tank filled with water that was 35 cm deep (out of 40 cm) and low enough to prevent the rats from drowning. The water temperature was maintained at about 25°C to prevent heat effects. Rats were dried with a towel and returned to their cages following each experiment [16].

Behavioral tests (OF, EPM)

Anxiety-related behaviors were measured using the open field test (OF) followed by the elevated plus maze (EPM) on days 4 and 21 of the experiment to examine the effects of the treatment on locomotor activity, learning ability, and memory performance. To ensure experimental consistency, all groups were tested on the same day between 9:00 a.m. and 5:00 p.m., and behavioral observations were manually recorded using a digital camera (Samsung HMX-F90, Algeria). Data analysis was conducted with investigators blinded to group assignments.

Open field test

Rats were measured for mobility, exploration, and anxiety using an open-field test [17]. The arena is made up of 2 zones: the center and peripheral zones, and a square base 70×70 cm in diameter that is encircled by 40 cm high Plexiglas parapets. The test took 5 min. The animal was placed in the middle of the field. Quantitative parameters were recorded, including the time spent in the center, the time spent in the corners, immobility time, and the number of crossings (total distance). After every recording, the field is cleaned with a 70° alcohol solution to remove residual odors.

Elevated plus maze

The maze consisted of two open arms (50×10 cm) perpendicular to two closed arms (50×10×40 cm) and a small central square between the arms. The maze was raised 50 cm above the floor. Each rat was positioned individually in the middle of the labyrinth, facing an open arm, and allowed to spend 5 min. The number of entries into open and closed arms, the times spent in open and closed arms, and the time and number of rearing (hind limb standing) were recorded. The arena was cleaned with 70% alcohol following every rat trial [18].

Biochemical assays

Following behavioral testing, on day 22 of the trial, each rat was humanely euthanized by cervical dislocation. Blood was collected from the retro-orbital sinus of the animals into ethylenediaminetetraacetic (EDTA) tubes and immediately centrifuged (3000×g, 15 min, 4°C) to obtain plasma, which was then stored at -20°C to analyze adrenocorticotropic hormone (ACTH). The rat brain and adrenal glands were then rapidly removed and weighed after washing with 0.9% NaCl solution. The brain was then cut lengthwise into 2 equal slices: 1 slice was submerged in a 10% formaldehyde solution for histological analysis. The 2nd slice was kept at -20°C to assess the oxidant/antioxidant status. The adrenal glands were prepared for histological examination.

Adrenocorticotropic hormone assay

Adrenocorticotropic hormone (ACTH) levels were measured using a commercial ACTH assay kit with an automated analyzer supplied by BioSystems S.A. (Costa Brava, Spain). The kit is based on a sandwich immunoradiometric technique that uses sACTH, a modified version of ACTH generated via succinylation [19]. The assay components included sodium acid pyrophosphate buffer, anti-sACTH monoclonal antibodies, anti-sACTH tracer, succinic anhydride, dimethylsulfoxide (DMSO), and wash solution. The concentration of ACTH was determined and reported in pg/mL.

Tissue preparation

One g of brain was homogenized in 3 mL of phosphate-buffered saline (PBS) (pH 7.4) and centrifuged (9000 ×g, 15 min, 4°C). The resulting supernatant was used to determine the malondialdehyde (MDA), reduced glutathione (GSH), total proteins, and the enzymatic activity of glutathione peroxidase (GPx) and glutathione- S- transferase (GST).

Estimation of the oxidant/antioxidant status of tissues

The level of GSH in the brain was determined using the method described in [20]. The tissue homogenate was mixed with 0.2 mL of 0.25% sulfosalicylic acid (SSA) for deproteinization. After 15 min in an ice bath, the mixture was centrifuged (1000 ×g, 5 min), and 0.5 mL of the supernatant was mixed with 1 mL of Tris-buffered saline (TBS) (0.4 M Tris, 0.02 M EDTA, pH 9.6) and 0.025 mL 5-5'-dithio-bis 2-nitrobenzoate (DTNB; 0.01 M). The absorbance was measured at 412 nm. The results were expressed in mmol/mg protein. The level of MDA was measured according to the method of [21]. Briefly, 0.5 mL of tissue homogenate was mixed with 0.5 mL of 20% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid (TBA). The mixture was incubated in a water bath (100°C, 15 min), then cooled and supplemented with 4 mL of n-butanol. After centrifugation (3000 ×g, 15 min), the supernatant was collected, and the absorbance was measured at 530 nm, and expressed in nmol/g tissue. The total protein content was quantified using the Bradford method [22], with Coomassie Brilliant Blue G-250 as the staining reagent and bovine serum albumin as the reference standard.

Estimation of antioxidant enzymatic activity

Glutathione peroxidase (GPx) activity was measured using the method of [23]. Briefly, 1 mL of the reaction mixture was prepared to contain 0.2 mL TBS (50 mM Tris, 150 mM NaCl, pH 7.4), 0.4 mL of glutathione (GSH; 0.1 mM), 0.2 mL of H_2O_2 (1.3 mM), and 0.2 mL of tissue supernatant. After incubation (37°C, 15 min), the reaction was stopped by adding 1 mL 1% TCA. The tubes were centrifuged (1500 ×g, 10 min) and the supernatant was collected. The reaction supernatant (0.48 mL) was mixed with 2.2 mL TBS and 0.32 mL of DTNB (0.4 mg/mL). The absorbance was measured at 420 nm, and results were expressed as µmol GSH/mg protein. GST activity was determined according to the method of [24]. The reaction mixture contained 830 µL of PBS (pH 6.5), 50 µL of 1-chloro-2,4-dinitrobenzene (CDNB; 0.02 M), 100 µL of GSH (0.1 M), and 20 µL of the brain supernatant. The change in absorbance was recorded at 340 nm for 5 min, and enzyme activity was expressed as nmol GSH-CDNB/mg protein.

Measurement of acetylcholinesterase activity (AChE)

Brain homogenate was made by finely reducing 100 mg of brain in 1 mL of PBS (pH 7.4) under freezing conditions. The method by which enzyme activity is measured is explained by [25]. The technique uses a 0.1 M phosphate buffered saline (PBS) pH 7.4 solution of acetylthiocholine (ACh) and 50 µL Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB), based on the reaction between acetylcholinesterase (AChE) present in the tissue fraction and acetylthiocholine (ACh), as well as the development of a yellow color that absorbs at 412 nm. The total AChE content was expressed as nmol/min/mg protein.

Statistical analysis

The data were reported as the mean±standard error of the mean (SEM) per animal with n=8 and analyzed using the F-test (one-way ANOVA) after verifying normality and homogeneity of variances, followed

Table 1. Body weight gain and absolute weights of the adrenal glands and brain in control and experimental groups over 21 days

Parameters	Control	H	EEC200	EEC500	H+EEC200	H+EEC500
Weight gain (g)	16.37±1.73	48.5±11.04	7.75±1.92$^{\#\#}$	68.12±6.93***	70.25±12.66***	59.37±6**
Absolute brain weight (g)	1.54±0.08	1.45±0.04	1.29±0.04	1.55±0.05	1.55±0.02	1.38±0.08
Absolute adrenal weight (g)	0.04	0.04±0.004	0.04±0.001	0.03 ± 0.004	0.4 ± 0.004	0.03 ± 0.001

Data are expressed as the mean±SEM, n=8 in each group. **P<0.01, ***P<0.001/control group. $^{\#\#}$P<0.01/harmine (H) group

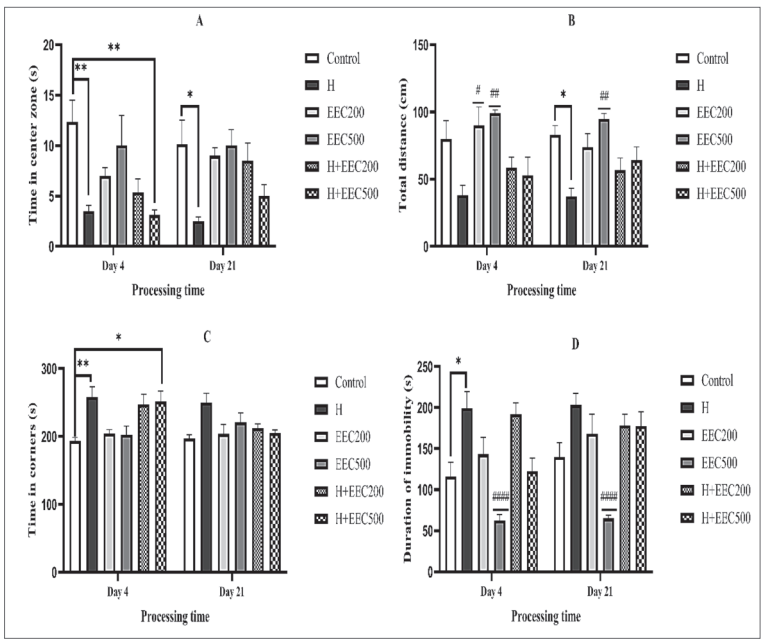

Fig. 1. Effect of treatments on the open field test (OF) anxiety parameters before and after stress induction: **A** – time spent in the center zone (s); **B** – total distance (cm); **C** – time spent in corners (s); **D** – duration of immobility (s). Data are presented as the mean±SEM (n=8). *P< 0.05, **P<0.01/control group, $^{\#}$P<0.05, $^{\#\#}$P<0.01,$^{\#\#\#\#}$P<0.0001/harmine (H) group.

by Tukey's post hoc test for pairwise comparisons when the ANOVA yielded significant results. Graphs were prepared using GraphPad Prism ver. 9 statistical software, and differences were considered statistically significant at P<0.05. Comparisons were performed only between the control group (*) and the harmine-treated group (#); no statistical analysis was conducted for the combined treatment group.

RESULTS

All treatment groups did not exhibit signs of intoxication or mortality during the study, except for the H group, which exhibited hypersalivation, tremors, decubitus, and rapid breathing for 2 h after intraperitoneal injection.

Body weight gain, absolute organ weights

Body weight did not significantly differ between the H and control groups over the 21 days. The EEC500 and H+EEC groups exhibited substantial recovery when compared to the control group (P<0.001). However, the EEC200 group showed lower body weight than the H group (P<0.01). These findings highlight that harmine caused transient toxicity signs, while *M. chamomilla* extract improved body weight gain in a dose-dependent manner. No weight change was observed in the absolute weights of the brain and adrenal glands between any treatment group (Table 1).

Effect of treatments on the open field test (OF)

Rats treated with H showed decreased locomotor activity and anxiety-like behavior, which was reflected in a significant reduction in time spent in the central area before (P<0.01) and after (P<0.05) stress induction compared to the control group, as well as by prolonged time spent in the corners and in the immobilization posture before but not after stress induction (Fig. 1C-D). A significant improvement was observed in the EEC and H+EEC groups, where the number of crossing and immobilization postures was reduced compared to the H group (Fig. 1B-D).

Effect of treatments on the elevated maze test (EPM)

H treatment significantly decreased the time spent and the number of entries in the open arms before and after stress (P<0.05) (Fig. 2A-B), while it prolonged the

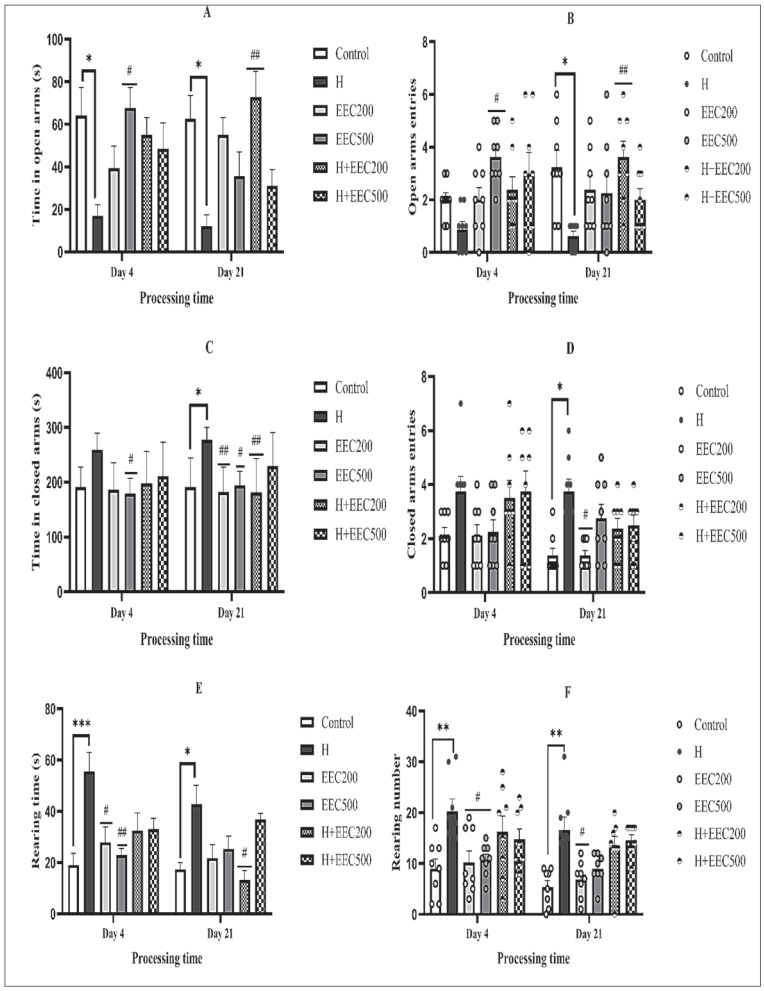

Fig. 2. Effect of treatments on exploratory behavior indicators in the EPM test before and after stress induction: **A** – time spent in open arms (s); **B** – open arms entries; **C** – time spent in closed arms (s); **D** – closed arm entries; **E** – rearing time (s); **F** – rearing number. Data are presented as the mean±SEM (n=8). *P<0.05, **P<0.01, ***P<0.01/control group, $^{\#}$P<0.05, $^{\#\#}$P<0.01/harmine (H) group.

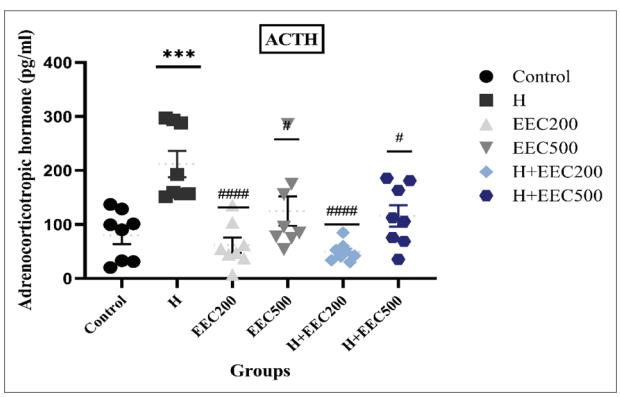

Fig. 3. Treatment effects on serum adrenocorticotropic stress hormone levels (ACTH). Data are presented as the mean±SEM (n=8). ***P<0.001/control group, $^{\#}$P<0.05, $^{\#\#\#\#}$P<0.0001/harmine (H) group.

duration and frequency of entries into the closed arms (P<0.05) and rearing behavior (P<0.01) compared to the control group (Fig. 2C-F). In contrast, EEC extract administration alone or in combination with H restored exploratory behavior by reversing harmine-induced alterations in anxiety-related parameters (Fig. 2A-F).

Treatment effects on serum adrenocorticotropic stress hormone levels (ACTH)

Fig. 3 highlights a significant increase in ACTH levels in the H-treated group compared to the control group (P<0.001). Oral administration of EEC at doses of 200 mg/kg and 500 mg/kg in H-treated animals significantly reduced plasma cortisol levels (P<0.0001 and P<0.05, respectively). Among the doses tested, 200 mg/kg of EEC extract was found to be the most effective, producing significantly greater reductions in stress response compared to the H-treated group.

Treatment effects on brain GPx, GST, GSH, and MDA

As presented in Fig. 4, the H-treated group exhibited brain oxidative stress, as evidenced by significantly lower levels of GPx (P<0.01) and GSH (P<0.001) compared to the control group. The MDA level of the H-treated group was significantly higher (P<0.001) than that of the normal control group. Furthermore, supplementation with 200 mg/kg and 500 mg/kg of EEC extract (H+EEC200) and (H+EEC500) significantly attenuated these adverse effects, resulting in increased GPx, GST, and GSH activity, as well as a marked reduction in MDA levels compared to the H group (P<0.0001). The 500 mg/kg dose was particularly effective, showing significant improvements in oxidative stress markers.

Brain AChE activity

AChE activity levels were considerably lower (P<0.01) in the H-treated group than in the control group, which suggests a disruptive influence on cholinergic balance. Comparing H and H+EEC groups revealed that treating rats with *M. chamomilla* significantly

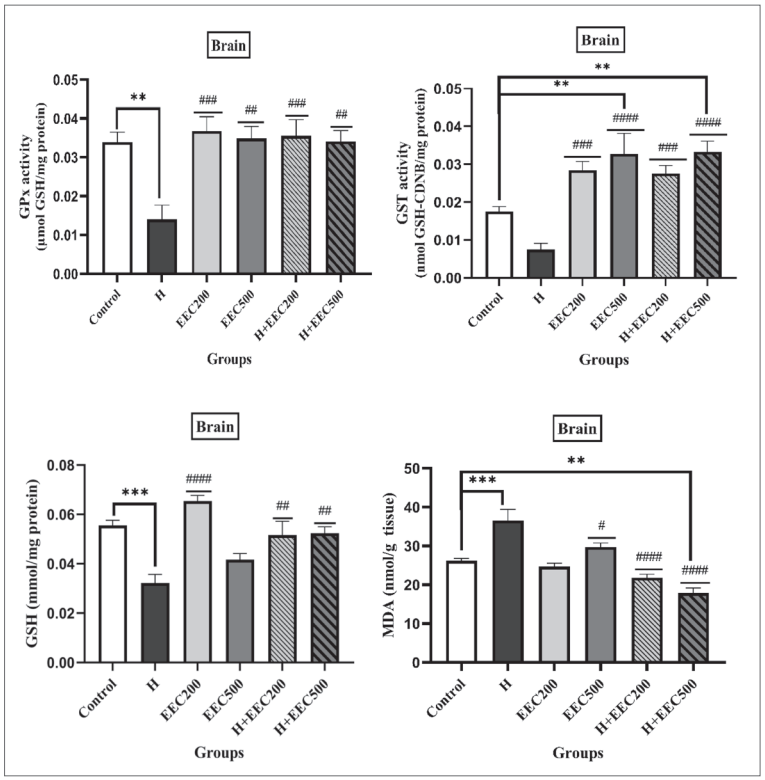

Fig. 4. Treatment effects on brain tissue, glutathione peroxidase, glutathione S-transferase, reduced glutathione, and malondialdehyde. Data are presented as the mean±SEM (n=8). **P<0.01, ***P<0.001/control group, $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001, $^{\#\#\#\#}$P<0.0001/harmine (H) group.

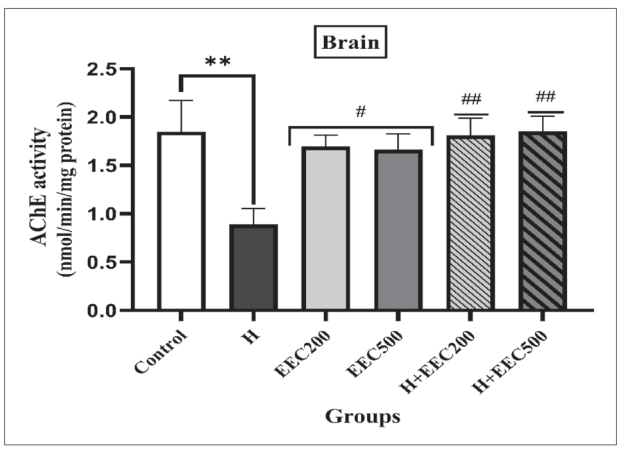

Fig. 5. Brain acetylcholinesterase activity (AChE). Data are presented as the mean±SEM (n=8). **P<0.01/control group, $^{\#}$P<0.05, $^{\#\#}$P<0.01/harmine (H) group.

reversed the harmine-induced decrease in AChE activity in the brain (P<0.01) (Fig. 5).

Histopathological examinations

Histological examination revealed that H-treated rats maintained preserved hippocampal and cortical architectures comparable to the controls. Both the control and EEC-treated groups (EEC200 and EEC500) exhibited intact lateral ventricles, ependymal layers, and choroid plexus structures, indicating protection against harmine-induced damage. Similarly, cerebellar sections from EEC co-treated rats showed well-organized cellular layers, supporting the restorative effect of chamomile extract (Fig. 6A-L).

Histological observation of the adrenal glands showed well-preserved cortical and medullary structures across all groups. In H-treated rats, the zonation of the adrenal cortex and the general architecture of the gland appeared intact, with no major degenerative changes. Similarly, the groups co-treated with *M. chamomilla* extract (EEC200 and EEC500) displayed normal cortical and medullary morphology, comparable to the controls, confirming the absence of structural alterations or cellular disorganization (Fig. 7A–L).

DISCUSSION

Anxiety and depression are closely linked to disturbances in neurochemical signaling. These conditions involve altered monoamine pathways and oxidative imbalance, which disrupt emotional regulation and increase vulnerability to stress. Even though there are many drugs available to treat anxiety, they frequently have negative side effects such as hypotension, sleeplessness, and sexual dysfunction. As a result, there is much greater interest in therapeutic alternatives, such as complementary treatments. In the present research, we investigated the protective effects of several dosages of the EEC on body weight, neurobehavioral scores, and biochemical and histological markers in

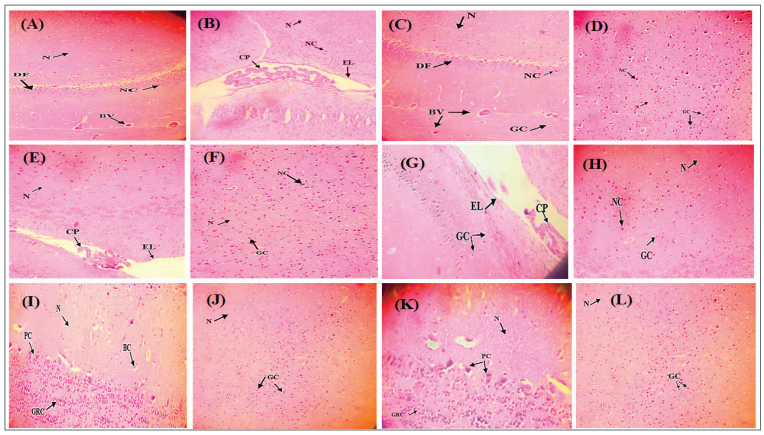

Fig. 6. Histopathological examinations using H&E staining (10X, 20X, 40X) of brain tissue sections from *Wistar* rats that served as controls (**A**-**B**), rats injected with harmine (**C**-**D**), rats treated with EEC 200 (**E**-**F**), EEC500 (**G**-**H**), the H+EEC200 group (I-J), and the H+EEC500 group (K-L). Areas of the lateral ventricle (**B**-**E**-**F**-**G**-**H**), cerebral cortex (**D**), hippocampal region (**A**-**C**) and the cerebellum region (**I**-**J**-**K**-**L**). Arrows point to: neuropil (N), neuronal cells (NC), glial cells (GC), blood vessel (BV), dentate fascia (DF), choroid plexus (CP), ependymal layer (EL), Purkinje cells (PC), basket cells: neuron (BC), granule cells (GRC).

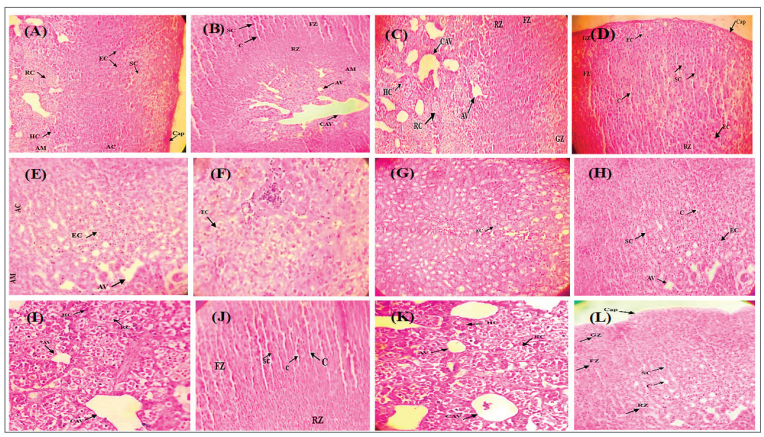

Fig. 7. Histopathological examinations using H&E staining (10X, 20X, 40X) of adrenal gland tissue sections from *Wistar* rats used as controls (**A**-**B**), rats injected with harmine (**C**-**D**), rats treated with EEC200 (**E**-**F**), EEC500 (**G**-**H**), the H+EEC200 group (**I**-**J**), and the H+EEC500 group (K-L). Arrows point to: capsule (Cap), adrenal cortex (AC), adrenal medulla (AM), glomerular zone (GZ), fascicular zone (FZ), reticular zone (RZ), capillaries (C), endocrine cells (EC), spongiocytic cells (SC), rhagiochromic cells (RC), haylochromic cells (HC), adrenal veins (AV), central adrenal veins (CAV).

rats administered an alkaloid in a FSS model of depression. Based on the available literature, no earlier investigations have reported on the combined effects of EEC and harmine. Our findings highlight the marked anxiolytic potential of the EEC at a dose of 200 mg/kg, as evidenced by the reduction of depressive-like behaviors in treated animals. This protective effect appeared to be independent of the extract's metabolic constituents. The EEC exerted a clear regulatory influence on the oxidative balance, as shown by the enhancement of key antioxidant enzyme activities and the consequent reduction in oxidative stress levels.

The experimental groups did not present any mortality. However, tremors and decreased locomotor and physical activity in rats were observed after intraperitoneal injection of harmine at a dose of 40 mg/kg. These observations suggest that harmine has significant secondary effects on the central nervous system, which is consistent with the findings described in [15], where administration of a total extract of *P. harmala* alkaloids induced tremors, a reduction in daily activity, and temporary paralysis. However, given that harmaline caused tremors [26], the tremorgenic effects of β-carboline may result from the excitability of the central nervous system, progressing to muscle rigidity. Earlier studies [27] have shown that β-carbolines can cause tremor via lowering gamma-aminobutyric acid (GABA). Previous research has shown that β-carbolines, including harmaline, cause impairments to rat motor and cognitive abilities [7]. The present study showed that the administration of harmine induced anxiety-like and hypoactive behaviors in the EPM and OF tests. Rats receiving harmine exhibited a marked preference for closed and peripheral areas, accompanied by reduced exploration and mobility, as evidenced by reduced transition to open or central areas and increased immobility. This result can be explained by its impact on monoamine (MA) levels in the brain and on GABAergic modulation, where reduced GABA activity decreases central inhibitory regulation in important areas of the brain, particularly the amygdala and hippocampus, and enhances anxiety responses. Our findings are consistent with those of [27], who found that rats given harmaline showed a significant motor deficit in the OF. In addition, the administration of harmane increased the duration of immobility [28], which explains the sedative properties of this substance. It has been shown that administration of harmaline decreased the distance traversed by rats in the OF test [7]. Previous studies have reported that harmaline interacts with 5-HT_2A

receptors, contributing to its psychostimulant and anxiogenic effects [29]. The role of 5-HT_2A in anxiety is well known, as its activation is associated with increased excitability and anxiogenic behavioral responses [30]. β-carbolines act as a reversible inhibitor of MAO-A, thereby modifying serotonergic neurotransmission [31], which corroborates the neurochemical basis of the anxiety-related responses observed in our study. Regarding depression-like responses, the HPA axis plays a central role in stress regulation through the secretion of corticosterone [32]. Herein, harmine produced a further elevation in circulating ACTH levels compared to the control group that was subjected to comparable stress conditions. Similar observations were previously reported, showing increased serum corticosterone after administration of the β-carboline harmane at 10 mg/kg [28]. Moreover, it has been shown that harmine can cross the blood–brain barrier [33], which may enhance the sensitivity of the HPA axis to stress-related stimuli [32].

Administration of *M. chamomilla* to rats exposed to harmine reversed anxious behavior. Open-arm explorations performed during the EPM test and the shorter immobility period both support this observation. This suggests that the chamomile treatment improved the locomotor activity by increasing the distance covered by the rats during the OF test. This improvement is mainly attributed to its bioactive flavonoids, particularly apigenin, which is known to improve motor deficits by interacting with various neurotransmission systems [34]. Additionally, chamomile extracts have been shown to influence the serotonergic system by regulating tryptophan metabolism, a precursor of serotonin, and to promote serotonin availability, contributing to emotional stabilization [35]. Our findings are in agreement with [36], which reported that chamomile improves locomotor activity by increasing tryptophan availability and reducing HPA axis activation, thereby restoring normal serotonergic signaling and lowering stress responses. In parallel, chamomile supplementation in our study also normalized ACTH levels. This effect may be attributed to its bioactive terpenoids, which have been shown to modulate neuroendocrine function and limit the release of stress-related hormones by reducing hypothalamic corticotropin-releasing factor (CRF), ultimately preventing excessive cortisol and ACTH secretion [33,37].

The brain is particularly vulnerable to oxidative damage due to its high content of polyunsaturated fatty acids, high oxygen demand, and relatively low antioxidant capacity [38]. Oxidative stress is also recognized as one of the early events contributing to anxiety and cognitive impairment through the activation of intracellular stress signaling pathways [39]. In this regard, our results suggest that harmine disrupted the redox balance, as indicated by elevated MDA levels and reduced major antioxidant defences (GSH, GPx, and GST). Harmine has been reported to undergo a redox cycle in mitochondria, promoting the formation of reactive oxygen species (ROS) and impairing mitochondrial electron transport [29,40]. Meanwhile, harmine inhibits monoamine oxidase A (MAO-A), which increases serotonin turnover and leads to the production of hydrogen peroxide (H_2O_2), further contributing to oxidative stress [31]. The resulting ROS overload consumes reduced glutathione (GSH) and decreases the activities of GPx and GST, which explains the significant depletion of antioxidant reserves observed in our study. Harmine-induced oxidative stress can also disrupt cholinergic transmission. The accumulation of ROS alters neuronal membrane integrity, impairing acetylcholine release and leading to compensatory changes in AChE activity [38,39]. Therefore, the variation in AChE observed in our study appears to be an indirect consequence of harmine-induced redox imbalance, which is consistent with reports that β-carbolines alter synaptic function through oxidative mechanisms [41]. In contrast, the absence of changes in organ weight or histological architecture indicates that harmine mainly caused functional (neurochemical and oxidative) disturbances rather than structural damage, likely due to the single-dose protocol allowing tissue recovery over time. This outcome is in line with other studies that found no change in body weight following the administration of three different dosages of harmine [42]. Treatment with *M. chamomilla* significantly reduced MDA levels in rats exposed to harmine, indicating a restoration of redox balance. This effect can be attributed to the plant's high content of flavonoids, polyphenols, and phenolic acids, that has proven powerful anti-free radical properties and support endogenous antioxidant defenses [34]. These bioactive compounds enhance the activity and synthesis of key antioxidant enzymes such as GSH, GPx, and GST, which were

depleted after harmine administration. Among these constituents, chlorogenic acid plays a central role in protecting cell membranes from lipid peroxidation and modulating GST-dependent glutathione signaling [34]. Furthermore, it has been reported that *M. chamomilla* influences the expression of antioxidant-related genes, thereby enhancing enzymatic defense capacity and reducing oxidative damage. The phenolic profile of the extract also contributes to the neutralization of H_2O_2, as previously demonstrated [13], providing an additional cytoprotective mechanism [43]. Consistent with this reparative biochemical effect, no histological alterations were observed in organ tissues, and treated rats maintained a stable physiological state. Furthermore, the extract's richness in flavonoids and polyphenols promotes metabolic regulation, as indicated in [15], which explains the marked improvement in body weight and stress tolerance in the chamomile-treated group.

In this study, *M. chamomilla* appeared to influence multiple neurobiological pathways and may represent a natural complementary approach to conventional anxiolytic treatments. While standard medications such as benzodiazepines and antidepressants are effective in managing acute anxiety, their long-term use can be restricted by tolerance, dependence, and adverse side effects. In this context, the anxiolytic and antioxidant properties of chamomile suggest a potential role as an adjunctive or alternative option, particularly for individuals seeking plant-based therapies. However, current evidence regarding its anxiolytic efficacy remains limited. Additional studies are still needed to clarify its underlying molecular mechanisms, determine optimal dosing strategies, and evaluate its long-term safety and effectiveness in clinical applications.

CONCLUSION

We report that harmine administration induced anxiety-like behavior and disrupted the cerebral redox balance, as evidenced by increased lipid peroxidation and reduced antioxidant capacity. Treatment with the *M. chamomilla* ethanolic extract, either preventively or curatively, significantly ameliorated these alterations by restoring oxidative defense markers and improving behavioral performance. These effects are likely related to the extract's high content of flavonoids and phenolic compounds that possess antioxidant and neuroprotective properties. A limitation of this study is the absence of a detailed phytochemical characterization of the *M. chamomilla* extract. While the presented findings suggest a potential role for *M. chamomilla* in mitigating harmine-associated oxidative stress and anxiety-like responses, further investigations, including extract standardization and broader dose-response analyses, and molecular pathway studies, are required to confirm these outcomes and clarify the underlying mechanisms.

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Conflict of interest disclosure: The authors declare no conflicts of interest.

Data availability: The data supporting this article are available in the online dataset: https://figshare.com/s/0b77264a41a5b6a04271

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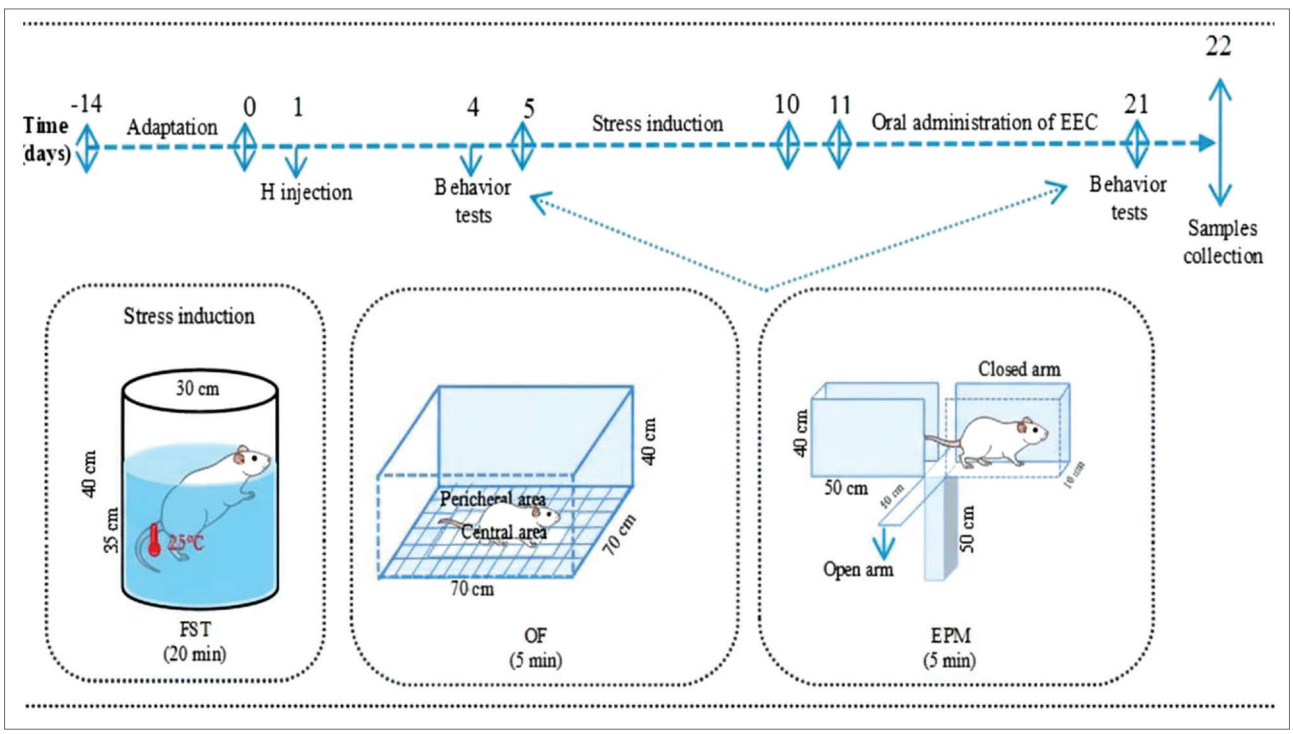
SUPPLEMENTARY MATERIAL



Supplementary Fig. S1. Schematic timeline of the experiment: two behavioral observations were recorded as follows: on the 4th day of the experiment and on the 21st day at the end of the experiment. FST – forced swim test; OF – open field; EPM – elevated plus maze.