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Eucalyptus alba Reinw Ex. Blume (Myrtaceae) extracts mitigate behavioral changes, neurohistopathological alterations, and hypothalamus-pituitary-adrenal axis dysregulation following prenatal stress in a rat model

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Abstract

Background Management as well as treatment of antenatal stress is a global concern and today, people tend to use alternative treatment through medicinal plants like *Eucalyptus alba (E. alba)*.

Objective This study investigates the potential of aqueous and ethanol leaf extracts of *E. alba* in management of the impact of antenatal stress in young rats.

Methodology Phytochemistry of the leaf extracts was conducted and forty-five gravid albino rats randomly assigned into 9 groups. All but group one was induced with stress before receiving treatments. Group 2 received 5mL/kg of distilled water; group 3 received 1 mg/kg of diazepam. Groups 4 through 9 received aqueous and ethanol extracts of *E. alba* (100 mg/kg, 200 mg/kg and 400 mg/kg respectively) until delivery. After 21 days, litters were subjected to behavioral tests, followed by sacrifice, then hormone levels evaluation and brain histological analysis conducted.

Results Flavonoids, saponins, phenols and tannins were present in both extracts. Motor dysfunction, anxiogenic like and depressive like behaviors were alleviated by both extracts. The plant extracts also antagonized the stress induced increase in corticotropin-releasing hormone, adrenocorticotropic hormone and corticosterone production. Histological analysis of the different structures of the brain cortex, hippocampal CA1 and CA3 regions, gyrus and the amygdala confirmed the obtained results as alteration of structures were prevented by both plant extracts.

Conclusion *Eucalyptus alba* extracts exhibited neuroprotective properties against prenatal stress by protecting brain damages, maintaining the hypothalamic-pituitary-adrenal axis integrity and consequently the level of hormones production by the axis.

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Keywords Prenatal stress, Anxiety, Phytochemistry, Eucalyptus alba, Neuroprotection, Hormones

Introduction

Stress is a physiological and psychological response to perceived threats or challenges that exceed an individual's coping capacity. It occurs when internal or external stressors disrupt homeostasis, leading to a cascade of behavioral and biological changes aimed at adaptation (Dchanche et al. 2024). If unresolved, stress can negatively impact the central nervous system, impair cognitive functions such as learning and memory, and contribute to the development of neuropsychiatric conditions including anxiety, depression, and sleep disorders (Nwogueze et al. 2020). At the neurobiological level, stress affects the structure and function of key brain regions such as the hippocampus, prefrontal cortex, and amygdala areas critical for cognition, emotion regulation, and memory formation. It also disrupts the hypothalamic-pituitary-adrenal (HPA) axis, altering cortisol levels and contributing to long-term neuroendocrine imbalance (Mcewen 2010; Pondeljak and Lugovic 2020).

In pregnant individuals, chronic stress has particularly harmful implications for fetal brain development. Maternal stress can impair neurogenesis, reduce synaptic plasticity, and alter fetal neural circuitry by triggering inflammatory responses, oxidative stress, hormonal imbalances, and neuronal apoptosis (Dchanche et al. 2024). These alterations have been linked to long-term behavioral and emotional disturbances in offspring, including heightened susceptibility to anxiety, depression, attention deficits, and impaired social interactions. Recent research highlights the ability of stress hormones, particularly glucocorticoids, to cross the placenta and directly influence fetal brain development, thereby increasing the risk of neurodevelopmental disorders (van den Bergh et al. 2018). Epidemiological data indicate that maternal stress during pregnancy is highly prevalent, with reported rates ranging from 5.5% to 78% (Futterman et al. 2023; Lynn et al. 2011; Vijayaselvi 2015). These numbers have significantly increased during global crises such as the COVID-19 pandemic (Boekhorst 2021; Pope et al. 2021). The timing, severity, and duration of prenatal stress exposure are critical variables influencing fetal outcomes (Weinstock 2017). In particular, stress experienced during early to mid-gestation has been associated with more profound effects on fetal brain structure and function (van den Bergh et al. 2018).

Although pharmacological agents such as sertraline, citalopram, and imipramine are commonly prescribed to manage stress-related disorders (Harsha and Anilakumar 2013), they pose several limitations. These include undesirable side effects, high treatment costs, and contraindications during pregnancy (Hirsch al. 2019). As a result, there is growing interest in exploring safer, plant-based alternatives that may offer neuroprotective effects with fewer risks to maternal and fetal health. Medicinal plants rich in bioactive compounds particularly flavonoids, saponins, and phenolic acids have shown promise in mitigating neurobehavioral deficits associated with prenatal stress. For instance,*Ginkgo biloba*(Ali 2020) and *Ocimum gratissimum* (Dchanche et al. 2024) have demonstrated antioxidative, anxiolytic, and antidepressant properties in animal models of prenatal stress. Furthermore, several phytochemicals are known to modulate neurotransmitter systems and regulate the HPA axis, both of which play central roles in stress response and emotional regulation (Tkaczenko et al. 2025). The present study aims to investigate the therapeutic potential of *Eucalyptus alba* (Myrtaceae), a plant traditionally used in the North West Region of Cameroon for the management of neurological disorders, including epilepsy and mental health conditions. Specifically, the study seeks to evaluate the efficacy of both aqueous and ethanol extracts of *Eucalyptus alba* in mitigating the neurobehavioral and biochemical alterations induced by prenatal stress in young Wistar rats. The study further explores the plant's potential modulatory effects on oxidative stress markers, neurotransmitter activity, and the hypothalamic-pituitary-adrenal (HPA) axis key mechanisms implicated in stress response and emotional regulation.

Procedures

Gathering and categorization of plant specimen

Novel *Eucalyptus alba* leaves were assembled in October 2023 from Santa Subdivision in the North West region of Cameroon. A specimen was taken to Cameroon National herbarium and identified compared to Meijer's materials W.15,004 of specimen of the herbarium's collection (No: 59281 SRFCam).

Plant extraction

Freshly collected *Eucalyptus alba* leaves were gently rinsed with tap water and left to air dry at room temperature. Once fully dried and crispy, the leaves were ground into fine powder using an electric blender (Binatone model). For the aqueous extract, 200 g of the powdered leaves were added to 2 L of distilled water and boiled for 20 min (decoction). The mixture was then allowed to cool at room temperature and filtered using Whatman No. 1 filter paper. The resulting filtrate was evaporated in an oven set at 45 °C to obtain a brown extract, which was weighed, yielding an extraction efficiency of 10.85%.

For the ethanol extract, 200 g of the same powdered leaves were macerated in 2 L of ethanol for 48 h. After

maceration, the mixture was filtered through Whatman No. 1 filter paper. The solvent was then removed by evaporation in an oven maintained at 40 °C. The resulting dry brown extract was weighed, with a calculated yield of 10.23%.

Phytochemical screening of the extracts of *Eucalyptus Alba*

Phytochemical study was conducted to appreciate tannins, phenols, saponins and flavonoids contents of the extracts. The assays were conducted following the reported methods of (Akinseye et al. 2017), (Selvakumar et al. 2019) and Panchal and Jha (2021) .

Experimental animal

Mature virgin females (*Rattus norvegicus)* Wistar albino rats (weight 150 ± 4 g) were obtained from Yaoundé, Cameroon. Animals were kept in standard conditions at room temperature, with natural dark-light cycle and free access to water and food under hygienic condition. All experiments were accepted by the Cameroon National Veterinary ethic committee (testimonial by the approval and health control No 003/19 CCS/MINEPIA/RD-NW/DDME/SSV) and, the study was carried out in line with the ethical standards as emphasized in the 1964 Declaration of Helsinki and its later adjustments. Animals were acclimatized for one week before initiation of the experiments.

Preselection tests

Preliminary tests were conducted to identify non-stressed animals for inclusion in subsequent experiments. The Forced Swim Test (Arbabi et al. 2014; Kada et al. 2022) and the Sucrose Preference Test (Bhagya et al. 2011) were employed for this purpose. Animals that did not exhibit signs of stress were selected and housed overnight with males at a 1:1 male-to-female ratio. The following morning, vaginal smears were collected and examined microscopically on a daily basis to detect the presence of sperm. The day sperm was first observed was designated as gestation day zero, and those animals were included in the experimental study.

Animal grouping and stress induction

Forty-five gravid females were assigned to 9 groups at random containing 5 animals each.

- Group 1 (Normal control group (NCG); not stressed and received no treatment).
- The group 2, (Vehicle control group (VCG); stressed and administered 5 mL/kg of distilled water daily.
- Group 3 (positive control group (DZP); stressed and received 1 mg/kg diazepam intramuscularly) every after a day.
- Groups 4, 5 and 6 (aqueous test groups; stressed and received 100 mg/kg, 200 mg/kg and 400 mg/kg aqueous extract respectively) and.
- Groups 7, 8 and 9 (ethanol test groups; stressed and received 100 mg/kg, 200 mg/kg and 400 mg/kg of ethanol extract respectively).

Apart from group 1, all animals in the other groups were exposed to a stressor of congestion and sunlight at alternated times every day as described by (Dchanche 2024) while being treated with the plant extracts except for the vehicle group that was not treated until the day of delivery. The doses of 100, 200, and 400 mg/kg body weight for the aqueous and ethanolic extracts of *Eucalyptus alba* were selected based on previous studies involving extracts from Eucalyptus species and other medicinal plants with similar phytochemical profiles (Nikhil et al. 2021; Tangu et al. 2025). Additionally, a preliminary dose-finding study conducted in our laboratory showed that these doses were effective and did not induce signs of toxicity in test animals. These doses represent low, medium, and high ranges typically used in in vivo pharmacological evaluations. Each mother and its litters were isolated and kept for 21 days after which litters were subjected to behavioral assessments.

Behavioral tests

Assessment of motor coordination using the beam walking test

The Beam Walking test was used to evaluate locomotor function and motor coordination deficits in rodents. The procedure was conducted as described by (Kada et al. 2022), with minor modifications. Briefly, each rat was placed at one end of a one-meter-long wooden beam elevated approximately one meter above the ground and encouraged to cross to the other side. During the task, the number of foot slips, total distance covered, and number of turns made were recorded by an observer. Motor incoordination was inferred from the number of foot slips. To eliminate olfactory cues between trials, the beam was wiped with 95% ethanol after each animal completed the task.

The open field test (OFT)

The Open Field Test (OFT) was used to assess spontaneous locomotor activity and the behavioral response of rats to a novel or anxiety-inducing environment, following the method described by (Abramova et al. 2021). The apparatus consisted of an open arena measuring 24 × 24 × 23 cm, with the floor divided into 16 equal squares (6 × 6 cm) and illuminated with direct lighting at 140 lx. At the beginning of each trial, a rat was placed at a designated starting point within the arena, and its behavior was observed and recorded for 5 min. The parameters

measured included time spent in the central square, number of lines crossed, total distance traveled, grooming frequency, and grooming duration (in seconds). Grooming behavior was interpreted as a displacement activity commonly observed in response to unfamiliar environments. To minimize olfactory cues, the arena was cleaned with 95% ethanol after each trial.

Force swim test (FST)

Forced Swim test evaluates depression and was carried out using the method of (Dchanche 2024). The apparatus was made up of a plastic cylinder of 25 cm wide and 50 cm height, filled with 30 cm water in depth at a temperature of 25 °C ± 2 °C. A pre-test was conducted a day before the test was carried out where each animal was led to swim individually in a swim tank for 10 min, a towel used to dry them, followed by placing them in the sun for another 10 min to prevent hypothermia before being taken back to their home cages. The water was replaced after each trial with an animal to prevent any influence on the subsequent animal. After 24 h, the same procedure was used to conduct a 5 min swim test session with an observer who was blind to the treatment regimen recording the activity from a top view. The duration of immobility was measured with a stopwatch.

Sacrifice and biospecimen collection

At the end of behavioral assessments, rats were weighed and anesthetized with the combination of diazepam (10 mg/kg) and ketamine (50 mg/kg) prior to sacrificed. Venous blood was collected for serum hormone analysis, brains were dissected out and their weights taken. Homogenates were prepared from part of the brains of each animal for biochemical evaluation while the others parts were preserved in 10% formalin for histological examination.

Evaluation of the effects of aqueous and ethanol leaf extracts of *E. alba* on serum levels of Corticotropin releasing hormone, adrenocorticotropin and corticosterone of prenatally stressed litters

Serum concentrations of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and corticosterone (CORT) were measured using rat-specific ELISA kits, according to the manufacturer's instructions (Elabscience, Wuhan, China).

Table 1 Concentrations of different phytochemicals found in different extracts of the leaves of *E. alba*

Molecules extracts	Tannins (mg/g)	Phenols (mg/g)	Flavonoids (mg/g)	Saponins (mg/g)
Aqueous	3.98 ± 0.40	0.04 ± 0.00	0.05 ± 2.80	32.4 ± 0.05
Ethanol	3.07 ± 0.03	0.03 ± 0.00	0.05 ± 1.40	29.6 ± 0.23

Values in Table 1 represent means ± standard error of means (SEM)

Evaluation of the effects of aqueous and ethanol leaf extracts of *E. alba* on the brain histology of prenatally stressed litters

Following sacrifice, specific brain regions including the prefrontal cortex, CA1 and CA3 regions of the hippocampus, the gyrus, and the amygdala were carefully dissected and fixed in 10% neutral buffered formalin. The samples were stored in separate containers based on experimental groups until further histological analysis. At the Animal Physiology Laboratory of the University of Yaoundé 1, tissue samples underwent dehydration through two changes of 95% ethanol followed by three changes of 100% ethanol, each change occurring every two hours. Clearing was carried out using two changes of xylene over a 24-hour period. The tissues were then infiltrated with molten paraffin wax in two changes over another 24 h, after which they were embedded and blocked. Sections of 3 to 4 µm thickness were obtained using a rotary microtome and mounted onto glass slides using a collagen adhesive solution (prepared by dissolving 500 mg of collagen in 1000 mL of distilled water). The mounted slides were deparaffinized by passing through three changes of xylene and then rehydrated through three changes of ethanol, each for 10 min, followed by rinsing in distilled water for 5 min. Staining was performed using hematoxylin and eosin (H&E), following the protocol described by Bancroft and Gamble (2008). Stained sections from both control and treated animals were examined and imaged using an OLYMPUS BX51 light microscope at 100X magnification.

Statistical analysis

Graph Pad Prism version 8.01 software was used to analyze all data via one way ANOVA prior to Turkey pairwise-test and the results displayed as mean ± standard error mean (SEM). A value at $p < 0.05$ was considered to have significant difference and a t test was further carried out to differentiate the means between different groups.

Results

Phytochemicals present in the aqueous, ethanol, extracts of *E. alba*

Quantitative phytochemical analysis of the aqueous and ethanol extracts of the leaves of *E. alba* revealed that tannins, saponins, phenols and flavonoids were abundant in aqueous and ethanol extracts, measuring 3.98 ± 0.40 mg/g, 0.04 ± 0.00 mg/g, 0.05 ± 2.80 mg/g and 32.4 ± 0.05 mg/g respectively for aqueous extract while 3.07 ± 0.0 mg/g, 0.03 ± 0.00 mg/g, 0.05 ± 1.40 mg/g and 29.6 ± 0.23 mg/g for the ethanol extract (Table 1).

Effects of aqueous and ethanol leaf extracts of *E. alba* on motor coordination using beam walking test

Rats exposed to prenatal stress in the vehicle control group (VCG) exhibited a significant reduction in distance traveled, along with increases in foot slips, number of turns, and pauses ($p < 0.001$) when compared to the normal group (Table 2). However, these adverse effects of prenatal stress were mitigated by treatment with the plant's aqueous extract ($p < 0.001$), bringing the measured parameters closer to those of the normal group. Administration of varying doses (100 mg/kg, 200 mg/kg and 400 mg/kg) of both the aqueous and ethanol extracts significantly increased distance traveled, along with decreases in foot slips, number of turns, and pauses ($p < 0.001$) compared to the vehicle control group, thereby improving behavioral outcomes.

Furthermore, different doses of the plant extracts (both aqueous and ethanol) did not show significant differences when compared to the positive control group ($p < 0.001$), indicating that both treatments were similarly effective in regulating the effects of prenatal stress in young rats. Although there was a slight difference between the effects of the aqueous and ethanol extracts, this variation was not statistically significant as determined by the unpaired t-test (Table 2).

Effects of the aqueous and ethanol leaf extracts of *E. alba* on exploration and anxiety using open field test (OFT)

In Table 3, prenatally stressed rats without treatment (VCG) exhibited a significant reduction in total distance traveled, number of lines crossed, and time spent in the central square ($p < 0.001$), alongside an increase in grooming frequency and grooming duration ($p < 0.001$), compared to the normal control group (NCG). Treatment with both aqueous and ethanol plant extracts appeared to mitigate the effects of prenatal stress ($p < 0.001$), thereby increasing total distance traveled, number of lines crossed, and time spent in the central square ($p < 0.001$), alongside a decrease in grooming frequency and grooming duration ($p < 0.001$) compared to the vehicle control group. Additionally, no significant differences were found between the various doses of the plant extracts and the positive control (DZP) ($p < 0.001$).

When comparing different doses of the plant extracts, no significant variation in therapeutic efficacy was observed relative to the positive control group. Specifically, analysis using the unpaired t-test showed that for distance traveled and grooming frequency, the different extract doses did not significantly differ from the positive control. For the number of lines crossed, all three doses (100 mg/kg, 200 mg/kg and 400 mg/kg) were comparable to the positive control group ($p < 0.001$). Regarding time spent in the central square, the 100 mg/kg dose was slightly lower ($p < 0.05$), while the 200 mg/kg and

Table 2 Effects of aqueous and ethanol extracts of *E. alba* in the beam walking test parameters

Treatments	Controls				Doses			F; *P* value
Test Parameters		NCG	VCG	DZP	100 mg/kg	200 mg/kg	400 mg/kg	
Distance (cm)	Aqueous	$1968 \pm 0.26^{\dagger}$	863.3 ± 0.48^{ns}	$1842 \pm 0.65^{\dagger}$	$1657 \pm 0.57^{\dagger}$	$1853 \pm 0.42^{\dagger}$	$1642 \pm 0.59^{\dagger}$	$F_{(5, 32)} = 368{,}898$ $P < 0.001$
	Ethanol	$1968 \pm 0.26^{\dagger}$	863.3 ± 0.48^{ns}	$1842 \pm 0.65^{\dagger}$	$1682 \pm 0.41^{\dagger}$	$1923 \pm 0.54^{\dagger}$	$1672 \pm 0.58^{\dagger}$	$F_{(5, 39)} = 376{,}193$ $P < 0.001$
T-test/P					36.88/0.00	92.92/0.00	36.30/0.00	
Foot slips	Aqueous	$0.67 \pm 0.21^{\dagger}$	5.25 ± 0.63^{ns}	$0.50 \pm 0.22^{\dagger}$	$0.60 \pm 0.27^{\dagger}$	$0.17 \pm 0.17^{\neq}$	$1.55 \pm 0.39^{\neq}$	$F_{(5, 41)} = 19.80$ $P < 0.001$
	Ethanol	$0.67 \pm 0.21^{\dagger}$	5.25 ± 0.63^{ns}	$0.50 \pm 0.22^{\dagger}$	$0.67 \pm 1.62^{\dagger}$	$0.92 \pm 0.39^{\dagger}$	$1.11 \pm 0.26^{\neq}$	$F_{(5, 54)} = 17.26$ $P < 0.001$
T-test/P					0.23/0.82	1.25/0.23	0.88/0.39	
Turns	Aqueous	$4.5 \pm 0.56^{\dagger}$	17.8 ± 1.11^{ns}	$4.8 \pm 0.29^{\dagger}$	$4.1 \pm 0.31^{\dagger}$	$3.8 \pm 0.31^{\dagger}$	$5.4 \pm 0.34^{\neq}$	$F_{(5, 40)} = 95.6$ $P < 0.001$
	Ethanol	$4.5 \pm 0.56^{\dagger}$	17.8 ± 1.11^{ns}	$4.8 \pm 0.29^{\dagger}$	$5.7 \pm 0.23^{\dagger}$	$6.1 \pm 0.84^{\neq}$	$6.2 \pm 0.55^{\neq}$	$F_{(5, 54)} = 34.36$ $P < 0.001$
T-test/P					4.24/0.00	1.76/0.09	1.31/0.21	
Pauses T-test/P	Aqueous	$2.3 \pm 0.42^{\dagger}$	3.0 ± 0.41^{ns}	$2.1 \pm 0.39^{\dagger}$	$2.3 \pm 0.26^{\dagger}$	$2.3 \pm 0.42^{\dagger}$	$2.7 \pm 0.38^{\neq}$	$F_{(5, 40)} = 0.6051$ $P = 0.6963$
	Ethanol	$2.3 \pm 0.42^{\dagger}$	3.0 ± 0.41^{ns}	$2.1 \pm 0.39^{\dagger}$	$2.4 \pm 0.25^{\dagger}$ 0.88/0.88	$1.8 \pm 0.20^{\dagger}$ 1.31/0.19	$2.4 \pm 0.34^{\dagger}$ 1.76/0.59	$F_{(5, 49)} = 1.33$ $P = 0.2678$

Values in Table 2 represent means ± standard error of means (SEM), Values not sharing a common character differ significantly with each other at $p < 0.001$

† = ***

$^{\neq}$ = **

ns = non significance

NCG normal control group, *VCG* vehicle control group, *DZP* diazepam for positive control group $n = 5$

Table 3 Effects of aqueous and ethanol extracts of *E. alba* in the open field test parameters

Treatments	Controls				Doses			F; *P* values
Test parameters		**NCG**	**VCG**	**DZP**	**100 mg/kg**	**200 mg/kg**	**400 mg/kg**	
Distance (cm)	Aqueous	1968 ± 0.26†	863 ± 0.48 ^{ns}	1842 ± 0.65†	1657 ± 0.57†	1853 ± 0.42†	1642 ± 0.59†	$F_{(5,32)} = 368{,}898$ $P < 0.001$
	Ethanol	1968 ± 0.26†	863 ± 0.48 ^{ns}	1842 ± 0.65†	1682 ± 0.41†	1923 ± 0.54†	1673 ± 0.58†	$F_{(5,39)} = 376{,}193$ $P < 0.001$
t-test/p					36.88/0.00	92.92/0.00	36.30/0.00	
Lines crossed(cm)	Aqueous	122.6 ± 0.18†	53.6 ± 0.18 ^{ns}	115.4 ± 0.16†	103.1 ± 0.26$^{\neq}$	115.8 ± 0.16†	102.7 ± 0.19$^{\neq}$	$F_{(5,49)} = 14{,}804$ $P < 0.001$
	Ethanol	122.6 ± 0.18†	53.6 ± 0.18 ^{ns}	115.4 ± 0.16†	103.7 ± 0.25$^{\neq}$	119.7 ± 0.19†	103.4 ± 0.34$^{\neq}$	$F_{(5,58)} = 9194$ $P < 0.001$
t-test/p					1.47/0.15	14.66/0.00	1.92/0.07	
Time in central sq (s)	Aqueous	14.3 ± 0.33†	3.5 ± 0.29 ^{ns}	12.6 ± 0.43†	10.2 ± 0.31†	12.5 ± 0.43†	9.1 ± 0.34†	$F_{(5,33)} = 69.81$ $P < 0.001$
	Ethanol	14.3 ± 0.33†	3.5 ± 0.29 ^{ns}	12.6 ± 0.43†	8.8 ± 0.48†	12.9 ± 0.61†	12.4 ± 0.29†	$F_{(5,41)} = 38.71$ $P < 0.001$
t-test/p					2.35/0.04	0.45/0.66	7.36/0.00	
Grooming (s)	Aqueous	1.5 ± 0.22†	5.7 ± 0.48 ^{ns}	1.7 ± 0.21†	1.5 ± 0.17†	1.4 ± 0.20†	1.9 ± 0.23†	$F_{(5,41)} = 29.82$ $P < 0.001$
	Ethanol	1.5 ± 0.22†	5.7 ± 0.48 ^{ns}	1.7 ± 0.21†	1.4 ± 0.14†	1.3 ± 0.14†	1.9 ± 0.26†	$F_{(5,53)} = 32.86$ $P < 0.001$
t-test/p					0.49/0.63	0.39/0.69	0.86/0.97	
Groom duration (s)	Aqueous	16.3 ± 0.80†	53.3 ± 0.85 ^{ns}	16.5 ± 0.50†	16.7 ± 0.52†	17.2 ± 0.70†	20.1 ± 0.37†	$F_{(5,41)} = 374.3$ $P < 0.001$
	Ethanol	16.3 ± 0.80†	53.3 ± 0.85 ^{ns}	16.5 ± 0.50†	21.9 ± 0.24†	16.7 ± 0.38†	21.5 ± 0.57†	$F_{(5,49)} = 467.1$ $P < 0.001$
t-test/p					10.22/0.00	0.60/0.56	2.18/0.04	

Values in Table 3 represent means ± standard error of means (SEM). Values not sharing a common character differ significantly with each other at $p < 0.001$

† = ***

$^{\neq}$ = **

ns = non significance

NCG normal control group, *VCG* vehicle control group, *DZP* diazepam for positive control group, $n = 5$

Table 4 Effects of aqueous and ethanol leaf extracts of *E. alba* on the immobility time of the force swim test (s)

Treatments		Controls			Doses			F; *P* values
Plant		**NCG**	**VCG**	**DZP**	**100 mg/kg**	**200 mg/kg**	**400 mg/kg**	
Extracts	Aqueous	18.80 ± 0.97†	92.67 ± 1.45 ^{ns}	18.71 ± 0.97†	24.00 ± 0.50†	20.00 ± 1.00†	23.50 ± 0.64†	$F_{(5,33)} = 644.7$ $P < 0.001$
	Ethanol	18.80 ± 0.97†	92.67 ± 1.45 ^{ns}	18.71 ± 0.97†	23.73 ± 0.38†	19.89 ± 0.59†	24.09 ± 0.48†	$F_{(5,40)} = 841.3$ $P < 0.001$
t-test/p					0.44/0.66	0.10/0.92	0.75/0.46	

Values in table 4 represent means ± standard error of means (SEM). Values not sharing a common character differ significantly with each other at $p < 0.001$

† = ***; ns = non significance

The asterisks (*$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$) indicate the significant differences as compared to the vehicle

NCG normal control group, *VCG* vehicle control group, *DZP* diazepam for positive control group, $n = 5$

400 mg/kg doses were statistically similar to the positive control. Finally, for grooming duration, the 100 mg/kg dose matched the positive control ($P < 0.001$), whereas the 200 mg/kg and 400 mg/kg doses were slightly lower ($P < 0.05$).

Effects of aqueous and ethanol leaf extracts of *E. alba* on depression using force swim test (FST)

Rats exposed to prenatal stress without treatment (vehicle control group, VCG) exhibited a significantly increased immobility time compared to the normal group ($p < 0.001$). Treatment with both the aqueous and ethanolic extracts of *Eucalyptus alba* at doses of 100, 200, and 400 mg/kg resulted in a significant reduction in immobility time compared to the vehicle control group ($p < 0.001$; Table 4). Similarly, administration of the reference drug also significantly decreased immobility duration ($p < 0.001$). Statistical analysis using an unpaired t-test revealed that while slight variations were observed among the different extract doses, these differences were

not statistically significant when compared to the reference drug.

Effects of the aqueous and ethanol extracts of *E. alba* on prenatal stress induced brain histological changes in litters

Effects of the aqueous and ethanol extracts of E. alba on prenatal stress induced changes in the brain cortex region of litters

Figure 1 presents the cortical regions of the brain of rats prenatally exposed to stress. As seen below, rats of the normal group (NCG) have a higher density of normal neurons (Nn) with strands of myelin sheaths. Rats of the vehicle group (VCG) demonstrated chromatolized neurons (Cn) with swollen cell bodies, hyperchromic neurons (Hn) with increased neuron sizes and necrotic neurons (nn). Both extracts *E. alba* evaluated prevented all these alterations in the normal morphology of the neuron compared to what was observed in the vehicle group. The group that received the reference drug (DZP) also prevented the alterations in the neuron morphology.

Effects of aqueous and ethanol extracts of E. alba on prenatally stress induced changes in the CA1 hippocampal region of litters

As seen on Fig. 2, rats of the normal group (NCG) have normal neurons (Nn) with well-defined morphology in cellular layers. Rats of the vehicle group (VCG) demonstrated chromatolized neurons (Cn) with swollen cell bodies, hyperchromic neurons (Hn) with increased neuron sizes and necrotic neurons (nn). Even though the groups administered the different doses of both extracts of *E. alba* presented some traces of necrotic neurons, this was to a very limited extend suggesting that the plant

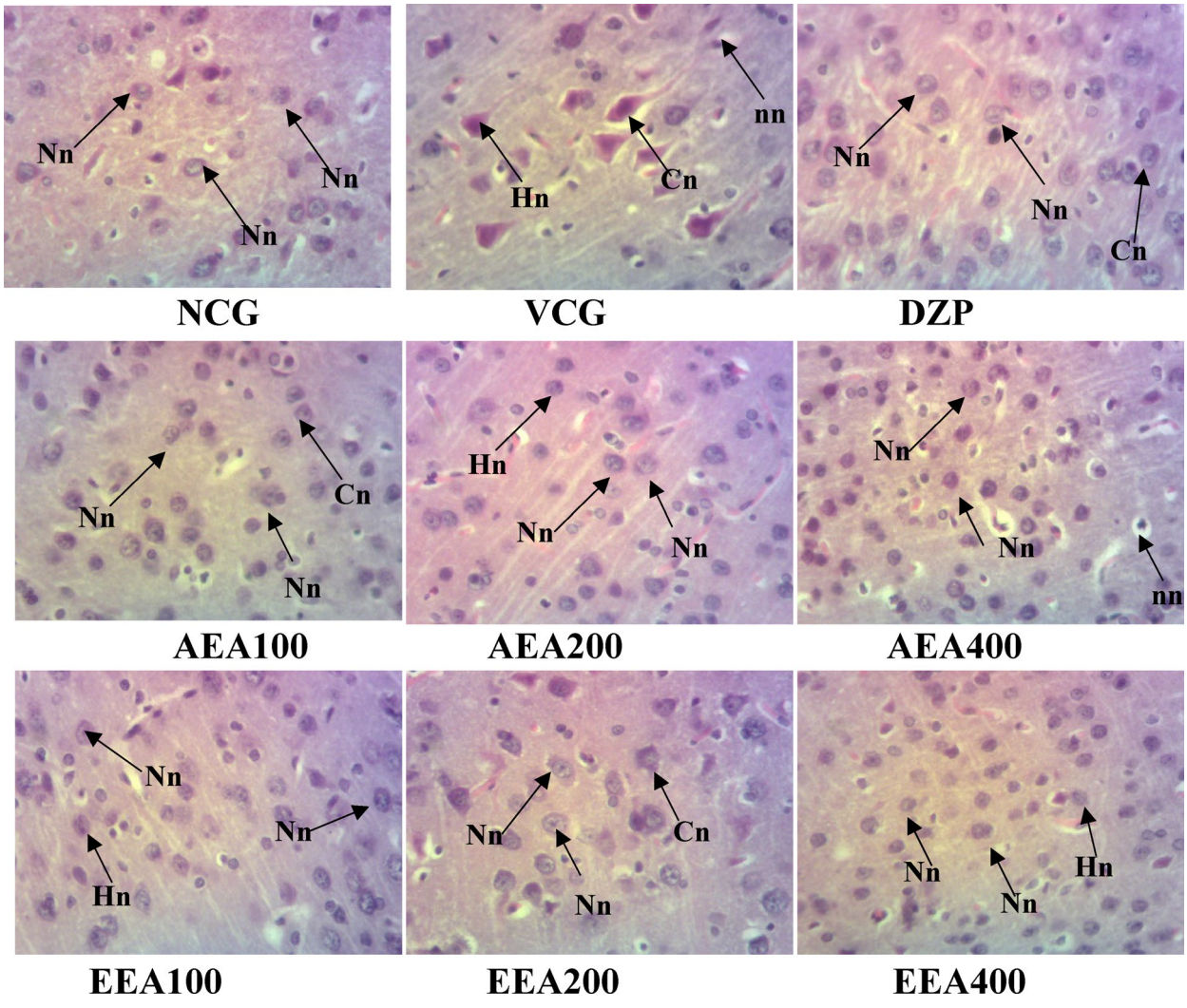

Fig. 1 Microphotographs showing the effects of the aqueous and ethanol extracts of *E. alba* on the brain cortex of litters prenatally stressed. H&E, 400X. *Nn* normal neurons, *Hn* hyperchromic neurons, *Cn* chromatolyzed neurons, *nn* necrotic neuron, *NCG* normal control group, *VCG* vehicle control group, *DZP* positive control group, *AEA* aqueous extract of *E. alba*, *EEA* ethanol extract of *E. alba*

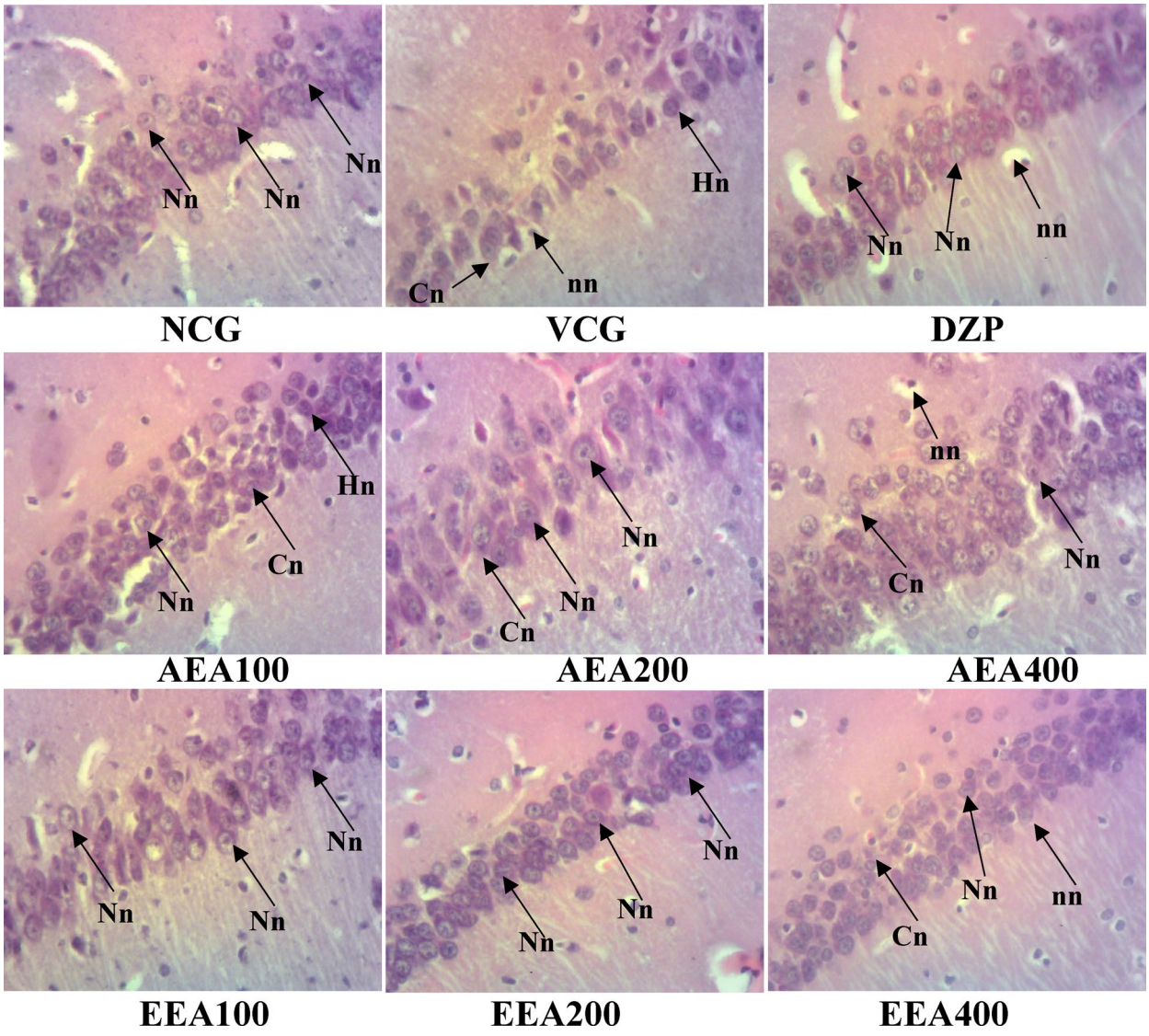

Fig. 2 Microphotographs showing the effects of the aqueous and ethanol extracts of *E. alba* on the hippocampal CA1 region of litters prenatally stressed. H&E, 400X. *Nn* normal neurons, *Hn* hyperchromic neurons, *Cn* chromatolized neurons, *nn* necrotic neuron, *NCG* normal control group, *VCG* vehicle control group, *DZP* positive control group, *AEA* aqueous extract of *E. alba*, *EEA* ethanol extract of *E. alba*

extracts had the therapeutic potential to alleviate the effects of prenatal stress compared to the vehicle group.

Effects of aqueous and ethanol extracts of E. alba on prenatally stress induced changes in the CA3 hippocampal region of litters

The CA3 region of the hippocampus presents normal morphology of neurons in the normal group compared to the vehicle control group which portrayed similar neuronal alterations as observed from the previous results (Fig. 3). Apart from dose 400 mg/kg of the aqueous extract that had few dead neurons, other doses of the plant extract ameliorated the effects of prenatal stress in this region of the brain compared to the vehicle control group. Also, rats of the positive control group (DZP) demonstrated a few hyperchromic neurons but not as those of the vehicle group.

Effects of aqueous and ethanol extracts of E. alba on prenatally stress induced changes in the gyrus region of litters

Figure 4 portrays a well dentate gyrus in the normal control group, having live neurons with normal morphology. The vehicle control group showed loss of neuronal density, loss of hilum of the dentate gyrus vacuolations, neuronal dispersal, neuronal atrophy, presence of eosinophilic necrotic cells and chromatolysis. Both extracts of *E. alba* evaluated as well as the positive control group showed the restoration of neuronal density, preserved

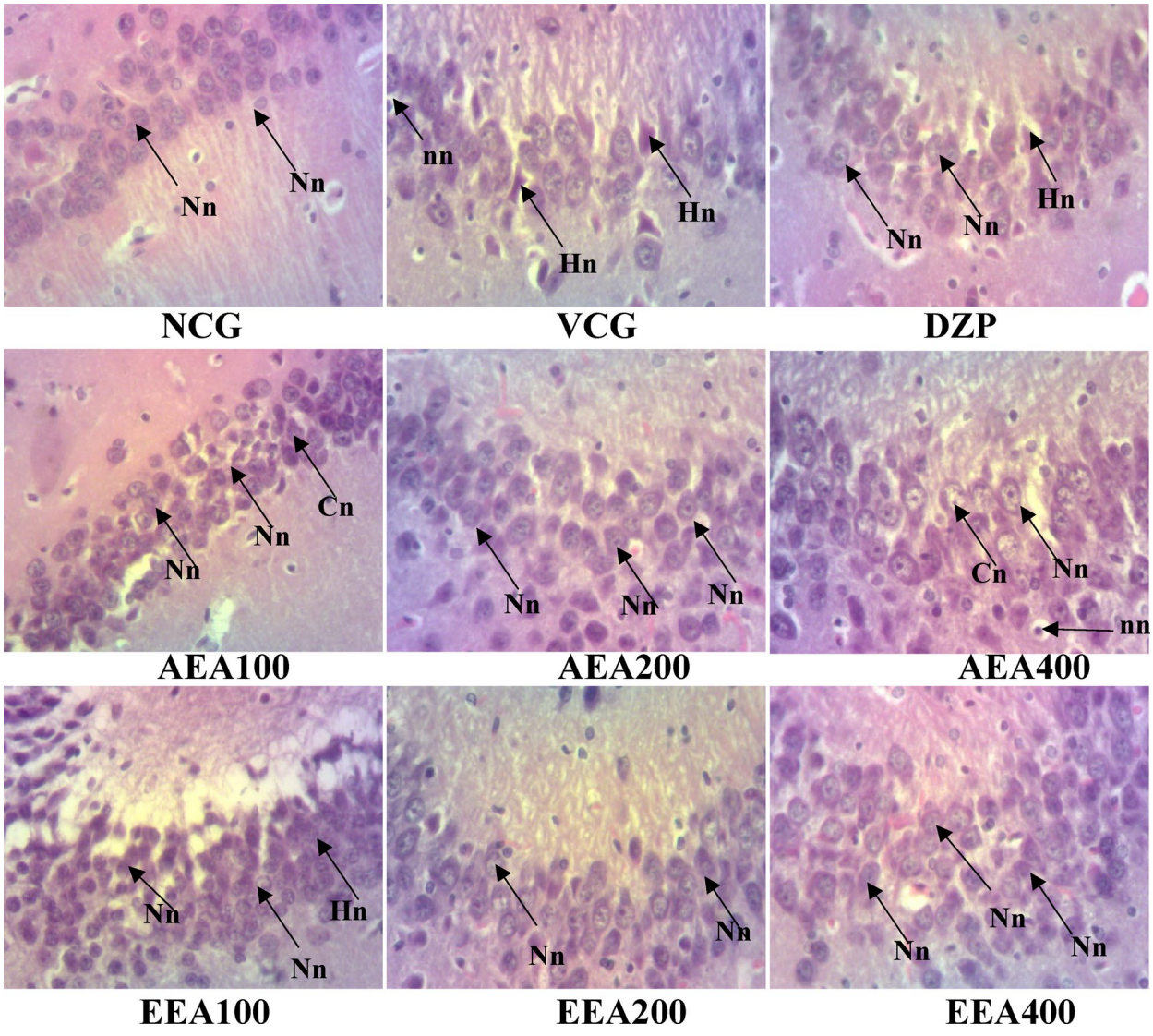

Fig. 3 Microphotographs showing the effects of the aqueous and ethanol extracts of *E. alba* on the hippocampal CA3 region of litters prenatally stressed. H&E, 400X. *Nn* normal neurons, *Hn* hyperchromic neurons, *Cn* chromatolized neurons, *nn* necrotic neuron, *NCG* normal control group, *VCG* vehicle control group, *DZP* positive control group, *AEA* aqueous extract of *E. alba*, *EEA* ethanol extract of *E. alba*

dentate gyrus hilum and numerous granular cells in comparison to the vehicle group.

Effects of aqueous and ethanol extracts of E. alba on prenatally stress induced changes in the amygdala region of litters

The amygdala of rats of the normal group (NCG) had normal neurons with well - defined morphology (Fig. 5). The amygdala region of the brain of rats in the vehicle group (VCG) demonstrated chromatolized neurons with swollen cell bodies, hyperchromic neurons with increased neuron sizes and necrotic neurons. Administration of both extracts of *E. alba* prevented all the alterations in the normal morphology of the neuron compared to the vehicle group. The reference drug (DZP) also alleviated the observed alterations in the neuron morphology recorded in the vehicle group.

Effects of the aqueous and ethanol leaf extracts of *E. alba* on some serum hormones of prenatally stressed litters

Effects of the aqueous and ethanol extracts of E. alba on CRH level in prenatally stressed young rats

A significant increase of CRH concentration was observed in the vehicle group ($p < 0.001$) in comparison to the normal group (Fig. 6). The different doses of both plant extracts prevented the effects of prenatal stress by decreasing ($p < 0.001$) the level of serum CRH compared to the vehicle control group. The positive control group (DZP) also decreased the level of serum concentration of CRH. The unpaired t test revealed that even though there

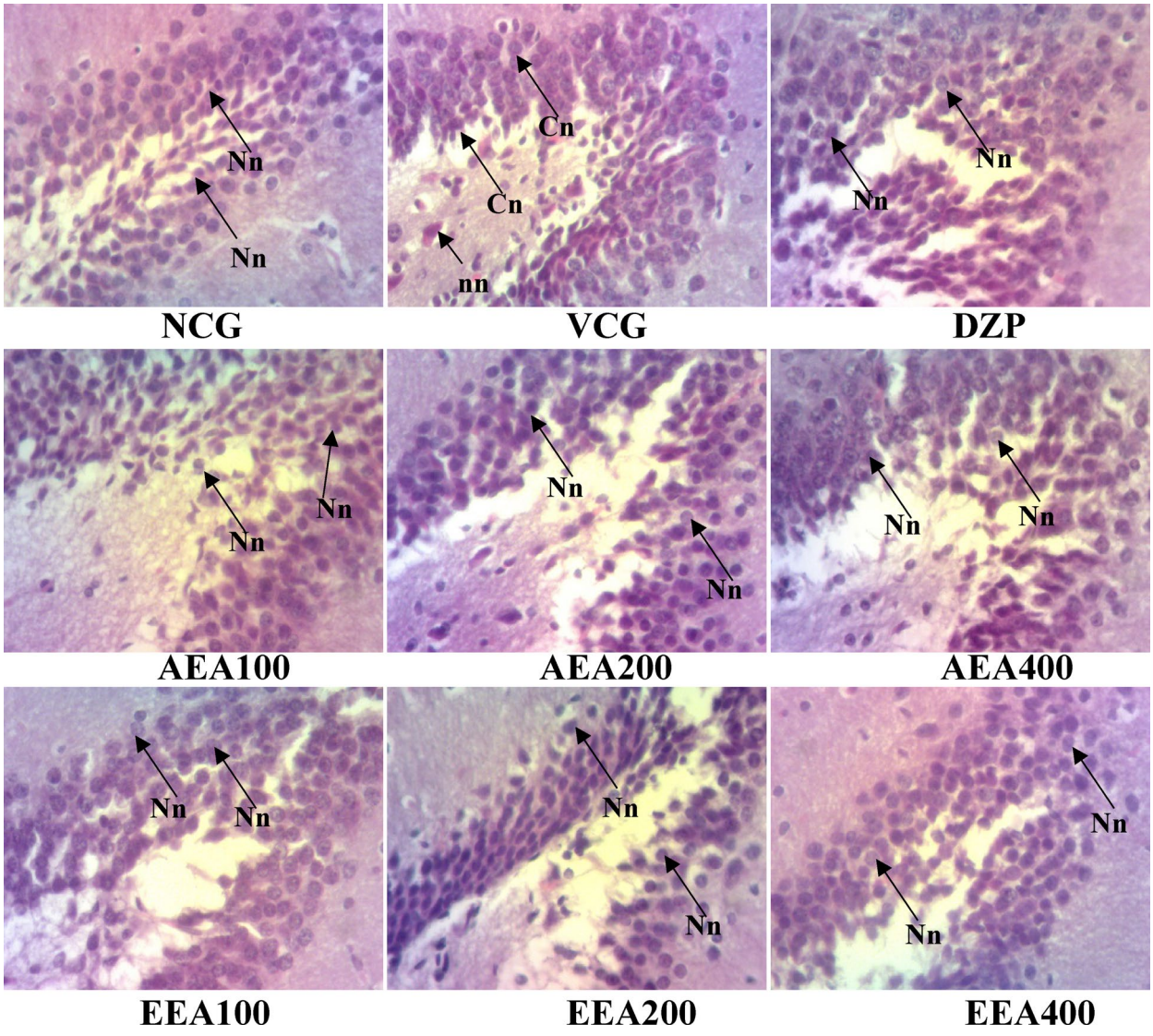

Fig. 4 Microphotographs showing the effects of the aqueous and ethanol extracts of *E. alba* on the gyrus region of litters prenatally stressed. H&E, 400X. *Nn* normal neurons, *Hn* hyperchromic neurons, *Cn* chromatolized neurons, *nn* necrotic neuron, *NCG* normal control group, *VCG* vehicle control group, *DZP* positive control group, *AEA* aqueous extract of *E. alba*, *EEA* ethanol extract of *E. alba*

could be a slight difference in the therapeutic potential of both extracts, the difference was not statistically significant compared to the positive control.

Effects of aqueous and ethanol extracts of E. alba on serum ACTH level in prenatally stressed young rats

The concentration of ACTH in prenatally stressed rats portrayed a significant increase ($p < 0.001$) in the vehicle group (VCG) compared to the normal (NCG). This increase in the concentration of ACTH caused by prenatal stress was prevented by the administration of both extracts of *E. alba* evaluated thereby reducing the concentration of serum ACTH compared to the vehicle group. The reference drug (DZP) also reversed the effects of prenatal stress by reducing the serum concentration of ACTH relative to the vehicle group. Taking a look at the therapeutic potential of the aqueous and ethanol extracts and their different doses, it was observed that both extracts are statistically indifferent in comparison to the positive group (Fig. 7).

Effects of aqueous and ethanol extracts of E. alba on CORT production in prenatally stressed young rats

As illustrated in Fig. 8, the concentration of corticosterone (CORT) was significantly elevated ($p < 0.001$) in the vehicle control group compared to the normal control group. This elevation was notably attenuated by treatment with various doses of *Eucalyptus alba* extracts (both aqueous and ethanol), reducing CORT levels compared to the vehicle control group. Similarly, the positive

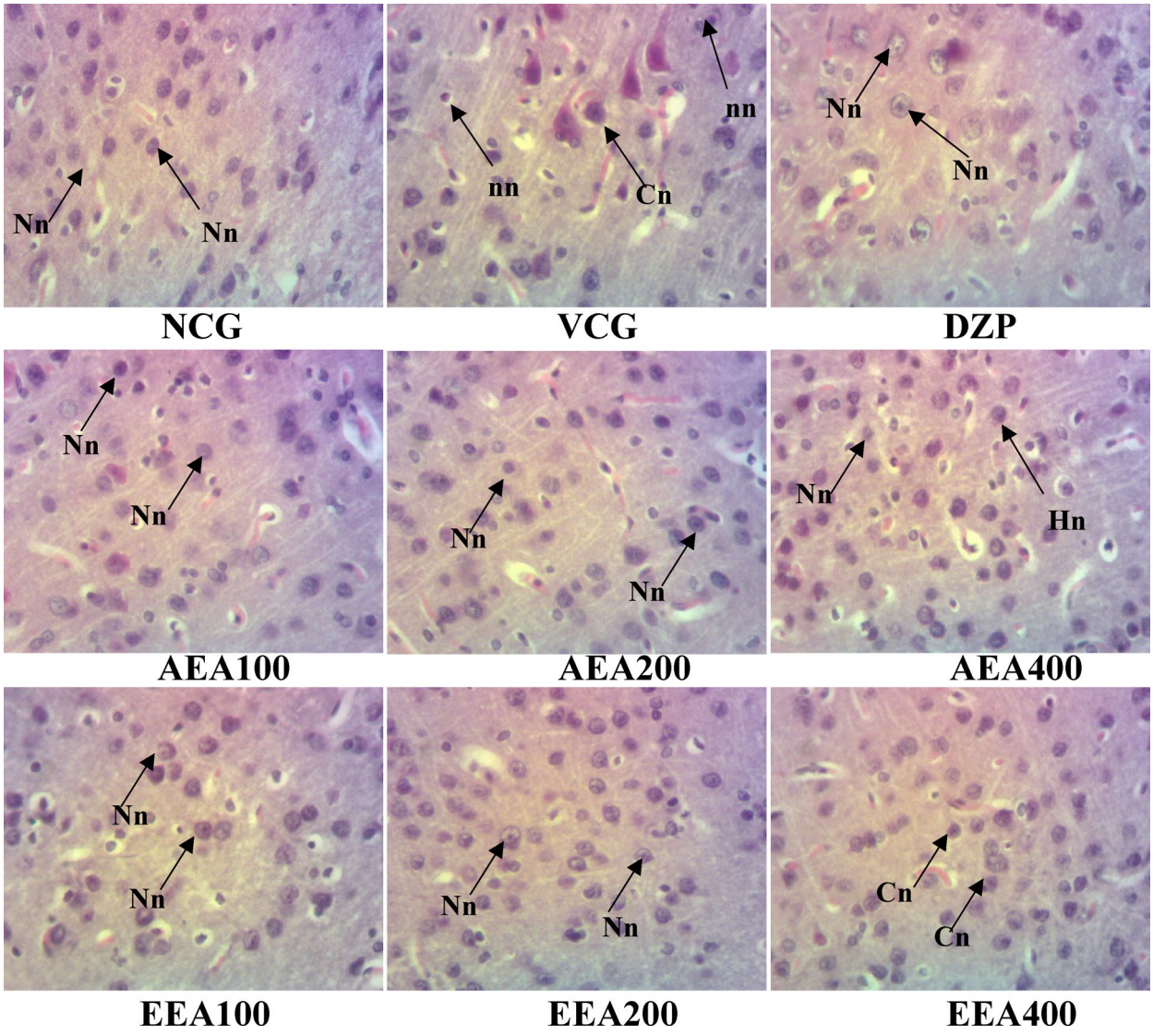

Fig. 5 Microphotographs showing the effects of the aqueous and ethanol extracts of *E. alba* on the amygdala of litters prenatally stressed. H&E, 400X. *Nn* normal neurons, *Hn* hyperchromic neurons, *Cn* chromatolized neurons, *nn* necrotic neuron, *NCG* normal control group, *VCG* vehicle control group, *DZP* positive control group, *AEA* aqueous extract of *E. alba*, *EEA* ethanol extract of *E. alba*

control group treated with diazepam (DZP) exhibited a reduction in CORT levels relative to the vehicle group. Furthermore, unpaired t-test analysis revealed no statistically significant differences between the lower and higher doses of either *E. alba* extract and the positive control group, suggesting comparable therapeutic efficacy.

Discussion

Phytochemicals that is naturally occurring compounds in plants are widely recognized for their diverse therapeutic properties (Baptista-silva et al. 2020; Jin 2021; Khan 2020). These bioactive compounds are broadly classified into primary metabolites, which are essential for growth and development, and secondary metabolites, which support defense, signaling, and adaptation to environmental stress. In the present study, phytochemical analysis of *Eucalyptus alba* leaf extracts revealed that the aqueous extract contained higher concentrations of tannins, phenols, flavonoids, and saponins compared to the ethanol extract. Tannins are known for their astringent, antimicrobial, and antioxidant properties and may also exert neuroprotective effects by enhancing cognitive function and memory (Nasr et al. 2019). Phenolic compounds, particularly abundant in the aqueous extract, are powerful antioxidants linked to reduced oxidative stress and chronic disease prevention (Oszmiański et al. 2020). Flavonoids are associated with neuroprotection, mood regulation, and cognitive enhancement, while saponins contribute to anti-inflammatory, immunomodulatory, and cholesterol-lowering activities (Ghosh 2021; Salehi et

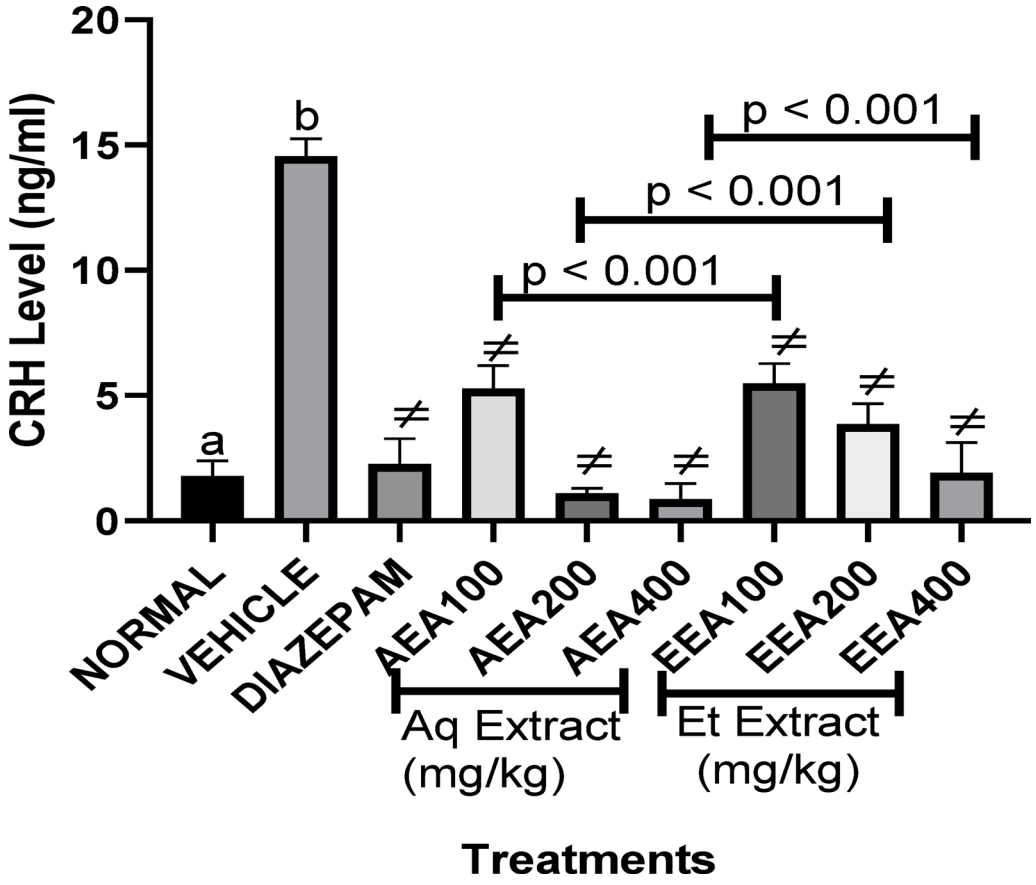

Fig. 6 Effects of aqueous and ethanol extracts of *E. alba* on serum CRH concentration in prenatally stressed young rats at $p < 0.001$. Bars represent means ± standard error of means (SEM). Letters a and b indicate the difference between normal group (NCG) and vehicle control group (VCG). $^{\neq}p < 0.001$ indicate the difference of extract treated groups compared to the vehicle group (VCG). *DZP* diazepam for positive control group, *AEA* aqueous extract. *EEA* ethanol extract; $n = 5$

al. 2019; Tiwari and Ajay 2020)Jiang et al., (Jiang Wang et al. 2024). These findings indicate that *E. alba* is a rich source of phytochemicals with potential neurotherapeutic benefits.

The Beam Walking Test, a sensitive tool for detecting motor impairments in rodents, revealed pronounced motor deficits in rats exposed to prenatal stress. These animals (vehicle control group) exhibited reduced distance traveled, along with increased foot slips, turns, and pauses, indicating impaired motor coordination and balance. These results are consistent with previous reports by (Kada et al. 2022), who demonstrated similar effects of prenatal stress on locomotor performance. However, treatment with *E. alba* extracts (aqueous and ethanol) significantly improved performance, suggesting that the phytochemicals present in the extracts counteracted prenatal stress-induced motor dysfunction. This improvement supports the presence of neuroprotective agents in *E. alba*, capable of restoring motor function and coordination (Dchanche et al. 2024).

The Open Field Test further confirmed the anxiogenic effects of prenatal stress. The vehicle control group exhibited reduced center square entries, decreased distance traveled, and increased stretch-attend postures indicative of heightened anxiety levels (Kada et al. 2022; Kaddour et al. 2016). Treatment with *E. alba* extracts led to a dose-dependent reversal of these anxiety-related behaviors, underscoring the plant's anxiolytic potential. These effects likely result from phytochemicals such as flavonoids and saponins that modulate stress-related pathways and enhance emotional resilience.

In the Forced Swim Test, prenatal stress significantly increased immobility time a hallmark of behavioral despair and depressive-like symptoms in rodents (Kada et al. 2022; Szczesny 2014). This behavioral outcome aligns with existing evidence linking prenatal stress to oxidative damage and impaired neurogenesis, which contribute to neuropsychiatric disorders such as depression and anxiety (Godoy 2018; Jagtap et al. 2023; Meli 2020). Treatment with *E. alba* extracts markedly reduced immobility time, suggesting antidepressant-like effects and neuroprotective action. These findings highlight the plant's potential to modulate mood-related behaviors

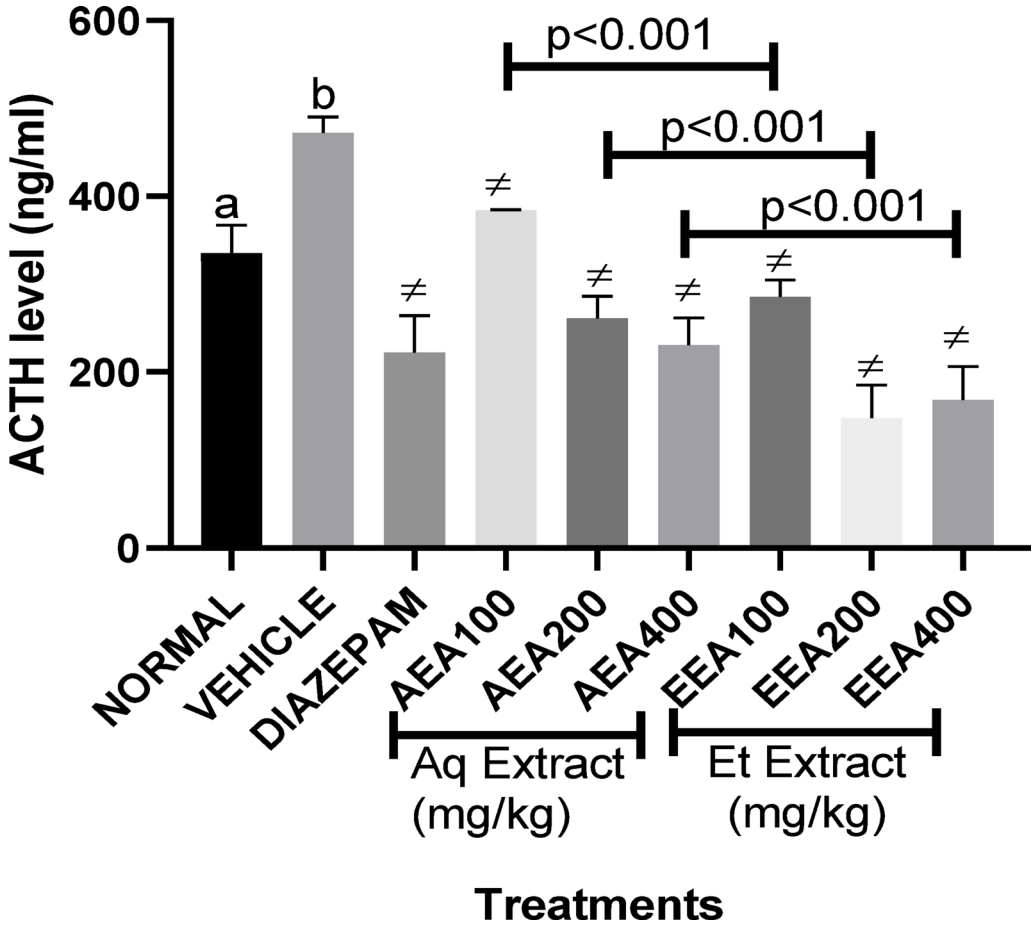

Fig. 7 Effects of aqueous and ethanol extracts of *E. alba* on serum ACTH concentration in prenatally stressed young rats at $p < 0.001$. Bars represent means ± standard error of means (SEM). Letters a and b indicates the difference between normal group (NCG) and vehicle control group (VCG). $^{\neq}p < 0.001$ indicate the difference of extract treated groups compared to the vehicle group (VCG). *DZP* diazepam for positive control group, *AEA* aqueous extract. *EEA* ethanol extract; $n = 5$

and mitigate stress-induced emotional dysregulation (Dchanche et al. 2024).

Brain histology supported the behavioral data. Prenatally stressed rats without treatment showed widespread neuronal damage, including chromatolysis, hyperchromic neurons, and cell death in the cortex, hippocampus, dentate gyrus, and amygdala. These structural changes are consistent with amygdala hyperactivity and hippocampal dysfunction, which impair learning, memory, and emotional regulation (Huang et al. 2025; Kozareva et al. 2019; Moreno-Jimenez et al. 2019; Patel et al. 2018). Such damage is commonly observed in stress-related neuropathologies (Bellini and Labombarda 2023; Chen et al. 2023). Conversely, treatment with *E. alba* extracts, particularly at higher doses, significantly reduced neuronal damage. Only minimal chromatolytic and hyperchromic changes were observed, with no evidence of neuronal death. This suggests that *E. alba* may protect neural tissue by modulating stress responses and preventing neurodegeneration. These findings are in agreement with previous reports on other plant-based neuroprotective agents, such as *Ocimum gratissimum* (Dchanche et al. 2024).

The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in stress responses. In this study, prenatal stress caused significant increases in serum CRH, ACTH, and corticosterone levels in the vehicle control group, reflecting HPA axis hyperactivation (Fotsing et al. 2017). These stress hormones can cross the placenta and negatively impact fetal brain development, predisposing offspring to neuropsychiatric disorders (Lopresti et al. 2021). Treatment with *E. alba* extracts effectively normalized CRH, ACTH, and CORT levels, indicating suppression of HPA axis overactivity. This suggests that the plant extracts modulate the neuroendocrine response to stress, thereby offering protection during critical periods of brain development. Similar outcomes were observed in studies involving *Scutellariae Radix* and *Ocimum gratissimum*, which also attenuated prenatal stress-induced

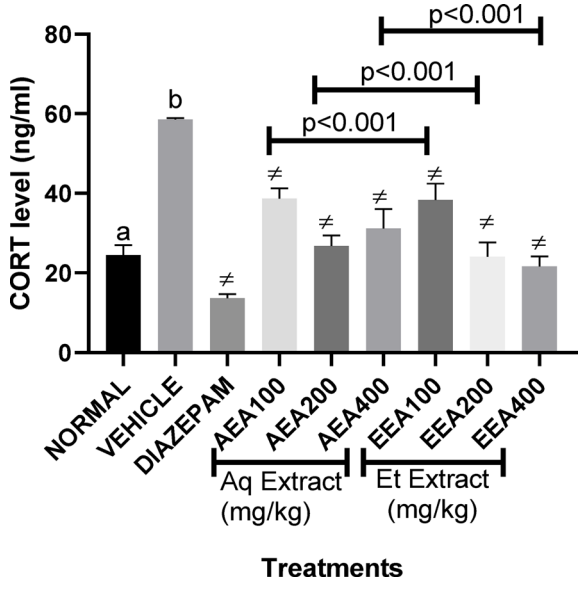

Fig. 8 Effects of aqueous and ethanol extracts of *E. alba* on serum CORT concentration in prenatally stressed young rats at $p < 0.001$. Bars represent means ± standard error of means (SEM). Letters a and b indicates the difference between normal group (NCG) and vehicle control group (VCG). $^{\neq}p < 0.001$ indicate the difference of extract treated groups compared to the vehicle group (VCG). *DZP* diazepam for positive control group, *AEA* aqueous extract. *EEA* ethanol extract; $n = 5$

hormonal imbalances (Dchanche et al. 2024; Li et al. 2024a; Li et al. 2024).

The neuroprotective effects of *Eucalyptus alba* may also be mediated through modulation of key neurotransmitter systems involved in stress, anxiety, and mood regulation. Flavonoids and other phytochemicals found in *E. alba* have been shown to interact with central neurotransmitters such as serotonin (5-HT), dopamine (DA), gamma-aminobutyric acid (GABA), and norepinephrine (NE), all of which play crucial roles in emotional and cognitive processes. By enhancing serotonergic and GABAergic signaling while downregulating hyperactive dopaminergic and noradrenergic pathways, these compounds may help restore neurochemical balance disrupted by prenatal stress (Kaurinovic 2019; Li et al. 2024a; Li et al. 2024). For example, (Li et al. 2024a; Li et al. 2024) demonstrated that essential oil from Eucalyptus species exerted anxiolytic and sedative effects via increased brain GABA levels and altered gut microbiota–brain axis signaling. Therefore, the behavioral improvements observed in this study such as reduced anxiety, improved motor coordination, and decreased immobility may be attributed, in part, to the extract's ability to influence neurotransmitter systems involved in neuroendocrine and emotional regulation.

Among the tested formulations, the ethanol extract of *E. alba* demonstrated greater efficacy than the aqueous extract, with the 200 mg/kg dose producing the most pronounced improvements across behavioral, hormonal, and histological parameters. This dose significantly increased distance traveled, reduced foot slips, and lowered grooming frequency, highlighting its potential as an optimal therapeutic dose. The superior efficacy of the ethanol extract may be attributed to the enhanced solubility and extraction of lipophilic phytochemicals with potent neuroactive effects. Considering its natural origin, affordability, availability, and multi-targeted actions, *Eucalyptus alba* especially the ethanol extract at 200 mg/kg emerges as a promising candidate for managing prenatal stress-related neurodevelopmental disturbances.

Conclusion

This study demonstrates that *Eucalyptus alba* leaf extracts, particularly the ethanol extract at 200 mg/kg, exert significant neuroprotective, anxiolytic, and antidepressant effects in a rodent model of prenatal stress. These therapeutic benefits are likely attributable to the plant's rich phytochemical profile comprising tannins, flavonoids, phenols, and saponins which may act by modulating oxidative stress, hormonal imbalance, and preserving neuronal integrity. However, a key limitation of this study is the absence of direct assessment of brain neurotransmitter levels, which restricts a more precise understanding of the underlying neurochemical mechanisms. Future studies will address this limitation to further elucidate the pathways involved. Overall, these findings highlight the potential of *E. alba* as a natural and accessible intervention for counteracting the detrimental effects of prenatal stress on brain development and behavior.

Abbreviations

E. alba	*Eucalyptus alba*
HPA	hypothalamic-pituitary-adrenal
OFT	The Open Field Test
FST	Force Swim Test
CRH	corticotropin-releasing hormone
ACTH	adrenocorticotropic hormone
CORT	corticosterone

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Author contributions

All authors have accepted responsibility for the entire content of this manuscript and therefore approve its submission. Kada Sanda Antoine, Oumar Mahamat, Tangu Patience Neng and Guessom Kamgue Oulianovie contributed to the study conception and design. Material preparation, data collection and analysis were performed by Bih Belta Lilian Fubi, Mumbi Laurantine Ngenteh, Kiafon Betrand Nsah and Ndifor Rose Nchang. The first draft of the manuscript was written by Antoine Kada Sanda Antoine and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability
All data sets generated during and/or analyzed during the present study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Procedures involving laboratory animals in this study were performed in conformity with the ethical standards of the Cameroon veterinary national research committee and with the 1964 Helsinki Declaration and its later adjustments. The study was approved by the Cameroon veterinary ethic committee (No 003/19 CCS/MINEPIA/RD-NW/DDME/SSV).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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