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Effects of *Khaya anthotheca* against behavioral disorders and oxidative stress induced by repeated variable stress

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Abstract

Aim: Menopausal women are suffering from stress-related disorders, and in the previous studies, *Khaya anthotheca* (*K. anthotheca*) decoction exhibited estrogenic and anxiolytic properties. Taken together, the aim of this study was to evaluate the effects of *K. anthotheca* decoction on behavioral disorders and oxidative stress induced by repeated variable stress in ovariectomized Wistar rats.

Methods: Forty-two female Wistar rats (10–12 weeks old; 145 ± 10 g) were used. They were ovariectomized (except those from the sham operated group). Fourteen days after ovariectomy, animals were randomly distributed into 7 groups (n = 6): sham operated and negative control groups receiving distilled water; two positive control groups receiving estradiol valerate and diazepam (1 mg/kg each), and three other groups receiving the tested doses of *K. anthotheca* extract (125, 250, and 500 mg/kg each). The treatment was applied every week. Anxiety, depression, and motor coordination were assessed throughout the experimental procedure. The anti-oxidative potential of the extract was evaluated in rat brain homogenate.

Results: It was noted that *K. anthotheca* extract induced anxiolytic effects marked by an increase in the locomotory activity during open field, light/dark, and elevated plus maze tests. Besides, its anti-depressive effects were shown by a significant ($p < 0.05$) decrease in the immobilization time during the forced swimming test. By improving the suspension time during grid and wire grip tests, the distance covered, and the number of switch directions during the beam walking test, the extract increased motor coordination. The antioxidant potential of the extract was marked by a significant decrease ($p < 0.01$) in malondialdehyde level and an increase ($p < 0.05$) in reduced glutathione level.

Conclusions: These results provide valuable insights into the potential therapeutic application of the *K. anthotheca* extract; however, more studies are needed to elucidate mechanisms of action.

Keywords

menopause, ovariectomy, repeated variable stress model, *Khaya anthotheca*, behavioral improvement, motor coordination improvement, antioxidant activity

Introduction

Menopause is a period in women characterized by changes in the menstrual cycle and a drop in estrogen levels. This reduction in estrogen production can lead to physical/physiological changes. Climacteric psychological changes include anxiety, depression, and irritability [1, 2]. In a menopause-induced ovariectomized rat model, estrogen depletion promotes the onset of anxiety and memory loss [3–6]. Examining stress and menopausal symptomatology, it has been reported that the severity of symptoms was directly related to life stressors and not to menopausal status, and an additive effect for the type of life event stressors commonly occur during the midlife years [2]. Low levels of stress might be useful and even healthy. However, high levels of stress could result in biological and psychological problems [7]. Stress can be caused by external or internal factors, and menopausal women can be more affected due to the lack of estrogen. In society, these disorders significantly affect socioeconomic performance. The stress factors can be family or personal, such as the needs of teenage children, career change, busy jobs, partner's challenge (or the stress of not having a partner), and health issues [2]. According to exposure duration to stressors, we can have acute stress (short-term) or chronic stress (long-term) [8]. In chronic stress, the patient is subjected to stress every day, and this stress is associated with negative effects on health. It is well known that chronic exposure to variable (type of chronic stress) stressors in an unpredictable manner provokes anxiety. The chronic variable stress (CVS) model, in which different stressors are applied in random, unexpected order, is non-habituating [9]. The paradigm of CVS, even when performed in many different schedules, produces a chronic state of hypothalamo-pituitary-adrenocortical hyperactivity [10].

The therapy to manage stress at the menopausal stage exists, but it presents many secondary effects [11, 12]. So, the alternative can be phytotherapy [13]. Furthermore, in order to cope with the various stressful situations experienced by menopausal women and to avoid polytherapy based on estrogens and anxiolytics, it is important to have a single treatment that could combine these two properties. The Cameroonian population uses the *Khaya anthotheca* (*K. anthotheca*, KA) extract in traditional medicine to manage anxiety in menopausal women [3]. In our previous work, KA showed estrogenic and anxiolytic properties in acute and sub-acute administration [3, 5]. It has also been shown that this extract is endowed with ameliorative effects in vanadium-induced anxiety, memory loss, and neuronal necrosis, and induced neuroprotection against estrogen depletion-induced neurodegeneration [14, 15]. The KA aqueous extract contains polyphenols such as flavonoids, and it is non-toxic after a sub-acute treatment [5, 15]. Because at menopause women experience different stress situations, and the estrogen-like, anti-anxiety-like, and neuroprotective effects of this extract plant, the aim of this study was to evaluate the protective effects of the KA decoction on behavioral disorders and oxidative stress in ovariectomized rats subjected to repeated variable stress.

Materials and methods

Animals

Female Wistar rats (145 ± 10 g; 10–12 weeks old) were used. Animals were handled according to the guidelines of the institutional Ethic Committee of Cameroon's Ministry of Scientific Research and Technological Innovation, which has equally adopted the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The study was carried out according to the Ethical Clearance Reference No: BTC-JIRB2023-079 of the Joint Institutional Review Board for Animal and Human Bioethics (JIRB) from the Center for Research and Graduate Studies in Life, Health and Environment Sciences—University of Yaounde I. The animals were housed in an environmentally controlled room (temperature 25°C; humidity 50–80%; 12 light-dark cycles). They had free access to tap water and a standard soy-free rat diet (SSniff GmbH, Soest, Germany).

Plant material and extraction

The stem barks of KA were collected in Mamougnam (District of Massangam, Department of Noun, West Region of Cameroun). The plant sample was identified at the National Herbarium of Cameroon (HNC) by comparison to the specimens deposited under voucher number 4230/HNC. The decoction and tested doses were obtained as previously described [3]. Briefly, the stem bark was dried and crushed. 250 g crushed bark was carried to ebullition for 30 min in 5 L of tap water. The supernatant was filtered and lyophilized, and 26.87 g of the dried extract was obtained (10.75% yield).

Chemicals

Diazepam (Diaz, Valium® 10 mg/2 mL, provided by Laboratoire Roche, Fontenay-sous-Bois, France) and estradiol valerate (E2V, Progynova® 2 mg, provided by DEL-PHARM, Lille, France) were used as the reference drugs. For this study, the use concentration of Diaz and E2V was 0.1 mg/mL.

Behavior assessment

For each stress behavioral assessment, all animals were acclimated at the laboratory for 72 h. The tests/stress were conducted in a calm room between 8 and 16 h under normal daylight [except beam walking (BW) test: 19 to 22 h]. To avoid perturbation, due to urine and feces, the different apparatuses were cleaned with a 70% ethanol solution and a dry cloth. During the behavioral test sessions, the data were collected with a video-camera system.

Open field

The open field (OF) apparatus described by Zemo et al. [4] was used. The OF test was used to evaluate the anxiolytic-like effect of the plant extract in animals. They were placed one by one at the center of the OF and allowed to explore it for 5 min. The time spent at the center, weight of fecal boli, number of rearing (number of times the animals stood on their hind legs), and number of crossings (number of square floor units entered) were recorded.

Light/Dark

The light/dark (L/D) apparatus used was made of wood and consisted of two rooms: a small black room (45 cm length, 30 cm broad, and 30 cm height) and a large light room (45 cm length, 45 cm broad, and 30 cm height). The two rooms were connected by a door (10 cm length, 10 cm broad) at the center of the separation wall (adapted from Arrant et al. [16]). The L/D test was used to evaluate the anxiolytic-like effect of the plant extract in animals. They were placed one by one at the center of the light room, looking toward the dark room, and allowed to explore the apparatus for 5 min. The escape latency time (latency time of entry into the dark room), number of transitions, total number of crossings (total number of square floor units entered in the two rooms), total number of rearing (total number of times the animals stood on their hind legs in the two rooms), and time spent in the dark room were recorded.

Forced swimming

The forced swimming (FS) apparatus used was made with a transparent cylindrical plastic bucket (30 cm diameter and 30 cm height). It was filled with tap water (25°C) at 23 cm high. The FS test (FST) was used to evaluate the effect of the plant extract on depression. Twenty-four hours before the test, each animal was subjected to a 15-minute training session (adapted from Yankelevitch-Yahav et al. [17]). During the test session, the immobilization time of each animal was recorded for 5 min. Immobility is defined as a stop of all movements, except those necessary to float, like splashing slightly with a foot. To preserve the temperature at 25°C, the clearness of the water in the bucket and to avoid any influence, water was renewed after the passage of each animal.

Grid suspension

The grid suspension (GS) apparatus used was made by a metal grid (55 cm length, 45 cm broad) assembled on a wooden framework. The stitch measured 1.5 cm on the sides. The grid was maintained horizontally at

100 cm height from the ground. A damping support was placed at 80 cm under the grid. The GS grip-strength test was used to evaluate the effects of the plant extract on grip strength. Each animal was placed at the center of the grid, and the apparatus was slowly turned over, so that the animal remained suspended under the device. The duration of the test was fixed at 60 seconds, and the latency time until the animal fell off the grid was recorded. The score of 60 seconds was allocated to the animals that succeeded in turning above the grid before the end of the session. This test was repeated in 3 sessions for each rat after 1 minute of rest. The average latency time of the three sessions was used for statistical analysis.

Beam walking

The beam used was adapted from Nicole et al. [18]. The apparatus was made from wood (120 cm length, 3.5 cm broad, 3 cm thickness), painted black, and the walking surface was graduated using a white line (5 cm interval). The beam was maintained horizontally at 88 cm height from the floor. The BW test was used to evaluate the effects of the plant extract on both locomotion and muscular coordination. Each animal was placed on the right end of the beam and spent 2 minutes on it. The distance covered on the beam, the number of switch directions, and the number of slips were recorded.

Stress induced by immobilization, inclined cage, and flooded cage

The immobilization of the animals was carried out using adhesive tape. The four paws of the rat were linked to abrogate mobility, and each animal was immobilized for 6 h [19]. To stress animals in an inclined cage, the cages used were inclined at 30°, and animals stayed there for 24 h. The method of stress induced by the flooded cage was done by introducing water into the cage at 2 cm with tap water, and the animals stayed there for 24 h.

Wire-suspension

The wire-suspension (WS) apparatus used was adapted from Baiba et al. [20]. It was made of a metal wire of 120 cm in length and 2 mm in diameter. The wire was maintained horizontally by two bars at a height of 100 cm above the ground. A damping support was placed at 80 cm under the wire. The animals were placed in the middle of the wire with their two forward paws. The WS grip agility test was used to evaluate the effects of the plant extract on grip and agility. The duration of the test was fixed at 60 seconds, and the latency time until the animal fell off the wire was recorded. The score of 60 seconds was allocated to the animals that succeeded in moving through the horizontal bar before the end of the session. This test was repeated in 3 sessions for each rat after 1 minute of rest. The average latency time of the three sessions was used for the statistical analysis.

Elevated plus maze

The elevated plus maze (EPM) apparatus described by Ketcha et al. [3] and Zemo et al. [4] was used. The EPM test was used to evaluate the anxiolytic-like effect of the plant extract in animals. They were placed one by one at the central square of the maze and allowed to explore it for 5 minutes. The number of rearing, the number of grooming, and the total number of entries into arms were recorded.

Experimental design

For this experiment, 36 female Wistar rats were subjected to the bilateral ovariectomy using the dorsal approach under Diaz (10 mg/kg, i.p.) and ketamine (ketamine hypochloride, Rotex Medica, Tritau, Allemagne; 50 mg/kg, i.p.) anesthesia [3, 6, 21], and 6 others were used as sham operated (SHAM). After 14 days of endogenous hormonal decline, animals were randomly distributed into seven groups (n = 6): the SHAM and negative control (OVX) groups receiving distilled water; two positive controls groups receiving E2V and Diaz at the dose of 1 mg/kg each and three other groups receiving the KA extract at the doses of 125, 250 and 500 mg/kg (KA 125, KA 250, and KA 500) each. The treatment was applied every week (the day of the stress situation). The substances were administered per os except Diaz (i.p.). The weight of the animals was taken every 7 days to evaluate the impact of the repeated variable stress on the metabolism. The stressful process or test was carried out one hour after the administration of the substances (except for

the L/D test: 30 min). To avoid habituation, to reduce mortality due to stress, and to diversify the neurological mechanisms involved in nervous pathologies, the animals were subjected to different unpredictable stressful processes every week. In the literature, there are several methods for exposing rodents to stress, and our laboratory has used an adapted protocol of repeated variable stress. After 14 days of endogenous hormonal decline, animals were subjected to different stress situations described above according to the following procedure in Figure 1.

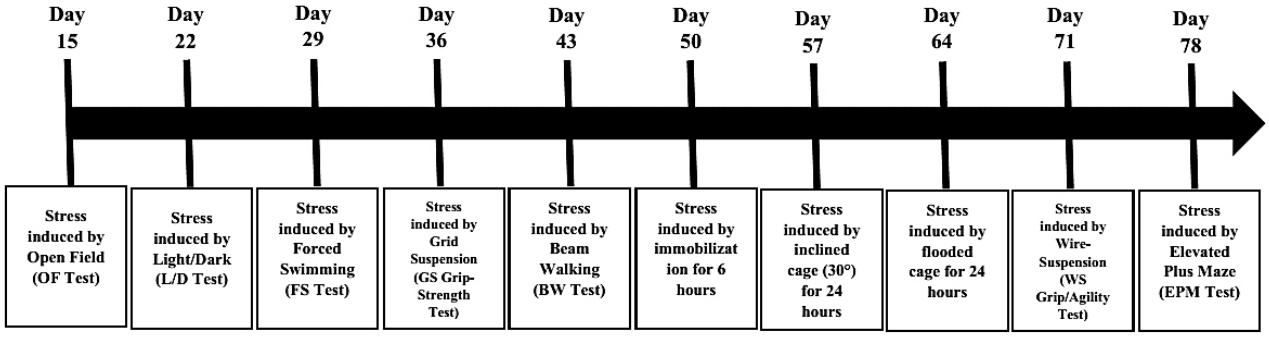

Figure 1. Repeated variable stress procedure.

On day 84 of estrogenic depletion, the animals were sacrificed by quick decapitation without anesthesia by a skilled operator, and the brain was immediately taken to prepare the 10% homogenate that was used for the determination of some oxidative stress biomarkers [MDA (malondialdehyde), GSH (reduced glutathione), and FRAP (ferric reducing antioxidant power)].

Biochemical parameter assay

MDA, GSH, and FRAP levels were determined using the method described by Wilbur et al. [22], Ellman [23], and Benzie and Strain [24], respectively. The absorbances were read at 532, 412, and 593 nm, respectively, for MDA (MAK568, Sigma-Aldrich®), GSH (MAK440, Sigma-Aldrich®), and FRAP (MAK509, Sigma-Aldrich®) using a UV spectrophotometer.

Statistical analysis

Results were expressed as the mean ± standard error of the mean (SEM). Unpaired *t*-test was used to determine the significance of the difference between the OVX group and SHAM group, and one-way ANOVA followed by Dunnett's test was used to determine the significance of the difference between treated groups and OVX group (Graph Pad Prism, version 5.03). A p-value < 0.05 was considered significant.

Results

Anxiolytic effects of *Khaya anthotheca* extract on ovariectomized rats evaluated by the open field test, light/dark test, and by the elevated plus maze test

In the OF test done after fifteen days of estrogenic depletion, compared to SHAM, ovariectomy led to a significant decrease ($p < 0.001$) in time spent in the center of the OF (Figure 2A). The treatment with KA, as well as E2V and Diaz, induced an increase of this parameter in comparison with the OVX group. It increased from 13.00 ± 1.44 s for the OVX group to 16.83 ± 1.33 s, 17.17 ± 0.98 s, and 17.17 ± 1.33 s, respectively, for the doses of 125, 250, and 500 mg/kg of KA. E2V and Diaz induced a significant ($p < 0.05$) increase in this parameter (Figure 2A).

As shown by the results in Figure 2B, ovariectomy induced a significant ($p < 0.001$) increase in the weight of fecal boli produced in comparison with the SHAM group. The treatment with KA, as well as E2V and Diaz, induced a significant ($p < 0.05$) decrease in this parameter compared to the OVX group. The results presented in Figure 2C showed that, compared to SHAM, ovariectomy induced a significant ($p < 0.01$) decrease in the number of rearing. The KA treatment, as well as E2V and Diaz, induced a significant (p

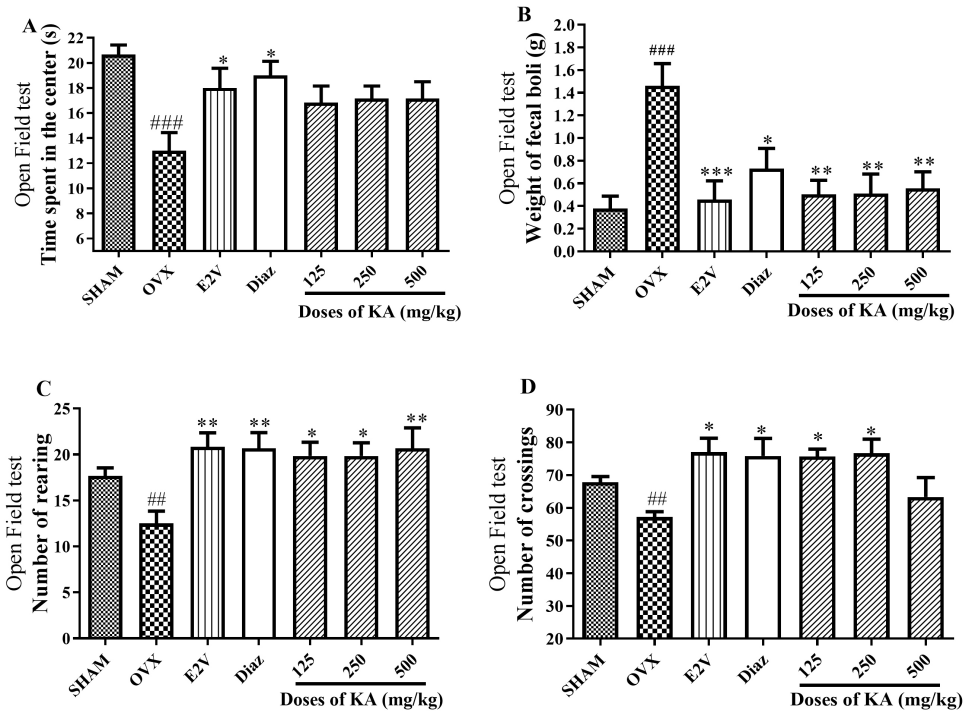

Figure 2. Effects of *Khaya anthotheca* extract on the behavior of ovariectomized rats evaluated by the open field test after fifteen days of estrogenic depletion. (**A**) Time spent in the center; (**B**) weight of fecal boli; (**C**) number of rearing; (**D**) number of crossings. Bars represent the mean ± SEM, $n = 6$. ##: $p < 0.01$, ###: $p < 0.001$ vs. SHAM (unpaired *t*-test); *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ vs. OVX (one-way ANOVA followed by Dunnett's test). Diaz: diazepam; E2V: estradiol valerate; KA: *Khaya anthotheca*; SEM: standard error of the mean; SHAM: sham operated; OVX: negative control.

< 0.05) increase in this parameter compared to the OVX group. The analysis of results presented in Figure 2D revealed that estrogen depletion induced a significant ($p < 0.01$) decrease in the number of crossings in comparison to the SHAM group. The KA treatment, as well as E2V and Diaz, induced an increase in the number of crossings. This increase was significant ($p < 0.05$) at the doses of 125 and 250 mg/kg for the extract and also with the reference drugs.

In the L/D test done after twenty-two days of estrogenic depletion, the results presented in Figure 3A revealed that, compared to SHAM, ovariectomy induced a significant ($p < 0.001$) decrease in escape latency time of entry into the dark room. The KA treatment, as well as E2V and Diaz, induced a non-significant increase in this escape latency time in comparison with the OVX group. It passed from 19.83 ± 1.38 s at the OVX group to 21.50 ± 1.72 s, 29.00 ± 5.13 s, and 31.00 ± 1.06 s, respectively, for the doses of 125, 250, and 500 mg/kg.

The analysis of results presented in Figure 3B revealed that ovariectomy induced a decrease in the number of transitions between the light and dark room compared to the SHAM group. This value was 3.17 ± 0.40 for the SHAM and 2.33 ± 0.33 for the OVX group. The treatment with KA extract, as well as E2V and Diaz, induced an increase in the number of transitions. It passed from 2.33 ± 033 for the OVX group to 3.67 ± 0.33, 2.50 ± 0.34, and 2.83 ± 1.16, respectively, for the doses of 125, 250, and 500 mg/kg. Compared to the OVX group, E2V and Diaz induced a significant ($p < 0.05$) increase in this same parameter.

The results presented in Figure 3C revealed that, compared to the SHAM group, ovariectomy induced a decrease in the total number of crossings. It passed from 45.17 ± 2.48 for the SHAM group to 39.00 ± 3.25 for the OVX group. The treatment with KA extract, as well as E2V and Diaz, induced a non-significant increase in this parameter in comparison with the OVX group. The total number of crossings passed from 39.00 ± 3.25 at the OVX group to 45.83 ± 5.72, 43.83 ± 1.45, and 43.83 ± 3.02, respectively, for the doses of 125, 250, and 500 mg/kg of KA.

The results presented in Figure 3D revealed that, compared to the SHAM group, ovariectomy induced a significant ($p < 0.05$) decrease in the total number of rearing. The treatment with KA extract at all tested doses, as well as E2V and Diaz, induced a significant ($p < 0.05$) increase in this parameter in comparison with the OVX group.

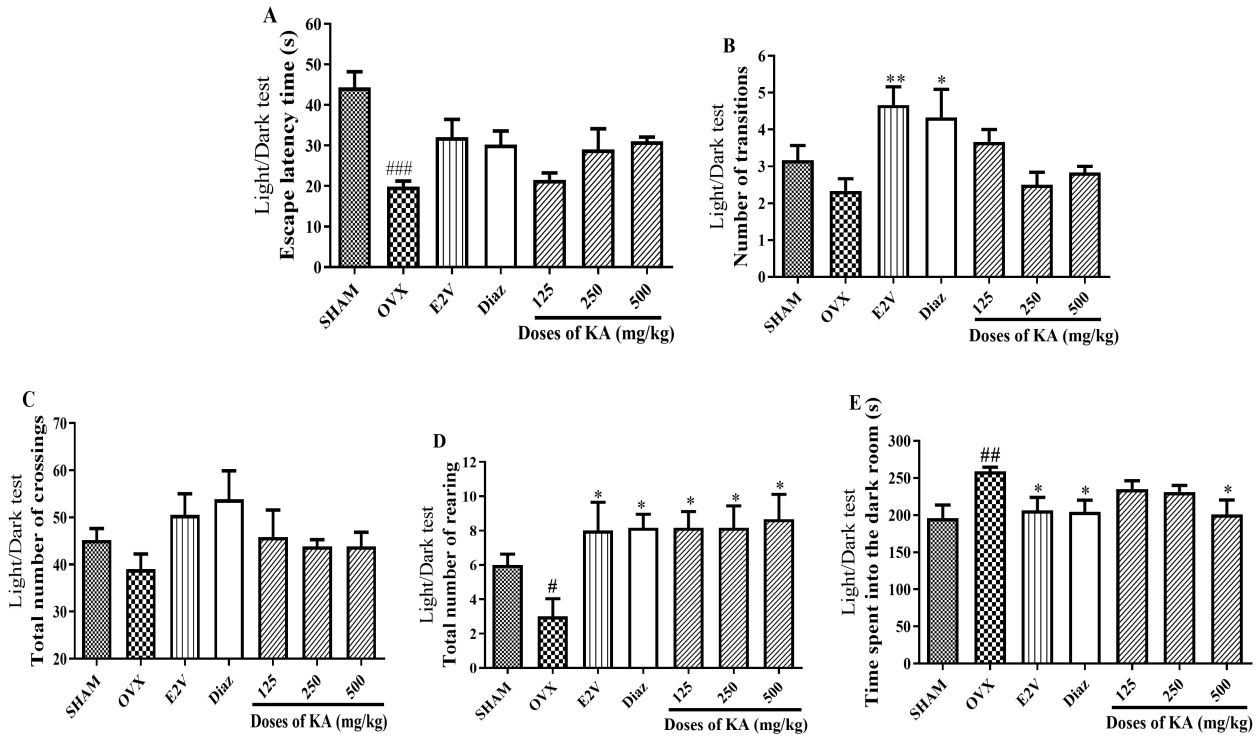

Figure 3. Effects of *Khaya anthotheca* extract on the behavior of ovariectomized rats evaluated by the light/dark test after twenty-two days of estrogenic depletion. (**A**) Escape latency time; (**B**) number of transitions; (**C**) total number of crossings; (**D**) total number of rearing; (**E**) time spent in the dark room. Bars represent the mean ± SEM, $n = 6$. #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$ vs. SHAM (unpaired *t*-test); *: $p < 0.05$, **: $p < 0.01$ vs. OVX (one-way ANOVA followed by Dunnett's test). Diaz: diazepam; E2V: estradiol valerate; KA: *Khaya anthotheca*; SEM: standard error of the mean; SHAM: sham operated; OVX: negative control.

The analysis of results presented in Figure 3E revealed that, compared to the SHAM group, ovariectomy induced a significant ($p < 0.01$) increase in the time spent in the dark room. The treatment with KA extract, as well as E2V and Diaz, induced a decrease in this time in comparison with the OVX group. This decrease was significant ($p < 0.05$) with the extract at the doses of 500 mg/kg, E2V, and Diaz.

In the EPM test done after seventy-eight days of estrogenic depletion, in comparison with the SHAM group, ovariectomy induced a significant ($p < 0.01$) decrease in the number of rearing (Figure 4A). The treatment with KA extract, as well as E2V and Diaz, induced an increase in the number of rearing in comparison with the OVX group. This increase was significant ($p < 0.05$) at the doses of 250 and 500 mg/kg.

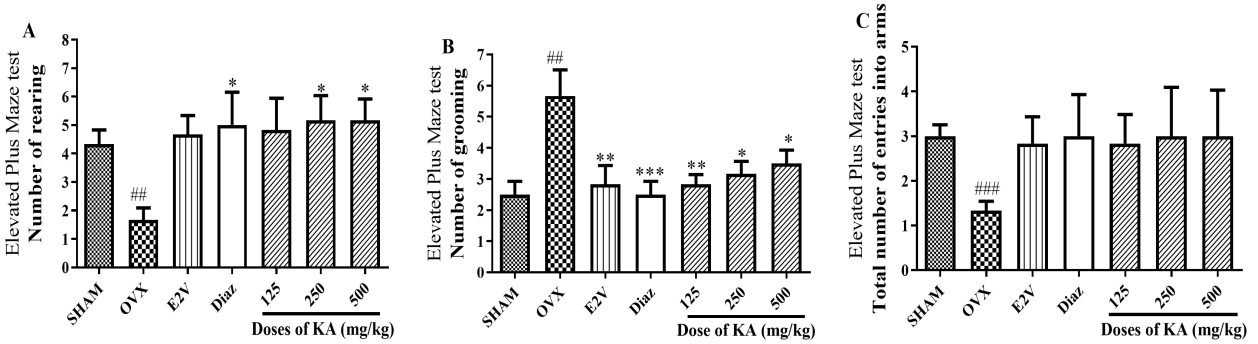

Figure 4. Effects of *Khaya anthotheca* extract on the behavior of ovariectomized rats evaluated by the elevated plus maze test after seventy-eight days of estrogenic depletion. (**A**) Number of rearing; (**B**) number of grooming; (**C**) total number of entries into arms. Bars represent the mean ± SEM, $n = 6$. ##: $p < 0.01$, ###: $p < 0.001$ vs. SHAM (unpaired *t*-test); *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ vs. OVX (one-way ANOVA followed by Dunnett's test). Diaz: diazepam; E2V: estradiol valerate; KA: *Khaya anthotheca*; SEM: standard error of the mean; SHAM: sham operated; OVX: negative control.

The results presented in Figure 4B revealed that ovariectomy induced a significant ($p < 0.01$) increase in the number of grooming compared to the SHAM group. The treatment with KA extract at all tested doses, as well as E2V and Diaz, induced a significant ($p < 0.05$) decrease in the number of grooming in comparison with the OVX group.

The analysis of Figure 4C revealed that ovariectomy induced a significant ($p < 0.001$) decrease in the total number of entries into arms of EPM in comparison with SHAM. Compared to the OVX group, the treatment with KA extract, as well as E2V and Diaz, induced a non-significant increase of this parameter. It passed from 1.33 ± 0.21 for the OVX group to 2.83 ± 0.65, 3.00 ± 1.10, and 3.00 ± 1.03, respectively, for the doses of 125, 250, and 500 mg/kg of KA (Figure 4C).

Antidepressant effects of *Khaya anthotheca* extract on ovariectomized rats evaluated by the forced swimming test after twenty-nine days of estrogenic depletion

The results presented in Figure 5 revealed that, compared to the SHAM group, ovariectomy induced a significant ($p < 0.001$) increase in the immobilization time. Compared to the OVX group, the treatments with KA at all tested doses and E2V induced a significant ($p < 0.05$) decrease in this parameter.

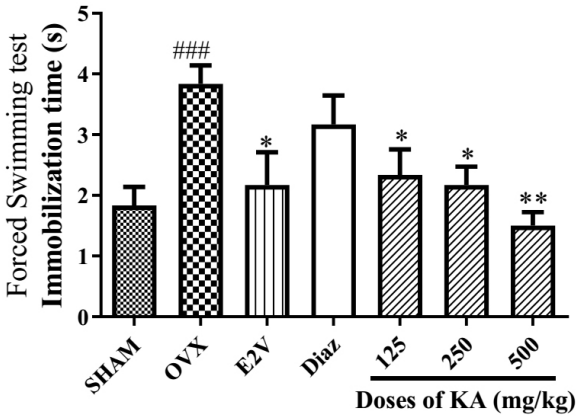

Figure 5. Effects of *Khaya anthotheca* extract on immobilization time of ovariectomized rat evaluated by the forced swimming test after twenty-nine days of estrogenic depletion. Bars represent the mean ± SEM, $n = 6$. ###: $p < 0.001$ vs. SHAM (unpaired *t*-test); *: $p < 0.05$, **: $p < 0.01$ vs. OVX (one-way ANOVA followed by Dunnett's test). Diaz: diazepam; E2V: estradiol valerate; KA: *Khaya anthotheca*; SEM: standard error of the mean; SHAM: sham operated; OVX: negative control.

Motor coordination improvement effects of *Khaya anthotheca* extract on ovariectomized rat evaluated by the grid suspension grip-strength test, beam walking test, and by the wire-suspension grip agility test

In the GS grip-strength test done after thirty-six days of estrogenic depletion, the analysis of the results in Figure 6A revealed that ovariectomy induced a significant ($p < 0.01$) decrease in the latency time to fall in comparison with the SHAM group. Compared to the OVX group, the treatments with KA at all tested doses and E2V induced a significant ($p < 0.05$) increase in the latency time to fall.

In the BW test done after forty-three days of estrogenic depletion, the results presented in Figure 6B revealed that ovariectomy induced a decrease in the covered distance on the bar in comparison to the SHAM group. It passed from 85.00 ± 9.13 cm at the SHAM group to 65.00 ± 11.11 cm in the OVX group. Compared to the OVX group, the treatments with KA, as well as E2V and Diaz, induced an increase in this parameter. This increase was significant ($p < 0.05$) with the extract at the doses of 250 and 500 mg/kg.

The analysis of Figure 6C revealed that ovariectomy induced a decrease in the number of switch directions on the bar in comparison to the SHAM group. This value passed from 2.83 ± 0.40 for the SHAM group to 1.83 ± 0.48 for the OVX group. Compared to the OVX group, the treatments with KA at all tested doses and E2V induced a significant ($p < 0.05$) increase in this parameter.

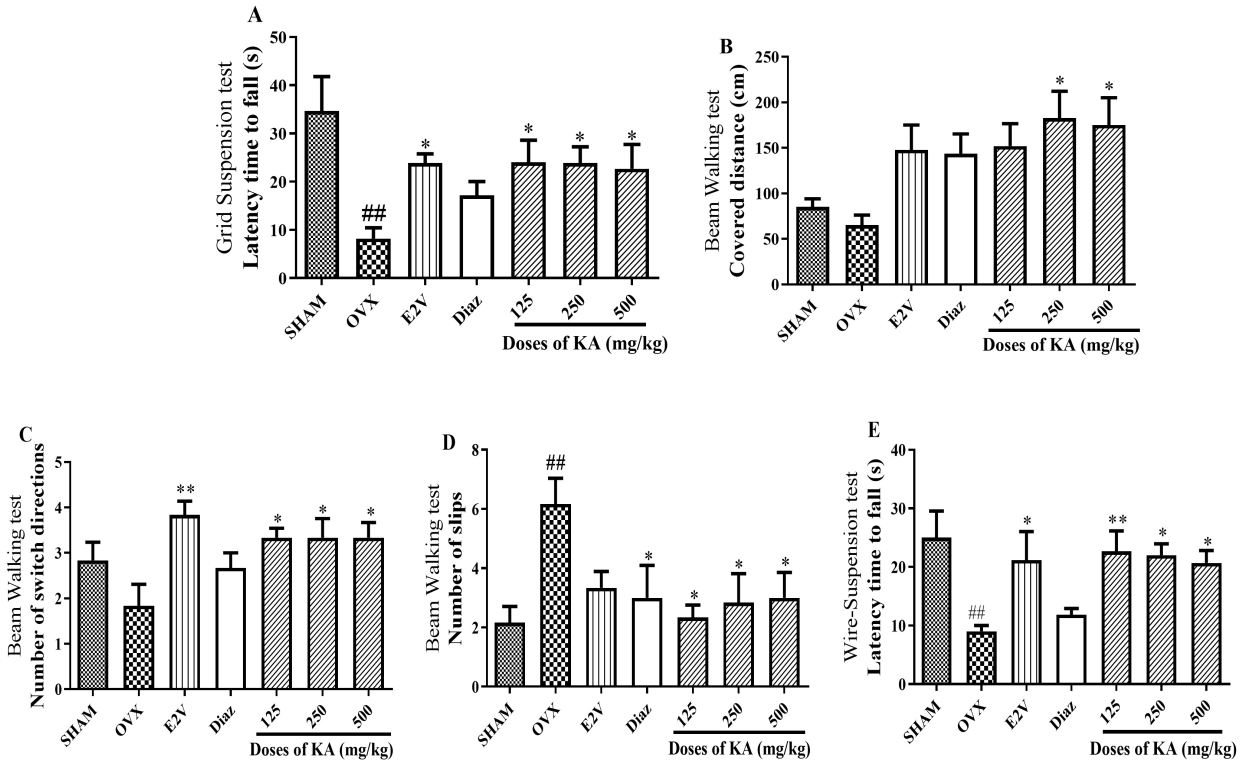

Figure 6. Effects of *Khaya anthotheca* extract on motor coordination of ovariectomized rats. (**A**) Grid suspension grip-strength test (after thirty-six days of estrogenic depletion); (**B**, **C**, and **D**) beam walking test (after forty-three days of estrogenic depletion); (**E**) wire-suspension grip agility test (after seventy-one days of estrogenic depletion). Bars represent the mean ± SEM, $n = 6$. ##: $p < 0.01$ vs. SHAM (unpaired *t*-test); *: $p < 0.05$, **: $p < 0.01$ vs. OVX (one-way ANOVA followed by Dunnett's test). Diaz: diazepam; E2V: estradiol valerate; KA: *Khaya anthotheca*; SEM: standard error of the mean; SHAM: sham operated; OVX: negative control.

The results presented in Figure 6D revealed that, compared to the SHAM group, ovariectomy induced a significant ($p < 0.01$) increase in the number of slips on the bar. The treatments with KA extract at all tested doses and Diaz induced a significant ($p < 0.05$) decrease in the number of slips in comparison with the OVX group.

In the WS grip agility test done after seventy-one days of estrogenic depletion, the analysis of Figure 6E revealed that ovariectomy induced a significant ($p < 0.01$) decrease in the latency time to fall in comparison with the SHAM group. The treatments with KA extract at all tested doses and E2V induced a significant ($p < 0.05$) increase in the latency time to fall in comparison with the OVX group.

Anti-oxidative activity of *Khaya anthotheca* extract on the brain of ovariectomized rats submitted to repeated variable stress and after eighty-four days of estrogenic depletion

The results presented in Figure 7 revealed that, compared to the SHAM group, ovariectomy and repeated stress induced a significant ($p < 0.001$) increase in MDA level (Figure 7A) and a significant ($p < 0.01$; $p < 0.05$) decrease in GSH (Figure 7B) and FRAP (Figure 7C) levels in the OVX group. Compared to the OVX group, the treatment with KA extract at all tested doses, as well as E2V and Diaz, induced a significant ($p < 0.05$) decrease in MDA level. The extract treatment, at all tested doses, induced a significant ($p < 0.05$) increase of GSH and FRAP levels in comparison with the OVX group.

Effects of *Khaya anthotheca* extract on the body weight of ovariectomized rats submitted to repeated variable stress

The results presented in Figure 8A revealed that repeated stress induced a decrease in body weight of the ovariectomized rat in comparison with the SHAM group. This decrease was significant ($p < 0.05$) at the 50th to 78th day of estrogen depletion. At day 36, the treatment with KA extract at all tested doses, as well as E2V and Diaz, reversed this loss of body weight observed in the OVX group (Figure 8B).

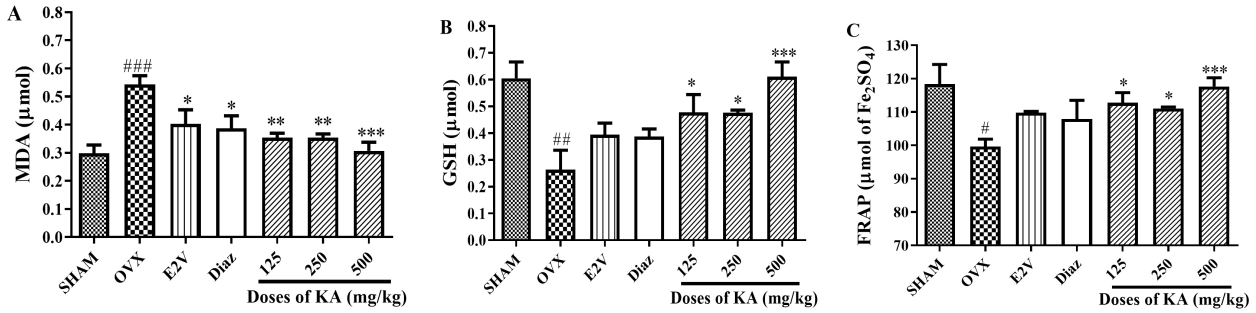

Figure 7. Effects of *Khaya anthotheca* extract on oxidative stress biomarkers in the brain of ovariectomized rats submitted to repeated variable stress and after eighty-four days of estrogenic depletion. (**A**) MDA; (**B**) GSH; (**C**) FRAP. Bars represent the mean ± SEM, $n = 6$. #: $p < 0.05$, ##: $p < 0.01$, ###: $p < 0.001$ vs. SHAM (unpaired *t*-test); *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ vs. OVX (one-way ANOVA followed by Dunnett's test). Diaz: diazepam; E2V: estradiol valerate; FRAP: ferric reducing antioxidant power; GSH: reduced glutathione; KA: *Khaya anthotheca*; MDA: malondialdehyde; SEM: standard error of the mean; SHAM: sham operated; OVX: negative control.

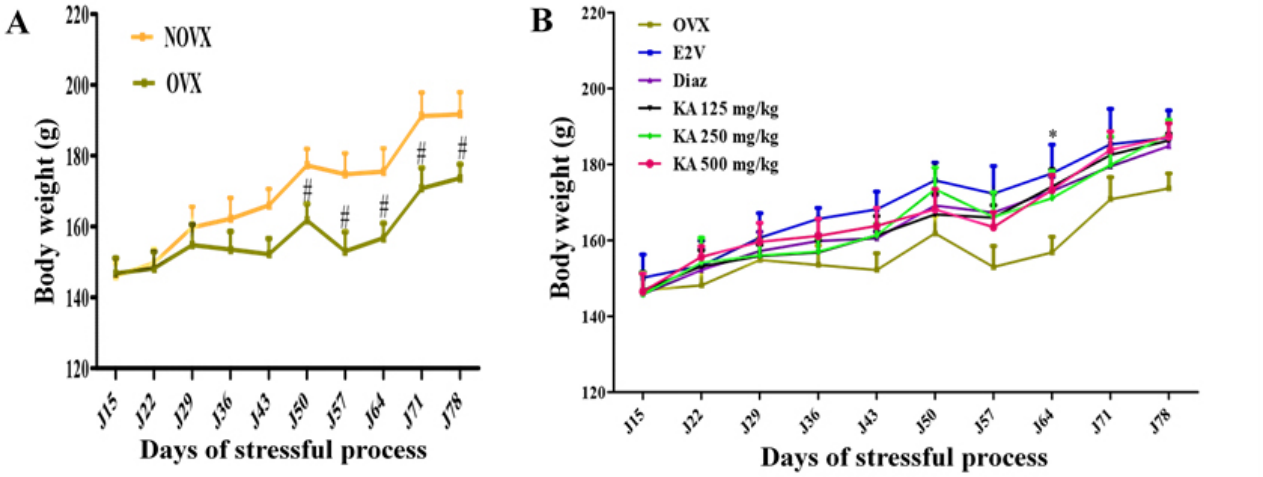

Figure 8. Effects of *Khaya anthotheca* extract on the body weight of ovariectomized rats submitted to repeated variable stress. Each point represents the mean ± SEM, $n = 6$. #: $p < 0.05$ vs. SHAM (unpaired *t*-test); *: $p < 0.05$ vs. OVX (one-way ANOVA followed by Dunnett's test). Diaz: diazepam; E2V: estradiol valerate; KA: *Khaya anthotheca*; SEM: standard error of the mean; SHAM: sham operated; OVX: negative control.

Discussion

Menopause is a permanent stop to menses that marks the end of a woman's reproductive cycle. This is due to the drastic drop in levels of endogenous estrogen. Sometimes called climacteric, menopause is marked by metabolic and physiological changes, causing several disorders in various tissues and systems, such as mood and behavior disorders in the nervous system [25–27]. Daily, menopausal women are subjected to various stressful situations. Indeed, menopausal symptom experience is impacted by lower income, more perceived stress, and a more negative attitude toward aging, according to Nosek et al. [28]. The aim of this study was to evaluate the effects of the KA aqueous extract on behavioral disorders and oxidative stress in ovariectomized rats submitted to repeated stress. Thus, ovariectomized rats were exposed to various unpredictable stressful situations.

The OF test executed after fifteen days of estrogen depletion revealed that ovariectomized animals (OVX) are more anxious than SHAM animals. In this study, compared to SHAM, this anxious status of ovariectomized rats was marked by a significant decrease in time spent in the center, number of rearing, and number of crossings. A significant increase in the weight of fecal boli was also observed in the OVX group. Indeed, Zemo et al. [4] showed that fourteen days of estrogen depletion is associated with anxiogenic effects. The treatment of ovariectomized rats with the KA extract at all tested doses, as well as E2V and Diaz, corrected all these effects induced by ovariectomy. These effects induced by the extract suggest that it is endowed with anxiolytic properties. Bum et al. [29] and Wanda et al. [30] showed that the substances that induce an increase in the time spent in the center, number of rearing, and number of crossings, and a

decrease in the weight of fecal boli possessed anxiolytic properties. According to these results, the KA extract shows anxiolytic effects in OF.

The L/D test executed after twenty-two days of estrogen depletion showed that treatment of animals with the KA extract at the dose of 500 mg/kg induced a significant decrease in the time spent in the dark room in comparison to the OVX group. As reported in the literature, this effect induced by the extract revealed a drop in the anxiety level [31]. Some studies showed that a decrease in the number of transitions between the dark and light rooms of the L/D box is an indication of an anxious state of the animals, and an increase in this parameter induced by a substance shows the anxiolytic properties of the substance [16, 32, 33]. Thus, KA extract at the dose of 125 mg/kg, by inducing an increase in the number of transitions, could possess anxiolytic effects. As presented during the OF test, anxious animals are characterized by a decrease in crossings and rearing. During the L/D test, the increase of these parameters induced by the treatment with KA extract showed that the extract is endowed with anxiolytic properties. Compared to the OVX group, the extract at the doses of 250 and 500 mg/kg also revealed its anxiolytic effects by inducing an increase in the latency time of entries in the dark room. As in the OF test, all these effects induced by the extract on the L/D test support its anxiolytic properties. These results suggest that the KA extract could contain compounds such as polyphenols endowed with anxiolytic effects.

Depression is a common mental disorder promoted by stress that is a leading cause of disability around the world and contributes greatly to the global burden of disease [19, 34]. In the FST, one sign of behavioral despair is a long immobility time, which matches the symptom of depression. So, this parameter is used as an indicator of depressed or not depressed/stressed or unstressed animals [17, 35]. The results obtained with the FST revealed that, compared to the SHAM group, twenty-nine days of estrogen depletion associated with repeated stress significantly increased the immobilization time. Compared to the OVX group, the treatments with KA extract at all tested doses, as well as E2V, induced a significant decrease in this immobilization time. According to Dar and Khatoon [35] and Walia [36], any substances that reduce the immobilization time of the animals subjected to the FST possess anti-depressive properties, and the effect could be mediated by the binding of bioactive compounds on the $GABA_A$ receptor. Thus, KA extract could contain compounds like polyphenols endowed with anti-depressive properties.

The evaluation of motor coordination is a considerable part of animal behavioral evaluation. To attain that, the GS grip-strength test, the BW test, and the WS grip agility test were performed after thirty-six, forty-three, and seventy-one days of estrogen depletion, respectively. The GS grip-strength test and WS grip agility test revealed that ovariectomy induced a significant decrease in the latency time to fall in comparison to the SHAM group. During the BW test, ovariectomy induced a decrease in the covered distance and the number of switch directions and a significant increase in the number of slips in comparison to the SHAM group. The treatments with KA extract, as well as reference drugs, reversed all these effects induced by estrogen depletion. It was observed a drop in motor coordination in the animals of the OVX group. According to Priya et al. [37], the substances that increase the level of activity during the grip test would be beneficial against neurodegenerative diseases such as Parkinson's disease. It was shown that forty-two days of estrogen depletion induced neurodegeneration in rats [15]. This neurodegeneration in ovariectomized rats could justify the drop in motor coordination observed during the BW test. Thus, KA extract could contain compounds that improve motor coordination.

After seventy-eight days of estrogen depletion, the ovariectomized animals subjected to the EPM test were more anxious than the SHAM group, as previously established with OF and L/D tests. Compared to the SHAM group, this anxious status of OVX animals was marked by a significant decrease in the number of rearing and total number of entries into the arms and a significant increase in the number of grooming. These effects induced by ovariectomy were corrected by the treatment with KA extract at all tested doses, as well as E2V and Diaz. The animals that received the extract treatment presented an increase in locomotor activity and level of exploration. According to some authors, the substances that induce such effects have anxiolytic properties [5, 27, 29, 30, 38]. These results obtained after seventy-eight days of estrogen depletion suggested that the weekly administration of the extract conserved its anxiolytic properties in the post-menopausal stage.

An important mechanism in the process of cell death is oxidative stress. In fact, in the cell membranes, the reactive oxygen species (ROS) attack the polyunsaturated fatty acids, thus causing lipid peroxidation and MDA formation, which is associated with damage to cell structures and functions [39]. After eighty-four days of estrogen depletion, compared to the SHAM group, ovariectomized rats presented a significant increase in MDA level in the brain and a significant decrease in GSH and FRAP levels. As reported by Ozgönül et al. [40] and Zemo et al. [15], estrogen depletion induced by ovariectomy is responsible for oxidative stress in the brain of rodents. The weekly treatment with KA extract, at all the tested doses, significantly reversed these effects induced by ovariectomy by inducing a significant decrease in MDA level and a significant increase in GSH and FRAP levels. These results suggest that KA extract is endowed with neuroprotective effects after repeated variable stress. This neuroprotection could be due to the estrogenic properties of this extract [3]. Indeed, estrogens have neuroprotective effects and play a significant role in the prevention of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, as well as depression and anxiety [41, 42].

It was shown that there exists a relationship between stress and metabolism [9]. In this study, body weight was analyzed to evaluate the impact of the repeated variable stress on the metabolism. As a result, compared to the SHAM group, the OVX animals presented a loss of body weight. This study revealed that the treatment with KA extract, as well as reference drugs, prevented the drop in body weight as observed in ovariectomized rats. In the literature, it was reported that exposure to chronic stress leads to a decrease in body weight [43]. Thus, the decrease in the body weight of OVX's animals could be due to the repeated variable stressful process. Repeated variable stress is known to induce a decrease in body weight and locomotor activity and affect fuel utilization [9]. The mechanisms involved are still unclear.

The beneficial effects of the aqueous extract of KA could be due to the estrogen-like compound present in this plant. Zemo et al. [15] showed that this plant extract contains polyphenol class compounds (flavonoids). It is well known that the polyphenols could act as endocrine estrogen and have estrogenic, anxiolytic, antioxidant, and neuroprotective effects [3, 14, 15, 44–46]. Polyphenols such as flavonoids found in this plant could induce these beneficial effects on the nervous system. This class of compound could act by binding to GABA and/or Erβ receptors, which play a major role in the regulation of brain function [15, 45, 46].

This study was carried out to evaluate the effects of KA aqueous extract on behavioral disorders and oxidative stress induced by repeated variable stress in ovariectomized Wistar rats. Results obtained in the current study suggest this model of repeated variable stress induced behavioral disorders and oxidative stress in the brain. The KA extract could be endowed with anxiolytic, anti-depressive, and antioxidant properties in ovariectomized rats exposed to repeated variable stress. The extract could also improve motor coordination in the same conditions. The results obtained show that all tested doses (125, 250, and 500 mg/kg) presented beneficial effects. According to the variation of the effects, the dose-effect relationship cannot be established. In the context of repeated and long-term treatment, small doses will be recommended. These results could justify the use of KA extract by traditional healers in Cameroon to manage stress conditions in menopausal women. This study provides valuable insights into potential therapeutic applications of KA extract. However, a limitation in this study could be the lack of determination of complete phytochemical standardization and the mechanisms of action. So, in the future, more studies are needed to standardize the extract and elucidate the mechanisms of action. The study was reported in accordance with the ARRIVE guidelines.

Abbreviations

BW: beam walking

CVS: chronic variable stress

Diaz: diazepam

E2V: estradiol valerate

EPM: elevated plus maze

FRAP: ferric reducing antioxidant power

FS: forced swimming

FST: forced swimming test

GS: grid suspension

GSH: reduced glutathione

HNC: National Herbarium of Cameroon

KA: *Khaya anthotheca*

L/D: light/dark

MDA: malondialdehyde

OF: open field

OVX: negative control

SHAM: sham operated

WS: wire-suspension

Declarations

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Author contributions

FGZ: Conceptualization, Methodology, Investigation, Formal analysis, Writing—original draft. SD: Conceptualization, Writing—review & editing. YSNM: Formal analysis, Writing—review & editing. CFA, RNTD, and CAP: Methodology, Investigation. DN: Supervision, Validation, Writing—review & editing. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Animals were handled according to the guidelines of the institutional Ethic Committee of Cameroon's Ministry of Scientific Research and Technological Innovation, which has equally adopted the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. The study was carried out according to the Ethical Clearance Reference No: BTC-JIRB2023-079 of the Joint Institutional Review Board for Animal and Human Bioethics (JIRB) from the Center for Research and Graduate Studies in Life, Health and Environment Sciences—University of Yaounde I.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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