Beppe GJ *et al.* (2023)
Notulae Scientia Biologicae
Volume 15, Issue 1, Article number 11459
DOI:10.15835/nsb15111459
Research Article

Antidepressant and anxiolytic-like activities of the dichloromethane/methanol extract of *Crateva adansonii* in mice exposed to chronic mild stress

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Abstract

Crateva adansonii (CA) is traditionally used in the treatment of epilepsy and memory loss. This work aims to evaluate the antidepressant and anxiolytic activities of the dichloromethane/methanol extract of *CA* trunk bark in a chronic unpredictable stress-induced depression (UCMS) model in mice. After exposure of mice to UCMS for 42 days, anhedonia was assessed using the sucrose preference test, antidepressant effects by the forced swim and caudal suspension tests, anxiolytic effects by the light/dark compartment (LDB) and open arena (OF) tests. Oxidative stress parameters Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), and Reduced Glutathione (GSH) were assessed. The results showed that multiple administrations of *C. adansonii* extract (150 and 300 mg/kg, resulted in a significant increase from 38.5% to 64.9% ($p<0.001$) in sucrose intake and a decrease from 47 seconds to 14 seconds in the immobility time in the forced swim test compared to the UCMS group. The extract significantly ($p<0.001$) reversed the time spent in the dark box at 150 mg/kg, and the number of groomings at 150 and 300 mg/kg compared to the UCMS group in the LDB and OF test. There was also a significant ($p<0.001$) improvement in SOD, GSH, and a reversal of MDA. The extract of *CA* improved symptoms of depression and anxiety in mice treated with different dose. The effects observed would be due to the presence in the extract of polyphenols such as flavonoids. These effects would justify the use of this extract in traditional medicine.

Keywords: antidepressant; antioxidant; anxiolytic; chronic stress; *Crateva adansonii*

Introduction

Depression is considered a psychiatric disorder characterized by sadness, loss of interest, and enjoyment, feelings of guilt, disturbed sleep, appetite, feeling tired, and lack of concentration (Moreno, 2017). It is considered a major public health problem; nearly one in five people have experienced, are experiencing, or will experience depression in their lifetime (Pelluet, 2019). Despite advances in the detection of this disease and the

Received: 26 Jan 2023. Received in revised form: 09 Mar 2023. Accepted: 10 Mar 2023. Published online: 16 Mar 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

discovery of new therapeutic strategies for its treatment, depression leads to numerous complications (Roopa *et al*., 2019). This pathology affects approximately 9% of the world's population and evolves in 20 to 30% of cases to relapse and then recurrence (Senova *et al*., 2019). Depression is linked to high mortality, contributes to suicide, disruption of relationships, memory impairment, and loss of work time, and often leads to abuse of certain medications (Halverson, 2010). Anxiety, which is often considered one of the symptoms of depression, is an emotion provoked by an observed or experienced threat, which most often leads to avoidance or evasion (Waraich *et al*., 2004). Permanent exposure to stress is the real cause of neuropsychiatric disturbances (Ricardo *et al*., 2010). These disturbances begin at the molecular level through stimulation of the hypothalamic-pituitary-adrenal complex or oxidative stress. Indeed, the involvement of stress and glucocorticoids in behavioral aspects is very important as they act on several vital components such as sleep (Born *et al*., 1989), mood disorders (Papadopoulou *et al*., 2015), food intake (Ulrich *et al*., 2015) or social behaviors (Cavigelli and Caruso, 2015). The stress axis regulates stress, and its non-regulation results in depression and its associated disorders (Prévôt, 2015). Several therapies are now used in the treatment of nervous system pathologies in general and depression in particular. Antidepressants currently used as treatment act through one or more of the following mechanisms: either through inhibition of serotonin or norepinephrine and dopamine reuptake, antagonism of serotonergic or noradrenergic presynaptic inhibitory receptors, or inhibition of monoamine oxidase (Mazelin, 2019). However, these classes of drugs have many serious side effects such as hallucinations, memory impairment, anxiety, and even depression (Sehonou and Dodo, 2018). Basic and clinical research is very interested in exploring new therapeutic targets, and new molecules acting on the central nervous system. *Crateva adansonii* DC is a tree widespread in the Sahelian and Sudanian zones. This plant is used in traditional medicine to treat epilepsy. Its bactericidal, anti-inflammatory actions and effects against yellow fever, hemorrhoid, indigestion, and gastritis have been scientifically proven (Zingue *et al*., 2016b). This study was undertaken to investigate the neuroprotective effects of Dichloromethane/Methanol (DCM/MeOH) extract of *C. adansonii* DC. bark on chronic mild stress induced in mice.

Materials and Methods

Chemical substances

Clomipramine (Anafranil®) was obtained from Alfa-sigma (France), and was dissolved in 2% ethanol. It was administered orally at a dose of 20 mg/kg, and a volume of 10 ml/kg.

Plant material and extraction protocol

The bark of the trunk of *Crateva adansonii* (Capparaceae) was collected in April 2020 in the locality of Moutourwa in the Far North of Cameroon. It was recognized by Professor TODOU Gilbert, a botanist at the University of Maroua, and authenticated at the National Herbarium of Cameroon by comparison with a sample that was there under the reference number HNC 36359. The bark was dried and ground. The powdered bark of *C. adansonii* (2000 g) obtained was recollected in 5 L of DCM/MeOH mixture (v/v : 1/1) for 72 h at room temperature and the obtained macerate was filtered with Whatman paper N°4. The filtrate obtained was concentrated in a rotavapor (BUCHI R-300) at 60 °C. A dry extract (33.2 g) was obtained, representing an extraction yield of 1.66%.

Quantitative phytochemical analysis of dichloromethane/methanol extract of C. adansonii trunk bark

Determination of total polyphenols

Total polyphenols were performed according to the method of Folin-Ciocalteu (Mahmoudi *et al*., 2013). The extract was dissolved in methanol at a concentration of 10 g/L. The assay consisted of taking a volume of 0.5 mL of the solution or standard solution with 1 mL of 1/10th Folin- Ciocalteu. After 5 minutes,

1 mL of 7.5% sodium carbonate was added to the solution, the tubes were then placed in the dark for 30 minutes at a temperature of 37 °C, and the absorbance was measured at a wavelength of 750 nm. The concentrations of polyphenols in the sample were determined from a calibration range established with gallic acid (0-125 µg/mL).

Determination of flavonoids

In an acidic medium, and the presence of aluminum chloride ($AlCl_3$), flavonoids give a red coloration with an absorption maximum of 430 nm. The assay consisted in mixing 1 mL of extract dissolved in methanol at a concentration of 10 g/L or the standard solution with 1 mL of $AlCl_3$, after shaking, 2 drops of acetic acid were added and then after a second shaking, the absorbance was measured at a wavelength 430 nm. The concentrations of flavonoids in the sample were determined from a calibration range established with quercetin (0-100µg/mL) (Mimica-Duckic, 1999).

Determination of tannins

In acidic media, tannins react with vanillin to form a complex that exhibits an absorption maximum at 500 nm. The assay consisted in mixing a volume of 0.2 mL of methanolic extract at a concentration of 10 g/L or of the standard solution with 2 mL of reagent (1 g of vanillin/100 mL concentrated HCl), the mixture was shaken and incubated at 30 °C for 5 min then the OD was read at 500 nm. Flavonoid concentrations in the sample were determined from a calibration range established with catechin (0-50 µg/mL) (Bainbridge *et al*., 1996).

Evaluation of the in vitro antioxidant activity of the Dichloromethane/Methanol extract of the bark of the trunk of C. adansonii

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing power was determined according to the method recommended by Benzie and Strain (1996). The assay consisted of mixing a volume of 0.1 mL of extract dissolved in methanol (10g/L) or standard solution with 1 mL of Fe(III)-TPTZ solution (Acetate buffer/TPTZ/$FeCl_3$=10:1:1), the mixture was stirred and then 5 min after, the OD was read at 593 nm. The reducing powers of the sample were determined from a calibration range established with vitamin C (0-125 µg/mL). An increase in the absorbance corresponded to an increase in the reducing power of the extracts tested (Hubert, 2006).

Evaluation of the antiradical activity with DPPH

2,2-Diphenyl-2-picrylhydrazyl (DPPH) is a stable purplish free radical that absorbs at 517 nm. This method is based on measuring the ability of antioxidants to scavenge the DPPH radical (Sun *et al*., 2005). The assay consisted of mixing a volume of 0.2 mL of extract dissolved in methanol (10 g/L) or standard solution with 2 mL of DPPH solution, the mixture was stirred, and then 5 min after, the OD was read at 517 nm. The antioxidant powers of the sample were determined from the calibration range established with Trolox (0-125 µg/mL).

Animal material and experimental protocol

Thirty male mice aged 8-12 weeks and weighing between 20-30 g were randomly distributed into 5 groups of 6 animals each. They were housed in plastic cages and acclimated for two weeks before the start of the experiment. The animals had free access to food and water, except on days when food and water deprivation were used as stressors. Animals were subjected to UCMS daily for 42 days and 30 minutes after treatment administration. Preliminary tests were carried out in order to choose the different doses. A normal control group received 2% ethanol (10 mL/kg, p.o), a negative control group received 2% ethanol (10 mL/kg, p.o), a positive control group received Clomipramine (20 mg/kg, p.o) and two test groups that received *C. adansonii*

extract at different doses (CA 150 and 300 mg/kg; p.o). Only animals in the normal control group were not subjected to UCMS. Behavioural tests were started after 30 days of treatment.

UCMS were induced according to the method described by Kuegong *et al.* (2020) with a slight modification (Table 1).

Table 1. Chronology of exposure to stressors and their duration

Week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Week 1	Weighing Social stress (4h) Litter removal (2h)	Night (3h) Restraint (2h) Day (6h)	Changes to Litter Social stress (4h)	Restraint (2h) Wet bedding (6h)	Tilt (3h) Social stress (4h)	Food deprivation (24h)	Sound stimulation (2h)
Week 2	Weighing Restraint (3h) Tilt (2h) Mouse feces (2h)	Wet bedding (12h) Forced swimming (10 min) Litter changes bedding	Social stress (4h) Restraint (1h30)	Sound stimulation (2h) Cold bath (5min)	Water deprivation (24)	Day/night alternation every 30 minutes (6h)	Day/night alternation every 30 minutes (6h)
Week 3	Weighing Insulation (24h)	Insulation (24h)	Social stress (4h) Bath (2h)	Wet litter (12h) Litter changes	Restraint (6h)	Food deprivation (24h)	sound stimulation (3h)
Week 4	Weighing Wet litter (4h) Cold bath (5min)	Social stress (4h) Forced swim at 45°C (5min)	Change of bedding Day/night alternation (4h)	Wet litter (24h)	Changes in bedding Bath (2h)	Day (12h)	Day/night alternation every 30 minutes (4h)
Week 5	Weighing Wet litter (4h) Forced swimming (10 min)	Tilt (3h) Social stress (3h)	Restraint (2h) Litter removal (4)	Mouse feces (2h) Litter change	Cold bath (10 min)	Day/night alternation every 30 minutes (6h)	Food deprivation (24h)
Week 6	Weighing	Restraint (4h)	Forced swimming 45°C (5 min) Sound stimulation (2h)	Insulation (24h)	Restraint (1h) Wet bedding (6h)	Tilt (3h) Night (3h)	Sucrose preference test
	Forced swimming test	Tests of the light/dark compartment box and Open Field Test	Sacrifice				

Behavioural tests

Depression Tests

Sucrose preference test

Anhedonia allows the effectiveness of the UCMS protocol to be monitored. For each isolated mouse, two water bottles were provided, one containing tap water and the other a 1% sucrose solution. The positions of the water bottles were changed 12 h later to avoid the lateralization effect (Liu *et al*., 2016). The sucrose solution was prepared in advance, and the solution temperature was equal to room temperature (Chang *et al*., 2012). The water provided to the animals was at room temperature. The sucrose preference was determined as follows:

Sucrose preference (%) = (amount of 1% sucrose solution) / (Volume of sucrose solution + volume of water) × 100

Forced swimming test

The forced swimming test is used to measure depressive-like behavior in animals (Porsolt, 1979). Each animal is placed in a device consisting of a transparent glass cylinder (30 cm high × 20 cm diameter and 20 cm deep) containing water maintained at 25 ± 2 °C (Kitada *et al*., 2017). The aim of the test is to leave the animal for 6 minutes and attempt to climb the wall or remain immobile. A resigned or depressed animal showing behavioral despair will spend more time immobile than a control animal (Beppe *et al*., 2015). The animal is then removed from the cylinder, dried with a towel, and placed under a heat lamp until recovery.

Anxiety tests

Lighted/dark box test

The lighted/shadowed box test was carried out according to Rebai's (2017) method with small modifications. The lighted/obscure box (45 × 27 × 27 cm) was made of polywood and consisted of two pieces connected by an opening (7.5 × 7.5 cm) located at floor level in the center of the partition. The floor was divided into 9 × 9 cm squares and covered with Plexiglas. The small room (18 × 27 cm) was painted black and the large room (27 × 27 cm) white. Lighting was provided by a 60-watt table lamp located 40 cm above the center of the white chamber (Foyet *et al*., 2012). During the test, mice were individually placed in the center of the light room with their backs opposite to the light room, and the behaviors observed were latency, number of transitions, time spent in the lightroom, and time spent in the darkroom.

Open field test

The open space arena was constructed of white polywood and square in shape measuring 72 × 72 cm with a height of 36 cm. Three red lines were drawn with a marker and were visible through the clear Plexiglas floor delineating the central zone, intermediate zone, and peripheral zone (Foyet *et al*., 2012). Mice were placed one at a time in the open field box for 6 min, and the behaviors recorded were time spent in the central square, number of lines crossed, the number of dressings, number of groomings, and time spent at the edge of the arena. After each trial, the mouse was removed and returned to its cage, and the entire field was cleaned with a 70% ethanol solution before the next animal was tested.

Biochemical analysis of oxidative stress parameters

Brain sample preparation

On the last day of the experiment, the mice were anaesthetized and decapitated. The whole brain was removed for biochemical studies. Part of the brain was ground and homogenized with 0.1M phosphate buffer (pH 7.4), the homogenate was centrifuged (3000 rpm for 15 min at 4 °C), and the supernatant was collected and stored at -20 °C for biochemical analyses.

Determination of Malondialdehyde (MDA)

The determination of the amount of MDA in the hippocampus was performed following the technique described by Wilbur *et al.* (1959). In each test tube, 0.5 mL of an iron chloride solution (2 mM) was added to 0.5 ml of the homogenate. The reaction medium was incubated for 1 h at room temperature and then centrifuged at 1000 rpm for 10 min. Subsequently, 100 µL of supernatant was mixed with 500 µL of 1% phosphoric acid and 500 µL of thiobarbituric acid in a 1% TCA solution. The mixture was homogenized by vortexing, passed through a boiling water bath for 20 min, cooled in an ice bath, and centrifuged at 3000 rpm for 10 min. The supernatant was collected again, and the absorbance was read at 532 nm against the blank. The amounts of MDA were estimated and expressed as Mm/mg of an organ.

Superoxide Dismutase (SOD) assay

The assay of SOD was performed according to the principle described by Mishra in 1972, which is based on the ability of SOD to inhibit or retard the auto-oxidation of adrenaline to adrenochrome in basic media. For this, 140 µL of the homogenates were added to 1660 µL of carbonate buffer (pH=10.2) followed by 200 µL of freshly prepared adrenochrome (0.3 mM). Auto-oxidation was then measured by OD reading at 480 nm at t=30 s and t=90 s. SOD activity was expressed in units/mg of an organ.

Determination of reduced glutathione (GSH)

The GSH assay is performed according to the method described by Ellman in 1959, which is based on the reaction of 2,2-dinitro-5,5-dithiodibenzoic acid (DTNB) with the SH group of glutathione to form a yellow complex, the yellow complex absorbing at 412 nm. In each assay, 200 µL of homogenate and 3 mL of Ellman's reagent are added to the tube. After homogenisation, the mixture is incubated at room temperature for 60 min. Prepare blank tubes under the same experimental conditions, replacing the homogenate with phosphate-buffered saline (0.2 M, pH 7.4, pKa 7.2). Read the absorbance of each tube at 412 nm against the blank and the amount of MDA expressed in mM/mg of organ.

Histology of the tissues

Histological analysis of the brain (hippocampus) was assessed using 5 µm sections of paraffin-embedded tissue. Coronal slices were obtained from the brain (left hemisphere) in the hippocampal region using the Mouse Brain Atlas with the following coordinates (anterior/posterior D 2.0 mm, medial/lateral D 1.5 mm and dorsal/ventral AP D 2.0 mm) (Smith and Bruton, 1977). After hematoxylin-eosin staining, micrographs of brain sections were evaluated using a digital camera (Scientico, Haryana, India) attached to a light microscope.

Statistical analysis

The results obtained were expressed as mean ± SEM. Data were analyzed by one-way ANOVA (Force Swimming Test, Light and Dark Box, and Open Arena Test), and two-factor ANOVA (TPS) followed by Dunnett's and Bonferroni's post-tests, respectively. All analyses were performed using Graph Pad Prism version 8.0.1 for Windows. Results were considered significant for $p<0.05$.

Results

Total polyphenols content (TPC), total flavonoids content (TFC), and total tannins of the DCM/MeOH extract of C. adansonii bark

The phytochemical screening performed on the DCM/MeOH extract of *C. adansonii* showed that flavonoids are the most abundant polyphenols, with an amount of 211.47 ± 11.66 mg quercetin equivalent

(eq) /g dry extract compared to tannins with an amount of 152.96 ± 5.12 mg catechin eq /g dry extract (Table 2).

Table 2. Total phenol, total flavonids and total tanin quantity of the DCM/MeOH extract of *C. adansoni*

Compounds	Concentration in the DCM/MeOH of *C. adansonii*
Total phenol	384.02 ± 6.27 (mg EGA/100 g DW)
Total flavonoids	211. 47 ± 5.38 (mgEQ/100 g DW)
Total tanin	152. 96 ± 5.12 (mg EC/100 g DW)

Results are expressed as mean ± MSE.mgEGA/100 g DW: milligram equivalent gallic acid per 100 g Dry Weight; mg EQ/100 g DW: milligram equivalent quercetin per 100 g Dry Weight; mg EC/100 g D W: milligram equivalent catechin per 100 g Dry Weight.

In vitro antioxidant activity of the DCM/MeOH extract of C. adansonii bark

The antioxidant potential of the DCM/MeOH extract from the bark of the trunk of *C. adansonii* was measured by two tests (DPPH and FRAP) and is correlated with their contents in total phenols and flavonoids. Figure 1 below shows the reducing power of iron and DPPH of the DCM/MeOH extract of the bark of the trunk of *C. adansonii.* The results revealed an inhibition of 60.36% for the DPPH test and 42.630% of the FRAP test, they are lower compared to butylhydroxytoluene (BHT) which showed 75.453% inhibition.

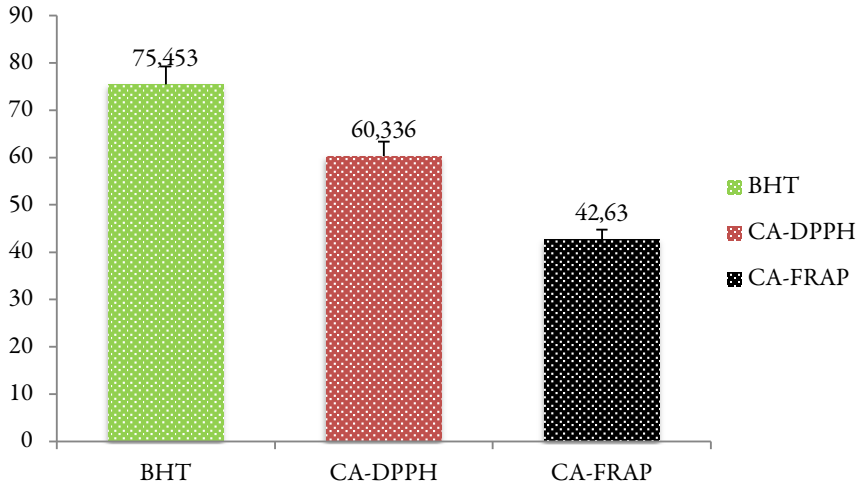

Figure 1. *In vitro* antioxidant activity at DPPH and FRAP of the DCM/MeOH extract of *C. adansonii*

Effects of DCM/MeOH extract of C. adansonii bark on sucrose preference and immobility time in the Forced Swimming Test

After 30 days of treatment, UCMS induced a significant decrease ($p<0.001$) in sucrose preference (Figure 2A) and significantly increased ($p<0.001$) immobility time (Figure 2B) of animals in the negative control group compared to animals in the normal control group. The administration of the extract at the doses of 150 and 300 mg/kg caused a significant increase ($p<0.001$) in sucrose preference and a significant decrease ($p<0.001$) in the immobility time of the animals compared to the negative control group. The immobility time thus decreased from 46,8±0,689 s in the UCMS control group to 13,9±0,688 s in the test group treated at a dose of 150 mg/kg. Similarly, there was a significant increase ($p<0.001$) in sucrose preference and a significant decrease ($p<0.001$) in immobility time in animals in the positive control group compared to animals in the negative control group.

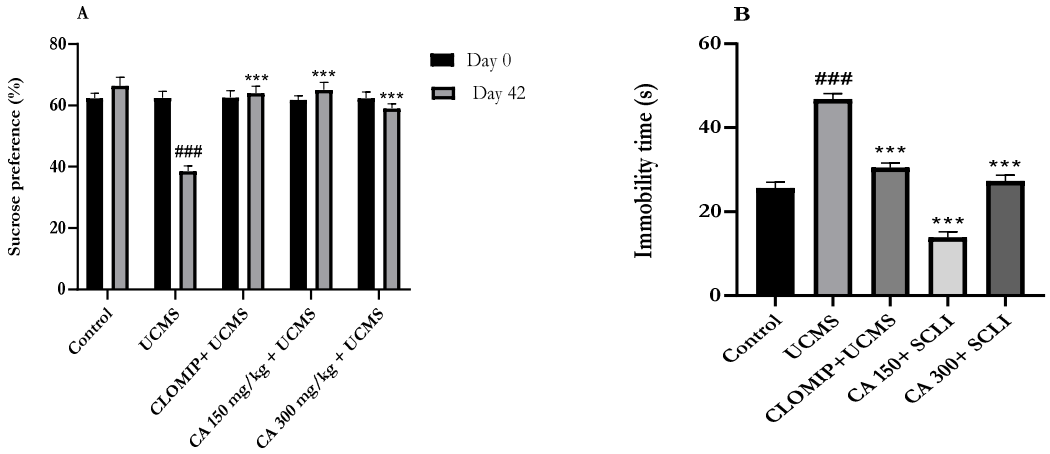

Figure 2. Effect of DCM/MeOH extract of *C. adansonii* on sucrose preference (A) and immobility time in the FST (B) of mice before and after 30 days of treatment

Each bar represents the mean ± MSE, UCMS: chronic mild unpredictable stress; CLOMIP + UCMS: positive control receiving clomipramine (20 mg/kg); CA + UCMS: animals receiving the DCM/MeOH mixture extract (v/v: 1/1) of *C. adansonii* at doses of 150 and 300 mg/kg. $^{***}p < 0.001$ compared to UCMS group. $^{###}p < 0.001$ compared to the normal group.

Effects of DCM/MeOH extract of C. adansonii bark on time spent in the LDB and number of groomings in the OFT

Figure 3 below represents the time spent in the dark compartment in the LDBT (Figure 3A) and the number of grooming in the OFT (Figure 3B) of the animals after 30 days of treatments. UCMS induced a significant ($p<0.001$) increase in the time spent in the dark compartment, increasing this time from 122±1.56 s in the normal control group to 164±1.55 s in the UCMS control group, and from the grooming count of 3.00±0.0632 in the normal control group to 6.00±0.0816 in the UCMS control group. In the UCMS extract-treated groups, there was a significant ($p<0.001$) decrease in the time spent in the dark compartment (105± 1.87 s) at the dose of 150 mg/kg and the number of groomings (3.00±0.0816 s; 3.80±0.122 s) at the doses of 150 and 300 mg/kg respectively compared to the UCMS control group. Clomipramine induced a significant decrease ($p<0.001$) in the time spent in the dark compartment of the positive control group compared to the negative control group.

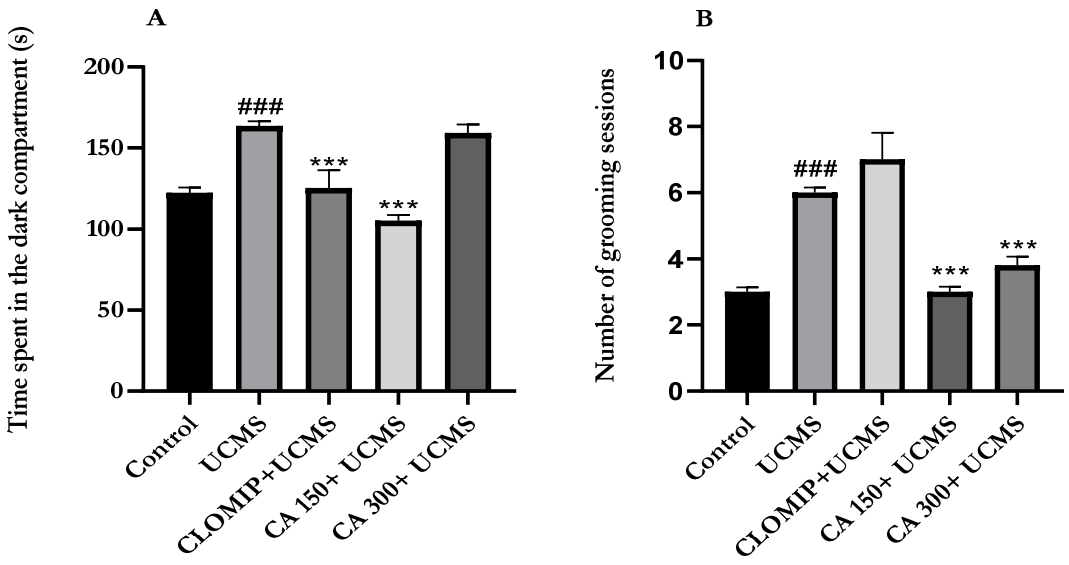

Figure 3. Effect of DCM/MeOH extract of *C. adansonii* on time in the LDBT (A) and number of groomings in OAT (B) after 30 days of treatment

Each bar represents the mean ± MSE, UCMS: chronic mild unpredictable stress; CLOMIP + UCMS: positive control receiving clomipramine (20 mg/kg); CA + UCMS: animals receiving the DCM/MeOH mixture extract (v/v: 1/1) of C. adansonii at 150 and 300 mg/kg. ***p < 0.001 compared to UCMS group. ###p < 0.001 compared to the normal group.

Effect of DCM/MeOH extract of C. adansonii bark on Malondialdehyde (MDA) concentration, superoxide dismutase (SOD) activity, and reduced glutathione (GSH) concentration

Figure 4 below shows the concentration of MDA (Figure 4A), SOD activity (Figure 4B) and GSH concentration (Figure 4C) in the hippocampal homogenate of mice after 30 days of treatments. UCMS induced a significant increase ($p<0.001$) in MDA concentration and a significant decrease ($p<0.001$) in SOD activity in negative control animals compared to normal control animals. The extract caused a significant decrease (150 mg/kg, $p<0.001$; 300 mg/kg, $p<0.01$) in MDA concentration and a significant increase ($p<0.001$) in SOD activity and GSH concentration at doses of 150 and 300 mg/kg in treated animals compared the control group. Similarly. There was also a significant ($p<0.01$) decrease in MDA concentration and a significant ($p<0.001$) increase in SOD activity in positive control animals compared to negative control animals.

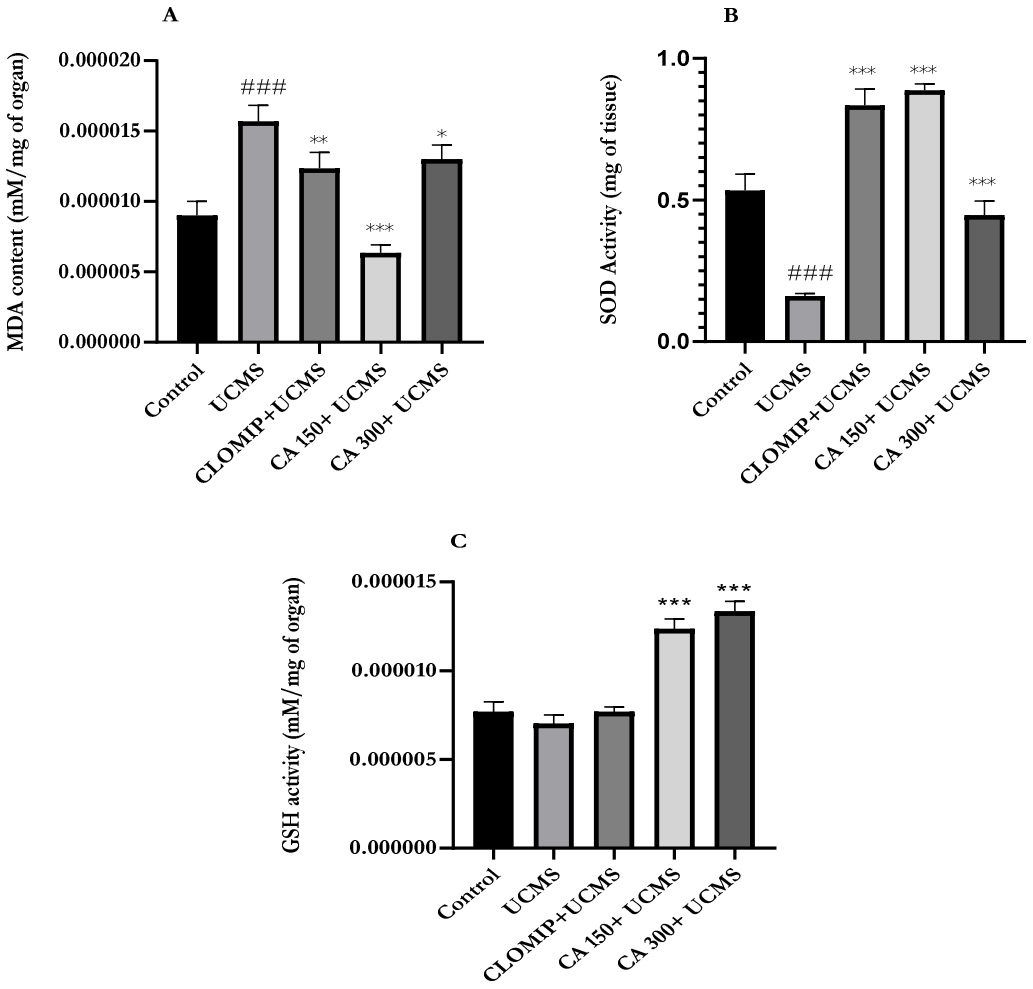

Figure 4. Effects of DCM/MeOH extract of *C. adansonii* on MDA concentration (A), SOD activity (B) and GSH concentration (C) in the hippocampus of mice after 30 days of treatment

Each bar represents the mean ± MSE, UCMS: chronic mild unpredictable stress; CLOMIP + UCMS: positive control receiving clomipramine (20 mg/kg); CA + UCMS: animals receiving the DCM/MeOH mixture extract (v/v: 1/1) of *C. adansonii* at doses of 150 and 300 mg/kg. ***p < 0.001 compared to UCMS group. ###p < 0.001 compared to the normal group.

Effects of DCM/MeOH extract of C. adansnii bark on hippocampal microarchitecture

The hippocampal microarchitecture depicted in Figure 5 shows that the negative control exhibits several histopathological changes in the hippocampus, marked by a decrease in the density of neuronal cells in Ammon's corns (CA1, CA2, and CA3), leukocyte infiltration (CA1), and vacuolization of gyrus dente cells compared to the normal control. The extract at doses of 150 and 300 mg/kg reversed all hippocampal structures, close to those of the normal control. Similarly, clomipramine caused a restructuring of all hippocampal structures.

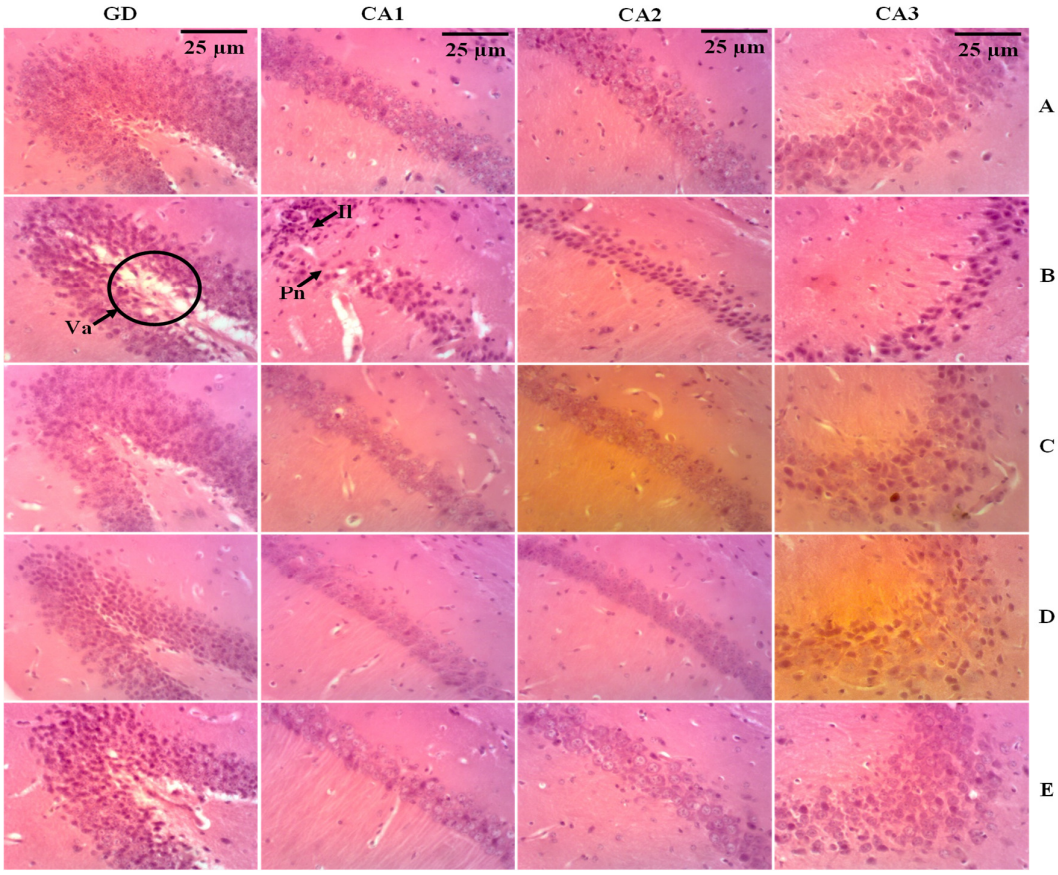

Figure 5. Microphotographs of the gyrus dente (X100) and Ammon horns 1, 2 and 3 (X200) of the hippocampus of male mice; Hematoxylin-Eosin staining.
A = Normal control; B = Negative control; C = Positive control; D, E = Batches receiving DCM/MeOH extract of *C. adansonii* at doses of 150 and 300 mg/kg, respectively; GD = Gyrus dente; CA1, 2, 3 = Ammon horns 1, 2, and 3; Pn = Neuronal loss; Va = Neuronal vacuolation; Il = Leukocyte infiltration.

Discussion

The current study aimed to demonstrate to evaluate the effects of a DCM/MeOH extract of *C. adansonii* bark on depression and anxiety in mice exposed to chronic unpredictable mild stress (UCMS). Sucrose preference and forced swimming tests were used to assess antidepressants, while the caudal suspension and dark/light compartment tests were used to assess anxiolytic effects. Anhedonia is one of the primary symptoms of depression in humans and the sucrose preference test is an indicator of anhedonic behavior (Rygula *et al*., 2005). We observed that exposure of mice to UCMS for 42 days induced a change in behavior by a significant decrease in sucrose preference. Pretreatment with DCM/MeOH extract of *CA* at doses of 150 and 300 mg/kg significantly ($p< 0.001$) increased sucrose preference in animals. The extract may act by stimulating the reward and motivation centers, resulting in increased dopamine levels in the animals' brains (Murray *et al*., 2008). The DCM/MeOH extract of *C. adansonii* could therefore have an antidepressant effect. Numerous studies have shown that there is a comorbidity between depression and anxiety, giving them common symptoms (Koprdová *et al*., 2016). The light and dark box test is widely used to assess the effect of drugs on the general behavior and excitability level (Foyet *et al*., 2014).

In this test, anxiety is generated by the conflict between the desire to explore and the fear of the lighted, unfamiliar space (Crawley, 1985). In this study, the UCMS induced a state of anxiety in mice that was reflected in a significant increase in time spent in the dark compartment. Chang *et al*. (2012) showed that UCMS contributed to the development of anxiety in animals in the elevated cross-maze test. Pretreatment with the DCM/MeOH extract of *C. adansonii* at the 150 mg/kg dose reportedly inhibited amygdala hyperactivity while protecting the prefrontal cortex, resulting in a significant decrease in time spent in the dark box of animals exposed to UCMS. The extract might act through a non-selective antagonism mechanism at 5-HT1 and 5-HT2 receptors which are involved in anxiety-like behaviors (Bourin and Hascoët, 2010; Rynn *et al.,* 2003). In this work, UCMS also induced a significant increase in grooming. This is thought to be due to a decrease in serotonin concentration in the limbic system and dysfunction of the GABAergic system (Švob *et al*., 2016). GABA increases chlorine ion transport in the intracellular medium, inducing hyperpolarization of the latter, making the cell refractory to certain stimuli thus decreasing hyperactivity in the central nervous system (Lopes *et al*., 2012). Pretreatment with DCM/MeOH extract of *C. adansonii* at doses 150 and 300 mg/kg protected the serotonergic and GABAergic systems of the central nervous system, resulting in a significant decrease in the number of grooming events. These results confirm that the DCM/MeOH extract of *C. adansonii* could have an anxiolytic activity.

Glucocorticoids accelerate cellular metabolism, which consequently increases free radical formation via the mitochondrial electron transport chain (McIntosh *et al*., 1998). Excessive generation of free radicals such as reactive oxygen and nitrogen species can potentially damage fatty acids, proteins, and DNA through oxidative stress (Hazel *et al*., 2021). Chronic stress can also lead to the production of substances that can activate glutamate NMDA receptors thus leading to excitotoxicity and consequently oxidative stress (Banasr *et al.,* 2010). Pretreatment with DCM/MeOH extract of *C. adansonii* protected the mouse brains from the subcortical low intensity (UCMS)-generated free radicals, resulting in a significant decrease (at 150 mg/kg and 300 mg/kg) in lipid peroxidation, and a significant increase in SOD activity and GSH levels at 150 and 300 mg/kg. The analysis of the antioxidant activity *in vitro* showed that the extract has more anti-radical activity than reducing activity, with a percentage of inhibition to DPPH of 60.36%. The improvement of enzymatic and non-enzymatic defense against free radicals would be due to the presence in the *Creteva adansonii* extract of some secondary metabolites such as flavonoids. Flavonoids act mainly as primary antioxidants, stabilizing peroxide radicals but can also deactivate reactive oxygen species and inhibit lipoxygenase or chelate metals (Sarni-Manchado and Cheynier, 2006). Hypersecretion of corticosteroids following chronic stress leads to impaired brain function and inhibition of hippocampal stem cell proliferation, resulting in reduced production of new neurons (Gold, 2015). UCMS resulted in alterations in the various structures of the hippocampus. Pretreatment with the DCM/MeOH extract of *C. adansonii* protected the hippocampus from the neurotoxic effects of UCMS: this, therefore, supports the neuroprotective effect of the extract. Flavonoids in the extract may protect the brain in several ways, including protecting vulnerable neurons, enhancing existing neuronal function, and stimulating neuronal regeneration (Vauzour *et al.,* 2010).

Conclusions

This study revealed that the DCM/MeOH extract of *C. adansonii* has antidepressant effects and anxiolytic activities. Moreover, the extract decreased lipid peroxidation and increased the activity of SOD and the level of GSH. These antidepressant, anxiolytic, and antioxidant effects would be due to the presence of phenolic compounds (flavonoids) in the extract. These results would justify at least partially the use of this extract in traditional medicine. Studies are underway to determine the possible mechanisms of action of this extract.

Authors' Contributions

NGA-D: Investigated the traditional healers, to choose the plant, **BPB**: Provided an extract and proposed the methodology, **GJB:** Validated the methodology and wrote the manuscript. **AIF**: analyzed the data and revised the English version of the manuscript, **ABD**: Corrected the protocol and brought expertise to the whole manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Animals were handled according to the guidelines of the Cameroon Bioethics Committee (reg. no. FWA-IRB00001954). The protocol was approved by the ethics committee of the Faculty of Sciences of the University of Maroua (ref. no. 14/0261/Uma/D/FS/VD-RC). Each animal was tested in only one behavioral test and tests were made to minimize animal suffering.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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