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Neuroprotective activity of *Conyza aegyptiaca* hydroethanolic extract against ethanol-induced neurodegeneration in adolescent Wistar rat's brain

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Alcohol consumption is a common well-known source of brain damages. This work aimed to evaluate the neuroprotective effect of *Conyza aegyptiaca* against ethanol-induced neurodegeneration in a Wistar rat binge drinking model. Animals, pretreated orally once a day during 3 days with the hydroethanolic extract of *C. aegyptiaca* (HECA 250 and 500 mg/kg), received daily intraperitoneally 7 g/kg of ethanol for 3 more days, concomitantly with HECA. Behavioral studies, brains tissue oxidative stress markers (MDA, GSH) assays, blood cell count and biochemistry analysis were performed. *In vitro* antioxidant activity using DPPH and FRAP tests and phytochemical tests were also realized. The negative geotaxis test showed that HECA 500 rats took significantly less time (3.24 ± 0.49 s, P<0.05 vs Ethanol control) compared to their congeners of Ethanol control group (6.80 ± 1.267 s, P<0.01 vs Sham). HECA treatment lowered significantly MDA levels (P<0.05 vs Ethanol control) and significantly increased GSH levels (P<0.05 vs Ethanol control). HECA treatment also significantly reduced the platelet count (P<0.001 vs Ethanol control). Ethanol 7 g/kg i.p raised hepatic function markers ALT and AST (respectively +27%, P<0.01 and +58.29%, P<0.01 vs Sham), effects significantly reversed by HECA treatments. DPPH scavenging activity with an IC50 of 368.769 ± 16.467 µg/mL and ferric ion reducing power with an EC50 of 139.65 ± 7.14 µg/mL should be related to the polyphenolic compounds found in HECA, tannins and flavonoids in particular. *C. aegyptiaca* could therefore potentially protect the brain against situations of neurodegeneration such as those observed in acute ethanol neurotoxicity.

Key words: *Conyza aegyptiaca*, ethanol, brain, antioxidant, neuroprotection, neurodegeneration.

INTRODUCTION

Degenerative damages to brain represent a major societal challenge because more than 50% of neurodevelopmental disabilities are due to perinatal or subsequent exposure to toxins or oxidants such as environmental pollutants, tobacco and stress, sources of free radicals harmful to biomolecules, membrane structures, and leading to a multitude of neuro-degenerative pathologies (Wang et al., 2021).

Among these potentially brain-damaging xenobiotics is ethanol. Alcohol is a neuroteratogen and its consumption during pregnancy can cause fetal alcohol syndrome (FAS) (Jones and Smith, 1973; Botia et al., 2011). FAS is characterized by growth deficit, mental retardation, and irreversible learning deficits (Chen and Luo, 2010).

In adolescence, massive binge drinking episodes are responsible for cognitive deficits (Lees et al., 2019). According to Cohen-Kerem and Koren (2003), the cerebral toxicity of alcohol would pass through oxidative-type mechanisms with a massive production of free radicals, the consequences of which can be summarized in two major pathways: an indirect pathway characterized by the reduction of glutathione peroxidase activity, and a direct pathway with the peroxidation of lipids, nucleic acids and proteins by free radicals.

In these conditions, the use of neuroprotective agents with antioxidant capacities could represent a very interesting therapeutic alternative to protect the brain against alcohol-induced oxidative damages. Plants often rich of antioxidant substances are, therefore, excellent candidates for neuroprotection studies. In Togo, a large part of the population continues to use plants remedies for their primary healthcare (Kpodar et al., 2016), including the management of central nervous system disorders (Kantati et al., 2016). *Conyza aegyptiaca* is one of these medicinal plants of Togolese flora, whose scientific studies have revealed its antioxidant potential through its antimicrobial (Anani et al., 2000), antihypertensive and cardioprotective (Kpegba et al., 2011), antimalarials and insecticides (Mansour and Mohamed, 2013), and antihyperglycemic's properties (Akakpo et al., 2016, 2017). However, none of these scientific studies have focused on its neuroactive potential. This study was then initiated in order to evaluate the neuroprotective effect of *C. aegyptiaca* in a model of cerebral damage induced by ethanol.

MATERIALS AND METHODS

Plant

The plant material is composed of whole plants of *C. aegyptiaca* harvested in September 2020 in the locality of Danyi located at 90 km northwest of the capital Lomé. A plant specimen was identified with skill assistance of botanists of Botany and Plant Ecology Laboratory, University of Lomé (Togo). The plants were then washed, dried under air conditioning at 20°C for one week, and then powdered.

Preparation of *C. aegyptiaca* hydroethanolic extract (HECA)

Four hundred grams of the powder were macerated in a volume of 4 L of an ethanol-distilled water mixture (50:50, v/v) for 72 h with intermittent manual stirring. The macerate obtained was filtered and evaporated under vacuum at 45°C using a rotary evaporator. The evaporated extract was lyophilized to obtain a dry extract stored in the refrigerator at 4°C until use.

In-vivo protocols

Animals

The animal material consisted of 24 rats of the Wistar strain aged of three (3) weeks and weighing between 70 and 123 g. Animals were raised in the animal house of the Department of Animal Physiology of the University of Lomé, housed in standard polypropylene cages and maintained under standard laboratory conditions (temperature 24-25°C, relative humidity and a 12 h/12 h light-dark cycle). They had free access to food and water. Institutional guidelines and ethical principles of Physiology/Pharmacology laboratory (University of Lomé-Togo, ref: 001/2012/ CB-FDS-UL) were followed.

Experimental design

The protocol used in this test is inspired by studies of Botia et al. (2011), slightly modified. Four groups of five rats each were constituted for this test.

The pretreatment phase lasted three days during which the animals of the first 2 groups received distilled water by gavage while the animals of the last two groups received HECA once a day at the respective doses of 250 and 500 mg/kg by gavage as shown in Figure 1. At the end of this pretreatment phase, animals were treated for 3 additional days during which the first group (Sham) received distilled water and NaCl 9‰, the second group (Ethanol control) received distilled water and 15% ethanol (diluted in NaCl 9‰) twice. Two doses spaced of 2 h (5 g/kg + 2 g/kg) by intraperitoneal injection to reach a final dose of 7 g/kg. The animals of the last two groups continued to receive simultaneously HECA 250 and 500 mg/kg, respectively once a day and ethanol 7 g/kg i.p, in two successive doses as indicated.

Behavioral studies

Negative geotaxis test: 16 h after the end of the treatments, animals were subjected to behavioral tests. Negative geotaxis test was used to measure the ability of rats to orient and move directionally against gravity. Rats are placed head down on a surface inclined at 20°, as previously described by Botia et al. (2011). This situation of discomfort forces them to get back upside down. The time taken by the animals to complete a 90° rotation is reported. The maximum time allowed for rotation was 180 s. Tests were repeated three times.

Righting reflex test: According to the method of Botia et al. (2011), the surface righting reflex test was used to evaluate muscular development and vestibular functions. For this test, rats were placed on a flat surface in a supine position, maintained for 1 s and released. The time required to regain all 4 paws in contact with the testing plane surface was measured with a stopwatch. The tests were carried out three times.

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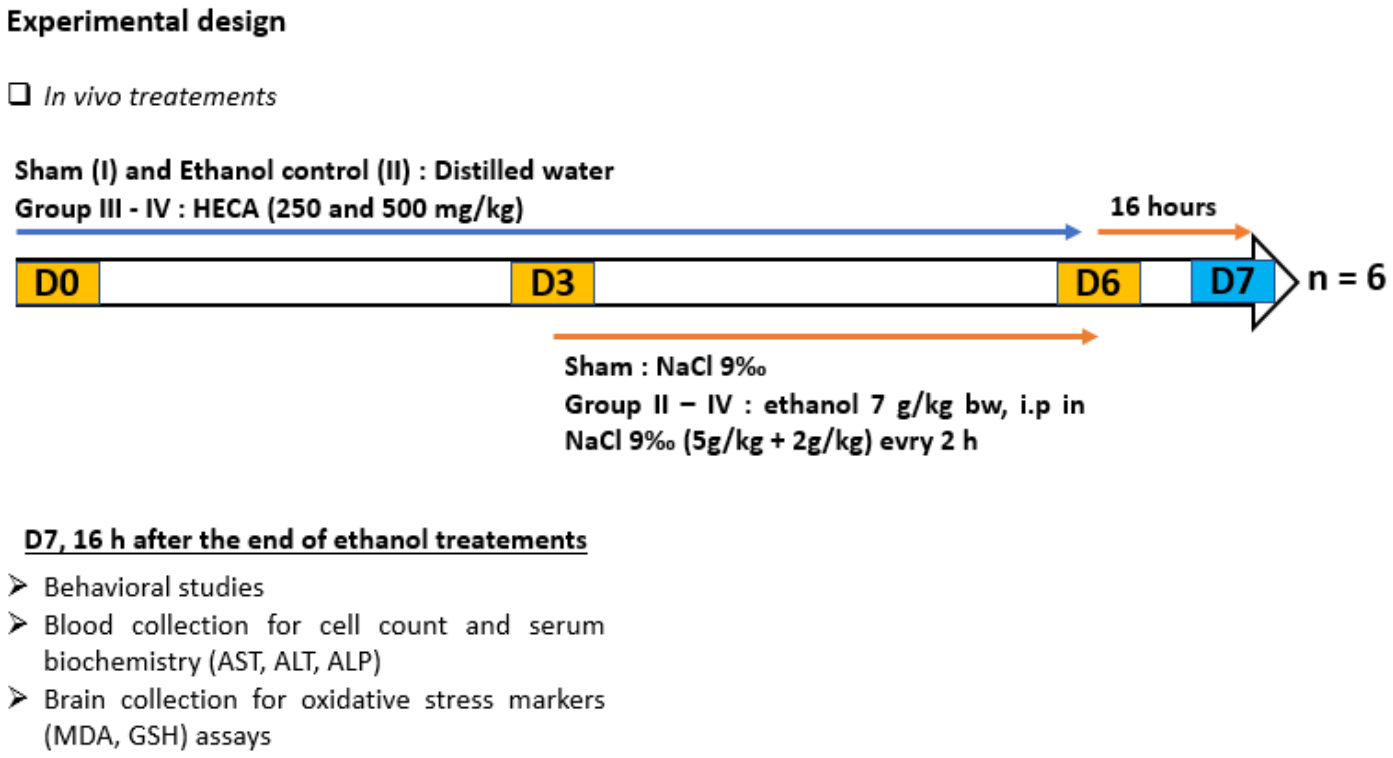

Figure 1: Experimental design of the study showing treatments and assays.
Source: Authors

Blood collection and analysis

At the end of the aforementioned tests, animals were anesthetized with diethyl ether by the open mask method, and the blood was collected by the retro-orbital sinus. Blood samples were taken in EDTA tubes for the blood count and in hemolysis tubes for the assay of biochemical parameters. Samples were centrifuged at 3,000 rpm for 10 min and supernatants collected were used for the determination of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) using an autoanalyzer (Prietest Touch Plus, Robonik India PVT LTD) and specific spectrophotometric diagnostic kits (PharmaLab, India).

Preparation of brain homogenate

After collecting the animals' blood, they were anesthetized with ether before being decapitated. Brains were quickly removed, rinsed in an ice-cold normal saline solution. 150 mg of brain tissues were then ground in 1 mL of 20 mM Tris-HCl solution, pH=7.4. The homogenates were centrifuged at 3,000 rpm for 10 min. MDA and GSH were assayed on the supernatant (Patsoukis et al., 2005).

Malondialdehyde (MDA) intracerebral level assay

The MDA rate was assessed using the method of Patlolla et al. (2009). A solution of 1-methyl-2-phenylindole (10 nM) was activated by mixing it with $FeCl_3$ (32 µM) in the respective proportions of 75 and 25% (45 mL of 1-methyl-2-phenylindole in acetonitrile + 15 mL of $FeCl_3$). The reaction medium contains 1.3 mL of the solution of 1-methyl-2-phenylindole activated, 500 µl of standard MDA or brain homogenate and 300 µl of HCl (37%). Reaction tubes were then incubated at 45°C for 60 min and centrifuged at 4,000 rpm for 10 min using an electric centrifuge. The blank is composed of 1.3 mL of activated 1-methyl-2-phenylindole, 500 µl of tris-HCl, pH 7.4 and 300 µl of HCl (37%).

A standard curve was realized using MDA solution at concentration points: 0.625, 1.25, 2.5, 5 and 10 nM. Absorbances were read at 586 nm using a UV-VIS spectrophotometer (Genesys 20, Thermo Scientific).

Intracerebral reduced glutathione (GSH) assay

For this assay, the method of Sedlak and Lindsay (1968) was used. Briefly, to 50 µL of the homogenate or standard GSH were added 150 µL of a Tris HCl solution (0.2 M, pH = 8.2), 10 µl of 5,5-dithiobis-2-nitrobenzoic acid (DNTB 0.01 M), and 790 µl of methanol. Reaction tubes were homogenized and then incubated for 30 min at room temperature.

The standard GSH concentrations used were 800, 600, 400, 200, 100, and 50 µg/mL. After this incubation time, tubes were centrifuged at 3,000 rpm before reading the absorbances at 412 nm using a UV-VIS spectrophotometer (Genesys 20, Thermo Scientific).

***In vitro* tests**

DPPH of antioxidant activity assay

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) test was realized according to the method of Sen and Dastidar (2010). To 1.5 mL of the DPPH

solution (100 µmol/l), 0.25 mL of methanol (white) or 0.25 mL of methanolic solution of HECA was added into tubes. 10 min after incubation at room temperature, absorbances were red at 517 nm. Ascorbic acid at different concentrations (12.5, 25, 37.5, 50 and 100 µg/mL) served as reference antioxidant. The percentage inhibition of the DPPH radical was calculated using the equation:

% inhibition = ((A0 - Ae) × 100) / Ac.

where A0 represents the absorbance of DPPH alone and Ae represents the absorbance in the presence of the extract or ascorbic acid. The tests were repeated three times.

Ferric ion reduction test (FRAP)

The ability to reduce ferric ions was measured using the method described by Nishaa et al. (2012) slightly modified. The FRAP reagent was generated by mixing 300 mM sodium acetate buffer (pH 3.6), 10.0 mM TPTZ (tripyridyl triazine) solution, and 20.0 mM $FeCl_3.6H_2O$ solution in a 10:1:1 volume ratio. HECA at different concentrations (50, 100, 250, 500 and 1000 µg/ml) were then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 37°C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working solutions of $FeSO_4$ were used for calibration. Antioxidant capacity based on the ability to reduce ferric ions in the sample was calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents (0.1, 0.2, 0.4, 0.6, 0.8 and 1 mM) per gram of the sample. The tests were repeated three times.

Phytochemical screening

The characterization of the major phytochemical groups was carried out by the colorimetric reactions described by Karumi et al. (2004) and Edeoga et al. (2005) using the appropriate reagents.

Research of tannins

HECA (1 mL) in a test tube was treated with 0.5 mL Molisch's reagent followed by three drops of ferric chloride, leading to a green color in a condensed form, thus indicating the presence of tannins.

Alkaloids

HECA (100 mg) was added to 5 ml of 10% sulfuric acid. The mixture was homogenized for 5 min and filtered, treated finally with 0.5 ml of the reagents (Bouchardat or Mayer) in different tubes. The appearance of an orange precipitate, a yellowish precipitate, and a brown precipitate, respectively, indicated the presence of alkaloids.

Saponins

HECA (4 mL) mixed with distilled water in a test tube was vigorously shaken for 2 min. A foam of about 5 cm formed and stable after about 10 min showed the presence of saponins.

Flavonoids

HECA spiked with hydrochloric acid (HCl 2.5 mL) was mixed with 1.5 mL of sodium hydroxide (NaOH) in a test tube. The appearance of a yellow color indicated the presence of flavonoids.

Polyphenols

A few drops of 5% ferric chloride $FeCl_3$ were added to 5 mL of HECA. The formation of an intense blackish coloration indicated the presence of polyphenols.

Sterols and triterpenes

1 ml of acetic anhydride and 1 ml of chloroform were added to 100 mg of HECA. After shaking, the resulting solution was separated into two test tubes. One part was used as a control. After addition of 1 ml of concentrated sulfuric acid, the formation of a brownish red or purple ring after a few minutes at the contact zone of the two phases indicates the presence of metabolites. The appearance of a gray or purple coloration indicated the presence of triterpenes, while a blue to gray-green coloration showed the presence of sterols.

Quantitative determination of total flavonoids

The determination of total flavonoids was carried out according to the method described by Kosalec et al. (2004) which is based on their ability to chelate metals including aluminium. To 2 mL of the ethanolic solution of HECA (1 mg/mL) or of rutin (1 mg/mL), were added 2 mL of aluminium chloride (20 mg/mL) and 6 mL of sodium acetate (50 mg/mL).

The blank was made in the same way without HECA, replaced by 2 mL of ethanol. After an incubation time of 150 min, absorbances were read at 440 nm. The total flavonoid content of HECA was determined from the linear regression equation of the calibration curve established with rutin (0-100 µg/mL) and expressed in µg of rutin equivalent per milligram of dry extract (µg Eq/mg of extract). Rutin served as a reference compound. The tests were repeated three times.

Quantitative determination of total phenols and tannins

The determination of total phenols is based on the Folin-Ciocalteu method described by Maksimović et al. (2005). Total phenols were assayed after fixing the tannins with polyvinylpolypyrrolidone (PVPP) following two steps. Firstly, to tubes containing 10 mg of PVPP diluted in 500 µL of methanol, 500 µL of HECA 1 mg/mL were added. The mixture thus obtained was homogenized and incubated on ice for 30 min. After centrifugation, 200 µL of the supernatant were transferred into dry tubes for the assay with the Folin-Ciocalteu reagent. The blank did not contain HECA, replaced by 500 µL of methanol. Secondly, Folin-Ciocalteu (200 µL) was added to 200 µL of HECA (1 mg/mL) or to 200 µL of the Gallic Acid solutions (200, 150, 100, 50, 25 and 0 µg/mL) or 200 µL of the solution obtained during the previous step (extract + PVPP). The mixtures were incubated at room temperature for 15 min. After incubation, 800 µL of sodium carbonate solution (700 mM) were added to the mixtures. The blank was prepared with 200 µL of Folin-Ciocalteu, 200 µL of methanol and 800 µL of the sodium carbonate solution (700 mM). Absorbances were read at 735 nm. The quantity of total phenol is expressed in mg equivalent of Gallic Acid/g of extract. The total amount of tannin was calculated using the formula:

DOT = DOExt – (DOExt + PVP)

where DOT = DO tannins, DOExt = DO extracted, and DOExt + PVP = DO extracted + PVP.

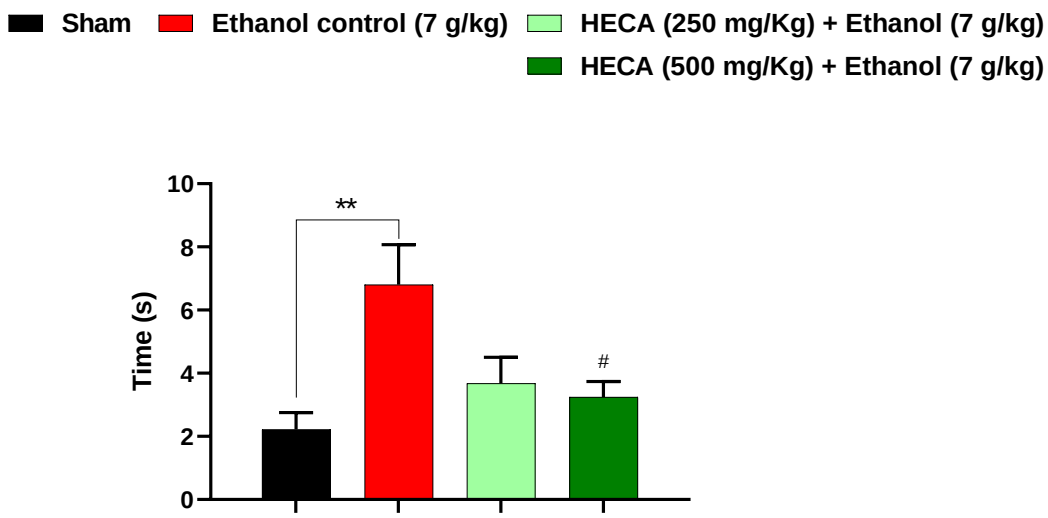

Figure 2. Effect of HECA on replacement time. The values are expressed as Mean ± SEM, n = 6. **p<0.01: significant when compared with Sham. #p<0.05: significant when compared with Ethanol control. Unit: seconds (s).
Source: Authors

Statistical analysis

Values are expressed as Mean ± SEM. GraphPad Prism 8.3.0 software (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analyses. Comparisons were made using ANOVA and results were considered significant when P< 0.05.

RESULTS

Behavioral studies

Negative geotaxis

Sham animals, injected with NaCl 9‰, turned 90° from a head-down position within less than 3 s (Figure 2), whereas ethanol (7 g/kg) treated animals (Ethanol control group) required 6.80 ± 1.267 s (P<0.01 vs Sham) to perform the same task. HECA 500 mg/kg treatment significantly reduced the deleterious effect of alcohol, bringing back this time to 3.24 ± 0.49 s (P<0.05 vs Ethanol control).

Righting reflex

Ethanol administrated intraperitoneally, either alone or in combination with HECA, did not affect the righting reflexes (Figure 3).

Blood biochemistry

Figure 4a and b shows that treatment with ethanol alone (7 g/kg) caused a significant increase in AST (+58.29%, P<0.01 vs Sham) and ALT levels (+27%, P<0.01 vs Sham). Concomitant administration of HECA 500 mg/kg significantly reduced the AST level to values close to normal (-54.71%, P<0.001 vs Ethanol control). For ALT, the two doses of HECA (250 and 500 mg/kg) significantly lowered the levels of this marker (-19.73 and -16.96%, respectively) when compared with ethanol control animals.

Blood cells count

The level of blood platelets increased significantly (+34%, P<0.001 vs Sham) in ethanol control animals. This rate experienced significant decreases in HECA treated rats (-5.80%, P<0.05 at the dose of 250 mg/kg, -12.43% P<0.05 at the dose of 500 mg/kg) when compared with ethanol control animals. For the rest of parameters, no significant variations were observed. The results are shown in Table 1.

Brain MDA levels

The basal level of MDA in brains of Shams was 74.37 ± 10.32 nM/g of tissue. In ethanol control animals, alcohol administration significantly increased lipid peroxidation rate (+22%, P<0.05 vs Sham).

This level was significantly lowered (-28%, P<0.05 vs Ethanol control) by HECA 500 mg/kg treatment (Figure 5).

Brain GSH levels

The basal level of GSH in brains of Shams was 0.38

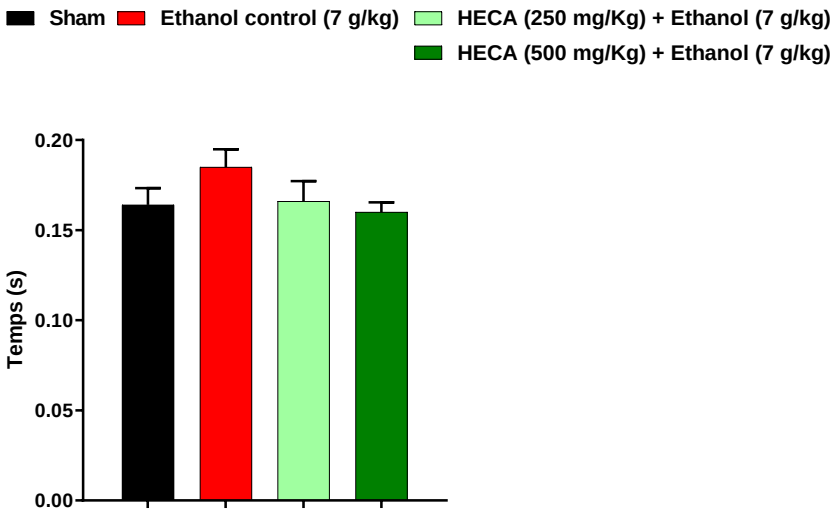

Figure 3. Effect of HECA on righting reflex time. The values are expressed as Mean ± SEM, n = 6. Unit: seconds (s).
Source: Authors

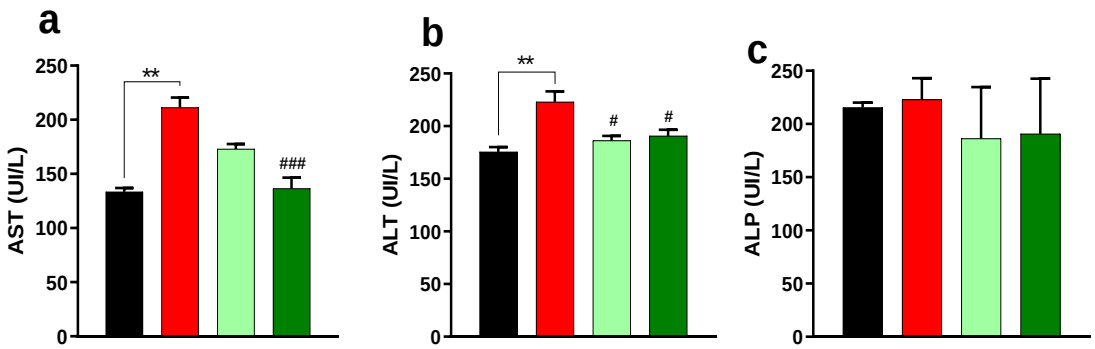

Figure 4. Effect of HECA on hepatic markers Aspartate aminotransferase (a) Alanine Aminotransferase (b) and Alkaline phosphatase ALP (c). The values are expressed as Mean ± SEM, n = 6. Units: U/L. **p<0.01: significant when compared with Sham. #p<0.05, ###p<0.001: significant when compared with Ethanol control.
Source: Authors

±0.013 nM/g of tissue (Figure 6). A significant reduction was observed in brain samples when animals received ethanol at dose of 7 g/kg (-26.66%, P<0.01 vs Sham). Treatment with HECA restored GSH levels. The best activity was recorded with HECA 500 mg/kg (+13.33 P<0.05 vs Ethanol control).

In vitro antioxidant studies

DDPH free radical scavenging capacity

HECA inhibited 50% of DPPH free radicals (IC50) at dose of 344.985 ± 17.139 µg/mL as presented in Figure 7, although lower than that of the reference molecule ascorbic acid which IC50 was 23.468 ± 0.091 µg/mL.

Ferric ion reducing power

HECA also clearly reduced ferric ions (Fe^{3+}) with an EC_{50} of 139.65 ± 7.14 µg/mL. Reference compounds quercetin and ascorbic acid, respectively presented EC_{50} of 48.52 ± 2.02 and 23.06 ± 0.21 µg/mL shown in Table 2.

Phytochemistry

Qualitative content

Table 3 summarizes the major chemical groups found in HECA.

Table 1. Effect of HECA on blood cells count.

Parameter	Sham	Ethanol control	HECA + Ethanol (7 g/kg)	
			250 mg/kg	500 mg/kg
WBC (10^6/µL)	7.64±0.61	7.12±0.41	6.43±0.81	7.19±1.69
RBC (10^6/µL)	7.08±0,46	8.25±0.29	8.40±0.26	7.58±0.40
HB (g/dL)	11.83±0.63	13.92±0.43	14.07±0.27	12.96±0.71
HT (%)	38.13±1.76	42.40±1.08	43.05±0.92	41.10±1.23
PLT (10^3/µL)	627.66±112.16	841.00±78.48***	794.66±70.05***#	748.00±23.26**#
Neu (10^3/µL)	4.19±1.70	2.66±0.48	2.30±0.53	5.15±1.57
Eos (10^3/µL)	0.36±0.24	0.20±0.03	0.10±0.03	0.16±0.06
Bas (10^3/µL)	ND	ND	ND	ND

The values are expressed as Mean ± SEM, n = 6. **p<0.01, ***p<0.001: significant when compared with Sham. #p<0.05: significant when compared with Ethanol control. Units: µL = microliter. WBC = White blood cells, RBC = red blood cells, HB = hemoglobin, HT = hematocrit, PLT = platelets, Neu = Neutrophils, Eos = eosinophils, Bas = basophils.
Source: Authors

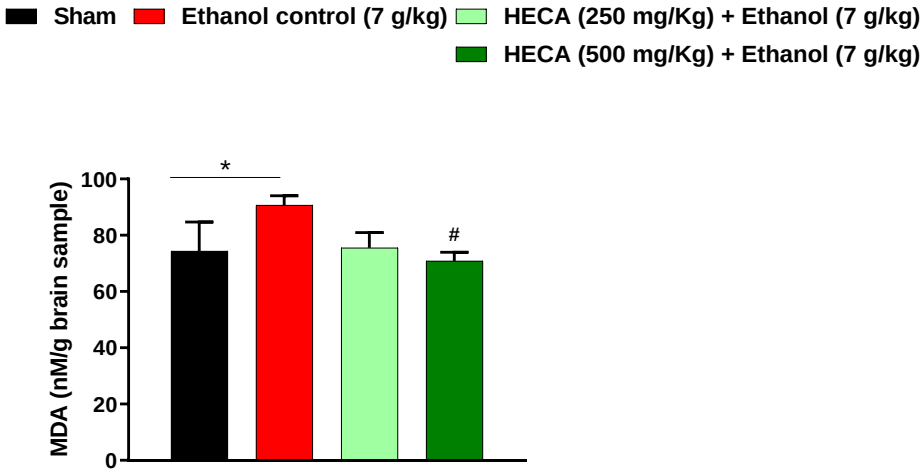

Figure 5. Effect of HECA on lipid peroxidation in brain. The values are expressed as Mean ± SEM, n = 6. Units: nM/mg of brain samples. *p<0.05: significant when compared with Sham. #p<0.05: significant when compared with Ethanol control.
Source: Authors

Quantitative content

The amount of flavonoids, total polyphenols and tannins found in the extract are shown in Table 4.

DISCUSSION

The present study aimed to evaluate the neuroprotective effect of HECA in an animal model of ethanol intoxication. Ethanol neurotoxicity is strongly linked to oxidative stress (Diallo et al., 2009) and inflammatory mechanisms. The review of Crews and Nixon (2009) described well preclinical rat experiments using a 4-day binge ethanol treatment known to induce brain damages. Binge drinking is defined as heavy alcohol consumption followed by periods of abstinence during adolescence (Reitz et al., 2021). In this model, the peak of neurodegeneration generally occurs on the 3rd day after the animals have received ethanol (Crews and Nixon, 2009). Neurodegeneration and cognitive deficits following binge treatment mimic the mild degeneration and cognitive deficits found in humans. Therefore, it is in a well-known model of neurodegeneration that HECA neuroprotective properties have been tested in this study. Ethanol heavy consumption, through the promotion of reactive oxygen species (ROS) production, causes damages or degradation of essential complex molecules in the cells

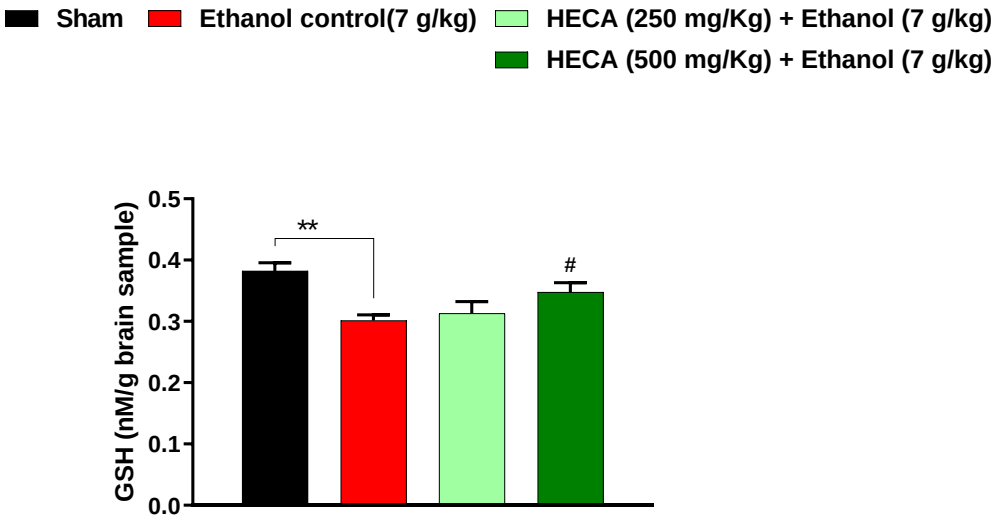

Figure 6. Effect of HECA on GSH levels in brain. The values are expressed as Mean ± SEM, n = 6. Units: nM/mg of brain samples. **p<0.01: significant when compared with Sham. #p<0.05: significant when compared with Ethanol control.
Source: Authors

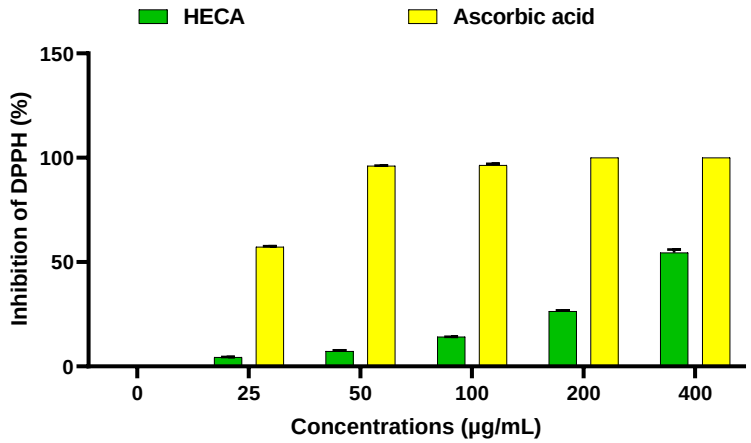

Figure 7. DPPH scavenging activity of HECA. The values are expressed as Mean ± SEM, n = 3. Units: µg/mL.
Source: Authors

(lipids, proteins, and DNA) and reduces the levels of antioxidants enzymes, glutathione (GSH) in particular (Wu and Cederbaum, 2003). Evaluation of neuroprotection by assessing lipid peroxidation (MDA) and GSH levels in the brain tissues show that HECA clearly protected animal's CNS from oxidative effects of ethanol. GSH is the most abundant thiol compound in the brain and is considered to be the major regulator of the intracellular redox state by participating in redox reactions.

It chelates free radicals or acts as a substrate for many antioxidant enzymes (Migdal and Serres, 2011). According to the pesent results, the level of GSH decreased significantly in ethanol control animals. This decrease could be explained by its participation in the inhibition of oxidative stress, but also by the indirect action of ethanol on glutathione levels as mentioned earlier in this paper (Cohen-Kerem and Koren, 2003). The increase in the intracerebral level of the marker of lipoperoxidation, MDA, indicates an increase in the production of free radicals in ethanol control animals. Excessive exposure to ethanol leads to cerebellar dysfunction with cerebellar ataxia and alterations in postural stability, motor skills and balance (Luo, 2015). Since ethanol-induced neurodegeneration is the cause of behavioral disorders, we investigated the neuroprotective

Table 2. EC50 of HECA, quercetin and ascorbic acid.

Parameter	HECA (µg/mL)	Quercetin (µg/mL)	Ascorbique acid (µg/mL)
EC_{50}	139.65±7.14	48.52±2.02	23.06±0.21

The values are expressed as Mean ± SEM, n = 3. Units: µg/mL.
Source: Authors

Table 3. Chemical groups found in HECA.

Compound	HECA
Alkaloids	+
Tannins	+
Flavonoids	+
Saponosides	+
Triterpenes	+
Sterols	+
Phenolic compounds	+
Glucides	+

(+) Presence; (-) Absence.
Source: Authors

Table 4. Flavonoids, tannins and total polyphenolic content of HECA.

Parameter	HECA	Reference compound
Flavonoids	53.86±0.901 mgER/g	Rutin
Tannins	41.43±1.846 mgEAG/g	Gallic acid
Total phenols	73.93±0.704mgEAG/g	Gallic acid

Values are expressed as Means ± SEM, n = 3. Units: Total phenols and tannins are expressed as mg Gallic acid equivalent/g of HECA (mgEAG/g), while Flavonoids are expressed as mg Rutin equivalent/g HECA (mgER/g).
Source: Authors

capacity of HECA by performing geotaxis and righting behavioral tests. The geotaxis test performed assesses motor coordination in rats. The rats are placed face down on a 20° slopping surface and due to the vestibular cues of gravity, they turn around to face the slope. The action of neurotoxins causing brain damage would alter this reflex and increase the turning time of animals (Ten et al., 2003; Elnar et al., 2012).

The results indicate a significant increase in the turning time for wthanol controls compared to Sham animals, characteristic of a brain lesion. The significant decrease in repositioning time of HECA 500 mg/kg animals indicates the capacity of this extract to exert neuroprotection, allowing this reflex to be maintained. In fact, HECA 500 mg/kg, in spite of ethanol 7 g/kg presence, lowered significantly the level of MDA and restored GSH levels, offering a good antioxidant protection in the brain. Interestingly, this study found that this protection was not only limited to brain. Ethanol, like many other chemicals, is mainly metabolized in the liver, making this organ a target of its peripheral toxicity (Comporti et al., 2010). Dosage of hepatic markers ALT, AST and ALP revealed a possible hepatoprotective effect of HECA, since alterations induced by alcohol have been reversed by the treatment with this plant extract. This also indicates a possible better alcohol elimination process.

These observations, linked to those of anti-lipoperoxidation and glutathione restoration, suggest that the neuroprotection confirmed in behavioral studies should be mediated by a general amelioration of animal's antioxidant status in the presence of HECA.

Moreover, a significant increase in the level of blood platelets in ethanol treated animals was observed. As Femia et al. (2013) have demonstrated in their studies in healthy patients that there is a correlation between platelet count and extent of platelet aggregation, the increased platelet count should be linked to an elevated risk of rat's blood platelet aggregation. This phenomenon called, "platelet rebound" is very well known in alcohol

studies such as binge drinking models. Many studies carried out both in humans and in animals define it as a mark of alcohol withdrawal (Renaud and Ruf, 1996). Ruf et al. (1995) showed earlier that alcohol deprivation of rats for 18 h was associated with an increase in the platelet response of 124% in those receiving 6% ethanol, of 46% with white wine but a decrease of 59% in those with red wine. This protection afforded by wine has been duplicated later in animals with grape phenolics added to alcohol (Ruf, 2004), confirming that phenolic antioxidants have protective effect on platelets rebound, a deleterious phenomenon characterized by higher responses of platelets to thrombin and ADP-induced aggregation, higher level of lipid peroxides and conjugated dienes in animals receiving alcohol (Ruf et al., 1995). Considering that high amounts of alcohol and its metabolites exert direct inflammatory effects on the brain and liver and induce free-radical production, therefore increasing lipid peroxidation and tissue inflammation (Jayatilleke and Shaw, 1998; Chen et al., 2016), we hypothesized that HECA should also possess anti-inflammatory property.

The hydroethanolic extract of *C. aegyptiaca* revealed a wide variety of major chemical groups with pharmacological activity.

Tannins and flavonoids in particular are considered as useful antioxidant and anti-inflammatory compounds (Yessoufou et al., 2013; Ferrini et al., 2021; Maliński et al., 2021). Regarding the antioxidant capacity evaluated by the DPPH and FRAP tests, HECA exhibited good free radicals scavenging capacities. Through these demonstrated antioxidant capacities, HECA would therefore have protected neuronal cells from death by inhibiting lipid peroxidation and restoring GSH levels in midbrain, and by ameliorating the total antioxidant status of animals as shown by peripheral tissues (blood and liver) function exploration.

Conclusion

The hydroethanolic extract of *C. aegyptiaca* showed interesting antioxidant activities *in vitro* and an ability *in vivo* to protect the brain against ethanol-induced neuronal damage. *C. aegyptiaca* therefore possesses neuroprotective activity in a model of ethanol neurodegeneration in the wistar rat. The presence of polyphenolic compounds, tannins and flavonoids, probably explains these activities. HECA should be then an excellent source of new pharmaceutical neuroactive agents. Furthermore, other studies are needed to validate these neuroprotective properties and verify its toxicological profile before recommending its use as possible neuroprotective natural remedy.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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