OPEN ACCESS
Saudi Journal of Biomedical Research
Abbreviated Key Title: Saudi J Biomed Res
ISSN 2518-3214 (Print) |ISSN 2518-3222 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Hydroethanol Extract of Cocktail Plants (*Spondias mombin, Vernonia amygdalina, Momordica charantia*) Prevents Abrupt Alcohol Withdrawal-Induced Cognitive-Affective Disorders in Mice Model

Habib Omar Yves Doumbia[1], Pacôme Kouadio N'Go[2*], Bessekon Denis Assi[1], Antoine Némé Tako[1]

[1]Biology and Health Laboratory, Biosciences Training and Research Unit, Félix HOUPHOUËT-BOIGNY University, 22 PO Box 582 Abidjan 22 (Côte d'Ivoire).
[2]Peleforo GON COULIBALY University, Training and Research Unit of Biological Sciences, PO Box 1328, Korhogo, Ivory Coast

DOI: 10.36348/sjbr.2021.v06i12.002 | **Received:** 19.10.2021 | **Accepted:** 24.11.2021 | **Published:** 14.12.2021

***Corresponding author:** Pacôme Kouadio N'Go

Abstract

A cocktail made with the extract of three medicinal plants (*Spondias mombin, Vernonia amygdalina and Momordica charantia*) is widely used in the traditional Ivorian pharmacopeia for the treatment of various illnesses and brain disorders. The present study aimed to evaluate the possible preventive effect of the hydroethanolic extract of the cocktail on anxiety and depression as well as memory disorders related to abrupt withdrawal from chronic alcoholism using a Mice model. Adult mice were either with vehicle (phosphate buffer saline, 1 ml/100 g, p.o.) or 20% of alcohol drink (2g/kg, p.o) for 28 days. Some alcoholic mice were concomitantly treated with cocktail extract (50 and 100 mg/kg, p.o). Twenty-four hours later, anxiety and depression-like behavior, and memory abilities were assessed with behavioral tests. The level of oxidative stress markers such as nitric oxide (NO), malondialdehyde (MDA) and non-protein-thiol (NP-SH) were assayed in hippocampus and prefrontal cortex. The results showed that the cocktail extract significantly prevented at dose-dependent manner the abrupt alcohol withdrawal-induced anxiety and depression-like behavior. In addition, the cocktail extract significantly improved the spatial learning and reference memory deficits observed in alcoholic mice. The results also revealed that the cocktail extract significantly mitigated the MDA and NO content, but in same time increased the level of NP-SH in both the hippocampus and prefrontal cortex when compared to chronic alcoholic mice. Our finding provides evidence that the cocktail of the three medicinal plants has neuroprotective effects against alcohol withdrawal symptoms and confirms its traditional use to relieve cognitive-affective disorders.

Keywords: Spondias mombin, Vernonia amygdalina, Momordica charantia, chronic alcoholism withdrawal, Neuroprotection, Memory, Anxiety and Depression-like behavior.

INTRODUCTION

International researches showed that common mental disorders such as depression, anxiety, social anxiety, stress, alexithymia and having insecure attachment styles are risk factors for alcohol- use disorders (AUD) (Obeid *et al*., 2020). AUD is a broad term for any drinking of alcohol that results in mental or physical health problems (Littrell, 2014). According to the Global Burden of Disease (GBD) 2016 Alcohol Collaborators (2018) the complications of health problems due to AUD led to 5.3% of global death during the 2016 year. The pathophysiology mechanisms of AUD include both neuroinflammation and subsequent oxidative stress processes leading to neuronal dysfunction (Crews *et al.*, 2015). For instance, chronic alcohol drinking is associated to peripheral pro-inflammatory cytokines release which could exacerbate brain inflammation (Neupane, 2016).

Otherwise, acute withdrawal from alcohol is among the criteria for an AUD, and results in a constellation of somatic and affective symptoms (American Psychiatric Association, 2013). The withdrawal from alcohol in dependent individuals can be associated with neurological manifestations of a consequent neurochemical imbalance. The underlying pathophysiology of alcohol withdrawal syndrome (AWS) is central nervous system hyperexcitation, whereas the initial withdrawal symptoms include hallucinations, seizures and delirium tremens *(Newman*

et al., 2021). It has been also reported that anxiety, depression and memory problems are symptoms of alcohol withdrawal that occur with abrupt cessation after chronic consumption (Arora & Vohora, 2016). However, the disorders associated to severe withdrawal from alcohol could be manage with psychotropic drugs to reduce the risk of seizures (Saitz, 1998). For this purpose, the benzodiazepine drugs seem to the best treatment since it is an gamma-aminobutyric acid (GABA) agonist, also anticonvulsants are needed (Kattimani & Bharadwaj, 2013; Long *et al.*, 2017). However, benzodiazepine use has revealed some side health problems (Votaw *et al.*, 2019). Therefore, it worthwhile to promote some alternative medical approaches based on traditional herbs drugs which are well-known for relieving various ailments.

In this context, we have conducted an ethnopharmacological survey, in south-west of Ivory Coast, which helped to collect the species namely *Spondias monbin L*. (Anacardiaceae), *Vernonia amygdalina Delile* (Asteraceae) and *Momordica charantia Linn* (Cucurbitaceae). In fact, the extract of those species is used as cocktail of drugs-based on their anxiolytic and antidepressant properties by local population to treat fear and depressive mood (Doumbia et *al*., 2021). Elsewhere, Asuquo *et al.* (2013) have reported in Wistar rat models of anxiety and fear that the ethanolic extract of *Spondias monbin L*. presented some potent anxiolytic and sedative effects compared to the aqueous extract. *Momordica charantia Linn* possesses an anxiolytic-like effect which may involve an action on benzodiazepine-like receptors as well as an antidepressant-like effect which depends on the serotonergic, noradrenergic, dopaminergic and muscarinic cholinergic systems (Ishola *et al.*, 2013). Finally, Onasanwo *et al.* (2016) have found that *Vernonia amygdalina Delile* and its bioactive compound reserpine exhibited some antidepressant effects mediated by serotoninergic, noradrenergic, and dopaminergic, when evaluated with several behavioral tests.

In present study, we investigated the possible neuroprotective effects of the drugs cocktail from the three traditional medicinal plants against neuroinflammation and oxidative stress mediated by chronic alcohol consumption. We also tested whether the cocktail could help to manage efficiently the neuropsychiatric and cognitive disorders related to chronic alcoholism and abrupt withdrawal from it.

MATERIEL AND METHODS

Plant materiel

Momordica charantia L. (cucurbitaceae), *Vernonia amygdalina Delile* (Asteraceae) and *Spondias monbin L*. (Anacardiaceae) leaves were obtained from the Department of Agboville, in the southeast of Côte d'Ivoire. Then these plants were identified and authenticated at the Centre National Floristique (CNF) of University Félix Houphouet Boigny under the following numbers: n° ucj004436 (*Momordica charantia L*.), n° ucj003893 (*Vernonia amygdalina Delile*) and n° ucj001020 (*Spondias monbin L*.). The cocktail extract was kindly donated by the Department of Galenics of Training and Research Unit of Pharmaceutical Sciences of Felix Houphouet Boigny University.

Animals and acclimation

The mice of two- to three-month-old of age (22-32 g) were obtained from the vivarium of the Ecole Normale Supérieure (ENS) of Abidjan. The temperature of the animal room was 27°C (± 3°C). The lighting was varied according to the sequence of 12 hours of light and 12 hours of darkness (lights on at 7 a.m., lights off at 7 p.m.). The animals were fed with standard laboratory food (pellets) and drinking water from the tap *ad libitum*. In order for the mice to become accustomed to the test environment, they were transported to it, in their home cage, at least 24 hours before the behavioral test in the waiting room. All experimental protocols were carried out according to NIH guide for the care and use of laboratory animals for minimizing the number of animals used and their suffering.

Experimental design

Symptoms of alcohol withdrawal syndrome may be occurred within 6-24h after the abrupt discontinuation or decrease of the consumption (Mirijello *et al.*, 2015). Alcohol withdrawal led to the anxiety (Xiao *et al*., 2018) or to the depression (Skuza, 2013). The experiment was carried out according to the method described by Arora and Vohora (2016) with some modifications. ─ Experimental animals were divided into four groups (n=5). Group 1 received PBS solution (1ml/100 g, p.o.) for 28 days, mice of group 2 were given 20% alcohol (2 g/kg, p.o.) for 28 days, and the group 3 and 4 were treated concomitantly with 20% alcohol and hydroethanolic extract cocktail (HEEC) of three medicinal plants (*Spondias mombin, Vernonia amygdalina and Momordica charantia*) at 50 and 100 mg/kg p.o., respectively for 28 days. 24 hours later, mice were submitted to cognitive-behavioral testing.

Behavioral assessment

Elevated Plus Maze (EPM)

The EPM is the most widely approved and used as test to evaluate animal's model of anxiety-like behavior (Asif *et al*., 2019). The EPM is performed according to the design of Komada et *al*. (2008), with some slight modifications. The maze consists of a wooden apparatus with four arms (two open arms of 50 cm × 10 cm and two closed arms of 50 cm × 10 cm × 40 cm) connected by a central one (10 cm × 10cm), raised up to 50 cm from the ground. On 15th day after the treatments, the mice were submitted to a testing with EPM by placing onto the central square facing to

an open arm. The free exploratory behavior of mice in the maze was recorded with a video tracking for 5 min. The parameters taken account the total time spent in the open arm to estimate the anxiolytic action of the substances tested. An open arm entry was considered when the mice crossed the arm with the four paws. The apparatus was cleaned with ethanol 70% between each test.

Open-field test (OFT)

The OFT was used to analyze locomotion, anxiety, and stereotypical behaviors such as grooming and rearing in rodents (Prut & Belzung, 2003). In this study, the OFT is based on the Walsh et Cumming (1976) model with some slight modifications. It is a straight vinyl chloride made in plywood block (60×60×30 cm), and the floor was divided by permanent black marks into 25 equal squares (12×12) with 9 central and 16 peripheral squares. The whole apparatus was lighted with a light of 120 lux. Each animal was left to move freely in the arena and behavior was videotaped for five minutes. Basal locomotor activity was expressed by the number of total squares crossed, and anxiety level was reflected by the time spent in the central area.

Forced swimming test (FST)

The FST is commonly the most used assays for the study of depression-like behavior in rodents (Porsolt *et al.*, 1977). It's based on the assumption that when placing an animal in a container filled with water, it will first make efforts to escape but eventually will exhibit immobility that may be considered to reflect a measure of behavioral despair. This study was realized with some modifications. There was one 6 min-session divided into pre-test (the first 2 min) and test (the last 4 min). Fill before the cylinders with tap water at 25°C and adjust the water depth according to the mouse' size, so that it cannot touch the bottom of the container with its hind legs. Then turn on the video camera placed on the level of water surface to clearly appreciate active and passive behaviors and then place each mouse in the water filled cylinder container for 6 min. After time elapsed, we turned off the camera off, removed the mouse from the container and placed it in the transient drying cage with the heat lamp above it and the heat pad under it. We changed the water after every session to avoid any influence on the next mouse. The stillness posture is characterized by floating in the water with only the movements necessary to keep the nose above the surface. The time of immobility was recorded.

Morris water maze (MWM)

MWM were conducted to evaluate learning and spatial memory as well as reference memory abilities according to the procedure described previously (Dagnas & Mons, 2013). The apparatus was a circular open pool (1 m in diameter and 55 cm in depth) filled with tepid water (21°C ± 1) to 30 cm of depth. The escape platform is submerged 1 cm below the surface of water. The tank was divided in four quadrants: a quadrant with the virtual position of platform (P), Right side of platform (RP), left side of platform (LP) and opposite side of platform (OP). Firstly, mice were submitted to swim freely for 60s in order to define the fixed position of the platform: habituation phase. Then, in acquisition training, for four consecutive sessions with an inter-session interval of 20 min, animals were left in the tank facing the wall and allowed to swim freely to the escape platform. If an animal did not find the platform after 90 s, it was gently guided to it, and allowed to remain on the platform for 20 sec after going back to the home cage. The procedure was repeated with 3 trials by sessions, and with at least 10 min interatrial. The probe session was conducted 24 h after the last training session. The test session consists of a single probe trial where the platform was removed from the tank and each animal was allowed to explore the virtual position of the platform for 90 min in the maze. All tracks of animal were recorded with a webcam video for analyses.

Oxidative stress markers assay

Tissue preparation

Twenty-four hours after the behavioral tests, the mice are euthanized with 7% chloral (400 mg/kg i.p). Then, hippocampal and prefrontal cortex (PFC) tissues from each mouse were isolated, weighed and homogenized in ice-cool 50 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm/min for 10 min. The supernatant (S1) was collected and kept at -20°C till the assay day.

Malondialdehyde (MDA) level

The MDA level is a biomarker of lipid peroxidation level. The determination of the MDA concentration is performed according to the method of Satoh *et al.* (1978) based on thiobarbituric-acid-reacting substance (TBAR). Briefly, the supernatant S1was mixed with 1 ml of 10% of trichloroacetic acid and 1 ml of TBAR (0.67%). After 15 min of incubation at room temperature, the mixture was heated in a boiling bath for 15 min, cooled on tap water and mixed with n-butanol (2:1 v/v). The solution was then centrifuged at 800 rpm for 5 min. Afterward, the supernatant was collected, and the absorbance read at 535 nm. Lipid peroxidation level was expressed as MDA µmol/g of tissue.

Non-protein thiol (NP-SH) level

NP-SH level was determined according to the method of Ellman *at al.* (1959). S1 was precipitated with 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. The thiol groups (-SH) were assayed from the supernatant. It was added 1M of potassium phosphate buffer pH 7.4 and 10 mM of Ellman reagent 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB). The NP-SH level was measured at 412 nm and expressed in µmol/g of tissue.

NO level assessment

The NO level was assayed using a modified Griess method (Touil-Boukoffa *et al.*, 1998). To the sample was added 50µl of Griess reagent (1%) sulphanylamide (A), and 0.1% N-1-naphtylethylenediamine dihydrochloride (B) in 2.5% orthophosphoric acid)], and the mixture was incubated at 37°C for 20 min in the dark. The reaction was performed in two steps. The first one consisted in a dinitrogenation reaction between the nitrite and Griess reagent A leading to a Diazonium salt by-product. The second step is the formation of stable chromophoric Azo product resulting by coupling between Griess reagent B and the Diazonium salt. The Azo product strongly absorbs at 540 nm at enzyme-linked immunosorbent assay (ELISA) reader. The NO content was expressed in µmol/g of tissue.

Statistical analysis

The experimental results data were expressed as means ± S.E.M (Standard Error of Mean). Statistical analyses were performed using one-way analyses of variance (ANOVA) or ANOVA repeated measure followed by Tukey post-hoc test for multiple comparison. P <0.05 was considered as statistically significant.

RESULTS

Effect of plant cocktail extract on cognitive-behavioral abilities in alcohol withdrawal mice

Evaluation of anxiety-like behavior level with EPM

Abruptly stopping the alcohol administration for 24 hours significantly increased anxiety-like behavior level in alcohol withdrawal mice. The cocktail of plants extract helped to prevent anxiety-like behavior related to alcohol withdrawal through a highly significant reduction of time spent in EPM (P < 0.001). However, the treatment with cocktail extract at 50 mg/kg was significantly more efficient than the 100 mg/kg dose (P < 0.01) (Fig.1).

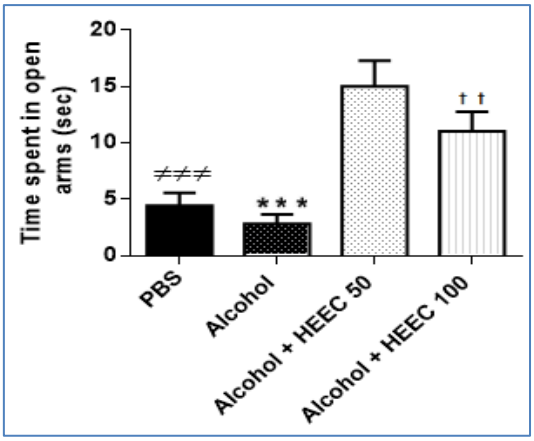

Fig-1: Effects of cocktail plants extract (*Spondias mombin, Vernonia amygdalina and Momordica charantia)* on chronic alcohol withdrawal-induced anxiety-like behavior.
Time spent in the open arms is expressed as mean ± SEM.
One way ANOVA / Tukey post hoc analysis
*** P < 0.001 (Alcohol vs. Alcohol + HEEC 50 or Alcohol +HEEC 100), ‡‡‡ P < 0.001 (PBS vs. Alcohol + HEEC 50 or Alcohol +HEEC 100), $^{++}$ P < 0.01 (Alcohol + HEEC 50 vs Alcohol +HEEC 100

Evaluation of mice's behavior in OF

The mice of Alcohol withdrawal group avoided the central anxiogenic part of the OF arena. This was expressed by a reduction of time in central area; however, the treatment with cocktail extract at 100 mg/kg increased significant this parameter (P < 0.05), but not the with dose of 50 mg/kg (Fig. 2a). Moreover, the basal locomotion activity was altered in alcohol withdrawal mice through a significant reduction of total squares crossed compared to both mice treated with cocktail at 50 mg/kg (P < 0.01) and 100 mg/kg (P < 0.001). However, the treatment with high dose of cocktail extract remained more efficient (P < 0.01) (Fig. 2b).

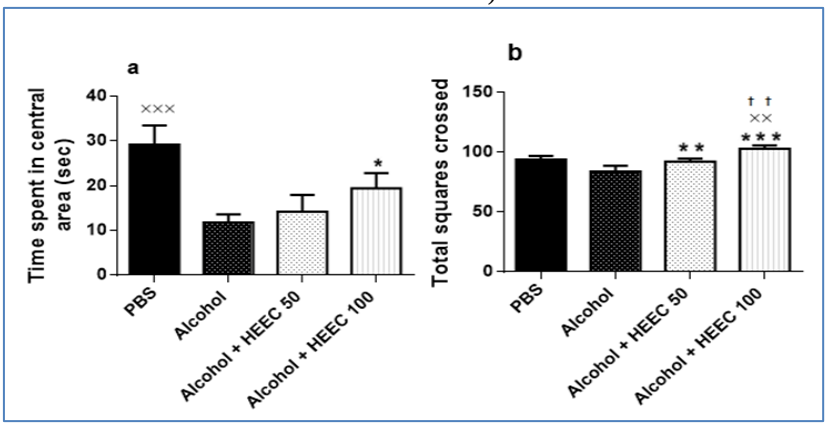

Fig-2: Effects of cocktail plants extract (*Spondias mombin, Vernonia amygdalina and Momordica charantia)* on chronic alcohol withdrawal-induced behavior impairment in OF. Time spent in central area (a) and Total squares crossed (b) are expressed as mean ± SEM.
One way ANOVA / Tukey post hoc analysis
* P < 0.055, **P < 0.01, *** P < 0.001 (Alcohol vs. Alcohol HEEC 50 or Alcohol HEEC 100); ^{xx} P < 0.01, ^{xxx} P < 0.001 (PBS vs. Alcohol or Alcohol + HEEC 100); $^{++}$ P < 0.01 (Alcohol + HEEC 50 vs Alcohol +HEEC 100)

Evaluation of Depression-like behavior level with FST

Our results revealed that a sudden weaning from alcohol lead to depression-like behavior in the mice. In FST, the immobility time was significantly increased in alcohol withdrawal mice ($P < 0.001$), and both treatment with 50 and 100 mg/kg of plants cocktail extract prevented almost at the same manner the depression-like behavior occurrence (Fig.3).

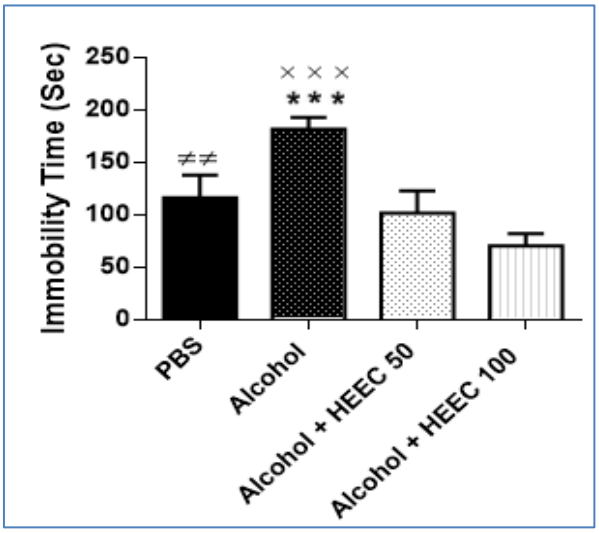

Fig-3: Effects of cocktail plants extract (*Spondias mombin, Vernonia amygdalina and Momordica charantia)* on chronic alcohol withdrawal -induced depression-like behavior.

Immobility time is expressed as mean ± SEM. One way ANOVA / Tukey post hoc analysis

*** $P < 0.001$ (Alcohol vs. Alcohol HEEC 50 or Alcohol HEEC 100); ^{xxx} $P < 0.001$ (PBS vs. Alcohol); $^{\pm\pm}$ $P < 0.01$ (PBS vs. Alcohol HEEC 50 or Alcohol HEEC 100)

Spatial learning and reference memory abilities evaluated with MWM

Alcohol drinking for 28 days mitigated the spatial learning and reference memory abilities when it evaluated 24 hours after sudden stopping (Fig.4). In fact, the latency to find the hidden platform was not significantly changed during trial-sessions learning in alcoholic mice as well as the time spent in platform quadrant during the probe day. If both treatments with cocktail extract 50 and 100 mg/kg significantly prevented the spatial learning capacity disrupted in the alcoholic mice, the high dose of 100 mg/kg seemed to be more efficient ($P < 0.05$). Otherwise, the alcoholic mice spent more less time in the target quadrant compared to other experimental groups. However, the plants cocktail treatment significantly improved the reminder of virtual platform position either for the dose of 50 mg/kg ($P < 0.01$) or for the dose of 100 mg/kg ($P < 0.001$). We noted also that the dose of 100 mg/kg exerted significant effect compared to 50 mg/kg

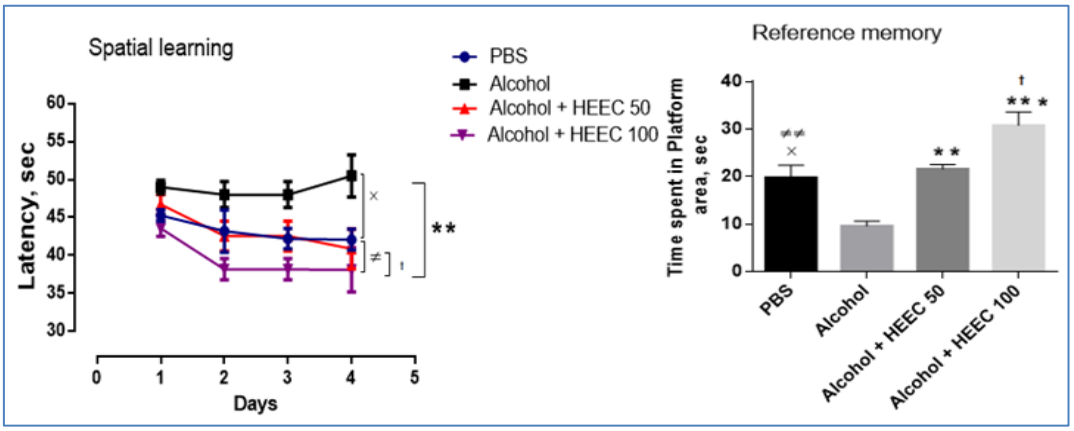

Fig-4: Effects of cocktail plants extract (*Spondias mombin, Vernonia amygdalina and Momordica charantia)* on chronic alcohol withdrawal-induced spatial learning and reference memory abilities.

Latency and Time spent in platform area are expressed as mean ± SEM. One way ANOVA / Tukey post hoc analysis

** $P < 0.01$, *** $P < 0.001$ (Alcohol vs. Alcohol HEEC 50 or Alcohol HEEC 100); ^{x} $P < 0.05$ (PBS vs. Alcohol); $^{\pm}$ $P < 0.05$, $^{\pm\pm}$ $P < 0.01$ (PBS vs. Alcohol HEEC 100); $^{+}$ $P < 0.05$ (Alcohol + HEEC 50 vs Alcohol +HEEC 100)

Effect of plant cocktail extract on oxidative stress markers level in alcohol withdrawal mice's model

NO level

The administration of alcohol for 28 days increased the NO content in hippocampus and PFC. The level of NO was significantly elevated in hippocampus of alcoholic mice ($P < 0.01$) when compared to those co-treated with the cocktail extract. Both 50 and 100 mg/kg acted at the same manner to reduce NO level. By contrast, in CPF, the dose of 100 mg/kg scavenged more efficiently the NO compared to that of 50 mg/kg ($P < 0.01$) (Fig.5).

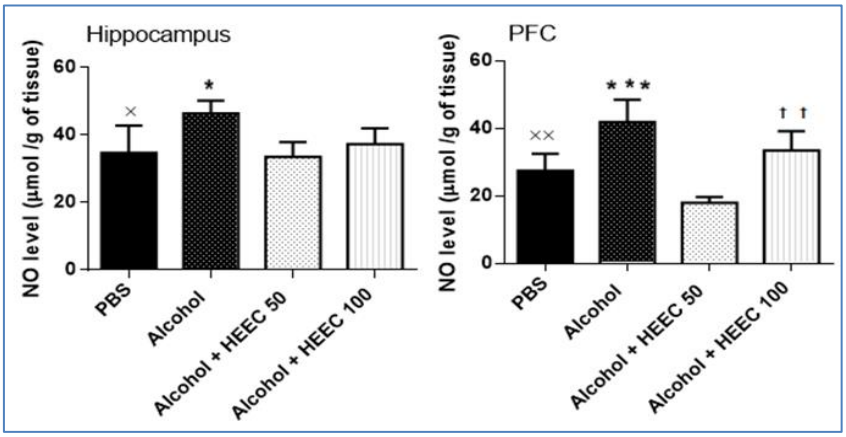

Fig-5: Effects of cocktail plants extract (*Spondias mombin, Vernonia amygdalina and Momordica charantia)* on chronic alcohol withdrawal-induced NO generation

NO level is expressed as mean ± SEM. One way ANOVA / Tukey post hoc analysis

* $P < 0.05$, *** $P < 0.001$ (Alcohol vs. Alcohol HEEC 50 or Alcohol HEEC 100); ^{x} $P < 0.05$, ^{xx} $P < 0.01$ (PBS vs. Alcohol); $^{++}$ $P < 0.01$ (Alcohol + HEEC 50 vs Alcohol +HEEC 100)

MDA level

Alcohol drinking significantly promoted the lipid membrane peroxidation in the brain of the Mice (Fig. 6). In the hippocampus of alcoholic mice, the level MDA biomarker was highly increased ($P < 0.001$) compared to those co-treated with the two dose plants cocktail extract acting at the same manner. In PFC, we noted also that alcohol consumption caused a high-level of MDA generated ($P < 0.001$), unless the co-treatment with the dose of 100 mg/kg demonstrated higher effect than the 50 mg/kg ($P < 0.001$).

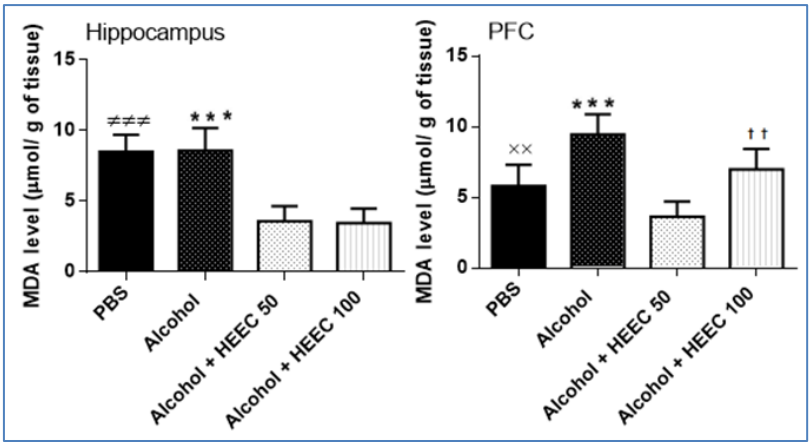

Fig-6: Effects of cocktail plants extract (*Spondias mombin, Vernonia amygdalina and Momordica charantia)* on chronic alcohol withdrawal-induced lipid membrane peroxidation

MDA *level* is expressed as mean ± SEM. One way ANOVA / Tukey post hoc analysis

*** $P < 0.001$ (Alcohol vs. Alcohol HEEC 50 or Alcohol HEEC 100); ^{xx} $P < 0.01$ (PBS vs. Alcohol); $^{\pm\pm}$ $P < 0.01$ (PBS vs. Alcohol HEEC 50 or Alcohol HEEC 100), $^{++}$ $P < 0.01$ (Alcohol + HEEC 50 vs Alcohol +HEEC 100)

NP-SH level

The level of natural antioxidant NP-SH was high significantly reduced in alcoholic mice in both hippocampus and PFC areas ($P < 0.001$) compared to other studied groups. By contrast, both doses of 50 and 100 mg/kg treatment with plants cocktail extract helped significantly to increase the NP-SH level (Fig.7).

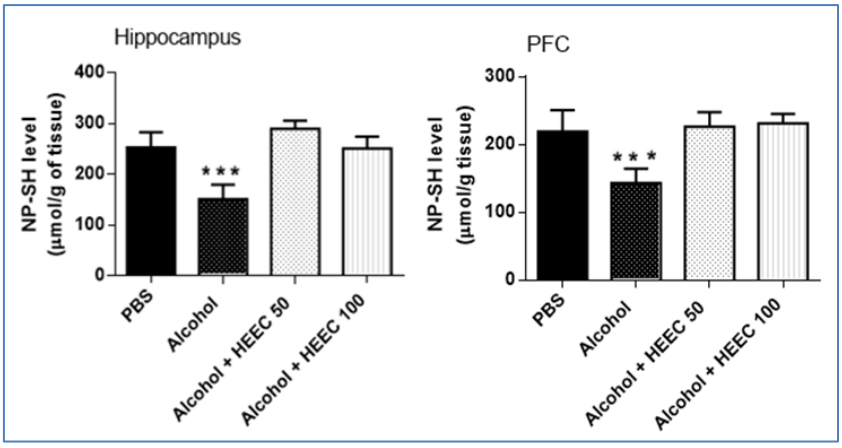

Fig-7: Effects of cocktail plants extract (*Spondias mombin, Vernonia amygdalina and Momordica charantia)* on chronic alcohol withdrawal-induced NP-SH level change

NP-SH level is expressed as mean ± SEM. One way ANOVA / Tukey post hoc analysis

*** $P < 0.001$ (Alcohol vs. other experimental groups)

DISCUSSION

This study was undertaken based on ethnopharmacological findings of the traditional use of a cocktail drug made with three medicinal plants (*Spondias mombin*, *Vernonia amygdalina* and *Momordica charantia*) for the treatment of anxiety and depression symptoms. In this preventive study, the hydroethanolic extract of those plants was tested to highlight the neuroprotective effects against a mice's model of chronic alcohol withdrawal-induced cognitive-affective disorders. As results, we found that the cocktail administration significantly attenuated at the dose-dependent manner the anxiety and depression-like behavior level as well spatial learning and memory abilities. It well-known that anxiety and depression represent recurrently negative affective disorders of alcohol withdrawal (Metten *et al*., 2018). Valcheva-Kuzmanova *et al.* (2013) showed with female rats' model that daily subchronic alcohol exposure at relatively high dose led to anxiety and depression. The chronic alcoholism or alcoholism withdrawal could alter the gut microbiota and affect the gut-brain axis involved in the regulation of anxiety level (Xiao *et al.*, 2018). Also, the relationship between chronic alcoholism and/or abrupt with withdrawal from it and anxiety or depression occurrence has been reported (Arora & Vohora, 2016; File *et al*., 1992; Zeigler *et al.*, 2005). In fact, the excessive alcohol consumption enables the activation of the mechanistic target of rapamycin complex 1 (mTORC1) pathway which is increasing the level of the Collapsin Response Mediator Protein-2 (CRMP-2) level in limbic area of rodent. It results of an assembly of microtubule which biding to CRMP-2 responsible to addiction leading to physical and / or psychological dependence (Sharma *et al.*, 2019). On the other hand, the medicinal plants are traditionally used to the relief of anxiety and depression in the world (Sarris *et al.*, 2011). The cotreatment with a chronic alcoholism and the cocktail extract has helped to manage efficiently the anxiety-like behavior. A past study reported that the extract of the *Spondias mombin* leaves exhibited some anxiolytic properties. The authors have attributed that to possible interaction between the *Spondias mombin* compounds and gamma-aminobutyric acid (GABA)ergic transmission (Ayoka *et al.*, 2005). On the other hand, the anxiolytic effects of *Vernonia amygdalina* extract has been examined using a Mice's model (Onasanwo *et al.*, 2016), and those effects seem to be due to the rich-antioxidant compounds such as flavonoids, saponins, terpenes, phenolic acids (Adedayo *et al.*, 2018). In the present study, we have examined the alcohol consumption-induced depressive-like behavior in mice. Our results revealed significant depressive-like behavior as measured the elevated of immobility time in the FST. According to Markianos *et al.* (2001), the ethanol facilitates the release of some neurotransmitters such as noradrenaline, serotonin, and dopamine in the central nervous system. Hence, at the pick concentration of those neurotransmitters, it results in the downregulation of both α-adrenergic and serotonin receptors. A sudden drop in the level of those neurotransmitters due to abrupt withdrawal is responsible for psychiatric disorders. This fact may explain the increased immobility time in FST observed in alcohol withdrawal group of rats. Interesting, we found that at dose-dependent manner the cotreatment of alcoholic rats with the cocktail extract decreased significantly the time spent to be immobile in the FST. This could suppose that our cocktail contains some antidepressant-miming psychotropic drug properties. Our results are in agreement with that of a previous one reporting the antidepressant effects of each specie used namely *Spondias mombin, Momordica charantia and Vernonia amygdalina* (Ayoka *et al.*, 2005). Clearly, *Vernonia maygdalina* acts as natural antidepressant-like potentials through its bioactive compound reserpine which is implicated in the functional interaction with monoaminergic systems (Onasanwo *et al.*, 2016). In addition, *Momordica charantia* antidepressant-like action is mediated by of the serotoninergic 5-HT2 receptor, α1- and α2-adrenoceptors and dopaminergic

D2 receptor as well as the muscarinic cholinergic systems, whereas its anxiolytic-like effect might involve an action on benzodiazepine-type receptor (Ishola *et al.*, 2013).

In the present study, we investigated the withdrawal from chronic alcoholism-induced cognitive impairment using Morris water maze. Our findings with results of previous study showed that alcohol withdrawal impacted progressively or seriously on memory abilities (Farr *et al.*, 2005). Here, we have reported that spatial learning and reference memory were significantly affected in alcoholic rats, when evaluated 24 h after the abrupt deprivation. By contrast, a past study indicated the impaired short-term memory during the alcoholism and withdrawal periods (Zeigler *et al.*, 2005). The chronic alcoholism seems to disturb the neurotransmission of acetylcholine which is considered as a major modulator of learning and memory, and that is well proved when using cholinesterase inhibitor as therapeutic drug against Alzheimer's disease (Reale *et al.*, 2020). Based on previous studied findings, some traditional folk medicines are used to improve learning and memory disorders by targeting acetylcholinesterase for the modulation acetylcholine neurotransmission (Choi *et al.*, 2011; Li *et al.*, 2017). The beneficial effects of our cocktail drug have been reflected by significant improvement of spatial learning and reference memory at dose-dependent manner after alcohol withdrawal. It suggests that our cocktail extract possesses some natural compounds regulating the bioavailability of acetylcholine. It has been elsewhere showed that *Vernonia amygdalina*, one of the constituents of our cocktail, improves memory by increasing the bioavailability of acetylcholine through the enhancement of the activity of the enzyme acetylcholinesterase (Ebuehi & Ajagun-Ogunleye, 2017). On other side, the 8 weeks-treatment with *Momordica charantia* extract improved the spatial memory performance of the rats after having received a high-fat diet (Sepehri *et al.*, 2019).

Finally, we investigated whether the neuroinflammation and subsequent oxidative stress in the brain of the alcohol withdrawal rats could be a possible explanation factor to the harmful cognitive-affective outcomes. In fact, chronic alcohol intake has been identified as a promoter of neuroinflammation (Lowe *et al.*, 2020), and ethanolic withdrawal facilitates prooxidant factors generation (Gonzaga *et al.*, 2015) which causes brain oxidative damage (Jung & Metzger, 2016). To this purpose, the approach consisted in the assay of neuroinflammation as well as oxidative stress biomarkers. As results, our cocktail extract significantly attenuated NO content, prevented the lipid membrane peroxidation, and helped to the increase of NP-SH level in both the hippocampus and PFC, when compared to alcohol-withdrawal rats. Importantly, the phytochemical studies on individual plant of the cocktail have identified some bioactive compounds with antioxidant properties such as flavonoids, and phenols for *Spondias mombin* (Nwidu *et al.*, 2018), Tannins and flavonoids for *Momordica charantia* (Manandhar *et al.*, 2019; Poovitha & Parani, 2020), and the polyphenols for *Vernonia amygdalina* (Erukainure *et al.*, 2019; Omojokun *et al.*, 2019; Poovitha & Parani, 2020).

In this study, the exact mechanism of action by which these beneficial cocktail activities occur is unclear and provides an important avenue for future research.

CONCLUSION

In conclusion, the study shows that the abrupt cessation of chronic alcohol consumption induces anxiety, depression and memory disorders. The use of the cocktail of three traditional plants helps to prevent those effects associated to abrupt alcohol withdrawal. The present study suggests that the HEEC of the three medicinal plants (*Spondias mombin, Vernonia amygdalina and Momordica charantia*) has some neuroprotective properties which need to be further elucidated by molecular, pharmacological, and clinical studies.

REFERENCES

1. Obeid, S., Akel, M., Haddad, C., Fares, K., Sacre, H., Salameh, P., & Hallit, S. (2020). Factors associated with alcohol use disorder: The role of depression, anxiety, stress, alexithymia and work fatigue- a population study in Lebanon. *BMC Public Health*, *20*, 245. https://doi.org/10.1186/s12889-020-8345-1
2. Littrell, J. (2014). *Understanding and Treating Alcoholism: Volume I: An Empirically Based Clinician's Handbook for the Treatment of Alcoholism:volume Ii: Biological, Psychological, and Social Aspects of Alcohol Consumption and Abuse*. Psychology Press. https://doi.org/10.4324/9781315807300
3. Global Burden of Disease (GBD) 2016 Alcohol Collaborators. (2018). Alcohol use and burden for 195 countries and territories, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet (London, England)*, *392*(10152), 1015–1035. https://doi.org/10.1016/S0140-6736(18)31310-2
4. Crews, F. T., Sarkar, D. K., Qin, L., Zou, J., Boyadjieva, N., & Vetreno, R. P. (2015). Neuroimmune Function and the Consequences of Alcohol Exposure. *Alcohol Research : Current Reviews*, *37*(2), 331–351.
5. Neupane, S. P. (2016). Neuroimmune Interface in the Comorbidity between Alcohol Use Disorder and Major Depression. *Frontiers in Immunology*, *7*, 655. https://doi.org/10.3389/fimmu.2016.00655
6. American Psychiatric Association, D.-5 T. F. (2013). *Diagnostic and statistical manual of mental disorders: DSM-5™, 5th ed* (pp. xliv, 947).

American Psychiatric Publishing, Inc. https://doi.org/10.1176/appi.books.9780890425596
7. Newman, R. K., Stobart Gallagher, M. A., & Gomez, A. E. (2021). Alcohol Withdrawal. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK441882/
8. Arora, S., & Vohora, D. (2016). Comparative Evaluation of Partial α2 -Adrenoceptor Agonist and Pure α2 -Adrenoceptor Antagonist on the Behavioural Symptoms of Withdrawal after Chronic Alcohol Administration in Mice. *Basic & Clinical Pharmacology & Toxicology*, *119*(2), 202–209. https://doi.org/10.1111/bcpt.12566
9. Saitz, R. (1998). Introduction to alcohol withdrawal. *Alcohol Health and Research World*, *22*(1), 5–12.
10. Kattimani, S., & Bharadwaj, B. (2013). Clinical management of alcohol withdrawal: A systematic review. *Industrial Psychiatry Journal*, *22*(2), 100–108. https://doi.org/10.4103/0972-6748.132914
11. Long, D., Long, B., & Koyfman, A. (2017). The emergency medicine management of severe alcohol withdrawal. *The American Journal of Emergency Medicine*, *35*(7), 1005–1011. https://doi.org/10.1016/j.ajem.2017.02.002
12. Votaw, V. R., Geyer, R., Rieselbach, M. M., & McHugh, R. K. (2019). The epidemiology of benzodiazepine misuse: A systematic review. *Drug and Alcohol Dependence*, *200*, 95–114. https://doi.org/10.1016/j.drugalcdep.2019.02.033
13. Doumbia, H. O. Y., Assi, B. D., & Koman, S. R. (2021). Etude Ethnobotanique De Plantes Medicinales Utilisees Dns Le Traitement De L'anxiete Et De La Depression A M'berie Et A Arraguie (Agboville, SudOuest De La Cote D'ivoire). *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, *16*(4), 12. https://doi.org/10.9790/3008-1604010112
14. Asuquo, O. R., Udonwa, U. N., Eluwa, M. A., & Ekanem, T. B. (2013). *Effects of Spondias Mombin Leaf Extract on the Cytoarchitecture of the Cerebal Cortex and on Learning and Memory in Wistar Rats*. *2*(9), 4.
15. Ishola, I., Akinyede, A., & Sholarin, A. (2013). Antidepressant and Anxiolytic Properties of the Methanolic Extract of Momordica charantia Linn (Cucurbitaceae) and its Mechanism of Action. *Drug Research*, *64*(07), 368–376. https://doi.org/10.1055/s-0033-1358712
16. Onasanwo, S. A., Aitokhuehi, G. N., Ajayi, O. T., & Faborode, S. O. (2016). The Anxiolytic Properties of Vernonia Amygdalina (Asteraceae) in Laboratory Mice. *African Journal of Biomedical Research*, *19*(3), 229–233. https://doi.org/10.4314/ajbr.v19i3
17. Mirijello, A., D'Angelo, C., Ferrulli, A., Vassallo, G., Antonelli, M., Caputo, F., Leggio, L., Gasbarrini, A., & Addolorato, G. (2015). Identification and management of alcohol withdrawal syndrome. *Drugs*, *75*(4), 353–365. https://doi.org/10.1007/s40265-015-0358-1
18. Xiao, H.-W., Ge, C., Feng, G.-X., Li, Y., Luo, D., Dong, J.-L., Li, H., Wang, H., Cui, M., & Fan, S.-J. (2018). Gut microbiota modulates alcohol withdrawal-induced anxiety in mice. *Toxicology Letters*, *287*, 23–30. https://doi.org/10.1016/j.toxlet.2018.01.021
19. Skuza, G. (2013). Ethanol withdrawal-induced depressive symptoms in animals and therapeutic potential of sigma1 receptor ligands. *Pharmacological Reports: PR*, *65*(6), 1681–1687. https://doi.org/10.1016/s1734-1140(13)71530-5
20. Asif, H. M., Hayee, A., Aslam, M. R., Ahmad, K., & Hashmi, A. S. (2019). Dose-Dependent, Antidepressant, and Anxiolytic Effects of a Traditional Medicinal Plant for the Management of Behavioral Dysfunctions in Animal Models. *Dose-Response: A Publication of International Hormesis Society*, *17*(4), 1559325819891262. https://doi.org/10.1177/1559325819891262
21. Komada, M., Takao, K., & Miyakawa, T. (2008). Elevated Plus Maze for Mice. *Journal of Visualized Experiments*, *22*, 1088. https://doi.org/10.3791/1088
22. Prut, L., & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology*, *463*(1–3), 3–33. https://doi.org/10.1016/s0014-2999(03)01272-x
23. Walsh, R. N., & Cummins, R. A. (1976). The Open-Field Test: A critical review. *Psychological Bulletin*, *83*(3), 482–504.
24. Porsolt, R. D., Le Pichon, M., & Jalfre, M. (1977). Depression: A new animal model sensitive to antidepressant treatments. *Nature*, *266*(5604), 730–732. https://doi.org/10.1038/266730a0
25. Dagnas, M., & Mons, N. (2013). Region- and age-specific patterns of histone acetylation related to spatial and cued learning in the water maze. *Hippocampus*, *23*(7), 581–591. https://doi.org/10.1002/hipo.22116
26. Satoh, K. (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *90*(1), 37–43. https://doi.org/10.1016/0009-8981(78)90081-5
27. Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, *82*(1), 70–77. https://doi.org/10.1016/0003-9861(59)90090-6
28. Touil-Boukoffa, C., Bauvois, B., Sancéau, J., Hamrioui, B., & Wietzerbin, J. (1998). Production of nitric oxide (NO) in human hydatidosis: Relationship between nitrite production and interferon-γ levels. *Biochimie*, *80*(8–9), 739–744. https://doi.org/10.1016/S0300-9084(99)80027-3
29. Metten, P., Schlumbohm, J. P., Huang, L. C., Greenberg, G. D., Hack, W. R., Spence, S. E., &

Crabbe, J. C. (2018). An alcohol withdrawal test battery measuring multiple behavioral symptoms in mice. *Alcohol*, *68*, 19–35. https://doi.org/10.1016/j.alcohol.2017.08.014

30. Valcheva-Kuzmanova, S., Eftimov, M., & Kuzmanov, K. (2013). An experimental model of alcohol-induced anxiety and depressive behaviour in rats. *Scripta Scientifica Medica*, *45*(3), 48. https://doi.org/10.14748/ssm.v45i3.305
31. File, S. E., Zharkovsky, A., & Hitchcott, P. K. (1992). Effects of nitrendipine, chlordiazepoxide, flumazenil and baclofen on the increased anxiety resulting from alcohol withdrawal. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *16*(1), 87–93. https://doi.org/10.1016/0278-5846(92)90011-3
32. Zeigler, D. W., Wang, C. C., Yoast, R. A., Dickinson, B. D., McCaffree, M. A., Robinowitz, C. B., Sterling, M. L., & Council on Scientific Affairs, American Medical Association. (2005). The neurocognitive effects of alcohol on adolescents and college students. *Preventive Medicine*, *40*(1), 23–32. https://doi.org/10.1016/j.ypmed.2004.04.044
33. Sharma, N., Zameer, S., Akhtar, M., & Vohora, D. (2019). Effect of lacosamide on ethanol induced conditioned place preference and withdrawal associated behavior in mice: Possible contribution of hippocampal CRMP-2. *Pharmacological Reports: PR*, *71*(5), 804–810. https://doi.org/10.1016/j.pharep.2019.04.009
34. Sarris, J., Panossian, A., Schweitzer, I., Stough, C., & Scholey, A. (2011). Herbal medicine for depression, anxiety and insomnia: A review of psychopharmacology and clinical evidence. *European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology*, *21*(12), 841–860. https://doi.org/10.1016/j.euroneuro.2011.04.002
35. Ayoka, A. O., Akomolafe, R. O., Iwalewa, E. O., & Ukponmwan, O. E. (2005). Studies on the Anxiolytic Effect of Spondias mombin l. (Anacardiaceae) Extracts. *African Journal of Traditional, Complementary and Alternative Medicines*, *2*(2), Article 2. https://doi.org/10.4314/ajtcam.v2i2.31113
36. Adedayo, L. D., Akintola, G. S., Ojo, A. O., Bamidele, O., Aitokhuehi, N. G., & Ayoka, A. O. (2018). Anxiolytic and Explorative Potentials of the Methanol Leaf Extract of Vernonia Amygdalina in Male Wistar Rats. *Annals of Depression and Anxiety*, *5*(1), 5.
37. Markianos, M., Lykouras, L., Moussas, G., & Hatzimanolis, J. (2001). Changes in dopamine receptor responsivity during alcohol detoxification may predict relapse. *Drug and Alcohol Dependence*, *64*(3), 363–365. https://doi.org/10.1016/s0376-8716(01)00139-9
38. Farr, S. A., Scherrer, J. F., Banks, W. A., Flood, J. F., & Morley, J. E. (2005). Chronic ethanol consumption impairs learning and memory after cessation of ethanol. *Alcoholism, Clinical and Experimental Research*, *29*(6), 971–982. https://doi.org/10.1097/01.alc.0000171038.03371.56
39. Reale, Costantini, Jagarlapoodi, Khan, Belwal, & Cichelli. (2020). Relationship of Wine Consumption with Alzheimer's disease. *Nutrients*, *12*(1), 206. https://doi.org/10.3390/nu12010206
40. Choi, J. G., Yang, W. M., Kang, T. H., & Oh, M. S. (2011). Effects of optimized-SopungSunkiwon on memory impairment and enhancement. *Neuroscience Letters*, *491*(2), 93–98. https://doi.org/10.1016/j.neulet.2010.12.058
41. Li, N., Liu, C., Jing, S., Wang, M., Wang, H., Sun, J., Wang, C., Chen, J., & Li, H. (2017). Compound Schisandra-Ginseng-Notoginseng-Lycium Extract Ameliorates Scopolamine-Induced Learning and Memory Disorders in Mice. *Evidence-Based Complementary and Alternative Medicine: ECAM*, *2017*, 8632016. https://doi.org/10.1155/2017/8632016
42. Ebuehi, O. A. T., & Ajagun-Ogunleye, M. O. (2017). Neurochemical Impact of the Aqueous Extract of *Vernonia amygdalina* and *Talinum triangulare* on Learning and Memory in Male Wistar Rats. *International Journal of Brain and Cognitive Sciences*, *6*(5), 81–88.
43. Sepehri, H., Hojati, A., & Safari, R. (2019). Effect of Bitter Melon on Spatial Memory of Rats Receiving a High-Fat Diet. *Journal of Experimental Pharmacology*, *Volume 11*, 115–119. https://doi.org/10.2147/JEP.S231260
44. Lowe, P. P., Morel, C., Ambade, A., Iracheta-Vellve, A., Kwiatkowski, E., Satishchandran, A., Furi, I., Cho, Y., Gyongyosi, B., Catalano, D., Lefebvre, E., Fischer, L., Seyedkazemi, S., Schafer, D. P., & Szabo, G. (2020). Chronic alcohol-induced neuroinflammation involves CCR2/5-dependent peripheral macrophage infiltration and microglia alterations. *Journal of Neuroinflammation*, *17*(1), 296. https://doi.org/10.1186/s12974-020-01972-5
45. Gonzaga, N. A., Mecawi, A. S., Antunes-Rodrigues, J., De Martinis, B. S., Padovan, C. M., & Tirapelli, C. R. (2015). Ethanol withdrawal increases oxidative stress and reduces nitric oxide bioavailability in the vasculature of rats. *Alcohol (Fayetteville, N.Y.)*, *49*(1), 47–56. https://doi.org/10.1016/j.alcohol.2014.12.001
46. Jung, M. E., & Metzger, D. B. (2016). A sex difference in oxidative stress and behavioral suppression induced by ethanol withdrawal in rats. *Behavioural Brain Research*, *314*, 199–214. https://doi.org/10.1016/j.bbr.2016.07.054
47. Nwidu, L. L., Elmorsy, E., Oboma, Y. I., & Carter, W. G. (2018). Hepatoprotective and antioxidant activities of Spondias mombin leaf and stem extracts against carbon tetrachloride-induced hepatotoxicity. *Journal of Taibah University*

Medical Sciences, *13*(3), 262–271. https://doi.org/10.1016/j.jtumed.2018.03.006

48. Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *Journal of Tropical Medicine*, *2019*, 1–5. https://doi.org/10.1155/2019/1895340
49. Poovitha, S., & Parani, M. (2020). Protein extracts from *Momordica charantia* var. *Charantia* and *M. charantia* var. *Muricata* show anti-lipidemic and antioxidant properties in experimental type 2 diabetic rats. *Journal of Food Biochemistry*, *44*(9). https://doi.org/10.1111/jfbc.13370
50. Erukainure, O. L., Chukwuma, C. I., Sanni, O., Matsabisa, M. G., & Islam, Md. S. (2019). Histochemistry, phenolic content, antioxidant, and anti-diabetic activities of *Vernonia amygdalina* leaf extract. *Journal of Food Biochemistry*, *43*(2), e12737. https://doi.org/10.1111/jfbc.12737
51. Omojokun, O. S., Famurewa, A. J., Jaiyeoba, O. A., Oboh, G., & Agbebi, O. J. (2019). Alkaloid extracts from Bitter leaf (*Vernonia amygdalina*) and Black nightshade (Solanum *nigrum*) inhibit phosphodiesterase-5, arginase activities and oxidative stress in rat's penile tissue. *Journal of Food Biochemistry*, *43*(6), e12889. https://doi.org/10.1111/jfbc.12889