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Amelioration of Chronic Unpredictable Mild Stress-Induced Behavioural Perturbations by Noni Juice in Mice: Possible Involvement of Antioxidant System

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BBA, IAO, AOA, SU, EOI and OAA designed the study. Authors BBA, IAO, AOA and OE wrote the protocol, managed the experimental processes and the analyses of the study. Authors BBA, IAO, AEOE and AMA performed the statistical analysis. Authors BBA, SU, DE and JJO did the literature searches and wrote the first draft of the manuscript. Authors BBA, IAO, AEOE and EOI wrote the final version of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Noni juice is a natural herbal formulation containing *Morinda citrifolia* widely acclaimed as an immune system stimulant and mood enhancer. The aim of the study was to explore the antidepressant- and anxiolytic-like behavioural effects of Noni juice and to further evaluate the possible mechanisms of action in terms of biomarkers of oxidative stress on chronic unpredictable mild stress (CUMS) induced depressive model in mice.

Methods: CUMS was used to induce behavioural deficits (depressive- and anxiety-like behaviours) and oxidative imbalance in mice. Mice were pretreated with Noni juice (2.5, 5 and 10 mL/kg, p.o) and thereafter subjected to different stress paradigms daily for a period of 21 days. Thereafter, sucrose preference test, behavioural despair tests, open field exploratory behaviour, Y-maze test as well as elevated plus maze, and light and dark maze tests were used to assess antidepressant- and anxiolytic-like behaviours, while standard biochemical protocols were used to assay for the biochemical alterations [Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA)].

Results: The results showed that Noni juice demonstrated significant ($P < 0.05$) inhibition of CUMS induced depressive- and anxiety-like behaviours in mice; by increasing the preference for sucrose, locomotor activity in the open field exploratory test, memory performance, decrease immobility in the behavioural despair tests and anxiety-related depressive behaviours. Furthermore, Noni juice decreased MDA concentration (33.2, 58.7 and 70.2%) in a dose-related manner. Moreover, Noni - juice significantly ($P < 0.05$) prevented the decrease in SOD and CAT activities, and increased GSH concentration in brain tissues.

Conclusion: These data provides a rationale for evaluating Noni juice as a novel psychotropic agent against depressive- and anxiety-related behaviours and suggest that its mechanisms of action may be related to the modulation of endogenous antioxidant defense system.

Keywords: Depression; anxiety; antioxidant; Morinda citrifolia; noni juice; chronic unpredictable mild stress.

1. INTRODUCTION

Depression and other related mood disorders are among the world's greatest public health menace. Depression is a psychiatric syndrome characterized by disturbances in mood, sleep, appetite, energy, motivation, hedonic capacity and thinking that are associated with behavioural despair [1]. Depression is one of the most common psychiatric disorders with a life time prevalence of 10 - 20% in the general population, with a higher disease risk in females than males [2] thus, making it the leading cause of disease burden for women in low, middle and high-income countries [3]. Its negative social impact on normal daily activities and wellbeing is characterized by impaired capability and loss of productivity [1]. Depression represents a heterogenous group of brain disorders characterized by wide range of symptoms reflecting alterations in cognitive, psychomotor and emotional processes, as well as anxiety-related behaviours [4]. Affected individuals differ remarkably regarding the profiles of clinical features, severity and course of illness, as well as their response to drugs treatment and reintegration efforts [5].

Converging lines of evidences suggest that depression may be accompanied by anxiety-related behaviours and may result from closely related mechanisms as indicated by their common symptomology, that suggest that vulnerability may result from common genetic factors, and comparable efficacy of antidepressants in the treatment of both disorders [4,6,7]. These observations further validate the appropriate use of anxiety components in antidepressant models, particularly when it is emanating from the angle of chronic stress-induced depression. Accordingly, the application of stress to rodents produces behavioural despair, a characteristic of depressive-like phenotypes and anxiety-like behaviours that are manifestations of the reduced exploration, freezing, etc., particularly since both types of behaviours are corrected with antidepressants [7]. Furthermore, in the perspective of the present study, both preclinical and clinical studies have shown a positive correlation between increased oxidative stress and major neuropsychiatric disorders, including depression [8,9]. Moreover, chronic unpredictable mild stress (CUMS)-induced depressive-like behaviour has been linked to

increased oxidative stress in different parts of the brain associated with mood regulation [8]. Oxidative stress is the condition arising from an imbalance between toxic reactive oxygen species (oxidants) and antioxidants defense system. Increased reactive oxygen species levels generate deleterious effects including lipid peroxidation [9]. Also, CUMS has been reported to deplete antioxidant enzymes like GSH, and therefore further damage brain cells through increased lipid peroxidation [8]. Increased lipid peroxidation and decreased antioxidant defense system have been found in the brain of depressive patients, which further support the role of oxidative stress in the pathophysiology of the disease [9].

Currently, the treatment for depression and anxiety mainly consists of chemical drugs, including tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), norepinephrine reuptake dual inhibitors (NARIs), selective norepinephrine reuptake inhibitors (SNRIs) and monoamine oxidase inhibitors (MAOIs) as well as benzodiazepines, respectively [10]. However, these drugs are ineffective in about 30% of patients and perhaps, only provide symptomatic relief; but not the underlying pathophysiological abnormalities and are also limited by their undesirable side effects such as psychomotor impairment, dependence liability etc, observed in clinical practice [8,9]. Thus, current research effort focuses on the development of natural products with multipronged mechanisms of action that could target various aspects of the underlying pathologies of these diseases [8,11]. Accordingly, medicinal plants have been used to treat psychiatric and behavioural conditions such as anxiety, depression, seizures, dementia and insomnia due to their phytochemical diversities [12]. Given that in recent decades, the number of patients with depression has continued to increase with the inadequacies of currently available orthodox anti-depressants therefore, the discovery or development of novel antidepressants for clinical usage continues to be a priority [10].

Noni fruit juice is a natural herbal formulation widely acclaimed as an immune system stimulant [13]. It is an extract of *Morinda citrifolia* Linn., which is from the family Rubiaceae, a pantropical bush or small tree [13,14]. In traditional pharmacopoeia, the fruit is claimed to prevent and cure several diseases [13]. It is primarily used to stimulate the immune system and thus to fight bacterial, viral, parasitic and fungal infections [11]; it is also used to prevent formation and proliferation of malignant tumors [9]. Noni juice is also claimed to relieve inflammation and depression [15]. It has also been reported to have a broad range of health benefits against arthritis, diabetes, asthma, hypertension, and pain [16]. Most Noni is consumed as juice, well tolerated, even at high oral doses equivalent to 80 mL/kg body weight/day [12,13,15,16]. Consumption of Noni juice was also associated with improvements in mood scores of postmenopausal women [15,16]. Among rural populations of the South Pacific, Noni fruit is thought to be useful for the treatment of anxiety and depression [17].

The search on the phytochemical compositions of Noni fruit has considerably increased over the years. Noni juice is known to contain several phytochemical constituents such as xeronine, terpenoids, arcubin, asperuloside, scopoletin and its isoform, isoscopoletin, anthraquinones, coumarins, flavonoids, iridoids, lignans [15,17,18], 3,4,3',4'-tetrahydroxy-9,7'"-epoxylignano-7",9'-lactone, 3,3'-bisdemethyltanegool, (-) pinoresinol, (-)-3,3'-bisdemethylpinoresinol, kaempferol, and vanillin [19], with different biological activities beneficial to mood disorders. The roots is known to contain a wide spectrum of anthraquinones derivatives such as rubiadin, damnacanthal and alizarin-1-methyl ether, naphthoquinone and sterols [17], while other constituents including iridoids, flavonol, triterpenes and glycosides were reported from the leaves [15]. Other classes of phytochemicals have been found including polysaccharides, fatty acid glycosides, phytosterols, carotinoids, and a range of volatile constituents including monoterpenes and short chain fatty acids and fatty acid esters [20]. Noni fruit contains numerous iridoids. Major compounds include asperuloside, asperulosidic acid and deacetylasperulosidic acid, while minor iridoids include deacetylasperuloside, dehydromethoxygaertneroside, *epi*-dihydrocornin, 6α-hydroxyadoxoside, citrifolinin B epimers a and b, and 6*b,7β*-epoxy-8-*epi*-splendoside [15,18].

A number of other compounds classes have been reported. These include flavonol glycosides (e.g., narcissoside, nicotifloroside, and rutin) [17,20]. Importantly, rutin is a phytochemical agent already implicated in the antidepressant property of the ethanolic extract of *Schinus molle* [21]. Several known and new lignans such as

3,3′-bisdemethylpinoresinol, americanol A, americanoic acid A, americanin A, morindolin, isoprincepin and balanophonin have also been isolated [15,20]. Furthermore, the coumarin scopoletin has been identified from the fruit and this has been found to control serotonin level in the body [22], and also increase long term potentiation (LTP) by potentiating acetylcholine release from synaptosomes [23]. Similar to other plant parts, the fruits also contain a wide spectrum of 1-hydroxyanthraquinones, though in much lower concentrations; including new compounds such as 2-methoxy-1,3,6-trihydroxyanthraquinone and 5,15-dimethylmorindol all of which have been found to possess antioxidant, radical scavenging, neuroprotective and anti-inflammatory properties beneficial to mood disorders [15,18,19]. Moreover, a detailed phytochemical study of Noni fruit juice by Deng and West [19] demonstrated the antidepressant effects of Noni fruits and its bioactive principles in terms of the monoamine oxidase (MAO) A and B bioassays for the first time, and reported that Noni is a natural MAO-A and MAO-B inhibitor; involving a synergistic effect from multiple active components such as kaempferol, quercetin, scopoletin, isoscopoletin, vanillin etc. [19]. In this context, the present investigation was designed to examine the effect of Noni juice on depressive- and anxiety-like behavioural phenotypes, as well as biomarkers of oxidative stress (neuroprotection) on chronic unpredictable mild stress (CUMS) model of depression in mice.

2. MATERIALS AND METHODS

2.1 Animals

Swiss Albino male mice (20–25 g) were purchased from the central animal house, College of Medicine, University of Ibadan. Animals were kept in the laboratory animal centre of the College of Medicine, Delta State University, Abraka in an air temperature controlled environment (23 ± 2℃) with 12-hr light:dark cycle, relative humidity 60 ± 5%, with food and water *ad libitum*. The experiments were performed in accordance with the regulatory protocol of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Also, efforts were made to minimize the suffering of the animals during CUMS experimental protocols and behavioural tests.

2.2 Drugs Preparation and Treatments

Pure, undiluted Noni fruit extract (Noni® fruit juice Innomark, Inc, USA), Imipramine (Sigma-Aldrich, St. Louis, MO, USA). Noni juice was administered *per oral* (p.o.) in three different doses (2.5, 5 and 10 mL/kg). The doses of Noni juice for this study were adopted from a previous work by West et al. [16]. Imipramine powder (15 mg/kg, p.o.) [24] was dissolved in distilled water and administered orally during the CUMS procedure. Distilled water (10 mL/kg, p.o.) was used as the control.

2.3 Chronic Unpredictable Mild Stress (CUMS)

2.3.1 CUMS-induced behavioural deficits and oxidative alterations

CUMS is an experimental procedure in which the mice are chronically exposed to variable unpredictable mild stressors to induce behavioural deficit (depressive- and anxiety-like behaviours) [25,26]. Prior to the start of the CUMS procedure, all of the mice were given 1% sucrose water for 24 hr to avoid neophobia for sucrose consumption training. After completion of the training, the mice were randomly assigned into six groups (n=8): Group 1: vehicle group (distilled water, 10 mL/kg, p.o.); Group 2: vehicle plus CUMS; Group 3: Noni juice (2.5 mL/kg, p.o.) plus CUMS; Group 4: Noni juice (5 mL/kg, p.o.) plus CUMS; Group 5: Noni juice (10 mL/kg, p.o.) plus CUMS; and Group 6: Imipramine (15 mg/kg, p.o.) plus CUMS. Briefly, mice were exposed to the following stressors twice daily for 21 days: 24 hr food deprivation, 24 hr water deprivation, 7 hr cage tilt (45° inclined), 24 hr exposure to a foreign object (piece of plastic and wood spread across the cage), 1 min tail pinch with push pine, 0.45 x 12 mm (1 cm from the end of the tail), 7 hr soiled cage (200 mL water in 100 g sawdust bedding), Hypoxia [15 min inside an air-tight hypoxic transparent plastic-container (height 23 cm, diameter 10 cm), overnight illumination (12 hr), 10 min cold stress (4-8℃) and hot stress (38-39℃), hot plate foot shock (50 ± 0.5℃) and 2 min oscillation (tail suspension on the edge of a table, 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail). Noni juice and other drugs were given orally 1 hr before each stressor once every day for 21 days. Control (unstressed) animals were undisturbed except for necessary procedures such as routine cage cleaning. For

ethical purpose, the following depressive- and anxiety-like behavioural tests were performed 24 hrs after the last treatments and unpredictable stressor on days 21 (open field, elevated-plus maze and tail suspension tests) and 22 (Y-maze, light/dark compartments and forced swim tests) of the experimental protocols; 5 min each, with 1 hr between successive tests in order to minimize stress resulting from behavioural tests by different observers who were blinded to the experiment. Also, all behavioural tests were performed between 8:00 am – 12:00 pm on days 21 and 22 in order to avoid behavioural alterations probably resulting from change in circadian rhythm. Thereafter, animals were euthanized for biochemical assays.

2.3.2 Behavioural tests

2.3.2.1 Sucrose preference test

Sucrose preference test was used to assess anhedonia-related behaviour [27]. The consumption of 1% sucrose was measured four times during the CUMS procedure. Mice had access to both water and a 1% sucrose solution in their home cages for a 48 hrs period prior to the start of the experiment. Before each test, the animals were deprived of water for 12 hr (8:00 pm – 8:00 am). Fluid consumption was recorded by re-weighing pre-weighed bottles of test solution post 1 hr test window (8:00-9:00 am) as originally described by Willner et al. [25]. Bottles were counter balanced across left and right sides of the cages throughout the experiment. Tests for fluid consumption were carried out weekly between 8:00 – 9:00 am on the same day each week (Friday) throughout the experiment. Sucrose preference was defined as percentage of consumed sucrose solution of the total amount of liquid drunk during the 1 hr test using the formula:

Sucrose preference (%)

$$= \frac{\text{Sucrose consumption}}{\text{Sucrose consumption + water consumption}} \text{ x } 100$$

2.3.2.2 Open field test (OFT)

The number of line crossing(s) and duration of immobility in open field paradigm were used to assess the locomotor activity as previously described by Mao et al. [28]. Before each test, each animal was kept in the test room at least 1 hour before the OFT for habituation. The main apparatus consisted of squared arena (50 cm × 50 cm × 40 cm) high with grey surface covering every wall. The floor of the arena was divided equally into twenty-five squares (10 cm × 10 cm) marked by black lines. Mice were placed individually into the centre of the arena and allowed to explore freely. The number of lines crossed with all paws (crossing) and duration of immobility were observed and counted in 5 minutes. Thereafter, the apparatus was cleaned with 70% ethanol following each mice session to remove residual odour.

2.3.2.3 Test for anxiety using elevated plus-maze (EPM) apparatus

This test was employed to assess the effect of Noni juice on stress-induced anxiety-related behaviour associated with depression in mice [6,7]. The apparatus validated for mice by Lister [29] was employed for this assessment. The apparatus consisted of open and closed arms, elevated to a height of 25 cm above floor level. Each mouse was placed at the center of the maze with its head facing the open arm and allowed to explore the maze for 5 min. The parameters measured were the number of entries and the time spent in the open and closed arms, and the exploratory behaviour (total number of arm entries). An entry with all feet put into one arm was defined as an arm entry in this experiment. 70% ethanol was used to clean the maze after each animal session to prevent odour bias. The results were expressed as time spent in arms and percentage of number of entries in arms (mean ratio of entries in an arm to total entries in both open and closed arms). The index of open arm avoidance [IOAA] was determined i.e. IOAA = 100 - (% time spent in open arms + % entries into open arms)/2.

2.3.2.4 Test for anxiety using light / dark compartment test

Another anxiety based model related to depressive-like behaviour was also engaged to further determine the effect of Noni juice on stress-induced anxiety-like behaviour in mice, according to the method described by Nina et al. [29]. The test box consisted of a small compartment painted black and a large compartment painted white. Each animal was individually placed in the center of the light compartment (facing away from the door between the light and dark compartments) and was observed for 5 min for exploration. The parameter measured was the time spent in each of the light and dark compartments. 70% ethanol

was also used to clean the test apparatus after each animal session to remove residual odour.

2.3.2.5 Test for memory performance using Y-maze test

The test for spatial working memory was assessed using the Y-maze test to evaluate the effect of Noni juice on stress-induced memory impairment associated with depression. The apparatus consisted of three equally spaced arms (120°, 41 cm long x 15 cm high) and 5 cm wide. Each mouse was placed in one of the arm compartments, usually arm A for consistency and was allowed to explore all three arms (A, B, C) freely for 5 min. An arm entry was defined as the body of a mouse except for its tail completely entering into an arm compartment. The sequence of arm entries was manually recorded. An alternation was defined as an entry into all three arms on consecutive devices. 70% ethanol was also used to clean the maze at intervals. The percentage correct alternations, which indicate cognitive searching behaviour and memory performance was calculated by dividing the total number of alternations by the total number of arm entries minus 2, multiplied by 100 [30].

2.3.2.6 Tail suspension test

The total duration of immobility following tail suspension was measured according to the method described for evaluating potential antidepressants [31]. Mice were suspended on the edge of a table, 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded for duration of 4 min after discarding the first 2 min during 6 min period in different groups. Mice were considered to be immobile when they hang passively and completely motionless.

2.3.2.7 Forced swimming test (FST)

The forced swim test was carried out according to the method described by Borsini et al. [32] with minor modification [30]. Mice were placed into Plexiglas cylinder (25 cm height, diameter 10 cm containing water to a height of 10 cm maintained at a temperature of 25℃) and forced to swim for 6 min. After an initial period of vigorous activity for two min, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling and making only minimum movements of its limbs necessary to keep its head above the water. The total duration of immobility was recorded during the next 4 min of the total test duration of 6 min.

2.3.3 Biochemical assays

Immediately after the behavioural tests, the animals were decapitated under ether anaesthesia and the brains were immediately removed and kept in the refrigerator with ice block for 30 min. Thereafter, the whole brain was weighed and homogenized with 5 ml of 10% w/v phosphate buffer (0.1M, pH 7.4). Each brain tissue homogenates was centrifuged at 10,000×g for 10 min at 4℃; the pellet was discarded and the supernatant was immediately separated into various portions for the different biochemical assays. Protein was measured with Biuret reagent assay using bovine serum albumin as standard.

2.3.3.1 Superoxide dismutase (SOD) assay

The level of SOD activity was measured by the method described by Misra and Fridovich [33]. This method is based on the inhibition of superoxide dependent adrenaline auto-oxidation in a spectrophotometer adjusted at 480 nm. Brain supernatant of 1 mL was diluted in 9 ml of distilled water to make a 1 in 10 dilution. An aliquot of 0.2 ml of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction was started by the addition of 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette (Blank) contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of distilled water. The increase in absorbance at 480 nanometer (nm) was monitored between 30 to 150 s. 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline [33].

2.3.3.2 Catalase (CAT) assay

CAT activity was assayed by the method of Sinha [34], which was based on the disappearance of Hydrogen peroxide (H_2O_2) in the presence of an enzyme source (catalase). Brain supernatant of the sample homogenate (1 ml) was mixed with 19 ml of distilled water to give a 1:20 dilution. Then, 1 mL of this was added to 5 ml of phosphate buffer (pH 7.0) and 4 ml of H_2O_2 solution (800 µmoles). The reaction mixture was mixed by a gentle swirling motion at room

temperature. Then, 1 ml of this portion of the reaction mixture was withdrawn and added into 2 ml dichromate/acetic acid reagent. The absorbance was measured using spectrophotometer at 570 nm and change in absorbance at 60 s interval was recorded. The catalase activity was expressed as µmoles of H_2O_2 decomposed per minutes per mg protein.

2.3.3.3 Glutathione (GSH) assay

This assay was performed as described by Aksenov and Markesbery [35] which was based upon the development of a relatively stable (yellow) colour when 5', 5'–dithiobis-(2-nitrobenzoic acid) (DTNB) is added to sulfhydryl compounds. Brain homogenates of 0.4 ml was added to 0.4 ml of 20% trichloroacetic acid (TCA) and mixed by a gentle swirling motion and then centrifuged in a cold (4°C) centrifuge at 10,000 rpm for 20 min. Then, 0.25 ml of the supernatant was added to 2 ml of 0.6 mM DTNB and the final volume of the solution was made up to 3 mL with phosphate buffer (0.2M, pH 8.0). Absorbance was read at 412 nm against blank reagent [2 ml of 0.6 mM DTNB + 1 ml phosphate buffer (0.2M, pH 8.0)] using a spectrophotometer. The concentration of reduced GSH in the brain tissues were expressed as nmole per gram tissue (nmol/g tissue).

2.3.3.4 Lipid peroxidation (malondialdehyde, MDA) assay

Lipid peroxide formation was analyzed by measuring the thiobarbituric acid reacting substances (TBARSs) according to the method described by Ohkawa et al. [36]. Briefly, an aliquot of 0.4 ml of the sample was mixed with 1.6 ml of Tris-potassium chloride (Tris-KCl) buffer to which 0.5 ml of 30% trichloroacetic acid (TCA) was added. Then, 0.5 ml of 0.75% TBA was added and placed in a water bath for 45 min at 80°C. This was then cooled in ice and centrifuged at 3000 rpm for 15 min. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm. The MDA concentration was calculated using a Molar extinction coefficient of 1.56×10^5 M^{-1} CM^{-1} and the value was expressed as nmole of MDA per gram tissue.

2.4 Statistical Analysis

Data were expressed as Mean ± S.E.M. (standard error of mean). The data was analyzed using one–way analysis of variance (ANOVA) followed by post–hoc test (Newman-Keul) for multiple comparisons where appropriate using Graph Pad Prism software version 5. A level of $P < 0.05$ was considered as statistically significant for all tests.

3. RESULTS

3.1 Effect of Noni Juice on CUMS-Induced Decrease Preference for Sucrose Consumption

Table 1 shows the % sucrose preference in the six groups of mice during the experimental period from week 0-3. After 3 weeks of CUMS, percentage of sucrose consumed by stressed animals were significantly (*P < 0.05*) lower than that of non-stressed animals. However, treatment with imipramine was found to significantly (*P < 0.05*) prevent the decrease in the % sucrose preference, as indexed by the increase in % preference for sucrose compared to the stressed group (CUMS group only) treated with vehicle. In the same pattern, administration of Noni juice (2.5, 5 and 10 mL/kg, p.o.) also demonstrated significant (*P < 0.05*) percentage increase for sucrose consumption (33.99, 39.36 and 44.62%) on the third week compared to the stressed mice without treatment.

3.2 Effect of Noni Juice on CUMS-Induced Hypolocomotion

CUMS significantly (*P < 0.05*) induced decrease in locomotion as indexed by the decrease in the number of line crossing and increase in the duration of immobility compared to the group treated with vehicle (10 mL/kg, p.o.) in mice. Administration of Noni (2.5, 5 and 10 mL/kg, p.o.) significantly (*P < 0.05*) prevented CUMS-induced decrease in spontaneous motor activity as indicated by the increase in the number of line crossing and decrease in the duration of immobility similar to imipramine (15 mg/kg, p.o.), as a positive control compared to CUMS group (Table 2).

3.3 Effect of Noni Juice on CUMS-induced Anxiety-based Depression in Mice on the EPM and Light and Dark Compartment Tests

In the EPM paradigm, CUMS significantly (*P < 0.05*) increased anxiety-like behaviours, as indicated by increase in the index of open arm avoidance (IOAA) compared to vehicle treated

group. However, the administration of Noni juice (2.5, 5 and 10 mL/kg, p.o.) significantly ($P < 0.05$) prevented CUMS-induced anxiety by decreasing index of open arm avoidance (IOAA) compared with the CUMS group. Similarly, effects was also observed in the group treated with imipramine, as it significantly ($P < 0.05$) prevented the increase in the % of close arm entry and IOAA by CUMS thus, causing an increase in the % of open arm entry and decrease in IOAA (Table 3).

Furthermore, the light and dark compartment test for anxiety also demonstrated that CUMS significantly ($P < 0.05$) increased anxiety-like behaviour, as indicated by the decrease in the time spent in the light compartment and increase in the time spent in the dark compartment compared to the group treated with vehicle, which is regarded as an index of anxiety-based behaviour related to behavioural despair. In contrast, administration of Noni juice (2.5, 5 and 10 mL/kg, p.o.) significantly ($P < 0.05$) prevented the anxiety-related behaviour in the animals, as indicated by the increase in the time spent in the light compartment and decrease in the time spent in the dark compartment similar to imipramine compared to CUMS group (Table 4).

3.4 Effect of Noni Juice on CUMS-induced Cognitive Impairment

The effect of Noni juice on CUMS-induced deficit on spatial working memory was also assessed based on the sequence and number of arm entries in the Y-maze test to ascertain for effect of Noni juice on cognitive performance as shown in Fig. 1. CUMS for 21 days significantly ($P < 0.05$) induced deficit on spatial working memory compared to the vehicle treated group (10 mL/kg, p.o.). In contrast, administration of Noni juice (5 and 10 mL/kg, p.o.) and imipramine significantly ($P < 0.05$) prevented the cognitive deficit induced by CUMS. However, treatment with lower dose of Noni juice (2.5 mL/kg) failed to prevent the deficit in cognitive performance compared to CUMS group.

Table 1. Effect of Noni juice on CUMS-induced decrease preference for sucrose consumption

Treatments	Dose	Week 0	Week 1	Week 2	Week 3
Vehicle	10 mL/kg, p.o.	77.40 ±2.40	75.60 ± 2.02	73.00 ± 3.78	56.75 ± 2.36
CUMS		76.00 ± 3.49	44.40 ± 1.54**	33.20 ± 1.59**	33.50 ± 2.02**
CUMS + NONI	2.5 mL/kg, p.o.	74.20 ± 2.48	59.20 ± 2.92*	48.80 ± 2.45*	50.75 ± 1.44*
CUMS + NONI	5 mL/kg, p.o.	77.00 ± 3.67	59.40 ± 1.91*	53.40 ± 2.34*	55.25 ± 1.84*
CUMS + NONI	10 mL/kg, p.o.	72.00 ± 3.11	67.60 ± 2.69*	59.40 ± 3.17*	60.50 ± 2.32*
CUMS + IMIP	15 mg/kg, p.o.	76.00 ± 2.45	67.80 ± 1.77*	62.40 ± 3.68*	61.50 ± 1.84*

Value represents the mean ± S.E.M (n=5). One way ANOVA revealed that there is no significant [F (5, 24) = 0.4564, P > 0.05] differences between various treatment groups at week 0, whereas there was significant [F (5, 24) = 23.72, P < 0.0001]; [F (5, 24) = 21.53, P < 0.0001] and F (5, 24) = 24.13, P < 0.0001] differences between various treatment groups at week 1; week 2 and week 3 respectively for % sucrose preference.
***Denotes P < 0.05 as compared to vehicle group. *Denotes P < 0.05 as compared to CUMS group.*
VEH = Vehicle, CUMS = Chronic unpredictable mild stress,
NONI = Noni juice, IMIP = Imipramine

Table 2. Effect of Noni juice on CUMS-induced hypolocomotion

Treatments	Dose	Number of line crossings	Duration of immobility (s)
Vehicle	10 mL/kg, p.o.	73.00 ± 2.702	163.80 ± 7.38
CUMS		31.00 ± 4.393**	235.80 ± 4.01**
CUMS + NONI	2.5 mL/kg, p.o.	68.40 ± 4.854*	198.40 ±4.96*
CUMS + NONI	5 mL/kg, p.o.	97.00 ± 4.207*	155.40 ± 14.13*
CUMS + NONI	10 mL/kg, p.o.	92.60 ± 4.082*	162.20 ± 7.13*
CUMS + IMIP	15 mg/kg, p.o.	96.00 ± 3.886*	149.30 ± 4.62*

*Value represents the mean ± S.E.M (n=5). One way ANOVA revealed that there was a significant [F (6, 28) = 38.64, P < 0.0001(line crossing) and F (6, 28) = 42.92, P < 0.0001 (immobility time)] differences between various treatment groups for locomotion, respectively. **Denotes P < 0.05 as compared to vehicle group.*
**Denotes P < 0.05 as compared to CUMS group.*
VEH = Vehicle, CUMS = Chronic unpredictable mild stress,
NONI = Noni juice, IMIP = Imipramine

Table 3. Effect of Noni juice on CUMS-induced anxiety-based depression in mice on the EPM

Group	Dose	Open arm entry	Open arm duration (s)	% Open arm entry	% Open arm duration	Index of open arm avoidance
VEH	10 mL/kg	1.60 ± 0.24	24.20 ± 2.57	35.00 ± 6.12	13.97 ± 2.62	75.20 ± 1.71
CUMS		0.00 ± 0.00**	0.00 ± 0.00**	0.00±0.00**	0.00 ± 0.00**	100.0 ±0,00**
NONI	2.5 mL/kg	2.60 ± 0.60*	19.40 ± 8.50*	34.93 ± 2.28*	6.89 ± 2.94*	79.08 ± 1.82*
	5	3.60 ± 0.40*	29.20 ± 4.85*	32.44 ± 2.27*	11.05 ± 1.83*	78.25 ± 1.82*
	10	4.60 ± 0.54*	41.20 ± 5.22*	47.52 ±7.13*	16.37 ± 2.05*	68.05 ± 4.14*
IMIP	15 mg/kg	5.00 ± 1.00*	29.20 ± 4.85*	48.89 ± 1.81*	7.182 ± 0.32*	71.96 ± 0.90*

*Results are expressed as Mean ± S.E.M (n=5). One way ANOVA revealed that there was a significant [F (5, 24) = 12.40, P < 0.0001 (open arm entry); F (5, 24) = 7.412, P < 0.0001(open arm duration); F (5, 24) = 18.34, P < 0.0001 (% open arm entry); F (5, 24) = 8.840 (% open arm duration); F (5, 24) = 27.19 P < 0.0001 (IOAA)] difference between the CUMS and treatment groups. The standard drug also revealed significance in all paradigms compared with the CUMS group in the closed arm entry. **Denotes P< 0.05 as compared to vehicle group. *Denotes P < 0.05 as compared to CUMS group.*
VEH = Vehicle, CUMS = Chronic unpredictable mild stress,
NONI = Noni juice, IMIP = Imipramine

Table 4. Effect of Noni juice on CUMS-induced anxiety-based depression in mice on light and dark compartment test

Treatments	Dose	Time spent in the light compartment (Sec)	Time spent in the dark compartment (Sec)
Vehicle	10 mL/kg, p.o.	204.80 ± 3.56	125.0 ± 4.30
CUMS		85.60 ± 5.56**	201.4 ± 5.73**
CUMS + NONI	2.5 mL/kg, p.o.	134.40 ± 6.35*	152.6 ± 5.60*
CUMS + NONI	5 mL/kg, p.o.	118.80 ± 7.30*	162.8 ± 5.07*
CUMS + NONI	10 mL/kg, p.o.	155.00 ± 5.83*	115.4 ± 7.93*
CUMS + IMIP	15 mg/kg, p.o.	131.40 ± 10.03*	156.4 ± 6.28*

*The results are expressed as Mean ± S.E.M (n=5). One way ANOVA revealed that there was a significant [F (6, 28) = 34.94, P < 0.0001 (Time spent in the light compartment) and F (6, 28) = 26.42, P < 0.0001 (Time spent in the dark compartment)] difference between CUMS and treatment groups in the time spent in light compartment. **Denotes P < 0.05 as compared to vehicle group. *Denotes P < 0.05 as compared to CUMS group.*
VEH = Vehicle, CUMS = Chronic unpredictable mild stress,
NONI = Noni juice, IMIP = Imipramine

3.5 Effect of Noni Juice on CUMS-induced Behavioural Despair on TST and FST

CUMS significantly (*P < 0.05*) enhanced immobility time in TST (Fig. 2) and FST (Fig. 3) in the animals compared to vehicle treated group. Administration of Noni juice (2.5, 5 and 10 mL/kg, p.o.) and imipramine (15 mg/kg, p.o.) significantly (*P < 0.05*) limited these CUMS-enhanced immobility time in TST and FST in a dose-dependent pattern compared to CUMS group.

3.6 Neuroprotective Effect of Noni Juice on Oxidative Stress Biomarkers in Mice Brains

The effect of Noni juice on antioxidant alterations by CUMS was also evaluated in the whole brain of the animals following a 3 weeks CUMS treatment protocols. Our data showed that CUMS administration significantly decreased SOD and CAT activities in the whole brain of the mice [F (5, 24) = 43.00, P< 0.0001] (SOD) and [F (5, 24) = 41.49, P < 0.0001] (CAT) compared to vehicle treated group without stress (Table 5). However, pretreatment with Noni juice (2.5, 5 and 10 mL/kg, p.o) and imipramine significantly (P < 0.05) increased SOD and CAT activities compared to the CUMS group. Furthermore, the administration of CUMS also significantly decreased the GSH concentration [F (5, 24) = 26.77, P < 0.0001] (GSH) (Table 5) compared to vehicle treated group. Pretreatment with Noni juice (2.5, 5 and 10 mL/kg, p.o) and imipramine also significantly prevented (*P < 0.05*) the decrease in GSH concentration induced by CUMS administration. The level of lipid peroxidation evaluated was increased by the 3 weeks of CUMS administration in the whole brain [F (5, 24) = 34.79, P < 0.0001] (Table 5), as the consequences of decrease in the endogenous

antioxidants (SOD, CAT, GSH) compared to the non-stress vehicle treated group. However, Noni juice (2.5, 5 and 10 mL/kg, p.o) treatments significantly ($P < 0.05$) prevented the increase in the lipid peroxidation produced by CUMS administration, by decreasing MDA-TBARS content in the whole brain similar to imipramine compared to the stressed animals in CUMS group.

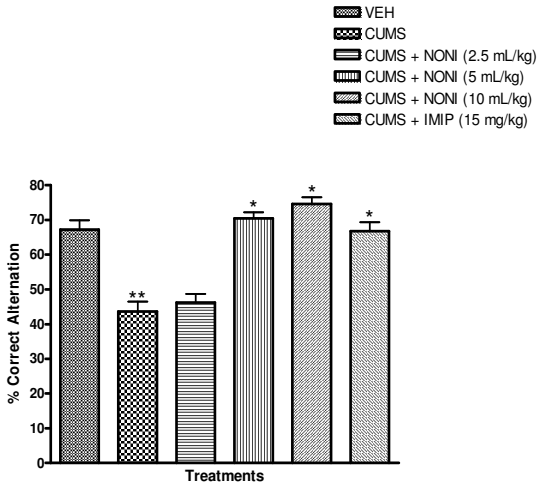

Fig. 1. Effect of Noni juice on CUMS-induced cognitive impairment

*Value represents the mean ± S.E.M (n=5). One way ANOVA revealed that there was significant [F (6, 28) = 30.29, P < 0.0001] differences between various treatment groups for percentage correct alternation. **Denotes P < 0.05 as compared to vehicle group. *Denotes P < 0.05 as compared to CUMS group. VEH = Vehicle, CUMS = Chronic unpredictable mild stress, NONI = Noni juice, IMIP = Imipramine*

4. DISCUSSION

In addition to the forced swim test (FST) and tail suspension test (TST), the CUMS model was also playing an important role in the scientific screening and evaluation of antidepressants in animal models of depression [37]. Since it is believed that long-term exposure to multiple, inescapable stressors can promote clinical depression as well as anxiety-related behaviours in humans, chronic unpredictable mild stress is considered a realistic animal model of behavioural deficits related to depressive- and anxiety-like symptoms [27]. CUMS paradigms are designed to model chronic depressive- and anxiety-related states that develops gradually over time in response to stress (a state of threatened homeostasis provoked by psychological, physiological and environmental stressors). Stressors are internal or external stimuli capable of activating the Hypothalamic-Pituitary-Adrenal (HPA) axis and sympathetic system resulting in physiological changes. CUMS depressive model is a well validated model with face and predictive validity [37]. Herein, we demonstrated that Noni juice prevented the depressive- and anxiety-related behaviours as well as oxidative damage induced by repeated chronic unpredictable mild stress in mice. Therefore, our data suggests that Noni juice may have novel therapeutic effects for the acute prevention and maintenance treatment phases of depression and related mood disorders.

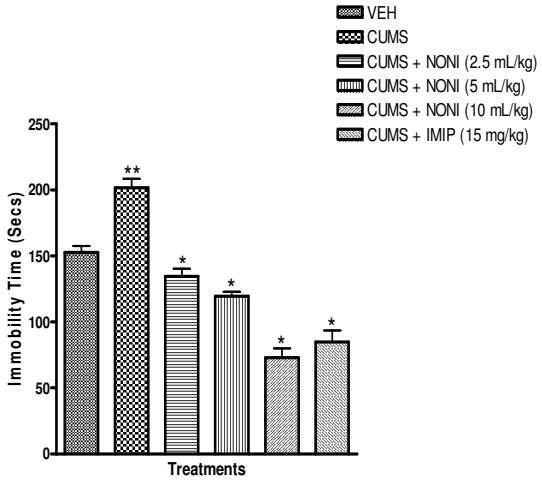

Fig. 2. Effect Noni juice on CUMS-enhanced immobility in TST

*Value represents the mean ± S.E.M (n=5). One way ANOVA revealed that there was significant [F (6, 28) = 54.47, P < 0.0001] differences between various treatment groups for immobility time, respectively. **Denotes P < 0.05 as compared to vehicle group. *Denotes P < 0.05 as compared with CUMS group. VEH = Vehicle, CUMS = Chronic unpredictable mild stress, NONI = Noni juice, IMIP = Imipramine*

In the present study, all CUMS-induced anhedonic-like behaviours (decreased preference for sucrose solution, locomotor activity, altered preference to the light compartment, increased index of open arm avoidance (IOAA), and increased behavioural despair) were prevented by Noni juice (2.5, 5 and 10 mL/kg). The reduced preference for sucrose we observed was not attributable to decreased thirst in general, because total fluid intake was not decreased in the stressed mice. It was also neither attributed to the slower weight gain in the stressed animals, as demonstrated in previous research [28]. A change in % sucrose preference

Table 5. Effect of Noni juice on CUMS-induced oxidative stress biomarkers in mice brains

Treatments	Dose	SOD (Unit/mg protein)	CAT (Unit/mg protein)	GSH (nmole/mg protein)	MDA TBARS (nmole MDA/mg protein)
Vehicle	10 mL/kg, p.o.	7.00 ± 0.22	4.38 ± 0.24	145.20 ± 4.64	15.58 ± 1.70
CUMS		2.58 ± 0.24**	1.28 ± 0.08**	83.02 ± 5.89**	30.20 ± 1.50**
CUMS + NONI	2.5 mL/kg, p.o.	4.60 ± 0.25*	2.54 ± 0.11*	127.80 ± 6.02*	20.16 ± 1.51*
CUMS + NONI	5 mL/kg, p.o.	5.84 ± 0.24*	3.50 ± 0.23*	158.40 ± 6.35*	12.48 ± 0.88*
CUMS + NONI	10 mL/kg, p.o.	5.60 ± 0.25*	3.48 ± 0.14*	166.80 ± 7.43*	9.00 ± 0.78*
CUMS + IMIP	15 mg/kg, p.o.	6.32 ± 0.23*	3.74 ± 0.17*	176.20 ± 8.33*	10.56 ± 1.38*

*The results are expressed as Mean ± S.E.M (n=5). **Denotes P < 0.05 as compared to vehicle group.*
**Denotes P < 0.05 as compared to CUMS group.*
VEH = Vehicle, CUMS = Chronic unpredictable mild stress,
NONI = Noni juice, IMIP = Imipramine

is clearly a valuable measure for lack of pleasure. Willner et al. [26] reported a statistically significant decrement in preference for a 1% sucrose solution, from a baseline value of around 80% to a value of 65% in stressed animals. In line with this context, the % sucrose preference in our study was about 75%, whereas there was a significant reduction in % sucrose preference to around 30% following the three weeks CUMS exposure.

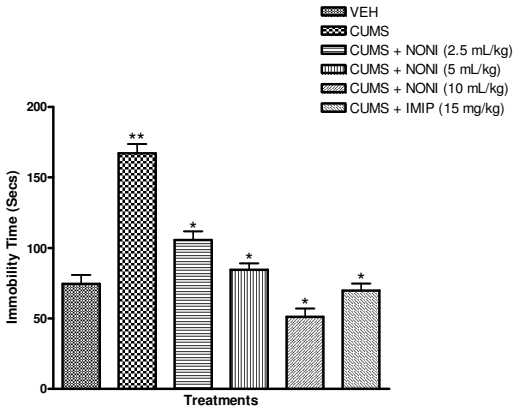

Fig. 3. Effect of Noni juice on CUMS-enhanced immobility in FST

Value represents the mean ± S.E.M (n=5). One way ANOVA revealed that there was significant [F (6, 28) = 49.67, P < 0.0001] differences between various treatment groups for immobility time, respectively.
***Denotes P < 0.05 as compared to vehicle group.*
**Denotes P < 0.05 as compared to CUMS group.*
VEH = Vehicle, CUMS = Chronic unpredictable mild stress, NONI = Noni juice, IMIP = Imipramine

In this study, the open field tests (OFT) for example, lethargy, apathy, and bodily neglect may be inferred from reduced exploration, rearing and grooming behaviours in the open field, respectively. So, anhedonia resulting from CUMS was also confirmed by the decrease in locomotor behaviour, decrease in the time spent in the light compartment, increase IOAA herein. Exploratory behaviours such as locomotion, rearing etc, is indicative of central excitatory behaviour [38]. Neurotransmitter interactions between dopaminergic, adrenergic, glutaminergic, serotonergic and cholinergic systems are reportedly involved in locomotion in mice [39]. Therefore, administration of Noni juice was found to prevent the decrease in exploratory behaviours in animals subjected to CUMS treated with vehicle, as indexed by increase in the number of line crossings and decrease in the duration of immobility compared to the CUMS group treated with vehicle, which is thus, suggestive of increased exploratory behaviours and anti-depressive-like effect.

The treatment with Noni juice was also found to prevent behavioural despair, as indexed by the decreased immobility time in the FST and TST respectively. Accordingly, it was shown from this study that CUMS induced prolonged immobility time in the depressed animals that underwent CUMS with vehicle treatment, which connotes behavioural despair, a symptom of depressive-like behaviour particularly attributed to low levels of serotonin and adrenaline [21,26]. Our finding thus, corroborates with previous studies on the use of CUMS as valid animal model predictive of human depressive behaviours [32,37]. Therefore, the decreased immobility time elicited by Noni juice treated animals that also underwent the stress protocol in this study, is indicative of an antidepressant-like activity, possibly due to the presence of phytochemical constituents like scopoletin, rutin, xeronine, flavonoids, quercetin, kaempferol etc capable of alleviating mood disorders [21,40]; thus, in part, contributing to the beneficial behavioural effects observed herein.

Interestingly, Machado et al. [21] demonstrated the antidepressant-like effect of the ethanolic extract of *Schinus molle,* due to the presence of rutin, which is a positive modulator of the serotonergic and adrenergic neurotransmissions. Moreover, the standard antidepressant agent, imipramine administered in this study, also prevented the behavioural despair induced by CUMS, by increasing locomotor activity in the OFT and decreasing immobility time in the FST and TST respectively.

Among the paradigms used in this study to evaluate anxiety-related behaviours include elevated plus maze (EPM) and light and dark compartment maze (LDM) paradigms [30]. The study demonstrated that the administration of Noni juice also significantly limited these anxiety-related behaviour evaluated both in the EPM, and light and dark compartments paradigms, respectively. EPM and LDM assessment revealed that CUMS significantly induced anxiety state characterized of decreased exploration similar to depressive-like behaviours in the experimental animals, as indexed by the increased in the numbers of entries and time spent in the closed arms, and increase in IOAA in the EPM, as well as increase in the duration of time spent in the dark compartment of the LDM compared to normal animals treated with vehicle [30]. This finding is in agreement with previous reports [8,25] which states that CUMS-induced depressive-like behaviours is accompanied by anxiety-related phenotype in mice. Accordingly, treatment with Noni juice significantly prevented the isolation of the animals from the closed arm of the EPM by decreasing the percentage of IOAA of the EPM test and increasing the time spent in the light part of the LDM than in the dark part. These indices suggest that Noni fruit juice possess the active principles to prevent anxiety-like behaviours associated with depression in animals.

Furthermore, previous studies have shown an association between depression and dementia (global cognitive impairment); and it has been suggested that lifetime depression is associated with a 2- to 4-fold increased risk of developing cognitive dysfunction related to Alzheimer's disease (AD) [41]. Accumulating evidence suggests that hyperphosphorylated tau sequestrates normal microtubule associated proteins (MAPs) and disrupts microtubule dynamics, a key hypothesis of AD [42,43]. Moreover, acute stresses, such as ether exposure, starvation, forced swimming in cold water, or restraint stress, induces tau phosphorylation in mice [42]. Also, accumulating evidences suggest that the action of CUMS on memory performance may be related to the upregulation of neuroregulatory tryptophan catabolite (TRYCAT) including kynurenic acid (KYNA), an endogenous N-methyl-D-arspatate receptor (NMDAR) antagonist, via diversion of tryptophan metabolism away from serotonin [44]. Notably, elevated levels of KYNA and its precursor, Kynurenine (KYN) have been shown in the brain of depressive and anxiety patients [45], and this increase have been attributed to the decline in cognitive function as a result of blockade of NMDAR and negative modulation of alpha-7 nicotinic acetylcholine receptor (α7nAchR) [44,45]. In line with this observation, treatment with Noni juice in CUMS-induced depression associated with memory impairment was found to prevent the cognitive deficit, as indicated by the increased correct alternations evaluated from the spatial-working memory apparatus (Y-maze). The result of this study is in accordance with the previously described memory enhancing effect of coumarin scopoletin [23], a key phytochemical ingredient of Noni fruit juice [15,18], which states that coumarin scopoletin potentiates acetylcholine release from synaptosomes, amplifies hippocampal long-term potentiation and ameliorates anticholinergic- and age-related memory impairments [44]. Also, there are increasing bodies of evidences that support the role of antioxidant molecules in the prevention of depressive-related diseases possibly associated with memory impairments [8,9]. Indeed, the presence of the rich antioxidant phytochemicals such as quercetin, kaempferol, rutin, arcubin, terpenoids, iridoids, carotinoids etc [15,18], in Noni fruit juice might at least in part, responsible for the beneficial memory enhancing effect demonstrated herein by Noni juice. Taken together, this result may have therapeutic implications for the treatment of cognitive deficits implicated in mood disorder-related diseases characterized by depression and anxiety.

In the perspective of the present study, it might be speculated that the mechanism of action involved in the anti-depressive- and anxiolytic-like effects of Noni fruit juice may also be mediated by the presence of these phytochemical constituents. Accordingly, quercetin, kaempferol, and 3,4,3',4'-tetrahydroxy-9,7'"-epoxylignano-7",9'-lactone (active principles) from Noni fruit juice have been found from the study of Deng and West [19], to be natural inhibitors of MOA-A and MOA-B,

involving a synergistic multipronged mechanisms of action from these active principles. Furthermore, scopoletin, another active principle isolated from Noni juice, have been found to control serotonin level in the body, an active ingredient that might be speculated to be partly responsible for the mood alleviating effect of Noni juice [22]. Also, Chan-Blanko et al. [46] reported the presence of a novel component, proxeronine in Noni fruit: an alkaloid and a precursor of xeronine that is suggested to improve the functionality of human protein, synaptic plasticity and neurotransmissions as ''messenger molecule''. In addition, the presence of rutin as a phytochemical ingredient of Noni fruit juice also strengthens the beneficial effect of Noni fruit juice as an antidepressant-like agent, as demonstrated in this study. Indeed, current therapeutic approach for the symptomatic treatment of depression and related mood disorders involves the use of agents capable of inhibiting synaptic neurotransmitter reuptake, particularly serotonin and noradrenaline; like imipramine, flouxetine and venlafaxine, which act by increasing the availability of synaptic serotonine and nonadrenaline in the brain [10]. However, these drugs had failed to correct the course of the disease and their clinical efficacy has also been limited by virtue of their side effects thus, instigating the search for more effective agents with little or no adverse effect for the treatment of patients with depression and related disorders [11,16]. In line with these observations, it is important to note that the LD_{50} of Noni fruit and its crude extracts are all greater than the minimum criteria for nontoxic status. In this context, Noni juice was found to be relatively safe at an equivalent dose of 80 mL/kg body weight per day [15]. Also, Noni fruit juice was considered nontoxic following an acute oral LD_{50} greater than 5000 mg/kg, or an acute intraperitoneal LD_{50} greater than 2000 mg/kg [15, 16]. Furthermore, another study elsewhere, has also confirmed through oral toxicity and allergenicity studies that Noni fruit juice was not toxic to human and as a result, has been approved by the European Commission as a safe food ingredient [47]. Moreover, Noni juice is generally regarded as a natural and safe source of juice for the Pacific Islanders and Southeast Asia [12].

Oxidative stress is an important factor involved in the pathophysiology of major neuropsychiatric disorders, including depression [8,9,48]. Increased reactive oxygen species levels generates deleterious effects on signal transduction, structural plasticity and cellular resilience, mostly by inducing lipid peroxidation in membranes, damage to proteins and nucleic acids [49]. Herein, the study also demonstrated that the beneficial effects of Noni juice, following the CUMS exposure-induced behavioural alterations were also accompanied by alterations in oxidative defense system. Our findings confirmed that the behavioural deficits evoked by CUMS was associated with increased oxidative stress, as shown by elevated brain levels of MDA and decreased antioxidant defense systems. Thus, the ability of Noni fruit juice to prevent CUMS-induced behavioural alterations in mice suggests an action involving prevention of oxidative stress. Hence, the administration of Noni juice via its active principles provided compensatory neuroprotective mechanisms of action on these antioxidants; thereby, prevented decrease in SOD, CAT and GSH levels, and also inhibited lipid peroxidation, as indexed by the decrease MDA content in the mice whole brain tissue comparable to imipramine.

Generally SOD, CAT and GSH constitute a mutually supportive enzyme system of the first line cellular defense against oxidative insult(s), decomposing O_2 and H_2O_2 prior to their interaction to form more harmful hydroxyl radicals [48]. Consequently, following CUMS administration, SOD and CAT activities, and GSH concentration were also decreased significantly in the experimental group that underwent the CUMS compared to non-CUMS vehicle treated group. This may in part, be largely due to excessive formation of superoxide anions in neuronal cells, including those from the mitochondrial leading to induction of lipid peroxidation that may trigger synaptic disconnections. Administration of Noni juice effectively prevented the decrease in SOD and CAT activities, and GSH concentration in treated mice brains, which can thus be correlated to the scavenging of oxidative free radicals of Noni juice [50]. Also, this finding supports that Noni juice treatments significantly prevented CUMS-induced lipid peroxidation through the increase in endogenous antioxidants, which might be a key factor responsible for the improvement in behavioural perturbations observed herein. Indeed, lipid peroxide products reportedly caused widespread neuronal injury [8]. Accordingly, increased MDA formation, a biomarker of lipid peroxidation, was observed in the CUMS group. Meanwhile, it appears apparently that the neuronal damage seems to be the prime culprit for the marked increase in the neuronal MDA

content and as such, may influence the severity of depressive-, anxiety-related symptoms as well as cognitive deficit [51]. However, Noni juice treatments prevented this lipid peroxidation process, by decreasing MDA formation in the brain of the animals.

Mechanistically, interactions between inflammations, oxidative stress and lowered antioxidant levels may occur via changes in the tryptophan catabolite (TRYCAT) pathway by diverting tryptophan metabolism away from serotonin, N-acetylserotonin and melatonin production. Moreover, increased activation of indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) have long been identified as being associated with depression and related mood disorders as well cognitive impairments [44,45]. Accordingly, the neuroprotective antioxidant beneficial effect of Noni juice observed in this study might be partly attributed to the redirection of tryptophan metabolism to serotonin, N-acetylserotonin, melatonin production, and decreased activation of TRYCATs (IDO, TDO) that leads to increase tryptophan concentration (possibly leading to increase serotonin synaptic concentration and post-synaptic connectivity), decrease oxidative-glutamate toxicity possibly through the modulation of GSH-glutamate pathway [51,52] largely via its antioxidant [50] and anti-inflammation properties [53]. This compensatory mechanism further reinforces the beneficial antidepressant property of Noni juice in previous studies by [19].

The neuroprotective property of Noni fruit juice might be attributed to the presence phytochemical antioxidant and non-antioxidant constituents including quercetin, kaempferol, scopoletin, vanillin, xeronine, coumarins, flavonoids [19,20,40]. Although more preclinical studies are needed on the antidepressant- and anxiolytic-like activities of Noni fruit juice in mice, the results of the present investigation provides more rationale for the traditional uses of Noni fruit juice as a natural remedy for mood disorders in humans [54], and also behavioural scientific evidence to the antidepressant-like activity of Noni in terms of MAO A and B inhibition by Deng and West [19]. This still needs to be confirmed further by human clinical studies.

5. CONCLUSION

This study provides valuable evidences that Noni (*M. citrifolia*) juice contains biologically active constituents that possess antidepressant-, anxiolytic-like and antioxidant properties, and may be useful to humans. The mechanisms of action of Noni fruit juice that prevented the development of depressive- and anxiety-related behaviours through CUMS may be related to increase in antioxidant defense systems (GSH, SOD and CAT) and inhibition of lipid peroxidation related activities. To this end, this study provides valuable justification to the ethnomedicinal claims of Noni fruit juice in the management of depression and related mood disorders. Further investigations are required to ascertain the exact constituent(s) exhibiting these pharmacologic effects for possible inclusion in our arsenal of antidepressant drugs that may benefit humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experiments were performed in accordance with the regulatory protocol of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Also, efforts were made to minimize the suffering of the animals during CUMS experimental protocols and behavioural tests.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ebmeier KP, Donaghey C, Steele JC. Recent developments and current controversies in depression. Lancet. 2006; 367:153–67.
2. Maciejewski PK, Prigerson HG, Mazure CM. Sex differences in event-related risk for major depression. Psychol Med. 2001; 31:593–604.
3. Driessen E, Hollon SD. Cognitive behavioral therapy for mood disorders: Efficacy, moderators and mediators. Psychiatr Clin North Am. 2010;33:537–55.
4. Szabo ST, Gould TD, Manji HK. Neurotransmitters, receptors, signal transduction, and second messengers in psychiatric disorders. In: Schatzberg AF, Nemeroff CB, eds. American Psychiatric

Publishing Textbook of Psychopharmacology. 3rd ed. Washington, DC: American Psychiatric Press. 2004;3-52.
5. Ehnvall A, Agren H. Patterns of sensitization in the course of affective illness: A life-charting study of treatment-refractory depressed patients. J Affect Disord. 2002;70:67–75.
6. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: Implications for neurocircuitry models of depression. Brain Struct Funct. 2008;213:93–118.
7. Serretti A, Chiesa A, Calati R, Perna G, Bellodi L, De Ronchi D. Common genetic, clinical, demographic and psychosocial predictors of response to pharmacotherapy in mood and anxiety disorders. Int Clin Psychopharmacol. 2009;24:1–18.
8. Nirmal J, Chidambaram SB, Thanukrishnan H, Muthiah R. Evaluation of behavioural and antioxidant activity of *Cytisus scoparius* Link in rats exposed to chronic unpredictable mild stress. BMC Complement Altern Med. 2008;8:15.
9. Michel TM, Dietrich P, Johannes T. The role of oxidative stress in depressive disorders. Curr Pharm Des. 2012;18:5890-9.
10. Kennedy SH, Rizvi SJ. Emerging drugs for major depressive disorder. Expert Opin Emerg Drugs. 2009;14:439–53.
11. Zhang ZJ. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci. 2004;75: 1659–1699.
12. Dixon AR, McMillen H, Etkin NL. Ferment this: The transformation of Noni, a traditional Polynesian medicine (*Morinda citrifolia*, Rubiaceae). Econ Bot. 1999;53: 51–68.
13. Krauss B. Plants in Hawaiian culture. Honolulu: University of Hawaii Press 1993; 103–252.
14. Hirazumi A, Furusawa E, Chou SC, Hokama Y. Immunomodulation contributes to the anticancer activity of *Morinda citrifolia* (Noni) fruit juice. Proc West Pharmacol Soc. 1996;39:7–9.
15. Potterat O, Hamburger M. *Morinda citrifolia* (Noni) fruit - phytochemistry, pharmacology, safety. Planta Med. 2007; 73:19–199
16. West BJ, Jensen CJ, White LD, Palu AK, Westendorf J. A double-blind clinical safety study of noni fruit juice. Pac Health Dialog. 2009;15:21–32.
17. Sang S, Ho CT. Chemical components of Noni (*Morinda citrifolia* L.) root. ACS Sym Ser. 2006;925:185–92.
18. Pawlus AD, Kinghorn DA. Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement *Morinda citrifolia* (noni). J Pharm Pharmacol. 2007;59:1587–1609.
19. Deng S, West, BJ. Antidepressant effects of noni fruit and its active principals. Asian J Med Sci. 2011;3:79–83.
20. Sang S, Wang M, He K, Liu G, Dong Z, Badmaev V, Zheng QY, Ghai G, Rosen RT, Ho C. Chemical components in noni fruits and leaves (*Morinda citrifolia* L.). ACS Sym Ser. 2002;803:34-50.
21. Machado DG, Bettio LE, Cunha MP, Santos AR, Pizzolatti MG, Brighente IM, Rodrigues AL. Antidepressant-like effect of rutin isolated from the ethanolic extract from *Schinus molle* L. in mice: Evidence for the involvement of the serotonergic and noradrenergic systems. Eur J Pharmacol. 2008;587:163-8.
22. Levand O, Larson HO. Some chemical constituents of *Morinda citrifolia*. Planta Med. 1979;36:186–87.
23. Hornick A, Lieb A, Vo NP, Rollinger JM, Stuppner H, Prast H. The coumarin scopoletin potentiates acetylcholine release from synaptosomes, amplifies hippocampal long-term potentiation and ameliorates anticholinergic- and age-impaired memory. Neuroscience. 2011; 197:280–92
24. Chen F, Madsen TM, Wegener G, Nyengaard JR. Imipramine treatment increases the number of hippocampal synapses and neurons in a genetic animal model of depression. Hippocampus. 2010; 20:1376–84.
25. Ramanathan M, Balaji B, Justin A. Behavioural and neurochemical evaluation of Perment an herbal formulation in chronic unpredictable mild stress induced depressive model. Indian J Exp Biol. 2011; 49:269–75.
26. Willner P. Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology. 2005;52:90-110.
27. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its

restoration by a tricyclic antidepressant. Psychopharmacology (Berl.). 1987;93: 358–64.
28. Mao QQ, Ip SP, Tsai SH, Che CT. Antidepressant-like effect of peony glycosides in mice. J Ethnopharnacol. 2008;119:272–75.
29. Lister RG. The use of a plus-maze to measure Anxiety in the mouse. Psychopharmacology (Berl.). 1987;111: 323–33.
30. Nina Dedic, Sandra M. Walser, Jan M. Deussing. Mouse models of depression, psychiatric disorders - Trends and Developments, Dr. Toru Uehara (Ed.); 2011.
ISBN: 978-953-307-745-1
31. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology. 1985;85:367-70.
32. Borsini F, Lecci A, Sessarego A, Frassine R, Meli A. Discovery of antidepressant activity by forced swimming test may depend on pre-exposure of rats to a stressful situation. Psychopharmacology (Berl.). 1989;97:183–88.
33. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972; 247:3170–75.
34. Sinha KA. Colorimetric assay of catalase. Anal Biochem. 1971;47:389–94.
35. Aksenov MY, Markesbery WR. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci Lett. 2001;302:141–45.
36. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351–58.
37. Willner P. The validity of animal models of depression. Psychopharmacology (Berl.). 1984;83:1–16.
38. Ajayi AA, Ukponmwan OE. Evidence of Angiotensin II and Endogenous opioid Modulation of NIR in the rat. Afr J Med Med Sci. 1994;23:287–90.
39. Svensson A, Carlsson ML, Carlsson A. Crucial role of accumbens nucleus in the neurotransmitter interactions regulating motor control in mice. J Neural Transm Gen Sect. 1995;101:127–48.
40. Heinicke RM. The pharmacologically active ingredient of Noni. Bull Nat Trop Bot Gard. 1985;15:10–14.
41. Green RC, Cupples LA, Kurz A, Auerbach S, Go R, Sadovnick D, et al. Depression as a risk factor for Alzheimer disease: The MIRAGE study. Arch Neurol. 2003;60:753–59.
42. Cuadrado-Tejedor M, Ricobaraza A, Del Rio J, Frechilla D, Franco R, Perez-Mediavilla A, et al. Chronic mild stress in mice promotes cognitive impairment and CDK5-dependent tau hyperphosphorylation. Behav Brain Res. 2011;220:338–43.
43. Wang JZ, Liu F. Microtubule-associated protein tau in development, degeneration and protection of neurons. Prog Neurobiol. 2008;85:148–75.
44. Maes M, Mihaylova I, Ruyter MD, Kubera M, Bosmans E. The immune effects of TRYCATs (tryptophan catabolites along the IDO pathway): Relevance for depression - and other conditions characterized by tryptophan depletion induced by inflammation. Neuro Endocrinol Lett. 2007;28:826-31.
45. Maes M, Mihaylova I, Ruyter MD, Kubera M, Bosmans E. The immune effects of TRYCATs (tryptophan catabolites along the IDO pathway): Relevance for depression - and other conditions characterized by tryptophan depletion induced by inflammation. Neuro Endocrinol Lett. 2007;28:826-31.
46. Chan-Blanko Y, Fabrice V, Ana MP, Max R, Jean-Marc B, Pierre B. The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. J Food Comp Anal. 2006;19:645–54.
47. European Commission. 2003/426/EC: Commission decision of 5 June 2003 authorising the placing on the market of"noni juice" (juice of the fruit of *Morinda citrifolia* L.) as a novel food ingredient under regulation (EC) No 258/97 of the European Parliament and of the council. Official J of the European Union L 144. 2003;0012–0012.
48. Maes M, Kubera M, Leunis JC, Berk M. Increased IgA and IgM responses against gut commensals in chronic depression: Further evidence for increased bacterial translocation or leaky gut. J Affect Disord. 2012;141:55–62.

49. Andrade L, Caraveo-Anduaga P, Berglund RV, Bijl R, De Graaf W, Vollebergh E. The epidemiology of major depressive episodes: Results from the International Consortium of Psychiatric Epidemiology (ICPE) surveys. Int J Methods Psychiatr Res. 2003;12:3–21.
50. Su BN, Pawlus AD, Jung HA, Keller WJ, McLaughlin JL, Kinghorn AD. Chemical constituents of the fruits of *Morinda citrifolia* (noni) and their antioxidant activity. J Nat Prod. 2005;68:592-95.
51. Bridges R, Lutgen V, Lobner D, Baker DA. Thinking out- side the cleft to understand synaptic activity: Contribution of the cystine-glutamate antiporter (systemxc) to normal and pathological glutamatergic signaling. Pharmacol Rev. 2012;64:780–802.
52. Schubert D, Piasecki D. Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. J Neurosci. 2001;21:7455–62.
53. Akihisa T, Matsumoto K, Tokuda H, Yasukawa K, Seino K, Nakamoto K, Kuninaga H, Suzuki T, Kimura Y. Anti-inflammatory and potential cancer chemopreventive constituents of the fruits of *Morinda citrifolia* (Noni). J Nat Prod. 2007;70:754-57.
54. Khairullah A, Zahi A. Review of studies on the biological activity and medical uses of *Morinda cirifolia* (Noni). Int J Trop Med. 2013;8:99–108.

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