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Neuropharmacological Evaluation of *Areca catechu* on Chronic Unpredictable Mild Stress Model of Depression in Mice: Behavioral and Biochemical Evidences

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ABSTRACT

Erratic chronic stresses can lead to the state identical to mental anxiety. The impact of free radicals in the pathogenesis of stress-induced depression is significant. Arecoline alkaloid found in kernels of the *Areca catechu* seeds, has wide spectrum of biological and pharmacological activities. The objective of this research was to evaluate the protective effect of *Areca catechu* on chronic unpredictable mild stress prompted variations in behavioral and brain oxidative stress parameters in mice. *Areca catechu* nuts' antidepressant effect could well be attributed to the alkaloids and saponins found in them, according to physicochemical research. For a period of 2 weeks, the animals were acclimatized and their baseline sucrose preference test was performed. At the termination of the study period, all the animal were exposed to behavioral and biochemical tests. The aqueous extract of *Areca catechu* nut displayed significant antidepressant activity by forced swim test that was additionally established by the inviolate locomotor actions of animals in the open field and actophotometer activity. Finally, it was concluded that the increase of serotonin and noradrenaline in the areca nut may have an antidepressant impact in chronic unpredictable mild stress.

Keywords: *Areca catechu*, alkaloids, chronic unpredictable mild stress, forced swim test, lipid peroxidation, antioxidants.

1. INTRODUCTION

As per the World Health Organization (WHO), depression is a severe illness that is one of the major risk factors globally. Chronic stress is a well-known cause or a consequence of a variety of psychiatric disorders in humans, particularly severe depression.[1] According to epidemiological analysis, stress is caused by the interaction of several genes with the surroundings or other variables. As per the monoamine theory, depression is caused by a lack of monoaminergic transmission in the central nervous system, while mania is caused by an overabundance.[2,3] Feeling of deep hopelessness and sorrow, psychological retardation and inability to focus, gloomy anxiety, lack of enjoyment, self-deprecation, and fluctuating anger or aggressiveness are all symptoms of major depression. Physical alterations include insomnia or hypersomnia; changed eating behaviors, including anorexia and losing weight or occasionally overeating; reduced energy and libido; and disturbance of the usual

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circadian and ultradian cycles of activities, body temperature, and altered endocrine processes.[4]

Tricyclic antidepressants, noradrenergic reuptake inhibitors, selective serotonin reuptake inhibitors, and other atypical antidepressant medications like monoamine oxidase inhibitors are presently accessible as pharmacological treatments for depression.[5,6] Yet, antidepressant effect is typically uneven, and most of them have unpleasant side effects including drowsiness, apathy, weariness, as well as sleep disturbances, memory deficits, and infertility. As a result, there is still a great need for novel antidepressants that are both effective and well tolerated.[7]

Earlier studies has shown that frequent and unexpected stress has a substantial influence on the generation of reactive oxygen species (ROS) in the brain, which leads to oxidative damage and dysregulation in CNS.[8] Numerous studies have shown that antioxidants have an essential role in alleviating chronic unpredictable mild stress caused (CUMS) depression, considering the aforesaid results.

Areca catechu (Arecaceae) is one of the most basic ingredients in Indian medicine. It grows on the arid plateaus of Southern India, Mysore, Malabar, Canara, and Assam.[9] Polyphenolic compounds, saponins, tannins, alkaloids, and fibers are the main components of *A. catechu*. Catechu, gum, tannin, gallic acid, and alkaloids (choline, arecoline, arecaidine, arecaine, guvacine, guvacoline) are found in the kernels. (+)catechin, & (–)epicatechin tetramers, along with procyandins A-1, B-1, and B-2 have been extracted from the seed of *A. catechu*.[10,11] The herb was acclaimed for its therapeutic qualities, which included antibacterial and antiviral activities. Areca nut is a frequent component in betel quid, which also includes piper betel leaf and lime with or without tobacco. It has antioxidant, antihypertensive, hypoglycemic, platelet aggregation, anti-HIV, proteasome inhibitors, molluscicidal, anti-venom, oxytocic and anti-fertility activities. It affects brain to induce antidepressant, anticonvulsant, and CNS stimulant activities.[9,12,13] The objective of this paper was to assess the impact of aqueous extract of *A. catechu* nut on chronic moderate stress-induced depression in mice.

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

Fluoxetine hydrochloride (Fludac) was supplied by Cadila Pharmaceuticals Ltd., India. Sigma Aldrich, Hyderabad, India provided the thiobarbituric acid (TBA) and trichloroacetic acid (TCA). Ellman's reagent, butanol, pyridine, hydrogen peroxide, EDTA, and other chemical were obtained from standard sources.

2.2 Experimental Animal

Swiss albino mice of either sex, weighing about 25-30 g, were used in this study. The animals were kept under conventional laboratory settings, which included a 12:12 hour light/dark cycle and a temperature of 22±2°C with a humidity of 60±10%. The animals were fed a normal food and given free access to water, and were housed in clean polypropylene cages. They were split into experimental groups after a week of laboratory acclimatization. All experimental methods were carried out in compliance with the guidelines established by the committee for the regulation and supervision of animal research. The Institutional Animal Ethics Committee, G. Pulla Reddy College of Pharmacy (GPRCP/IAEC/23/19/02/PCI/AE-4-Mice-M/F-30), Hyderabad, India authorized the study.

2.3 Collection and Extraction of Areca Nut

A. catechu nuts were collected from local market of Coimbatore, Inida. The extract was made by combining 20 g of well rinsed and coarsely diced nuts with 100 ml distilled water in a 250 ml Erlenmeyer flask and boiling the liquid for 10 minutes. After then, the solution was withdrawn from the head source and allowed to cool to room temperature. The extract was then filtered by a Whatman filter paper No. 1 The extract was stored at 4°C in the refrigerator for future studies.[14]

2.4 Qualitative Phytochemical Analysis

Phytochemical components of the aqueous extracts of *A. catechu* nut (ACAE) and betel leaf

were screened by using standard methods. The components analyzed were alkaloids, flavonoids, anthroquinone, saponins, phenol, protein, coumarin, reducing sugar, tannins, phytosterols, anthrocyanides, triterpinoids, phlobatannins, acids and glycosides.[15]

2.5 Experimental Protocol

Before beginning the Chronic Unpredictable Mild Stress (CUMS) regimen, the animals were acclimatized for one week and trained to consume a 1 percent (w/v) sucrose solution. A sucrose preference baseline test was done three days later. They are then separated into five groups (n=6) at random.

Group I (Normal Control): Received vehicle for 14 days

Group II (Disease Control): Received CUMS + vehicle for 14 days

Group III (Standard Control): Received CUMS + 20 mg/kg Fluoxetine HCl for 14 days

Group IV (Treatment I): Received CUMS + 10 mg/kg ACAE for 14 days

Group V (Treatment II): Received CUMS + 20 mg/kg ACAE for 14 days

Table 1: Schedule stressors to induce chronic unpredictable mild stress

Days	Schedule Stressors	Days	Schedule Stressors
1-7	Adaptation		
8	Exposure to empty water bottles	15	Immobilization for 2 hrs
9	Immobilization for 2 hrs	16	Exposure to empty water bottles
10	Overnight illumination	17	Overnight illumination
11	Tail pinch for 60 sec	18	Tail pinch for 30 sec
12	Exposure to foreign object for 24 hrs	19	Cage tilting at 45° for 7 hrs
13	Cage tilting at 45° for 7 hrs	20	Tail pinch for 30 sec
14	Tail pinch for 30 sec	21	Exposure to foreign object for 24 hrs

On the 15th day, all of the animals were exposed to different behavioral tests (Sucrose Preference, Forced Swim, Open Field, and Actophotometer Tests) at the end of the treatment period. On the 15th day, all of the animals were CO_2 asphyxiated and their brains were dissected to determine endogenous antioxidant levels of MDA and GSH.[16]

2.6 Induction of Chronic Unpredictable Mild Stress (CUMS)

The mice were treated to CUMS as previously described, with minor modifications. Animals were exposed to stress paradigm once a day for two weeks, with the following stressors employed in the order (Table 1).[17,18]

2.7 Behavioral Assessment

2.7.1 Body Weight Gain and Sucrose Consumption

Sucrose (1% sucrose solution) intake and weight gain were recorded once per week, following 18 hours of food and water restriction, during a 1-hour interval. At the end of the test, the pre-weighed bottle was weighed to determine consumption. The consumption was measured in grams per kilogram of body weight (g/kg). Less than a week before CUMS began, the baseline was taken. The interval of fasting and restriction of food and drink prior to sucrose intake assessment might be viewed as an additional stress on top of the CUMS procedure.[19]

$$\text{Sucrose Preference (\%)} = \frac{\text{Sucrose Solution Intake (g)}}{\text{Sucrose Solution Intake(g)} + \text{Water Intake(g)}} \times 100$$

2.7.2 Forced Swim Test (FST)

The FST swim test was carried out using Dhingra et al (2005) method. The mouse was taken in a beaker container of 22.5 cm diameter and 30 cm height with 15 cm of water level of 23±2°C temperature. The mice were submerged in water for 6 minutes and forced to swim. Each animal acquired a characteristic motionless position after a 2-minute period of intense exercise. When a mouse stayed afloat in water without moving, making just the smallest moves of its paws required to sustain its head above water, it was termed motionless. During the next 4 minutes of the 6-minute test, the total time of immobility was observed. Every animal was only used once. Between sessions, the water was replaced and the temperature was set at 23±2°C.[20]

2.7.3 Open Field Test

The open field test was conducted before one day following the previous stressor and drug exposure. The exposed area was split into 25 (5x5 cm) equal segments by white stripes (20x20 cm), with a foundation of 100x100 cm and black walls of 20 cm. The squares were separated into two sectors: peripheral and central, with the center sector containing the 9 central squares (3x3 cm) and the periphery sector containing the squares along the wall. For additional study, the animals were put in the center section and their activity was videotaped for 5 minutes. Across each test, the open field arena was completely cleaned. A faint crimson light illuminated the room. The animals were not exposed to any stressors for at least 24 hours before to the test. The action on the open field was manually scored. When the mice traversed a sector boundary with both hind limbs, it was given a score for mobility. During the 5-minute test, peripheral activity i.e. the number of peripheral sectors crossed, central activity i.e. the number of center squares crossed, and total activity i.e. overall activity in the peripheral and central regions were all assessed.[21]

2.7.4 Actophotometer Test

Animal locomotor activity was measured using an actophotometer equipped with a digital counter, photocell, and light source. For 5 minutes, each animal was inserted in the Actophotometer, and the baseline activity score was measured for each animal. After 30 minutes and 1 hour, every animal was given the appropriate medication and an activity score was taken. The score of stopped activity was used as a measure of CNS depression.[22]

2.8 Biochemical Assessment

2.8.1 Preparation of Tissue Homogenate

In an ice cold state, 10 mg of tissue was weighed and homogenized in 5 ml ODF buffer solution using a remimotor at a speed of 2500 rpm for 2 minutes.

2.8.2 Estimation of Malondialdehyde (MDA)

The amount of malondialdehyde in tissue is a marker of lipid peroxidation. Method of Ohkawa et al (1979) was used to assess it in brain tissue homogenate. Thiobarbituric acid (TBA) interacts with malondialdehyde (MDA) in an acidic medium at 95°C for 30 minutes to produce thiobarbituric acid reative product, which has a 534 nm absorbance.[23]

2.8.3 Estimation of Reduced Glutathione (GSH)

Ellman's technique was used to assay GSH. The disulfide chromogen 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) was easily converted to a brightly yellow molecule by the sulfahydryl group of GSH. The decreased chromogen's absorbance was measured at 412 nm and was directly proportional to the GSH content.[24]

2.9 Statistical Analysis

All data was presented as a mean ± standard error of the mean (SEM). Graph pad prism software was used to do a one-way analysis of variance (ANOVA) proceeded by a Tukey's post hoc test on the data (5.3 version). Statistical significance was defined as a value of $P < 0.05$.

3. RESULTS

3.1 Preliminary Phytochemical Analysis

The ACAE revealed the presence of alkaloids, tannins, saponins, phenol, reducing sugar, triterpenoids, glycosides and gums.

3.2 Effect on Sucrose Preference Test

In the baseline test, no significant difference was observed in sucrose preference (%) between any of the groups, as indicated in Table 2. When the mice were exposed to stress for 14 consecutive days, their sucrose preference fell considerably ($P < 0.01$) when compared to the vehicle control group. When compared to disease control, ACAE (10 & 20 mg/kg) and fluoxetine (20 mg/kg) significantly ($P < 0.01$; $P < 0.001$) enhanced sucrose desire. When compared to normal control, however, these alterations were more severe than typical value.

3.3 Effect on Duration of Immobility in FST

When compared to the normal control group, mice given CUMS had significantly longer periods of immobility ($P < 0.01$). In contrast to the disease

control animals, ACAE (10 & 20 mg/kg) and fluoxetine significantly ($P < 0.01$) reduced the length of immobility (Table 2).

3.4 Effect on Locomotor Activity in Open Field Test

When mice were given CUMS, the length of locomotor activity was significantly reduced ($P < 0.001$) compared to the normal control group. When compared to disease control, ACAE (10 & 20 mg/kg) and fluoxetine significantly ($P < 0.01$) increased the length of locomotor activity (Table 2).

3.5 Effect on Locomotor Activity in Actophotometer Test

When mice were given CUMS, the length of locomotor activity was significantly reduced ($P < 0.001$) compared to the normal control group. When compared to the control group, the ACAE (10 & 20 mg/kg) and fluoxetine (20 mg/kg) treated groups exhibited substantial CNS antidepressant effects. This depression was, however, less severe in the *A. catechu* nut-treated group than in the fluoxetine-treated group (Table 2).

3.6 Effect on Brain MDA Level

In contrast to the normal control group, induction of CUMS significantly ($P < 0.001$) raised MDA levels in the brain. ACAE (10 & 20 mg/kg) and fluoxetine (20 mg/kg) treatment significantly ($P < 0.01$) decreased MDA levels (Table 3).

3.7 Effect on Brain GSH Level

In contrast to the normal control group, induction of persistent CUMS resulted in a significant ($P < 0.001$) reduction in brain GSH concentration. In a dose-dependent manner, treatment with ACAE (10 & 20 mg/kg p.o) and fluoxetine (20 mg/kg p.o) dramatically enhanced reduced GSH in stressed mice when compared to illness control animals (Table 3).

4. DISCUSSION

The $GABA_A$ receptor complex engaged in physiological functions related to behavioral, psychological and neurological disorders is primarily responsible for anxiety in the CNS. Oxidative stress is linked to depression, according to previous researches.[25,26] The phytochemical analysis of ACAE resulted in the discovery of different components such as alkaloids, tannins, saponins, phenol, reducing sugar, triterpenoids, glycosides, and gums in the current study. The active principle underlying majority of the biological activity evoked by the nut has been attributed to arecoline alkaloid.[27]

Table 1: Effect of ACAE on various behavioral tests in mice

Groups	Sucrose Preference (%)		Forced Swim Test (Sec)	Actophotometer Test (Count)	Open Field Test		
	Before	After			Peripheral Square (Sec)	Central Square (Sec)	Central Crossing's (Count)
Normal Control	38.59 ± 6.27	35 ± 2.15	161.5 ± 14.58	338.5 ± 5.14	134.33 ± 2.36	45.66 ± 2.36	7.33 ± 0.61
Disease Control	49.79 ± 5.61	19.14 ± 2.83[b]	236 ± 5.08[c]	278 ± 1.58[c]	163.5 ± 2.37[c]	19.83 ± 3.38[c]	3 ± 0.57[c]
Standard Control	44.44 ± 10.38	39.5 ± 2.41***	109 ± 6.093**	317.83 ± 7.67***	131.83 ± 1.40***	49 ± 1.13***	6.67 ± 0.49***
Treatment I	38.89 ±5.93	35.33 ± 2.69**	181.66 ± 4.36**	304 ± 4.13**	152.16 ± 2.32*	31 ± 2.32*	5.16 ± 0.31*
Treatment II	52.42 ± 5.06	37.49 ± 2.16**	175.83 ± 3.31***	310.5 ± 5.23***	149 ± 3.09**	36 ± 0.87**	5.83 ± 0.48**

*Values were expressed as mean ± SEM (n=6). Data were analyzed by one way ANOVA followed by Tukey's Test. [b]P<0.01, [c]P<0.001 when compared to the normal control group; ***P<0.001, **P<0.01, *P<0.05 when compare to the disease control group.*

Table 3: Effect of ACAE on endogenous antioxidants in brain

Groups	MDA (nmol MDA/mg protein)	GSH (µmol/mg protein)
Normal Control	1.73 ± 0.16	0.25 ± 0.03
Disease Control	3.90 ± 0.26^{c}	0.08 ± 0.02^{c}
Standard Control	2.40 ± 0.26**	0.24 ± 0.03***
Treatment I	2.90 ± 0.25**	0.21 ± 0.02**
Treatment II	2.70 ± 0.21**	0.22 ± 0.04**

Values were expressed as mean ± SEM (n=6). Data were analyzed by one way ANOVA followed by Tukey's Test. ^{c}P<0.001 when compared to the normal control group; ***P<0.001, **P<0.01, when compare to the disease control group.

Treatment with ACAE reduces unpredictable chronic depression produced by mild stress in the current investigation, resulting in a shorter period of immobility as shown by higher sucrose intake, the forced swim test, and the oxidative stress measure. According to earlier studies, animals who were exposed to diverse stress conditions for 14 days acquired substantial depression symptoms. The administration of uncontrolled stressors is a sound animal paradigm for the preclinical assessment of antidepressants.[31] In earlier studies, the FST test and sucrose preference test were utilized to characterize CUMS-induced depressed behavior in mice.[28]

In a mouse model, the sucrose preference test is a useful and reliable behavioral indication of chronic stress. Sucrose preference is thought to be a sign of loss of interest or pleasure, which is a fundamental symptom of depression. Chronic unexpected stress, according to the research, destroys nerve cells in the neuronal system. The serotonergic and dopaminergic systems are considered to be damaged; resulting in a lack of capacity to perceive happiness.[29] In the current research, anxious mice consumed a smaller amount of sucrose solution than unstressed mice. This results supports prior reports that mice subjected to CUMS consumed not as much of sucrose than non-stressed mice, but treatment with ACAE and fluoxetine dramatically restores sucrose preference.[30] Reduced sucrose preference indicates *A. catechu* antidepressant-like effect. In the depression model of CUMS, catechu nut is used. Antidepressant therapy has been demonstrated in studies to be effective in reversing the chronic stress-induced decrease in sucrose intake.[31]

In the current study, the CUMS group had a longer period of immobility in FST, indicating a depressive-like condition. In FST, immobility is a sign of depression. Treatment with ACAE significantly improved sucrose preference and decreased immobility time in mice in the CUMS group, which was consistent with previous studies.[32] The effectiveness of *A. catechu* nut were equivalent to those of fluoxetine.

An open field test and an actophotometer were used to determine anxiety levels. In an open field test, ACAE therapy was shown to successfully counteract CUMS-induced increases in actophotometer count and decreased crossing and rearing numbers. Bende and colleagues (2016) also found that continuous treatment of *A. catechu* nut extract improved depressive symptoms, indicating that it has a neuroprotective impact.[33]

The development of neuropsychiatric diseases is influenced by reactive oxygen species (ROS). Increased generation of ROS can result in destruction of macromolecules such as DNA, proteins, and lipids, resulting in neuronal malfunction and depression. The antioxidant state of brain tissue was shown to be impaired by CUMS, probably due to the generation of increased ROS.[34-37] Other investigations have corroborated this finding. Because they recovered to normal ranges after antidepressant therapy, antioxidant enzymes and lipid peroxidation may be state indicators of severe depression.[38] Malondialdehy-

de, a by-product of lipid peroxidation, increased after 14 days of exposure to many stressors in the current investigation. Treatment with repeated ACAE extract prevented lipid peroxidation and CUMS-induced depressive-like behavior.

GSH is a non-enzymatic intracellular thiol antioxidant that helps to neutralize free radicals. Depressive-like behavior was produced by a decrease in antioxidant defense mechanisms.[39] In the CUMS model of depression, stressed mice exhibit decreased brain GSH levels, indicating a change in antioxidant brain defenses. The GSH level in the brains of stressed mice was recovered after treatment with ACAE. Similarly, earlier research have indicated that phytochemicals such as curcumin, punarnavine, mucunapruriens, and piperment have a protective effect against CUMS-induced depression by correcting behavioral and biochemical characteristics.[40-43]

5. CONCLUSION

Finally, the results of this investigation showed that treatment with an aqueous extract of *A. catechu* nut have protective effect against chronic mild stress-induced depression. The active components of areca nut extract, more probably alkaloid and saponin, evoked dose-dependent antianxiety and antidepressant action, which can be ascribed to atypical increase of serotonin and noradrenaline levels in the hippocampus of mice. As a result, *Areca catechu* can be utilized as an antioxidant and neuroprotective agent.

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