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ANXIOLYTIC AND ANTI DEPRESSANT ACTIVITY OF *ARECA CATECHU* LINN. IN MICE

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ABSTRACT

Seventy two Swiss adult albino mice weighing 25–30 grams were divided equally into three groups and allocated to Forced swim test (FST), Tail suspension (TST) and Elevated plus maize test (EPM). FST and TST were used to evaluate antidepressant activity and EPM for anxiolytic activity. Each group had 4 sub groups (control, standard and the 2 test groups) of 6 animals in each. The control group received the vehicle, gum acacia; standard group 1, Diazepam (for EPM), Standard group 2, Imipramine (For FST and TST) and the 2 test groups received the aqueous (AEAC) and methanolic extract (MEAC) of areca catechu. For acute study, single dose was given 60 min prior to the experiment and for chronic study the drugs were given once a day for 15 days. Statistical analysis was performed using Mean +/- SD. ANOVA followed by Student–Newman–Keuls as the post-hoc test. AEAC and MEAC have shown significant anxiolytic activity greater than diazepam in EPM test. The antidepressant activity of both AEAC and MEAC was comparable to Imipramine in FST and TST. The present study has shown that both AEAC and MEAC have anxiolytic and antidepressant activity. The anxiolytic activity was greater than Diazepam and antidepressant activity is comparable to Imipramine.

KEYWORDS: Areca catechu, Imipramine, Diazepam, Anxiety, Depression, EPM, FST, TST.

INTRODUCTION

According to WHO, Depression is one of the 10 leading causes of disability-adjusted life years (DALYs) lost globally and regionally and it is projected to be among the top three causes of DALYs lost by 2030. Globally, more than 350 million people of all ages suffer from depression. Less than 50 % of them and in many countries less than 10% only receive the care they need. [1] The failure to seek treatment could be due to the stigma attached to the depressive illness. Unless treated early depressive illness may lead to suicides and significant human loss.

Anxiety is a normal emotional response which when chronic or severe, becomes pathological and can aggravate cardiovascular & psychiatric disorders.

Despite the availability of wide range of drugs, the treatment of depression and anxiety is still challenging as these drugs produce various side effects or exhibit tolerance on continuous use. Many medicinal plant derivatives have been claimed to be effective in depression, free from side effects and comparable in efficacy to the available standard drugs. [2] E.g. Emblica officinalis [3-4], Nympheaba Alba [5], Ocimum santum [6], Passiflora incarnate [7], St John's wort [8], Ginkgo biloba [9], piper betle [10] etc.

Areca catechu. Linn (Family: PALMAE), popularly known as Betel nut is one of the medicinal plants used from ancient times for its medicinal properties. Chewing areca nut along with betel leaf and lime is a tradition in most parts of India by both men and women. The important constituents of areca nut are tannins, gallic acid, oils and alkaloids. The alkaloids include arecoline, arecaidine, guvacine and guvacoline. Arecoline has cholinomimetic actions. The pharmacological actions of areca nut are mainly due to its alkaloid content and resemble that of muscarine & pilocarpine. [11] Dried areca nut is reported to have hepatoprotective [12], hypoglycemic [13], antiulcer [14], antifertility [15], anti ovulatory & abortifacient activities. [16] It has stimulant, euphoric and digestive properties. [17] Abbas G et al (2012) have reported its anti depressant activity but the anxiolytic activity has not been reported. Hence this study was undertaken to find out the effect of areca catechu extract in animal models of both depression and anxiety.

MATERIALS AND METHODS

Animals: The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chettinad Hospitals and Research Institute, Chettinad University,

Chennai, India. 72 adult Swiss albino mice of either sex weighing 25-30 gms were obtained from the Institutional animal house. The animals were housed at 24±2°C with free access to food and water and acclimatized for a period of 7 days before the study. The animals were maintained according to the CPCSEA guidelines for the use and care of experimental animals.

Drugs & Chemicals: Imipramine and diazepam tablets and, aqueous & methanolic extracts of areca catechu (AEAC & MEAC).

Preparation of extracts

Methanolic Extract: The areca nuts were powdered using a mechanical grinder. Powder (100 g) was successively extracted with 95 % 500 ml of methanol by soxhlet extraction method.

Aqueous Extract: The areca nuts were powdered & extracted with water by maceration process.

Both the extracts were suspended in 1% gum acacia and used for the experiments.

EXPERIMENTAL DESIGN

72 adult swiss albino mice weighing 25–30 gms were selected. 24 animals were allocated to forced swim test, 24 animals to tail suspension test and remaining 24 to elevated plus maize test. In each model there were 4 groups of 6 animals in each. The control group received vehicle (1% gum acacia 10 ml/kg P.O), standard group 1, Diazepam (1mg/kg P.O.) and standard group 2 Imipramine (10 mg/kg P.O.) and the 2 test groups received the aqueous (AEAC) (300mg/kg P.O) and methanolic extract (MEAC) (250mg/kg P.O) of areca catechu. For acute study single dose was given 60 min prior to the experiment and for chronic study all the drugs were given PO, once daily for 15 days. Elevated plus maze (EPM), forced swim test (FST) and tail suspension test (TST) were used to assess the anxiolytic and antidepressant activities.

Groups	Drugs	Dose
Group 1	1% gum acacia	10 mg/kg P.O.
Group 2	Imipramine	10 mg/kg P.O.
Group 3	Diazepam	1mg/kg P.O.
Group 4	Aqueous extract of areca catechu	300 mg/kg P.O.
Group 5	Methanolic extract of areca catechu	250mg/kg P.O.

EVALUATION OF ANTIANXIETY ACTIVITY

Elevated Plus Maze Test: EPM consists of two open arms (50cm×10cm) and two closed arms (50cm× 10cm× 40cm).The arms same type were opposite to each other with a central square of 10cm.The maze was elevated to a height of 50cm above the floor. Each animal was placed in the centre square of plus maze, facing one of the open arms. The number of entries in open and closed arms by the animal in a 5 minutes period was noted. [18-19]

EVALUATION OF ANTIDEPRESSANT ACTIVITY

Tail Suspension Test: The mice were hung by tail on a plastic string, 75 cm above the surface with the help of an adhesive tape. They were observed for a period of 8 minutes and the duration of immobility was recorded in the last 6 minutes. Mice were considered immobile only when they hung passively and completely motionless. [20]

Forced swim Test: Each mouse was placed individually in a 5 liter glass beakers filled with water up to a height of 15 cm and were observed for 6 minutes. The duration of immobility was recorded during the last 4 minutes. The mouse was considered immobile when it floated motionlessly and made only those movements necessity to keep its head above the water surface. The water was changed for every animal after each test. [21]

STATISTICAL ANALYSIS

The mean ± SD. values were calculated for each group. The data were analysed using one-way ANOVA followed by Student–Newman–Keuls as the post-hoc test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Elevated plus-maze: Results are given in table-1 & 2. Administration of AEAC and MEAC (300 & 250 mg/kg) significantly increased the number of entries in open arms compared to control in acute study. In chronic study both the doses of AEAC and MEAC produced a greater increase in the number of entries in open arm compared to both the control and the standard drug diazepam.

Table 1: Acute effect of Areca Catechu Linn. on behaviour of mice in EPM

Groups	Number of Open Arm Entries	Number of closed Arm Entries	Percentage of Open/Closed Arm Entries
1% gum acacia 10ml/kg	3.33± 1.50	6.33 ± 2.58	40 ± 1.67
Diazepam 1mg/kg	6.33 ± 2.58*	11.83 ± 4.07*	97 ± 8.0*
AEAC 300mg/kg	8±3.63*	12.67 ± 5.85*	107.50 ± 8.06*
MEAC 250mg/kg	9.17 ± 1.72*	13 ± 4.98*	109.33 ± 13.76*

Values represented mean ± S.D (n=6), *P<0.05 vs. control.

Table 2: Chronic effect of Areca Catechu Linn. on behaviour of mice in EPM

Groups	Number of Open Arm Entries	Number of closed Arm Entries	Percentage of Open/closed Arm Entries
1% gum acacia 10ml/kg	2.17 ± 0.75	6.83 ±3.18	30.50 ± 3.56
Diazepam 1mg/kg	6.83± 2.22*	13.83 ± 3.71*	103± 5.06*
AEAC 300mg/kg	8.50± 4.97**	16.17 ± 4.26**	124.33± 9.30**
MEAC 250mg/kg	9.50± 2.95**	18.50 ± 2.34**	132.83± 7.22**

Values represented mean ± S.D (n=6), *P<0.05 vs. control, **P<0.05 vs. standard

Tail suspension test (TST) & Forced swim test (FST): Results are given in table-3 & 4. A significant decrease in the duration of immobility was seen in both FST & TST in all the animals treated with Imipramine and Areca nut extracts (AEAC 300 mg/kg and MEAC 250 mg/kg) compared to control in both acute and chronic studies, denoting antidepressant activity.

Table 3: Effect of Areca Catechu Linn. on immobility in TST using mice

Group (Drug Treatment)	Duration of Immobility (in sec)	
	Acute Study	Chronic Study
1% gum acacia 10ml/kg	204±11.01	199.6±11.3
Imipramine 10mg/kg	113.8±4.77*	165.8±3.59*
AEAC 300mg/kg	115.8±6.55*	163.5±5.96*
MEAC 250mg/kg	114.8±7.02*	166.1±6.93*

Values represented mean ± S.D (n=6), *P<0.05 vs. control

Table 4: Effect of Areca Catechu Linn. on immobility in FST using mice

Group (Drug Treatment)	Duration of Immobility (in sec)	
	Acute Study	Chronic Study
1% gum acacia 10ml/kg	95.32±8.19	137.5±5
Imipramine 10mg/kg	52.35±3*	117.3±2.5*
AEAC 300mg/kg	53.35±4.9*	118.5±4.9*
MEAC 250mg/kg	52.73±5.27*	118.8±5.2*

Values represented mean ± S.D (n=6), *P<0.05 vs. control

DISCUSSION

The present study has shown that areca catechu extracts, MEAC and AEAC have anxiolytic and antidepressant activity. Areca catechu contains alkaloidal and non-alkaloidal components. The alkaloids are arecoline, arecaidine, guvacine and guvacoline. The main non alkaloidal component is found to be dichloromethane fraction. [22] The alkaloids are cholinomimetic having actions similar to that of acetylcholine. Dichloromethane is used in industry as a paint stripper and a degreaser. In the food industry, it has been used to decaffeinate coffee and tea as well as to prepare extracts of hops and other flavourings. [23] Dichloromethane fraction has been found to be present in many plant extracts such as Pimenta pseudocaryophyllus [24], Cimicifuga heracleifolia [25], Laminaria japonica [26], Melissa officinalis [27], Stemona tuberosa Lour [28], areca catechu and several others.

Our study has shown for the first time that the methanolic and aqueous extracts of areca catechu have anxiolytic activity. The anxiolytic activity was found to be greater than even the prototype anxiolytic diazepam. Diazepam is a benzodiazepine and acts through GABAergic pathway as an agonist of GABA. GABA is an inhibitory neurotransmitter and inhibits neuronal hyper excitability. Joshi et al. [29] reported that areca catechu extract increased memory and learning in rats. Anxiety will interfere with learning and memory. It is reported that students who have academic anxiety also have a higher risk of developing depression. [30] Hence an anxiolytic with antidepressant activity may improve learning and memory. Cholinergic drugs are known for improving learning and memory and drugs like Rivastigmine and Donepezil are in use for Alzheimer disease to improve memory. The possibility of enhancing chloride ion conductance by acetyl choline has been studied by Janssen in guinea pig tracheal myocyte. [31] Whether acetylcholine has a similar action in the neuronal cells of the brain is not known. The prototype anxiolytic diazepam enhances chloride conductance by facilitating GABA. As arecoline has exhibited significant anxiolytic activity it might have chloride ion channel mediated activity in the brain but further studies are needed to establish this effect.

The ethanolic extract has been studied already and found to have antidepressant activity. [32] In our study the methanolic extract also has shown similar effect. The antidepressant activity of areca catechu was found to be equivalent to Imipramine. Imipramine is a tricyclic antidepressant which enhances synaptic nor epinephrine and also dopamine and serotonin by inhibiting their reuptake into the neurons. The antidepressant activity of areca catechu may

not be due to the alkaloids present but to a different constituent, dichloromethane. Dichloromethane is an inhibitor of mono amino oxidase - A (MAO-A) enzyme [33] and MAO-A inhibition results in antidepressant activity, but the alkaloids, arecaidine, arecoline do not inhibit MAO-A enzyme. MAO are group of enzymes which metabolize biogenic monoamines. [34] MAO-A metabolizes norepinephrene, epinephrine and serotonin whereas as MAO-B metabolizes mainly dopamine. MAO-A inhibition leads to increased levels of norepinephrene, epinephrine and serotonin which is responsible for antidepressant activity. MAO-A inhibition by dichloromethane in areca catechu thus results in antidepressant activity.

Thus areca catechu is found to have anxiolytic and antidepressant activity brought out by its two different constituents. Anxiolytic activity is due to the alkaloidal component and antidepressant activity is due to the non alkaloidal component. Facilitation of chloride ion conductance may contribute to anxiolytic activity similar to benzodiazepines whereas elevation of sympathetic amines in the brain to antidepressant activity. Both the actions can improve learning and memory significantly but further studies are needed to establish the combined effect.

CONCLUSION

The results of the present study suggest that the AEAC and MEAC have anxiolytic activity better than Diazepam and antidepressant activity comparable to Imipramine. These two activities are due to two different constituents of areca catechu. The alkaloids arecoline and arecaidine may be responsible for anxiolytic activity and the non alkaloidal component dichloromethane fraction could have contributed to antidepressant activity.

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