

Attenuation of depression by *Areca catechu* ethanol extract in swiss albino mice

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Abstract

Background: Mood or affective disorder is psychological manifestations, depression is now known as a complex disorder involving the whole body and the diagnosis of depression is based on a heterogeneous set of symptoms.

Objectives: To evaluate antidepressant activity of *Areca catechu* Ethanol Extract in Swiss Albino Mice.

Materials and Methods: Adult Swiss albino mice were selected and randomly divided into five groups as control which received normal saline, standard which received Imopramine 10mg/kg and three test groups which received *Areca catechu* ethanol extract at the dose of 40, 80 and 160 mg/kg respectively. All the drugs were administered intraperitoneally (0.1ml/10gm) 30 minutes before the experiment followed by testing with forced swim test and tail suspension test successively to note the duration of immobility with the observation as mean \pm SD and was compared with the control by using ANOVA followed by Dunnett's test. $p < 0.05$ was considered as significant.

Conclusion: We found that, acute treatment with *Areca catechu* ethanol extract at the dose of 80mg/kg and at the dose of 40 and 80mg/kg has shown significant antidepressant action by forced swim test and tail suspension test respectively.

Introduction

Disorders of mood, or affect, have been described since the 4th century BC. Despite this early acknowledgment, their aetiology is still a source of debate. There is a growing knowledge that, away from being purely psychological manifestations, depression has been now known as a complex disorder involving the whole body and the diagnosis of depression is based on a multiple set of symptoms. These criteria have gradually developed and documented by both the American Psychiatric Association and the World Health Organisation Geneva providing essential guidance for both clinicians and researchers. Major depression is defined as depressed mood on a daily basis for a minimum duration of two weeks. An episode may be characterized by sadness, indifference to surroundings, apathy and or irritability and is commonly associated with changes in sleep patterns, appetite, and weight, motor agitation or retardation, fatigue; impaired concentration and decision-making, feelings of shame or guilt, and thoughts of death or dying. Patients suffering from depression have a profound loss of pleasure in all enjoyable activities, exhibit early morning awakening, feel that the dysphoric mood state is qualitatively different from sadness, and often notice a diurnal variation in mood (worse in morning hours). Approximately 15% of the population experiences a depressive episode of major variety at one or other point in life, and 6-8% of all outpatients in primary care settings satisfy diagnostic criteria for the disorder.⁽¹⁻³⁾

About 60 to 70% of patients with depression respond to conventional antidepressants at a therapeutic dose for a period of 6-8 weeks. But unfortunately, there is no ideal antidepressant drug with rapid onset of action, moderate half-life, a low side/ adverse effect

profile with minimal interaction with other drugs, and also safety in overdose is a matter of concern. Hence the medical field needs newer, well tolerated and more efficacious drugs. Even today, herbal medicines are still the mainstay in primary health care especially in the developing countries like India, because of the belief that drugs of herbal origin are without any side / adverse effects and they are economical and also easily available.⁽⁴⁻⁶⁾

Areca catechu is the areca palm, is a species of palm which is usually grown in India, Malaysia, Taiwan and many other countries for their seeds. Betel nut is placed 4th next to nicotine, ethanol and caffeine, is chewed by millions of people living in east coast of Africa and also the western Pacific.

Areca catechu belongs to the family *Arecaceae* and is erroneously called as betel tree due to its fruit, the arecanut is always chewed along with the betel leaf, a leaf from the *Piperaceae* family. This is a medium sized and graceful palm tree growing straight to 20m tall with a trunk measuring 20-30 cm in diameter. The leaves are 1.5-2m long, pinnate with numerous crowded leaflets. *Areca catechu* is grown for its important seed crop, the *Areca nut* for commercial purposes. The seed contains alkaloids such as arecaine and arecoline. About nine alkaloids together constitute the active ingredients of betel nut, the most abundant of which is arecoline – a potent cholinergic agonist that crosses the blood-brain barrier (BBB) and includes a range of parasympathetic effects.

It is known as 'Akota' in Sanskrit and 'Adike' in Kannada. Ethanol extract of *Areca catechu* has been shown to have antidepressant properties in our previous study when given for 10 days.⁽⁷⁾

In view of this the present study was undertaken to compare the antidepressant activity of ethanol extract of

Areca catechu in Swiss albino mice when given as single dose as a part of acute study.⁽⁸⁻¹⁰⁾

Materials and Methods

This study was conducted in the Department of Pharmacology, A.J Institute of Medical Sciences, Mangalore, after getting approval from the Institutional Animal Ethical Committee. Adult Swiss albino mice (either sex) weighing 20-25 grams inbred in the central animal house of A.J Institute of Medical Sciences were used for the study. Mice were housed in clean polypropylene cages containing six mice in each cage, kept under a controlled environment (260-280c) with a 12 hour light and dark cycle. They were fed with commercial pellet chow and water *ad libitum*. The animals were acclimatized for these conditions for one week. Experiments were performed during the light phase of the cycle (10:00-17:00). All animals were treated and handled according to committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines.

Drugs and treatment:

1. Ethanol extract of areca catechu which was obtained using soxhlet apparatus and was procured in Srinivas College of Pharmacy.
2. Tablet Imipramine (Torrent pharmaceuticals) at the dose of 10 mg/kg, intraperitoneally (0.1ml/10g). 25mg tab dissolved in 25 ml of distilled water (1mg/1 ml concentration).

Study design: For the first phase of the study, animals were divided into five groups, comprising six mice in each. Group 1 was pre-treated with normal saline (0.1 ml/10 g) which served as control. Groups 2 were pre-treated with imipramine at the dose of 10 mg/kg. Group 3, 4 and 5 were pre-treated with three different doses (40, 80, and 160 mg/kg) of ethanol extract of *Areca catechu*.

Behavioural despair test: (Porsolt test or forced swim test)

This animal model is based on the principle that mice forced to swim in restricted space from which they cannot escape leads to characteristic behaviour of immobility. This behaviour reflects a state of despair, which can be reduced by several agents that are therapeutically effective in human depression.

Equipment: 5-litre glass beakers

Drug administration: For acute testing the drugs were given intra-peritoneal, 30min before the experiment.

Procedure: The duration of immobility in the forced swim test in male mice is measured to evaluate the antidepressant potential of compounds. After 30 minutes of drug administration, mice were placed individually in the 5L glass beakers, filled to a height of 15 cm with water (room temperature) and the duration of immobility was recorded during the final 4 minutes in a duration of 6 minutes test. A mouse was considered immobile when floating motionless or making only those movements necessary to keep its head above water surface. The water was changed after each experimental test. Antidepressants are known to decrease the immobility time. The test has been validated by most current types of antidepressants.

Tail suspension test in mice: This test is a variant of the behavioural despair test where immobility will be induced by simply suspending a mouse by tail. Mice provide better results than rats. This model for testing antidepressant activity is based on the principle that suspending mice suspended upside down leads to behaviour of continuous immobility after initial momentary struggle. This behaviour reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. In acute treatment, test was performed after 15 minutes of drug administration and in chronic treatment, on day 10 of treatment tail suspension test was conducted after 15 min of drug administration. Mice were suspended on the metal rod stand 50-75 cm above the table top by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was considered during 8 min period. Animal was considered to be immobile when it did not show any movement of body and hanged passively. A decrease in the immobility period is indicative of antidepressant like activity.⁽¹¹⁾

Statistical Analysis

Mean duration of immobility for each group was calculated. The data was presented as mean + SD. Comparison between experimental and control group was performed by one-way ANOVA. p value less than 0.05 was considered as significant.

Results

Table 1: Effect of acute treatment of *Areca catechu* on duration of immobility in forced swim test

Groups	Treatment (dose in mg/kg)	Duration of immobility in seconds (mean \pm SD)	p value of different groups when compared with the control
1	Control (normal saline), i.p	93.83 \pm 37.15	
5	Imipramine (10 mg/kg) , i.p	***25.5 \pm 6.41	0.001
2	<i>Areca catechu</i> (40 mg/kg) , i.p	*83.17 \pm 38.76	0.949
3	<i>Areca catechu</i> (80 mg/kg) , i.p	**39.83 \pm 12.32	0.009
4	<i>Areca catechu</i> (160 mg/kg) , i.p	*81.83 \pm 13.76	0.924

Observations are mean \pm SD. ANOVA followed by Dunnett's test.

*p>0.05- Not Significant, **p<0.01-Significant, ***p<0.001-Highly Significant,

AC- *Areca catechu*, i.p-Intraperitoneal, Dose-0.1ml/10gm

Areca catechu ethanol extract at the dose of 80mg/kg has shown significant antidepressant action by forced swim test in Swiss albino mice (Table 1 & Fig. 1).

Table 2: Effect of acute treatment of *Areca catechu* on duration of immobility in tail suspension test

Groups	Drugs / Dose	Duration of immobility in seconds (mean \pm SD)	p value of different groups when compared with the control
1	Control (normal saline 0.3ml/kg), p.o	206.83 \pm 33.31	
2	Imipramine (10mg/kg), i.p	***103.83 \pm 12.17	0.0001
2	<i>Areca catechu</i> (40 mg/kg), i.p	***147.50 \pm 27.97	0.001
3	<i>Areca catechu</i> (80 mg/kg), i.p	***117 \pm 18.08	0.0001
4	<i>Areca catechu</i> (160 mg/kg), i.p	*173.83 \pm 15.48	0.122

Observations are mean \pm SD. ANOVA followed by Dunnett's test.

*p>0.05- Not Significant, **p<0.01-Significant, ***p<0.001-Highly Significant,

AC- *Areca catechu*, i.p-Intraperitoneal, Dose-0.1ml/10gm

Areca catechu ethanol extract at the dose of 40 and 80mg/kg has shown significant tail suspension test in Swiss albino mice (Table 2 & Fig. 2).

Discussion

The present study was carried out to evaluate the antidepressant activity of *Areca catechu* in two different models of depression in animals. Both forced swim test and tail suspension tests are standard animal models with predictive of antidepressant activity. Since their introduction almost 20 years ago, the tail suspension test and forced swim tests have become the most widely used animal models for evaluating antidepressant activity in mice. These models were based on the fact that animals subjected to the short-term, inescapable stress of being suspended will develop an immobile posture. Indeed the sensitivity of these models to a broad range of antidepressants drug is the most important feature supporting its use in drug discovery of novel antidepressants. Even though rodent behavioural models have a good predictive validity for antidepressants and they are sensitive to the acute administration for these compounds, it is widely recognized that the symptoms of depression in patients

are only reduced after chronic drug treatment. Therefore, we decided to check whether the acute effects of antidepressants in the forced swim test and tail suspension tests are also effective.

The present study conclusively shows that *areca catechu* has significant antidepressant activity which was comparable with standard antidepressant drug imipramine. Our results confirm the literature data, by showing that *areca catechu* reduces the immobility time in both the models used. Antidepressant like effect of *areca catechu* in the forced swim test in mice has been reported.

The administration of *Areca catechu* produced a decrease in immobility time in both the models, a response that is consistent with an antidepressant like action. In forced swim test model, *Areca catechu* has shown significant antidepressant activity at the dose of 80mg/kg which was comparable with Imipramine 10mg/kg. In the tail suspension model, *Areca catechu* has shown significant antidepressant activity in 80 &

160mg/kg used in our experiment. The results of the present study were comparable with previously published data.⁽¹²⁾

However the molecular mechanism has to be still evaluated by comparing Imipramine as well as with other antidepressant drug.

Conclusion

Acute treatment with *Areca catechu* ethanol extract at the dose of 80mg/kg and at the dose of 40 and 80mg/kg has shown significant antidepressant action by forced swim test and tail suspension test respectively.

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