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Research Article

The Antidepressant like action of ethanolic extract of areca catechu on behavioral models of depression in rats

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ABSTRACT

Background: The objective was to investigate the anti-depressant like activity of areca catechu nut ethanolic extract (ACEE) using behavioural tests in rats.
Methods: Forced swim test (FST) and tail suspension test (TST) were used to assess the anti-depressant like effect of ACEE rats. Motor coordination was also assessing using rota-rod test in rats to see generalised CNS depression. Fluoxetine was the reference standard drug. Rats were divided into four groups with six rats in each group namely control group, fluoxetine 10 mg/kg group, ACEE 50 mg/kg group and fluoxetine 5 mg/kg plus ACEE 25 mg/kg. All treatments were administered orally.
Results: The areca nut ethanolic extract (ACEE) (50mg/kg oral) exhibited anti-depressant like activity i.e. decrease the duration of immobility time (sec) in acute forced swim test (FST) and in acute tail suspension test (TST) in rats (104±1.7, 95%CI 99.65 to 108.4, p <0.01) Vs control and (136.3±1.94, 95%CI 131.3 to 141.3, p<0.01) Vs control respectively. ACEE in low dose of 25 mg potentiated the anti-depressant activity of low dose fluoxetine 5 mg/kg in both the test 102.3±2.60, CI 95.64 to 109.0 p<0.01) Vs control. The ACEE did not produce motor incoordination in rats.
Conclusions: The results of present study suggest that the areca catechu nut ethanolic extract 50mg/kg possess potential anti-depression like effect without generalized CNS depression. Further studies are needed to confirm this.

Keywords: Areca catechu ethanolic extract, Fluoxetine, Forced swim test, Tail suspension test, Anti-depression activity, Immobility time (sec)

INTRODUCTION

The Major depression is a serious chronic mental illness with pessimism and low self-esteem. There occur functional deficiency of monoamine neurotransmission viz. noradrenaline, 5-HT, and dopamine. The known anti-depressants (TCA/MAO-I, SSRI, SNRI) facilitate monoamine neurotransmission (monoamine theory).[1] Other causes underlying major depression are under investigations. Anti-depressant drugs show side effect and effectiveness is limited, 30% may not respond. Hence the search for new anti-depressant without side effect is important.[9] Currently there is increasing research on traditional AYUSH system of medicine.[7,11] Traditional medicines on the basis of their known efficacy observations traditionally are considered safe and free from adverse effect and toxicity unlike allopathic medicines. Efforts are underway to develop natural herbs for their anti-depressant use.[8] India has reach herbs used traditionally for various ailments. Few have been explored by various researchers for anti-depressant activity. Areca catechu has been anecdotally reported for anti-depressant action.[9] Hence the aim of present study was to investigate anti-depressant activity of areca catechu nut in experimental rats.

METHODS

The experimental protocol was approved by IAEC (registration no.805/03/ca/CPCSEA).

Areca catechu nuts were collected locally and authenticated by local botanist.

a) ***Preparation of ethanolic extract:*** areca nuts (betel nuts) were crushed into fine powder. The powder was subjected to hot percolation using ethanol (1:4). The extract then passed through filter paper 1. Ethanol was evaporated by heating, drying was done in desiccator.

b) ***Rats and housing***: Male Sprague Dawley rats (150-250gm) were obtained from rats house facility of savangi meghe, Maharashtra. They were kept at (25 ± 1ºC), 12 hour light / dark cycle. Food and water were provided ad libitum. All experiments were performed as per CPCSEA guidelines between 9am to 5pm. All treatments drugs or chemical or vehicle were administered orally in rat. Control was tween 80.

- Total 24 rats were divided into 4 groups. In each group there were 6 rats.

c) ***Chemicals/drugs:*** Chemicals/drug was purchased from local market.

- Fluoxetine - Sun Pharma
- Dosing - doses for anti-depressant activity were selected from previous studies. Fluoxetine (10 mg/kg), Areca catechu ethanolic extract (ACEE) (50 mg/kg).[1]

d) ***Methods:***

- *Rota-rod test:* The effect on motor co-ordination was assessed using rota-rod individually in rats [17]. Rats were trained to remain on rota-rod for 3 minutes at speed 25 rpm. On the day of experiment either vehicle or test drug was administered orally, the ability of rats to remain on rota-rod was assessed 30 min after. Fall time was noted.
- *Forced swim test (FST):* (Porsolt et al l1977).[3] The Experiment was conducted as per the method previously described by porsolt et al.[3] Rats were placed in glass tank (15 X 15 X 50 cm) filled with water (at 23-25ºC) up to a level of 15 cm. Treatment was given 1 hour prior to test session of .6 min. Total duration of immobility time (in seconds) in 6 min was counted. In each rats immobility time (sec) i.e. rats remain static or making passive movements to keep its head above water level for 4 min were recorded after initial 2 min.
- *Tail suspension test (TST):* (Steru L et al.).[4] The test was performed according to method described by Steru L et al. Each rat was suspended by adhesive tape to a flat surface placed approximately 1 cm from tail tip and 50 cm below surface. Immobility time in seconds was measured for 6 min. Immobility was considered when the rats hung passively motionless without limb movement.

e) ***Statistical analysis***: The values were expressed as mean±SEM. The difference between various mean were tested by one way ANOVA followed by Dunnet multiple comparison test. P value <0.05 was considered statistically significant. Analysis was done by using graph pad prism version 5.

RESULTS

The rats treated with control/fluoxetine/ACEE/ fluoxetine + ACEE remained on rota-rod for 3 min. The above treatments did not induced motor in-coordination versus control (p > 0.05). Results are summarised in Figure 1.

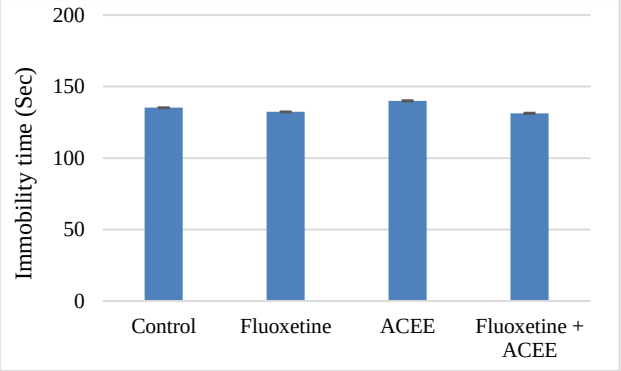

Figure 1: Effect of various treatments on rota-rod test in rats.

Results are summarised in Table1 and Figure 2.

Table 1: Forced swim test (FST).

Groups	Dose (mg/kg)	Immobility time (s) (M±SEM)	95% CI	p value
Control / Tween 80	1ml	144.7±17.35	100.1 to 189.3	
Fluoxetine	10	81.83±1.7	77.36 to 86.31	***
ACEE	50	104±1.7	99.65 to 108.4	**
Fluoxetine + ACEE	5 + 25	102.3±2.6	95.64 to 109	**

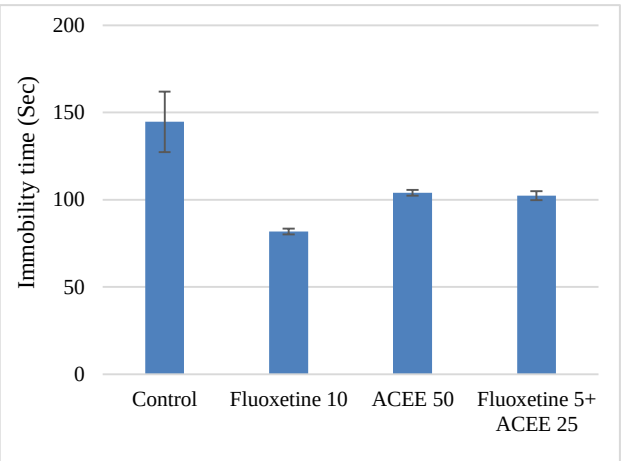

Figure 2: Effect of various treatments on Forced swim test (FST) in rats.

Effect of fluoxetine and ethanolic extract of areca catechu (oral) on immobility time (sec) in FST in rats <30 min Vs tween 80. Data are M ± SEM. Statistical analysis- one way ANOVA followed by post hoc dunnet test. **p <0.01, *** P <0.001 vs control; n= 6.

Results are summarised in Table 2 and Figure 3.

Table 2: Tail suspension test (TST).

Groups	Dose (mg/kg)	Immobility time (s) (M±SEM)	95% CI	p value
Control / Tween 80	1ml	142.8±2.55	136.3 to 149.4	
Fluoxetine	10	118.3±4.716	106.2 to 130.5	***
ACEE	50	136.3±1.94	131.3 to 141.3	**
Fluoxetine + ACEE	5 + 25	102.3±2.60	95.64 to 109	**

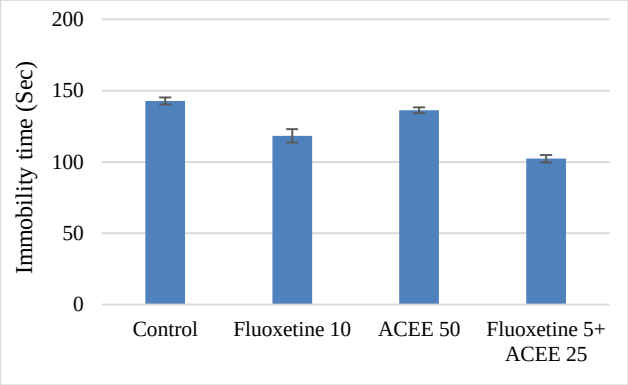

Figure 3: Effect of various treatments on tail suspension test (TST) in rats.

Effect of fluoxetine and ethanolic extract of areca catechu on immobility time (sec) in TST in rats <30min Vs tween 80. Data are M ± SEM. Statistical analysis -one way ANOVA followed by post hoc dunnet test. ** P <0.01, *** P <0.001 versus control. n = 6.

FST

The reference compound, anti-depressant fluoxetine (10 mg/kg) statistically significantly reduced immobility time (s) (81.83±1.7, CI 77.36 to 86.31, p <0.001) compared to control in rats. The efficacy was 22.73%.

ACEE at dose (50 mg/kg) in rats statistically significantly reduced immobility time (s) (104±1.7, CI 99.65 to 103.4, p <0.01) compared to control. The action was less than fluoxetine 10 mg/kg. The efficacy was 28.88%.

Combination of low dose fluoxetine 5 mg/kg and low dose ACEE 25 mg/kg statistically significantly reduced immobility time (s) (102.3±2.6, CI 95.64 to 109, p <0.01) compared to control in rats. The efficacy was 28.41%.

Low dose ACEE potentiated the action of low dose of fluoxetine implying that ACEE has anti-depressant like action which is less than fluoxetine.

TST

The reference compound, anti-depressant fluoxetine (10 mg/kg) statistically significantly reduced immobility time (s) (118.3±4.716, CI 106.2 to 130.5, p <0.001) compared to control in rats in TST test. The efficacy was 32.86%.

The ACEE at dose (50 mg/kg) in rats statistically significantly reduced immobility time (s) (136.3±1.94, CI 131.3 to 141.3, p <0.01) compared to control. The action was less than fluoxetine 10 mg/kg. The efficacy was 37.86%.

The Combination of low dose fluoxetine 5 mg/kg and low dose ACEE 25 mg/kg statistically significantly reduced immobility time (s) (102.3±2.6, CI 95.64 to 109, p <0.01) compared to control in rats. The efficacy was 28.33%. Low dose ACEE potentiated the action of low dose of fluoxetine, showing that ACEE has anti-depressant like action which is lower than fluoxetine.

DISCUSSION

The Results of present study proved that ACEE has anti-depression like action as it significantly reduced immobility time in animal models, which was lower to fluoxetine (FST) without motor incoordination proving that it is not a generalised CNS depressant. Two classical rat models (FST and TST) were used to evaluate antidepressant like effect of ethanolic extract of areca catechu nut.

Efficacy of fluoxetine 10mg/kg (a standard anti-depressant) on immobility time (sec) in FST was significant (81.83±1.7, 95% CI 77.36 to 86.31, p < 0.001) Vs control. The immobility time (sec) was lower than that of fluoxetine.

The FST was commonly used and validated test to assess anti-depressant like effect of AECC in previous studies [9]. In the present study also areca nut ethanolic extract (ACEE) (50mg) induced significant decline in immobility time (sec) in rats (104±1.7, 95%CI 99.65 to 108.4, p <0.01) Vs control, exhibiting potential anti-depressant action (Figure 2). This is in agreement with earlier reports but they got improved results on these tests using aqueous extract of areca catechu.[16] At dose of 50mg of ACEE however there was significant difference in reducing immobility time (sec) between fluoxetine and ACEE, indicating that the efficacy of fluoxetine was more than ACEE. The combination of fluoxetine and ACEE in low dose of 5mg and 25mg respectively potentiated the activity (immobility time (sec) 102.3±2.60, 95% CI 95.64 to 109.0, p < 0.01) Vs control suggesting that fluoxetine and ACEE may be working by different mechanisms.

Similar results were noted on TST. Fluoxetine 10mg/kg showed highly significant reduction in immobility time Vs control (142.8±2.55, 95% CI 136.3 to 149.4 p<0.001).

ACEE also showed significant reduction (136.3±1.94, 95%CI 131.3 to 141.3, p <0.01) Vs control but lower than Fluoxetine 10 mg/kg. Low dose of fluoxetine and ACEE potentiated the activity significantly (102.3±2.60, 95.64 to 109.0 p <0.01) Vs control.

The alkaloid of arecholine has been considered as active principle underlying most of the biological actions of areca catechu nut. Arecholine is choline esterase inhibitor with established efficacy in Alzheimer dementias, the hallmark of Alzheimer's disease is reduced cognitive behaviour.[2] Arecholine slows down improper memory. Areca catechu nut is definitely not a CNS stimulant.[15] Arecholine act on multiple muscarinic receptors (M_1, M_2, M_3, and M4). Arecholine improves memory in healthy volunteer.[4] Arecholine slows down improper memory (dementia) but is carcinogenic [13, 14]. Other alkaloids contained in areca nuts are pyridine, piperidine, guvacine, and guvacholine, the later showed antidepressant activity in experiments.[5,8] Areca catechu nut is definitely not a CNS stimulant.[15] Exactly which alkaloid is antidepressant and whether acetylcholine got any role underlying depression is unknown.

Pathophysiology of major depression is complex, not fully understood.[2] Many neurotransmitters are involved in depression.[26] Monoamine theory of depression says that monoamines are low in brains of major depression.[2] Fluoxetine increases serotonin levels in brain by selective serotonin reuptake inhibition.[10] Areca nut ethanolic extract may have similar effect on monoamine.[6] Dang 2009 reported natural saponin has anti-depressant action by increase NA, increase 5-HT), MAOI.[7] In the study by Ghulam Abbas et al using hippocampal slices, areca nut caused significant elevation of serotonin and noradrenaline proving this mechanism behind its antidepressant like action.

However further more elaborate investigations are required to confirm the exact mechanism of action ACEE.

CONCLUSION

Our study confirmed that areca catechu ethanolic extract has anti-depressant like effect as it statistically significantly reduced immobility time (sec) in forced swim test (FST) and in tail suspension test (TST) without impairment in motor coordination in rats can be attributed to enhanced serotonin and noradrenalin levels in brain. Though further studies needed.

Limitations

Anti-depressant effect was seen after acute tests. Conventional anti-depressant take 2-3 weeks to act, hence effect after chronic treatment is needed to be studied using chronic models of depression. Levels of different alkaloids contained in areca catechu nut are needed to be estimated to study their anti-depressant effect.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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