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Antidepressant Activity of *Alocasia Macrorrhizos* on Mice

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ABSTRACT

Currently there is considerable interest in the development of drugs from natural products for various diseases. The purpose of this study was to evaluate the antidepressant activity of hydroalcoholic extract of leaves of *Alocasia macrorrhizos*(AM), which is known to have anti-oxidant activity. In this study, mice were randomly assigned to four groups of six mice each. Group 1and 2 served as control and standard where as group 3 and 4 were treated with hydro alcoholic extract of *Alocasia macrorrhizos* at the doses of 250mg/kg and 500mg/kg respectively. Drugs were suspended in 1% gumacasia and administered to mice orally one hour before test procedure. After 1 hour mice were screened for its antidepressant property using two well to established models for antidepressant screening like Forced swim test and Tail suspension test. Study result showed that *Alocasia macrorrhizos* at 250mg/kg and 500mg/kg significantly reduced immobility duration in both the models when compared to control group showing its antidepressant property. Further when these groups were compared to standard imipramine there was significant difference in immobility duration with AM 500mg/kg where as AM 250mg/kg values were comparable to imipramine. Hence we have concluded that hydroalcoholic extract of leaves of *Alocasia macrorrhizos* is having antidepressant property which was comparable to Imipramine at 250mg/kg and significantly better results than imipramine at 500mg/kg dose.

Keywords: *Alocasia macrorrhizos*, FST, TST, imipramine

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INTRODUCTION

Major depression which is one of leading cause of disease worldwide reflects the extremes of affective disorder which refers to pathological changes in mood state[1]. It affects a person's mood, physical health and behavior. Patients with major depression have symptoms that reflect changes in brain monoamine transmitters, specifically norepinephrine, serotonin and dopamine[2]. Despite the advent of new molecules in the pharmacotherapy of depression, it is unfortunate that this disorder still goes undiagnosed and untreated. Although the currently prescribed molecules provide some improvement in the clinical condition of the patient, it is at the cost of having to bear the burden of their adverse effects[3]. The use of alternate medicines is increasing worldwide day by day in various diseases. Hence, finding newer and safer therapeutic agents would benefit the existing treatment modalities.

Alocasia macrorrhizos commonly known as elephant ear taro or giant taro is an indigenous herb belonging to the family Araceae. They are naturally grown on marshy land of tropical area in India. Bangladesh, China. It plant were used traditionally in inflammation[4]. The juice of leaves of the plant is used as a digestive, laxative, diuretic, astringent, antifungal agent and for the treatment of rheumatoid arthritis. The leaves of this plant are used to prevent iron deficiency, to enhance eye sight and as a good source of protein. Also the whole plant is used for jaundice and constipation[5].

Leaf extract of *Alocasia macrorrhizos* is proven to have anti oxidant[6], antinociceptive, anti-inflamattory[7], laxative, diuretic[8]and hepato protective[9] properties. Rhizome extract has shown antihyperglycemic,antioxidant and cytotoxic activity in various studies[10].

Hence we hypothesized that the natural compound *Alocasia macrorrhizos* having multiple pharmacological actions may also have antidepressant property and the hydroalcoholic extract of leaves of this plant has proven to have antioxidant properties. Since antioxidants are taken as a newer modality for the treatement of depression, this study was undertaken to evaluate the antidepressant property of *Alocasia macrorrhizos* in rodents.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Alocasia macrorrhizos*(Araceae) collected from different places at Mangalore were identified and authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poornaprajna College,Udupi, and a voucher specimen was deposited at the Herbarium of institute.

Preparation of Extracts (by cold maceration method) [11]:

Fresh leaves of *Alocasia macrorrhizos*were shade dried for about ten days and homogenized to get a coarse powder. Powder (500gm) was extracted with ethanol (99%) and distilled water in 1: 1 proportion at room temperature by cold maceration method. The filtrate was collected and concentrated on a heating mantle at 45 ˚C till a syrupy consistency was obtained. The extract was successively dried by using a rotary evaporator and preserved at <0˚C.The percentage yield of the extract was found to be 6.75%.

Experimental Animals:

Swiss albino mice of either sex, inbred in the institutional animal house were used for the study. Total of 48 mice were taken in to the study in which 24 were used for forced swim test which was divided in to 6 mice of 4 groups. Rest 24 was used for tail suspension test which was also divided into 6 mice of 4 groups. Mice were housed in clean polypropylene cages, six mice in each cage, in a controlled environment (24-26^{0}C) with a 12 hour light and dark cycle with standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4%. The mice were allowed to acclimatize to these conditions for one week. The experiment was performed during the light phase of the cycle (10:00-17:00hours). The animals were maintained as per the CPCSEA guidelines and regulations. The study was approved by the Institutional animal ethics committee.

Study procedure

Mice were randomly assigned to 4 groups of 6 mice each. Grouping and treatment schedule for each group is as shown in table 1.

Table1: Drug treatment schedule

	Group	Drug treatment
Group I	Control	gumacasia 10ml/kg p.o
Group II	standard	Imipramine 15mg/kg p.o[12]
Group III	test 1	*Alocasia macrorrhizos* (AM) hydroalcoholic extract 250mg/kg[6]
Group IV	test II	*Alocasia macrorrhizos* (AM) hydroalcoholic extract 500mg/kg[6]

All the drugs were suspended in 1% gumacasia and given orally once daily for all the animals

Evaluation of antidepressant activity

Forced swim test[13]

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

Respective drugs were administered to mice orally one hour before the test procedure. After one hour each animal was individually forced to swim inside a vertical Plexiglas cylinder (height 50 cm, diameter 20cm) containing water column of 15 cm of height. After an initial two-minute period of vigorous activity, usually each animal assumes a typical immobile posture. A mouse was considered immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 minute of the total six minutes of the duration of test[14].Durations of immobility period were compared with those of control and standards.

Tail Suspension Test in Mice[13]

This test is a variant of the behavioral despair test in which immobility is induced by simply suspending a mouse by tail. This test is reliable and rapid screening method for antidepressants, including those involving serotonergic system. This animal model for testing antidepressant activity is based on the principle that suspending mice upside down leads to a characteristic behavior of immobility after initial momentary struggle. This behavior reflects a state of despair, which can be reduced by several agents that are therapeutically effective in human depression[17].

Respective drugs were administered to mice orally one hour before the test procedure. In each animal tail suspension test was conducted after one hour 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 recorded during 8 min period. The immobility during the first two minute due to vigorous activity is not taken into account. Animal was considered to be immobile when it did not show any movement of body and hanged passively[14]

Statistical Analysis:

All results are expressed as Mean±SE. All the groups will be analyzed using one way ANOVA followed by Dunnets multiple comparison test. Data was analyzed using SPSS Software version 17. $P< 0.05$ was considered statistically significant

RESULTS AND DISCUSSION

Forced swim test

In forced swim test as shown in the table 2 control group showed immobility for the duration of 110.83±12.595 sec in a total duration of 4 minutes. Standard group of animals showed immobility for the duration of 36.50±12.748 sec which was significantly different from the control group ($p<0.001$). Whereas group 3 animals which were treated with AM at the dose of

250 mg/kg didn't not show any significance difference when compared to standard group, but both group values were comparable, total duration of immobility being 19.67±9.404 sec but these values were significantly different when compared to control group ($p<0.001$). Whereas *Alocasia macrorrhizos* treated animals at the dose of 500 mg/kg showed significance difference when compared to both control and standard group.

Table 2: Effect of *Alocasia macrorrhizos* on Forced Swim Test (FST)

Group	Duration OF Immobility (sec)
Control	110.83± 12.595
Standard	36.50±12.748[a]
Test 1 (AM-250)	19.67±9.404[a]
Test 2 (AM-500)	3.00±2.049[a,b]

Data expressed as mean ± SEM (Standard error of mean). [a]$p<0.001$ compared to group control, [b]$p<0.05$ compared to group standard

Tail Suspension Test

As seen in the table 3 there was a significance difference between control and standard group in total duration of immobility i.e 228.50± 18.749 sec and 139.00± 8 sec in control and standard group respectively.

Table 3: Effect of *Alocasia macrorrhizos* on Tail Suspension Test (TST)

Group	Duration of Immobility (sec)
Control	228.50± 18.749
Standard	139.00± 8.274[a]
Test 1 (AM-250)	143.00± 7.317[a]
Test 2 (AM-500)	106.17± 10.339[a,b]

Data expressed as mean ± SEM(Standard error of mean). [a]$p<0.001$ compared to group control, [b]$p<0.05$ compared to group standard

Where as in case of *Alocasia macrorrhizos* treated mice the low dose group ie which was treated with 250mg/kg of AM showed immobility for the duration of 143.00± 7.317 sec. These results were significant when compared to control group ($p<0.001$) but when compared to standard these were insignificant but the values were comparable to standard

Alocasia macrorrhizos at the dose of 500mg/kg showed a significance difference in total duration of immobility ($p<0.001$ v/s control $p<0.005$ v/s standard) when compared to both control and standard.

Above mentioned results show that hydroalcoholic extract of leaves of *Alocasia macrorrrhizos* has antidepressant property. At the dose of 500mg/kg it has shown significantly better results than standard Imipramine.

Oxidative stress represents a loss of balance in oxidation – reduction reaction. It is characterized by reduced ability of the antioxidant defense system to efficiently eliminate the excess of oxygen derived species production, eliciting the toxicity of oxygen and its detrimental effects. Increased oxidative stress is seen in patients suffering from depression[16]. N-acetylcystiene has been tried as a newer modality for treatment of depression with encouraging results[17]. Our test drug *Alocasia macrorrhizos* is recorded among cultivated medicinal as well as vegetable plants by the folklore of south Asia. This plant is proven to have many pharmacological actions including antioxidant properties[6]. Phytochemical screening of this plant has showed that it contains flavonoids, cynogenetic glycosides, ascorbic acid, gallic acid, mallic acid, oxalic acid, alocasin, amino acids, succinic acid, and β-lectines[15.] Hence the antidepressant activity of *Alocasia macrorrhizos* shown in our study may be because of its antioxidant property.

CONCLUSION

To conclude hydroalcoholic extract of *Alocasia macrorrhizos* has antidepressant property especially at the dose of 500mg/kg where it has shown significantly better result than standard Imipramine. Further studies are required to find out exact mechanism of action of this drug producing antidepressant property which may be because of antioxidant property of drug or any other mechanism. So results of this study guides further future studies to establish the mechanism of action of this drug.

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