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Research Article

Phytochemical Analysis and Antidepressant activity of Ethanolic Extract of *Amaranthus caudatus*

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ABSTRACT

Amaranthus caudatus belongs to the family Amaranthaceae, are spread throughout the world growing under a wide range of climatic conditions. It has been widely used in Mexican traditional medicine for the treatment of different central nervous system disorders. Nevertheless, the available scientific information about this species is scarce and there are no reports related to its possible effect on the CNS. In this work, the effects of ethanolic seed extract of *Amaranthus caudatus* were evaluated in mice using behavioral tests sensitive to clinically effective antidepressant compounds. The extract (100 and 200mg/kg), administered intraperitoneally, was able to decrease the immobility time of mice dose dependently when subjected to both tail suspension and forced swim tests and the effects are comparable to that of standard drugs fluoxetine (20mg/kg). Preliminary phytochemical analysis showed the presence of phenolic compounds, steroids, alkaloids, flavonoids, saponins and amino acids in ethanolic extract of *A. caudatus*. It is concluded that *A. caudatus* possess antidepressant activity may be due to the presence of polyphenolic compounds and flavonoids. However further study is needed to understand mechanism of action and to identify active component responsible for antidepressant activity.

INTRODUCTION

According to the World Health report, approximately 450 million people suffer from a mental or behavioural disorder. This amounts to 12.3% of the global burden of disease and will rise to 15% by 2020. ^[1,2] Psychiatric illness is also

often associated with suicide and there are between 10 and 20 million suicide attempts every year. Depression is one the most prevalent forms of psychiatric disorders and is a leading cause for mortality. ^[3] Lifetime prevalence of depression in

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the general population is 4.4- 20% and suicides occur is up to 15% of individuals with severe major depression. [4] Therefore, major depression is a serious public health problem and causes a considerable heavy psychological and economic burden for families. [5] In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline reuptake inhibitors (SNRIs), depression continue to be a major medical problem. [6] Basic neuroscience offers the promise of improving our understanding of disease pathophysiology, identifying novel mechanisms that can be targeted by more effective pharmacotherapies and screening of herbal sources of drugs. *Amaranthus caudatus* was investigation for antidepressant activity.

Amaranthus caudatus Linn, (Amaranthaceae), are spread throughout the world, growing under a wide range of climatic conditions and they are able to produce grains and leafy edible vegetables. [7] *A. caudatus* traditionally used in jaundice, amoebiasis, kidney diseases, [8,9] as blood purifier, diuretic, abortifacient, vermifuge and astringent. [10] *A. caudatus* is reported for its antiatherosclerotic, [11] hepatoprotective, [12] antihelmintic, antinociceptive and antipyretic activities. [13] *A. caudatus* seeds showed cholesterol lowering, *in vitro* antioxidant and alpha amylase inhibition activities. The Amaranth seed oil is used as nutraceutical resource from Ecuadorian flora. [14] *A. caudatus* contains antimicrobial peptides, agglutinin, triterpenoid saponins and ionol derived glycoside, vitamin E isomers, and amaranthin. [15,16] The main aim of the study is to investigate antidepressant activity in seed extract of *A. caudatus*.

MATERIALS AND METHODS

1 Collection of plant material

Amaranthus caudatus seeds was collected from the local market in the state of Madhya Pradesh District Bhopal during the Month of May. The seeds has been identified and authenticated by Dr. Saba Naaz Head of the Department Botany at the Safia college of science, Bhopal (M.P.). The plants parts were dried under shade. It was pulverized to coarse powder with the help of mixer grinder. The coarse powder was passed through sieve No. 20 to maintain uniformity and packed into airtight container and stored in cool and dry place. This material was used for the further study.

2 Preparation of *Amaranthus caudatus* Seeds extract

Extraction of *Amaranthus caudatus* was done by maceration method. Maceration Extraction method using 90% ethanol for extraction. Extract obtained was passed through the Whatman filter paper No.1 and the ethanol was evaporated (at 40°C) with the help of heating mantle and dried in a desiccator. Calculated percentage yield of the extract was 8.6%.

3 Preliminary phytochemical screening

The ethanol extract of *A. caudatus* was screened for the presence of various phytoconstituents like flavonoids, saponins, glycosides, terpenoids, aminoacids, alkaloids, carbohydrates, phenolic compounds and proteins as described by Kokate. [17]

4 Animals

Male or female Swiss albino mice weighing between 20 – 25 g were used for the present study. The animals were maintained under standard environmental conditions (25 ± 2° C and relative humidity of 45 to 55%) and were fed with standard pellet diet and water *ad libitum*. The study was approved by Institutional Animal Ethics Committee. CPCSEA guidelines were adhered during the maintenance and experiment.

5 Acute oral toxicity study

Acute oral toxicity study was evaluated as per OECD guidelines (425) on albino mice. Before



experimentation mice were fasted overnight with water ad libitum. Three animals were selected which receives dose of 2000 mg/kg. ^[18] All three animals were received dose of 2000 mg/kg body weight of ethanolic extract of plants extract + 1% aqueous CMC by gavage using oral cannula (limit test). Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 24 hours, with special attention given during the first 4 hours, and thereafter, 24 hours, administered dose was found tolerable (as no death found). Therefore, two dose levels 250, and 350 mg/kg was selected for CNS depressant activity.

6 Antidepressant activity

Antidepressant activity of EEAC was assessed using Forced swimming test and Tail Suspension Test.

6.1 Forced swimming test

Assessment of antidepressant activity using forced swimming test in mice. Swiss Albino mice weighing around 20 to 25 gm were used for this study. They were divided into four groups each group consist of 6 animals (n=6). Each animal was placed individually in a 5 liter glass beakers, filled with water upto a height of 15 cm and were observed for duration of 6 minutes. The duration of immobility was recorded during the last 4 minutes of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface. The water was changed after each test. A decrease in the duration of immobility is indicative of an antidepressant like effect. ^[19]

Group 1 Received - Normal saline (5ml/kg P.o)

Group 2 Received - Fluoxetine (20mg/kg P.o) (Standard)

Group 3 Received - EEAC 100mg/kg, P.o

Group 4 Received - EEAC 200mg/kg, P.o

6.2 Tail suspension test

Assessment of antidepressant activity using tail suspension test in mice.

Swiss Albino mice weighing around 20 to 25 gm were used for this study. They were divided into four groups each group consist of 6 animals (n=6). The animals were hung by the tail on a plastic string 75 cm above the surface with the help of an adhesive tape. The duration of immobility was observed for a period of 8 minutes. The duration of immobility was recorded during the last 6 minutes of the observation period. Mice were considered to be immobile only when they hung passively and were completely motionless. A decrease in the duration of immobility is indicative of an antidepressant effect. ^[20]

Group 1 Received- Normal saline (5ml/kg P.o)

Group 2 Received - Fluoxetine (20mg/kg P.o) (Standard)

Group 3 Received - EEAC 100mg/kg (t1)

Group 4 Received - EEAC 200mg/kg (t2)

7 Statistical analysis

All the values were expressed as Mean±S.E.M. The results were analyzed statistically by one-way ANOVA followed by Dunett Multiple comparison test, $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Depression is characterized by emotional symptoms such as hopelessness, apathy, and loss of self-confidence as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances and loss of appetite. Preliminary phytochemical analysis showed the presence of carbohydrates, steroids, alkaloids, phenolic compounds, flavonoids, saponins and amino acids in ethanolic seed extract of *A. caudatus* (Table 01). The study was conducted to assess the antidepressant activity of EEAC using FST and TST. Exposure to stress place an important role in depression. FST and TST models of depression which provides a rapid and reliable behavior screening test for anti- depressants.



The present study showed the effect of ethanolic seed extracts of *Amaranthus caudatus* on Antidepressant activity was Forced Swim Test in mice. The immobility period of mice in water is the parameter measured. The results show that Test Group III (100mg/kg) produced less immobility period while Test Group IV (200mg/kg) showed a significant immobility time compared to standard group (Fluoxetine – 20mg/kg). The % increase of immobility time in Group III, Group IV & standard groups are 47%, 65% and 71%. (Table no. 02)

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produced less immobility period while Test Group IV (200mg/kg) showed a significant immobility time compared to standard group (Fluoxetine – 20mg/kg). The % increase of immobility time in Group III, Group IV & standard groups are 69%, 78% and 88%. (Table no. 03)

In this study, treatment of EEAC decreased the duration of immobility in FST and in TST which reveals the antidepressant activity of EEAC. The presence of Phytoconstituents like saponins and flavonoids could attribute to the antidepressant activity of EEAC. Further studies would be necessary to evaluate the contribution of active chemical constituents for the observed antidepressant activity as it still remains to be determined which components were responsible for these effects.

Table 01: Phytochemical screening of Ethanolic extract of *Amaranthus caudatus*

S. No.	Identification Test	Test name	Results
1	Alkaloids	Mayer's test	+
		Dragendroff's test	-
		Wagner's test	+
2	Glycosides	Killer-killani test	+
3	Carbohydrate	Fehling test	-
4	Tannins & Phenols	Gelatine test	+
		Ferric chloride test	+
5	Flavonoids	Shinoda test	+
		Alkaline reagent test	+
6	Steroids	Libermann-Burchard test	-
		Salkowski test	-
7	Saponins	Foam test	+
8	Protein	Xanthoprotic	+

(+) Present, (-) Absent

Table 02: Effect of ethanolic extract of *Amaranthus caudatus* on Immobility Time in the Forced Swimming Test (FST) using mice

S. No	Treatment	Duration of immobility (seconds)		% Increase in immobility
		Day 1	Day 14	
1.	Group I (Normal saline -5ml/kg)	220.2±0.21	217.5±0.32	13 %
2.	Group II (Fluoxetine –20mg/kg)	56.3±0.31	52.4±0.48	71 %
3.	Group III (EEAC – 100mg/kg)	63.6±0.26	60.9±0.60	47 %
4.	Group IV (EEAC – 200mg/kg)	46.7±0.19	43.5±0.56	65 %

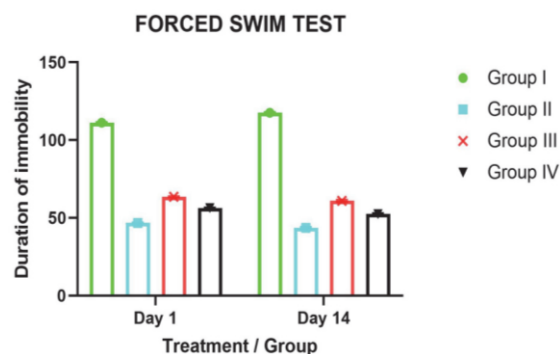
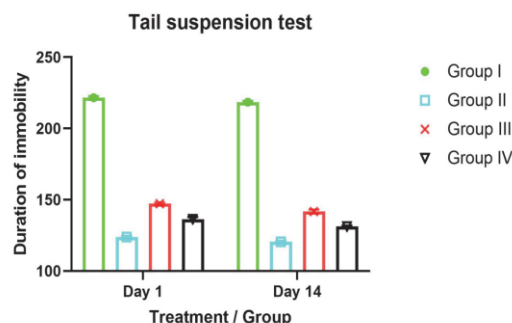
All values are expressed as mean ± S.E.M.; (n=6) animals in each group. **P<0.01



Table 03: Effect of ethanolic extract of *Amaranthus caudatus* on Immobility Time in the Tail Suspension Test (TST) using mice

S. No	Treatment	Duration of immobility (seconds)		% Increase in immobility
		Day 1	Day 14	
1.	Group I (Normal saline -5ml/kg)	221.6±1.06	218.4±0.96	13 %
2.	Group II (Fluoxetine 20mg/kg)	147.2±0.83	135.7±1.41	88 %
3.	Group III (EEAC-100mg/kg)	122.2±0.83	115.7±1.41	69 %
4.	Group IV (EEAC-200mg/kg)	123.3±1.45	114.2±0.71	78 %

All values are expressed as mean ± S.E.M.; (n=6) animals in each group. **P<0.01

**Figure 01: Graph of Effect of ethanolic extract of *Amaranthus caudatus* on Immobility Time in the Forced Swimming Test (FST) using mice****Figure 02: Graph of Effect of ethanolic extract of *Amaranthus caudatus* on Immobility Time in the Tail Suspension Test (TST) using mice**

CONCLUSION

Our study indicates that the Seeds of *Amaranthus caudatus* showed antidepressant activity. Further studies are required to know the phytochemical responsible for antidepressant activity.

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