ORIGINAL ARTICLE

Evaluation of antianxiety and antidepressant activity of aqueous extract of *Cynodon dactylon* (Doob grass) in Swiss albino mice

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Abstract

Objective: Anxiety and depression are common psychiatric conditions that cause changes in the lifestyle of humans and impose huge costs for the treatment to the patients. The present study was carried out to find antianxiety and antidepressant activity of aqueous extract of *Cynodon dactylon* (AECD) in mice. **Methods:** AECD was prepared by maceration method. The yield was 5% w/w. The AECD was screened for antianxiety activity by elevated plus maze (EPM) and light and dark box and for antidepressant activity by forced swim test (FST) and tail suspension test (TST) in mice. For each model, animals were divided in four groups of six animals. Group I served as control and received gum acacia aqueous suspension 10 ml/kg. Groups II and III served as test group and received AECD 200 and 400 mg/kg, respectively. Group IV served as standard group and received diazepam 1 mg/kg for antianxiety activity and fluoxetine (20 mg/kg) for antidepressant activity. All drugs were administered by gavage. **Results:** AECD 200 and 400 mg/kg showed significant ($P < 0.01$) dose-dependent increase in entries and stay in open arms in EPM and entries and stay in light compartment in light and dark box as compared to control. In both models effect of AECD at dose of 400 mg/kg was comparable with that of diazepam. AECD also produced significant ($P < 0.01$) antidepressant effect at all the doses, as indicated by reduction in immobility time as compared to control in both FST and TST. The efficacy of AECD at dose of 400 mg/kg was comparable ($P > 0.05$) with that of fluoxetine in both the models. **Conclusion:** Results of our study suggested that AECD possess significant antianxiety and antidepressant activity and the effect is dose-dependent.

Key words: Antianxiety, antidepressant, *Cynodon dactylon*, elevated plus maze

INTRODUCTION

Anxiety as well as depression presents the most widely recognized types of mental illnesses nowadays[1] that afflict many people and can be associated with symptoms such as tachycardia, palpitation, sweating, lack of concentration, respiratory dysfunction, and numbness.[2]

Anxiety disorder is one of the most common mental disorders which are experienced as a part of day-to-day life. It is a state of fear and worry, if persist longer, affects physical as well as mental health. It affects one-eighth of the total population worldwide.[3] Benzodiazepines are the mainstay of treatment of anxiety. Azapirones and some antidepressant drugs are also effective in the treatment of anxiety disorders. At present, approved anxiolytic drugs have several adverse effects including drowsiness, sedation, confusion, anterograde amnesia, dependence, abstinence syndrome, paradoxical reaction in humans, and decay of psychomotor functions.[4]

Depression is also one of the common psychiatric disorder which affects physical and mental health. The current modalities of the treatment for depression include tricyclic antidepressants, monoamine oxidase inhibitors and selective serotonin reuptake inhibitors, and selective noradrenaline reuptake inhibitors. They produce anxiety, diaphoresis, tachycardia, tremor, sedation, insomnia, serotonin syndrome,

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parkinsonism, postural hypotension, blurred vision, and so forth.[5] Because of these, there is a need of drugs which are devoid of above adverse effects.

Medicinal plants are the natural resources in developing of new drugs. It has been well documented that herbal plants and their derivatives play critical roles in modern drug development. There has been a shift in universal trend from synthetic to herbal medicines.[6] Use of herbal medicinal preparations is well established and widely acknowledged to be safe and effective.[7]

Cynodon dactylon also known as "Durva" is perennial, creeping grass growing throughout the country. It is the second most holy plant of Hindu religion after Tulsi (*Oscimum sanctum*). It has a variety of biological activities such as antiviral, antibacterial, antimicrobial, antidiabetic, immunomodulatory, antioxidant, hypolipidemic, and wound healing properties.[8] Alcoholic extract of leaves of C. *dactylon* exhibits anxiolytic effect in experimental rats.[9] Ethanolic extract of C. *dactylon* possess promising antidepressant activity in mice.[10] Literature survey revealed that antianxiety and antidepressant property of the aqueous extract of C. *dactylon* (AECD) is not studied in experimental animals. Therefore, the present study was carried out to find antianxiety and antidepressant activity of AECD in mice.

MATERIALS AND METHODS

Plant Materials

Whole plants of C. *dactylon* (without roots) were purchased from Aveek nursery situated at Gwalior in the month of February 2019. The plant was identified by Dr. Avinash Tiwari Professor Department of Botany Jiwaji University Gwalior Madhya Pradesh (M.P.) and a voucher specimen SKM/19 has been retained in our department.

Preparation of Extract

AECD was prepared by maceration. The collected plants were washed thoroughly with tap water and dried at room temperature in the absence of sunlight. The dried plants were powdered using the grinder. One kilogram of dry powder soaked into 5 L of distilled water for 3 days. The mixture was then filtered using the Whatman filter paper. The solution was finally evaporated to dryness using water bath. Dry powder was stored in amber color bottle for further use. The yield was 5% w/w.

Phytochemical Screening

AECD was subjected to phytochemical tests for presence of bioactive compounds by standard methods as described by Harborne.[11]

Drugs and Chemicals

Fluoxetine (Cap. Flunil 10 mg- Intas Pharmaceuticals Ltd.) and Diazepam (Tab. Valium 2 mg- Abbott Healthcare Pvt. Ltd.) were purchased from medical store of Gwalior, M.P. All drugs were administered as 2% gum acacia suspension by gavage.

Animals

6–8 weeks old male albino mice weighing 30–40 g used for study were available in animal house of the Department of Pharmacology, Gajra Raja Medical College Gwalior, M.P. All animals under experiment were kept in 12 h light dark cycle and were provided with food and water *ad libitum*. The care and maintenance of animals were as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals in India. The experimental protocol was approved by institutional Animal Ethics Committee of Gajra Raja Medical College, Gwalior, registration number 846/PO/Re/S/04/CPCSEA.

Methods

Study of antianxiety activity

The test compound was screened for antianxiety activity by elevated plus maze (EPM) and light dark box in albino mice.

EPM

The EPM consisting of two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) was used. The maze was elevated to height of 25 cm above the floor. Test solution was administered once daily at 10 AM for a period of 30 days. On the 30th day, animals were placed individually at the center of the EPM with their head facing towards the open arm. Total time of stay and number of entries in different arms were recorded during 5 min period. Number of rearing and head dipping were also counted. An arm entry is defined as the presence of all four paws in the arm.[12] EPM was cleaned with 70% ethanol after each reading to eliminate any possible bias due to odor.

Light and dark box

The light dark box apparatus consisted of two compartments (20 × 20 × 20 cm), one painted white and illuminated and other painted black which is kept dark. These two compartments connected to each other by a door (5 × 5 cm). The light source kept at 50 cm above the compartment's roof. Test solution was administered once daily at 10 AM for a period of 30 days. On the 30th day, animals were placed individually at the center of the illuminated white chamber (light compartment) with their head facing the side wall. Total time of stay and number of entries in light compartment were recorded during 5 min period. An entry is defined as the presence of all four paws in

the respective compartment.[13] The light and dark box were carefully wiped with 70% ethanol after each trial, to eliminate the possible bias due to the odor of the previous animal.

Study of Antidepressant Activity

The antidepressant activity was done using forced swim test (FST) and tail suspension test (TST) in albino mice.

FST

Mice were individually placed to swim in an open cylindrical container (diameter 13 cm, height 24 cm) containing water (22 ± 2°C) to a depth of 10 cm (Tail does not touch to the bottom of cylinder). When mice ceases to struggle and swim and remained floating in water, only moving to keep its head above water, it was considered immobile. Drugs were administered once daily at 10 AM for a period of 30 days. On the 30th day in a single session, mice were placed to swim in a narrow cylinder from which they cannot escape. Immobility period was recorded during the last 4 min of the 6 min test session by summing the total time spent immobile, which were the short periods of slight activity where the mice make those movements necessary to maintain its head above water. During the first 2 min of the test session, mice show a high frequency of exploratory and escape-directed behavior, the last 4 min is the time during which the mice show the most immobility.[14] Following every session the animals were removed from the cylinder, dried with towels and placed in heated cage for 15 min before returning to their home cage.

TST

This method is based on the assumption that when a mouse suspended by tail, it shows alternating agitation and immobility; the immobility is indicative of a state of depression. Drugs were administered once daily at 10 AM for a period of 30 days. Mice were suspended on the edge of a table, 50 cm above the ground, with the help of a tape placed approximately 1 cm from the tip of the tail on the 30th day. During a 6 min test session, immobility period was recorded. When mice did not show any movement of the body and hanged passively, it was considered to be immobile.[15]

Study Design

To study the antianxiety activity using EPM and light and dark box model mice were divided into four groups having six animals in each group as follows. Group1: Control received 2% gum acacia at the dose of 10 ml/kg. Groups 2 and 3: Test drug-treated group received AECD 200 mg/kg and AECD 400 mg/kg, respectively. Group 4: Standard drug-treated group received Diazepam 1mg/kg. Doses of AECD for antianxiety and antidepressant activity were selected on the basis of the previous studies using C. *dactylon*.[9]

To study the antidepressant activity using FST and TST model mice were divided into four groups having six animals in each group as follows. Group1: Control received 2% gum acacia at the dose of 10 ml/kg. Groups 2 and 3: Test drug-treated group received AECD 200 mg/kg and AECD 400 mg/kg, respectively. Group 4: Standard drug-treated group received Fluoxetine 20 mg/kg.

Statistical Analysis

The data collected after experiments were represented as mean ± SEM and were analyzed using SPSS software version 20. One-way ANOVA test was applied, followed by Tukey's multiple comparison tests. $P < 0.05$ was considered as statistically significant.

RESULTS

Assessment of Antianxiety Activity

AECD 200 and AECD 400 showed significant increase in entries and stay in open arms in EPM and entries and stay in light compartment of light and dark box as compared to GA10 ($P < 0.01$). AECD treated groups showed dose-dependent effect on open arm entries in EPM and entries in light compartment of light and dark box (AECD 200 vs. AECD 400 [$P < 0.01$]) and open arm stay in EPM (AECD 200 vs. AECD 400 [$P < 0.05$]) and stay in light compartment of light and dark box (AECD 200 vs. AECD 400 [$P < 0.01$]). AECD 200 and AECD 400 also showed significant increase in rearing in EPM as compared to GA10 ($P < 0.01$) and the effect was dose-dependent (AECD 200 vs. AECD 400 [$P < 0.01$]). In EPM head dipping was also significantly increased with AECD 200 ($P < 0.05$)] and AECD 400 ($P < 0.01$) as compared to GA10 and the effect was dose-dependent (AECD 200 vs. AECD 400 [$P < 0.05$]). Standard drug (Diazepam) group showed significant ($P < 0.01$) increase in rearing, head dipping, entries, and stay in open arms in EPM and entries and stay in light compartment of light and dark box as compared to GA10 and AECD 200. Rearing, head dipping, entries, and stay in open arms in EPM and entries and stay in light compartment of light and dark box with AECD 400 was comparable ($P > 0.05$) with Diazepam treated group [Table 1 and Figure 1].

Assessment of Antidepressant Activity

AECD 200 and AECD 400 significantly lowered the mean immobility time as compared to GA10 ($P < 0.01$) in both FST and TST. Effect of AECD 400 was more than AECD 200 and it was statistically significant in FST (AECD 200 vs. AECD 400 [$P < 0.05$]) and TST (AECD 200 vs. AECD 400 [$P < 0.01$]). Standard drug FXT20 significantly decreased the mean immobility time as compare to GA10 and AECD 200 ($P < 0.01$) in both FST and TST. Anti-depressant effect of AECD 400 was comparable with FXT20 ($P > 0.05$) in both FST and TST [Table 2 and Figure 2].

Table 1: Effect of aqueous extract of *Cynodon dactylon* on elevated plus maze parameters in mice

Groups	Number of entries		Time spent (s)		Rearing (no.)	Head dipping (no.)
	Open	Closed	Open	Closed		
GA 10	2.83±0.40	3.17±0.48	21.67±2.76	278.33±2.76	3.83±0.60	3.67±0.92
AECD 200	6.67±0.49[a]	7.17±0.60[a]	121.50±14.57[a]	178.50±14.57[a]	7.50±0.43[a]	8.50±0.76*
AECD 400	12.17±0.48[ab]	12.83±0.60[ab]	162.50±6.29[a]#	137.50±6.29[a]#	11.17±0.48[ab]	13.00±0.58[a]#
DZ1	12.33±0.67[ab]	13.00±0.63[ab]	173.17±4.36[ab]	126.83±4.36[ab]	12.00±1.15[ab]	14.50±1.65[ab]

GA10: Gum acacia 10 ml/kg; AECD 200 and AECD 400: AECD 200 mg/kg and 400 mg/kg, respectively; DZ1: Diazepam 1mg/kg (standard drug). Each group consists of 6 animals (n=6). Values are represented as mean±SEM. df=3, 20 F= 78.69 (open arm entries), 67.24 (closed arm entries), 68.51(open and closed arm stay), 26.65 (rearing), 21.32 (head dipping). [a]P<0.01 as compared to GA10, [b]P<0.01 as compared to AECD 200 mg/kg, *P<0.05 as compared to GA 10, #P<0.05 as compared to AECD 200 mg/kg. AECD: Aqueous extract of *Cynodon dactylon*

Table 2: Effect of AECD on immobility period in forced swim test in mice

Groups	Mean immobility period (s)
GA10	129.67±1.33
AECD 200	92.33±2.19[a]
AECD 400	83.50±2.80[a*]
FXT20	79.33±2.28[ab]

GA10: Gum acacia 10 ml/kg; AECD 200 and AECD 400: AECD 200 mg/kg and 400 mg/kg respectively; FXT20: Fluoxetine 20 mg/kg. Each group consists of 6 animals (n=6). Values are mean±SEM, df=3,20 F=107.63 [a]P<0.01 as compared to GA10 [b]P<0.01as compared to AECD 200 mg/kg *P<0.05 as compared to AECD 200 mg/kg. AECD: Aqueous extract of *Cynodon dactylon*

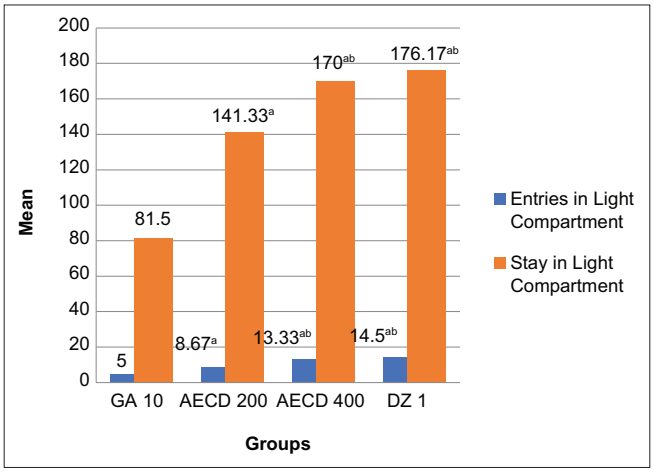

Figure 1: Effect of AECD on entries and stay of mice in light compartment of light dark box. GA10: Gum acacia 10ml/kg; AECD 200 and AECD 400: AECD 200 mg/kg and 400 mg/kg respectively; DZ1: Diazepam 1 mg/kg (standard drug) each group consists of six animals (n = 6). Values are represented as mean ± SEM. df=3, 20 F= 60.34 (Light compartment entries), F = 108.15 (Light compartment stay). [a]P < 0.01 as compared to GA10. [b]P < 0.01 as compared to AECD 200 mg/kg

DISCUSSION

C. *dactylon* is a medicinal plant of great value; it occupies a key position in ethno medicinal practices and traditional medicinal systems. It is extremely useful in various disorder such as diarrhea, vomiting, dysentery, dropsy, hemorrhages, and wounds.[16] Various pharmacological activities of this plant have been investigated by researchers all around the world and the research process is still ongoing.[17] Phytochemical analysis of AECD revealed presence of flavonoids, phenols, tannins, sterols, saponins, and glycosides and is in accordance with the previous study.[18]

In the present study, the AECD has shown antianxiety activity in EPM and light and dark box models used for the evaluation of antianxiety activity.

Elevated plus-maze is the simplest apparatus to study anxiolytic responses produced by the test drugs. It is used to test almost all types of anxiolytic agents. Open arms are more fear provoking than the closed arms. The number of entries and time spent in the open arms is found to be increased by anxiolytics.[19]

The light/dark box test is based on the inherent aversion of mice to brightly illuminated places. Anxiolytics reduce the natural aversion to light and increase the time spent in the light compartment.[20]

AECD 200 and 400 mg/kg showed significant dose dependent increase in entries and stay in open arms in EPM and entries and stay in light compartment in light-dark box as compared to control. These results are in accordance with the study done with alcoholic extract of C. *dactylon*.[9]

Flavonoids act as antianxiety agents by modulating the gammaaminobutyric acid (GABA) receptors similar to that of benzodiazepines, thereby increasing the frequency of chloride channel opening and resulting in the neuronal hyperpolarization.[21] Various other studies have also shown that flavonoids are responsible for antianxiety activity.[22,23] The antianxiety activity of AECD in our study might be due to flavonoids and increased GABA levels in brain as reported in earlier study.[24]

In the present study, the antidepressant activity of AECD in albino mice was evaluated by using FST and TST methods.

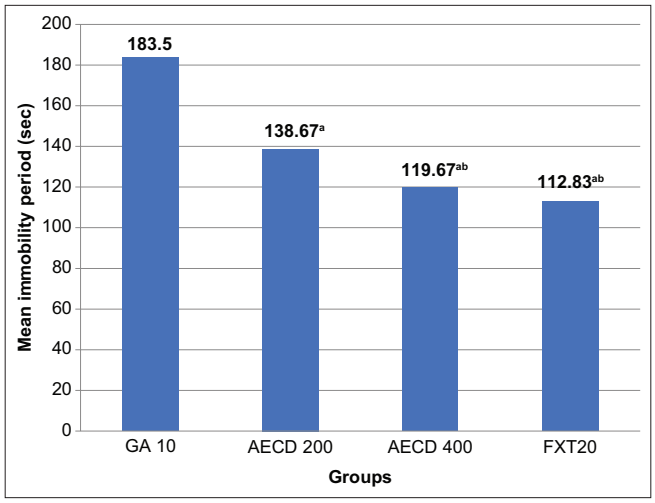

Figure 2: Effect of AECD on Immobility period in tail suspension test in mice. GA10= Gum acacia 10 ml/kg; AECD 200 and AECD 400: AECD 200 mg/kg and 400 mg/kg, respectively; FXT20: Fluoxetine 20 mg/kg. Each group consists of six animals (n = 6). Values are mean ± SEM, df= 3,20 F=163.57 [a]P < 0.01 as compared to GA10 [b]P < 0.01 as compared to AECD 200 mg/kg

Stress plays an important role in developing depression. FST and TST animal models are quite sensitive and create physical stress and there by leading to depression. These models of depression are rapid and reliable behavior screening test for antidepressants. The immobility has been expected to reflect a state of behavioral despair and failure to adapt to the stress. Both these models are widely used to screen new antidepressant drugs.

In our study, AECD 200 and 400 mg/kg showed significant dose-dependent antidepressant activity in both FST and TST models. Results of our study on depression are in accordance with the earlier study where inhibition of depression is reported with C. *dactylon*.[10] However, different results were found in EPM and FST models in a study with ethanol extract of C. *dactylon*. This study showed CNS depressant activity in mice.[25] Antidepressant activity in our study can be due to increased catecholamines level in brain with the use of C. *dactylon*.[24]

A wide range of plant-derived flavonoids can cross the blood-brain barrier and are able to influence brain function. Flavonoids also have been appeared to have depression reducing impact.[1,15,26] Therefore, in our study, antidepressant activity of AECD might be due to flavonoids and increased catecholamine levels.

In conclusion, AECD showed significant anxiolytic and antidepressant activity. Hence, it may be served as a potential resource for natural psychotherapeutic agent against stress related disorders such as anxiety and depression. However, the study has its share of limitations. There is need to conduct further *in vivo* study with more number of animals and more number of behavioral animal models and *in vitro* experiments such as GABA receptor binding assay, benzodiazepine receptor binding assay, serotonin receptor binding assay for antianxiety activity; and MAO inhibition assay, measurement of monoamines, and their metabolites levels for antidepressant activity to know the exact mechanism of AECD.

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