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EVALUATION OF ANTIDEPRESSANT ACTIVITY OF *DACTYLONIUM AEGYPTIUM* IN CHRONIC UNPREDICTABLE STRESS INDUCED DEPRESSION

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ABSTRACT

Aim: To evaluate the anti depressant activity of hydro alcoholic extract of *Dactylotenium aegyptium* (HEDA) in chronic unpredictable stress induced depression. **Methods:** Rats were subjected to chronic unpredictable stress for 14 days and treated with 250mg/kg and 500 mg/kg of HEDA using fluoxetine as standard. On 15th day rats were screened for behavioral parameters using open field test, despair swim test and plus maze test. Biochemical parameters like superoxide dismutase, reduced glutathione, lipid peroxidation and catalase were estimated from brain homogenate, followed by histopathology of brains. **Results:** Administration of HEDA at 400 mg/kg as well as 200mg/kg exhibited profound effect on behavioral parameters and biochemical parameters. Significant increase in the superoxide dismutase, catalase levels and reduced glutathione and decrease in lipid peroxidation were observed. The present study suggested that HEDA significantly reversed the stress induced depressive like behavior and oxidative damage. **Conclusion:** Treatment with HEDA had a beneficial effect on stress induced depression. These results may indicate that HEDA exerts potential anti depressant activity.

KEYWORDS: Anti depressant activity, chronic unpredictable stress, *Dactylotenium aegyptium*, stress induced depression.

INTRODUCTION

Depression can be defined as a complex syndrome which is characterized by mood disturbances and a variety of cognitive, psychological disturbances. It's the most common affective disorder that ranges from very mild to severe psychosis accompanied with hallucinations. There are so many factors which will affect a person's mood like biological factors, genetic factors,[1] physiological factors and social factors. At its severe stage it may lead to suicidal symptoms which include decreased interest or pleasure,[2] sleep disturbances, guilt or feeling worthless ,energy loss or fatigue, concentration problems or problems with memory, appetite disturbance, weight loss or gain, suicidal ideation and thoughts of death.[3] It is characterized by combination of symptoms that interfere with person's ability to pleasurable activities. At least 350 million people are suffering with depression according to WHO statistics.[4] Depression is more prevalent at an age of 45-65 of which women are generally more susceptible than men. Depression is associated with biochemical factors like decrease in the level of neurotransmitters like nor-epinephrine, serotonin and dopamine in the brain.[5,6] Currently the available antidepressant agents are with unwanted side effects; especially they cause hypertension, hyperthermia, insomnia, anxiety and weight gain. Since the depressive disorders are having a huge impact on our lives, it is worth evaluating the alternative forms of medicines which can be used for its treatment. So in this study, an effort was made to investigate the antidepressant effect of *Dactylotenium aegyptium*. It belongs to Poaceae family. It is commonly known as Crow foot grass or Egyptian grass. *Dactylotenium aegyptium* is very rich source of cyanogenic glycosides and also contains oxalic acids and oxalates, glutamic and aspartic acids and amino acids like cysteine and tyrosine.[7]

MATERIALS AND METHODS

Plant material and extraction

The fresh plants of *Dactylonium aegyptium* were collected from the agricultural areas of Tirumala, Andhra Pradesh, India, in the month of May, 2013, and they were authenticated by Dr. K. Madhava Chetty, Asst. Prof., Dept. Botany, S. V. University, Tirupathi, India. Voucher specimen of this plant bearing number 1184is kept for further reference in S.V. University.

Extraction
Aerial parts of the plant were air dried and grinded into fine powder. Powdered material was macerated with equal ratio of water and ethanol for 5-7 days with frequent agitation. Extract was filtered and concentrated [8]. Concentrate was preserved by adding 3-4 drops of chloroform.

Phyto chemical screening
The extract was screened for the presence of various phytochemical constituents. Procedure followed to perform the phytochemical screening.[9,10]

Acute toxicity study
Acute oral toxicity study was carried out in accordance with OECD guidelines No. 423 by using Wistar rats. Four dosing levels (5, 50, 300, and 2000 mg/kg p.o.) were considered to carry out acute oral toxicity study. The animals were observed for the signs of toxicity and mortality for a period of 14 days.[11]

***In vitro* antioxidant study**
The DPPH scavenging effect was assayed according to the method of Makris *et. al.*, 2007. 1ml of different concentrations (25µg, 50µg, 100µg, 200µg, 400µg, 800µg) of extract was prepared and added to 3ml of 0.1M methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 3 min at room temperature. Absorbance of the samples was measured at 517nm using UV spectrophotometer. Free radical scavenging activity was calculated by using the formula.

$I = Ao - A_1 / Ao \times 100$, where, Ao is absorbance of control, A_1 is absorbance of test compound.

Grouping of animals
Animal studies were conducted after approval (No: IAEC-RIPER-11/2013) from the Institutional Animal Ethics Committee (IAEC: 878/PO/RE/S/05/CPCSEA) of Raghavendra Institute of Pharmaceutical Education and Research, Ananthapuramu. Thirty healthy Albino Wistar rats of either sex were weighed and grouped randomly into five groups (n = 6). Group 1: Served as normal control receiving normal saline (p.o) Group 2: Animals were subjected to chronic stress. Group 3: and Group 4: Were considered as treatment groups, the animals were subjected to stress and received HEDA at doses of 250 mg/kg/day and 450 mg/kg/day (p.o), respectively. Group 5: The animals were subjected to stress and received Fluoxetine (10 mg/kg i.p). HEDA and Fluoxetine treatments were done for 14 days following stress induction.

Chronic unpredictable stress model used
Exposure to chronic stress was done for seven days. Stressors used were tail suspension, cold water immersion and social isolation.[12]

Screening models for anti depressant activity
Forced swim test
The animals were forced to swim in a glass cylinder measuring 25cm height, 12cm diameter containing water at room temperature to a depth of 15cm. After placing the animal in swim test apparatus initially the animal is very vigorous. After 2-3 minutes activity begins to subside, after 5-6 minutes immobility reaches a plateau. The rat was considered immobile when it remained floating in the water without struggling (Immobility time), making only minimum movements of its limbs necessary to keep its head above water. After 6 min rat was taken out, dried with a towel. After drug administration Immobility time of animal get reduced indicates an effective antidepressant activity.[13]

Open field method
In this test 2 days before the actual test every animal is placed in open field for 5 min/day separately. During experimentation the animal is placed in centre of the four corners of the field and the following parameters were noted for 5 min. Preference of the animal into central or peripheral arms, total no of entries in central or peripheral arms, average time spent in central or peripheral arms, rearing, grooming, urination & Defecation. Each animal was given a score for total locomotor activity as it was calculated by using above parameters.[13]

Plus maze test
Elevated plus maze was used to study anxiolytic & antidepressant property [13].Every animal was placed in the centre of the maze individually, facing towards open arm. The following parameters were noted. First preference of the animal to open or closed arm, no. of entries into open or closed arm, average time spent in each arm. The % of animal preference towards open /closed arm[14] was calculated.

Evaluation of antidepressant potential
Biochemical analysis
On the 15th day of experimental protocol, the animals were euthanized by cervical dislocation under mild ether anesthesia and blood was collected by cardiac puncture. Then, the blood was collected and coagulated by leaving them undisturbed for 1 h at4°C and it was centrifuged (REMI, R-8^{0}C laboratory centrifuge) at 3000 rpm for 15 min to separate serum. Brains were isolated and homogenated individually for estimation of antioxidant parameters.

Histopathology
The excised brains from sacrificed animals were fixed in 10% neutral formalin solution immediately for a period of 24 h. Then, they were processed for dehydration using absolute ethanol, cleaned in xylene, and embedded in paraffin. The sectioning was made by using microtome apparatus at a thickness of 4 µm and stained with eosin and hematoxylin. The histopathology changes of each section were observed and photographed.

Statistical Analysis
The results were expressed as mean ± standard error of mean (n = 6) and the statistical analysis was made by one way ANOVA followed by Dunnett's comparison test using Computerized Graphpad prism (version 5.0) software at a level of significance of $P < 0.01$.

RESULTS

Phytochemical screening of HEDA
Phytochemical evaluation revealed the presence of carbohydrates, flavonoids, glycosides, phytosterols, saponins and phenolic compounds in HEDA.

Acute oral toxicity study
It was observed that there were no clinical signs of toxicity and mortality for a period of 14 days at a testing dose of 2000 mg/kg. As a result of this, one tenth of the maximum tolerated dose of HEDA was selected as therapeutic low dose (200 mg/kg, HEDA 200) and double of this low dose was considered as highest dose (400 mg/kg, HEDA 400) for this study.

***In vitro* DPPH radical scavenging activity of HEDA**
The DPPH radical is considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid auto oxidation. The Inhibitory concentration 50 was found to be 811µg. The scavenging effects of HEDA on the DPPH radical are illustrated in [Table 1].

Antioxidant effect of HEDA
The animals in stress control group reported a significant increase in levels of lipid peroxidation as compared to the normal group. Simultaneously, the rats treated with HEDA 200mg/kg and 400 mg/kg significantly reduced the levels of lipid peroxidation that was comparable with fluoxetine. [Table: 5].

The levels of super oxide dismutase, reduced glutathione and catalase were found to be significantly increased when compared with the stress control group that was comparable with fluoxetine. The rise in superoxide dismutase level with both doses of HEDA was more significant ($P< 0.0001$) [Table: 5].

Effect of HEDA on behavioural models
At the dose 400 mg/kg, HEDA showed antidepressant effect which is comparable to that of fluoxetine at the dose of 10 mg/kg. The Anti depressant effects of HEDA on the behavioural models were illustrated in Table 3, 4 and 5.

Effect of HEDA on histopathology
In control group: neurons in brain tissue were normal with no evidence of degenerative changes.

In stress control: Indicating several patchy neuronal degenerative changes with aggregation, extensively dark pycnotic nuclei in neuron and reactive glyosis. In HEDA 200 mg/kg treated group exhibited patchy neuronal degeneratiove changes with dark pycnotic nuclei. In HEDA 400 mg/kg – Mild degenerative changes and inflammation were observed. In fluoxetine 10 mg/kg – No significant degenerative changes or inflammation was seen.

Table 1: *Invitro* DPPH radical scavenging activity of HEDA.

S. No.	Concentration (µg/ml)	Absorbance at 517nm	Percentage free radical scavenging activity (%)
1	25	0.80	8.2
2	50	0.77	11.9
3	100	0.73	16.6
4	200	0.69	20.5
5	400	0.64	26.1
6	800	0.44	49.3
7	1000	0.46	52.0

Biochemical parameters
Table 2: Effect of HEDA on superoxide dismutase (SOD).

Treatment	SOD (U/mg protein)	L PO (nmol of MDA/ mg protein)	GSH (nmol/mg protein)	Catalase (U/mg protein)
Control	1.9 ± 0	20 ± 0.9	91.9 ± 1.3	217.9±4.2
Stress	0.59 ± 0$^{\#\#\#\#}$	34 ± 3.3	57.03 ± 2.5$^{\#\#\#}$	147.3±3.9$^{\#\#\#\#}$
HEDA 200 mg/kg	1.3 ±0.1****	27.2 ± 3.9*****	71.7 ± 1.5*	168.2±1.2*
HEDA 400 mg/kg	1.4 ±0.1****	26.9 ± 2.4*****	75.2 ± 2.7*****	175.6±2.0**
Fluoxetine 10 mg/kg	1.6 ± 0.1****	22.6 ± 0.6******	83.4 ± 2.7**	189.2±3.1***

*The values are expressed as mean ± S.E.M.(n=6). ####P<0.0001, ###P<0.001 when compared with control group; ****P<0.0001, ***P<0.001, **P<0.01 and *P<0.05 when compared with stress group.*

Behavioral parameters

Table 3: Effect of HEDA on immobility time in forced swim test.

S. No.	Treatment	Immobility time (sec)	No. of Counts
1	Control	119.4 ± 4.7	26 ± 2.3
2	Stress control	180.4 ± 6.4$^{\#\#\#\#}$	13.6 ± 1.5$^{\#\#}$
3	HEDA 200 mg/kg	170.2 ± 5.1	22.6 ± 1.1*
4	HEDA 400 mg/kg	147.4 ± 4**	23.2 ± 1.8*
5	Fluoxetine 10 mg/kg	136.6 ± 6***	24.8 ± 2.6**

*The values are expressed as mean ± S.E.M.(n=6). ####P<0.0001, ##P<0.01 when compared with control group; ****P<0.0001, ***P<0.001, **P<0.01 and *P<0.05 when compared with stress control group.*

Table 4: Effect of HEDA in open field test.

S. No.	Treatment	No of entries	No of rears
1	Control	94 ± 1.7	28 ± 3.6
2	Stress control	67 ± 2.6$^{\#\#\#\#}$	49 ± 4.1$^{\#\#\#}$
3	HEDA 200 mg/kg	80 ± 2.1*	38 ± 3.7*
4	HEDA 400 mg/kg	83 ± 2.8**	36 ± 2.6*
5	Fluoxetine 10 mg/kg	88 ± 2.7***	32 ± 3.3**

*The values are expressed as mean ± S.E.M.(n=6). ####P<0.0001, ###P<0.001 when compared with control group; ****P<0.0001, ***P<0.001, **P<0.01 and *P<0.05 when compared with stress group.*

Table 5: Effect of HEDA on elevated plus maze test.

S. No	Treatment	Open arm exploration		
		%preference	No. of entries	time spent
1	Control	45 ± 2.9	14 ± 0.7	142.3± 3.2
2	Stress control	17 ± 1.3$^{\#\#\#\#}$	4 ± 0.7$^{\#\#\#\#}$	90.0 ± 4.3$^{\#\#\#\#}$
3	HEDA 200 mg/kg	24 ± 3.1*	9 ±1.4*	114.0 ± 1.6*
4	HEDA 400 mg/kg	28 ± 4.3**	10 ±1.1**	117.3 ± 2.2*
5	Fluoxetine 10 mg/kg	34 ± 4.1***	11 ± 1.1**	126.0 ± 2.8**

*The values are expressed as mean ± S.E.M. (n=6). ####P<0.0001 when compared with control group; ****P<0.0001, ***P<0.001, **P<0.01 and *P<0.05 when compared with stress group.*

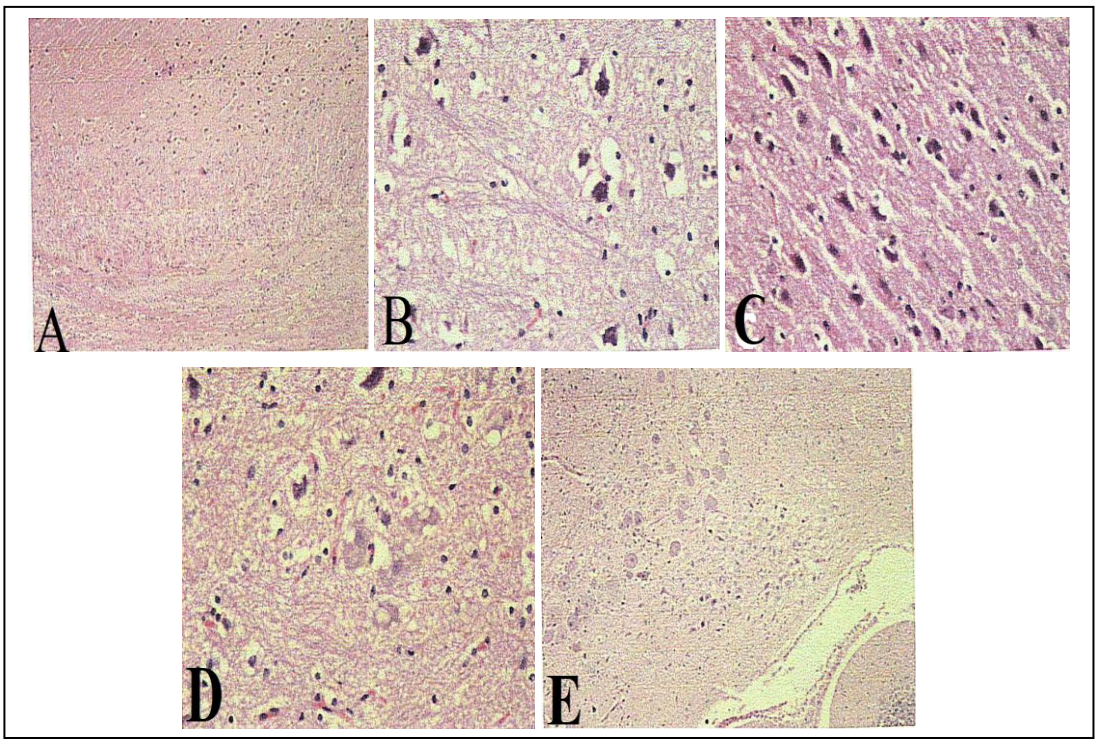

Fig. 1: Histopathology of brain. A: Control, B: Stress control, C: HEDA 200 mg/kg, D: HEDA 400 mg/kg and E: Fluoxetine 10 mg/kg.

DISCUSSION

In the present study HEDA was screened for its anti-depressant activity in chronic unpredictable stress induced depression. Results obtained from *Invitro* DPPH radical scavenging activity.[13] Reveals the potential antioxidant property of *Dactylotenium aegyptium*. Effect of free radicals generated from stress can be elleviated by HEDA. Initially the animal spend more time in the peripheral arms but after drug treatment there is reduction in the entries or time spent in peripheral arm indicates Antidepressant activity of given Plant Extract. The results of the behavioral models suggested the anti-depressant activity of HEDA. The comparable anti-depressant effect of HEDA with that of fluoxetine suggest possible involvement of either nor-adrenergic or serotonergic system. Levels of biochemical parameters like super oxide dismutase, catalase and reduced glutathione[15,16] are significantly increased when compared with the stress control group but when it comes to lipid peroxidation the values are significantly decreased. In neuronal diseases, brain is especially vulnerable to oxidative damage, due to the imbalance between the generation of oxygen free radicals and antioxidant defense system. In this study from the above results it was believed that HEDA has neuro protective properties as it restores the depleted anti-oxidant levels. Morphological changes caused by stress induced depression were significantly lowered by HEDA as evidenced by the histopathology.

The main focus of our work is to find out the anti-depressant activity of HEDA. From the presented data it was concluded that hydro alcoholic portion of this herb has significant antidepressant activity when compared with the standard drug fluoxetine.

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