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Pharmacological Evaluation of Antidepressant and Antianxiety Activity of *Desmostachya Bipinnata*.

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Abstract

Anxiety and Depression are widespread psychiatric disorders affecting around 5% of the population. Furthermore, it is difficult to predict which patient will respond to any given treatment. In the traditional systems of medicine, many plants have been used to treat anxiety and depression for thousands of years. The present study was designed to evaluate the antianxiety and antidepressant activity of the alcoholic and aqueous extracts of *Desmostachya Bipinnata* leaves in rodents. Antianxiety activity was tested by exposing rats to unfamiliar aversion in different methods like elevated plus maze model and actophotometer. The results infer that reduced aversion fear elicits antianxiety activity. The antidepressant activity was tested by using forced swim test and Open Field Test. The results infer that reduced immobility time elicits antidepressant activity. It was concluded that alcoholic and aqueous extracts of *Desmostachya Bipinnata* leaves have antianxiety and antidepressant activity. Alcoholic extract of *Desmostachya Bipinnata* leaves showing more significant activity over the aqueous extract.

Keywords

Desmostachya Bipinnata, Antianxiety activity, Antidepressant activity, Elevated plus maze, Actophotometer, Open Field Test

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INTRODUCTION

The present investigation explores the isolation and purification of another active compound from the aqueous and alcoholic leaves extract of Desmostachya Bipinnata, which was responsible for snake venom neutralization. Antagonism of both viper and cobra venom and antiserum action potentiation, antioxidant property of the active compound was studied in experimental animals. Recently, from this laboratory reported that an active compound from the Strychnus nux vomica seed extract, inhibited viper venom induced lipid peroxidation in experimental animals. The mechanism of action of the plant derived micromolecules induced venom neutralization needs further attention, for the development of plant-derived therapeutic antagonist against snakebite for the community in need. However, the toxicity of plants has known for a long period of time, and the history of these toxic plants side by side with medicinal ones are very old and popular worldwide, they considered the major natural source of folk medication and toxication even after arising of recent chemical synthesis of the active constituents contained by these plants. Before the introduction of modern medicines, disease treatment was entirely managed by herbal remedies. It is estimated that about 80% of the world population residing in the

vast rural areas of the developing and underdeveloped countries still rely mainly on medicinal plants.[1-3] Medicinal plants are the only affordable and accessible source of primary health care for them, especially in the absence of access to modern medical facilities. Studies reveal that there are more traditional medicine providers than allopathic providers, especially in the rural areas (WHO 2002). Increasing interest by multinational pharmaceutical companies and domestic manufacturers of herbal-based medicines is contributing to a significant economic growth of the global medicinal plants sector.[4-6.]

It focuses on aspects of medicinal plant research: from collection of plant material to efficacy and safety evaluation through preclinical studies and phytochemical standardization. Billions of dollars are spent for developing a new drug every year, but little is spent to know their exact pattern of use, and how much devastation it is causing at the user level. Isolation of the natural analgesic drug morphine from Opium, the latex of Papaver somniferum capsules, in 1804 is probably the first most important example of natural drugs which plants have directly contributed to modern medicine. Isolation of other important plant derived drugs of modern medicine rapidly followed and many useful drugs have since been discovered and introduced into modern medicine. Drugs like strychnine from Strychnos nuxvomica, emetine from Cephaelis ipecacuanha, caffeine from Camellia sinensis, quinie from Cinchona spp. and colchinine from Colchicum autumnale constitute some examples of such early drugs. The list of the plant derived medicinal substances occurring in modern medicine is very long now.[7-9.]

Desmostachya bipinnata, commonly known in English by the names Halfa grass, big cordgrass, and Salt reed-grass, [10] is an Old-World perennial grass, long known and used in human history. In India it is known by many names, including: *Daabh*, *Darbha*, *Kusha*, etc. The parts used are leaves, seeds and roots. It is used for dysentery and menorrhagia, and as a diuretic. *Desmostachya bipinnata* leaf extracts have anti-helicobacter, anti-microbial, anti-inflammatory, analgesic and anti-pyretic activity. The purpose of the present study was to evaluate the antianxiety and antidepressant activity of alcoholic and aqueous extracts of *Desmostachya bipinnata.*

Experience of mental illness is as old as human existence. Studies have reported that anxiety and depression may occur together with the association of sub threshold depressive symptoms. Anxiety may also predispose depression or symptoms of anxiety and depression may be external manifestations of one under cause. Thus, depression and anxiety issues are difficult enough to deal with without the added concern of side effects and cost. Though several drugs are available, many are associated with some limitations and drugs having properties to combat both anxiety and depression are very few.

PLANT MATERIAL COLLECTION

The leaves of *Desmostachya Bipinnata* were collected. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

Preparation of plant extracts

Preparation of Aqueous Extract:

Fresh leaves of *D.Bipinnata* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of water. The contents were mixed well and then the mixture was boiled up to 80-100^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiments to check the activities[11].

Preparation of Alcoholic Extract:

Fresh leaves of *D.Bipinnata* were collected and washed under tap water. The leaves extract used was prepared by taking 20gms of finely cut leaves into 250ml beaker containing 200ml of alcohol. The contents were mixed well and then the mixture was boiled up to 50-60^{0}C for 4-5hrs. Further the extract was filtered with whatmann filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiments to check the activities.

Selection of dose for animal study

The dose considered for the experiment on rats was obtained from conversion of human dose of *Desmostachya Bipinnata* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats and 0.002 for mice. Hence the calculated dose for the rats (considering human dose3 and 5 g/kg) is 200 mg/kg and for mice is 20 mg/kg. Acute toxicity was done at dose of 2000mg/kg body weight.[12-13.]

Pharmacological evaluation

Preparation of extracts:

The aqueous and alcoholic extracts of *Desmostachya Bipinnata* suspended in water in presence of 3%v/v Tween-80 solution. All the drugs were administered

orally for experimental purpose. Each time preparations of the extracts were prepared when required. The drugs were administered at a constant volume of 10ml/kg for each animal.[14]

Acute oral toxicity:

The acute oral toxicity of aqueous and alcoholic extracts of *Desmiostachya Bipinnata* was determined by using rats and mice which were maintained under standard conditions. The animals were fasted 12 hours prior to the experiment, up and down procedure OECD guideline no. 425 were adopted for toxicity studies. Animals were administered with single dose of individual extract up to 2000mg/kg and observed for its mortality during 2days and 7days study period (short term) toxicity and observed up to 7days for their mortality, behavioral and neurological profiles.[15-16.]

Screening For Antianxiety and Antidepressant Activity

The aqueous and alcoholic extracts of *Desmostachya Bipinnata* leaves were tested for antianxiety activity using elevated plus maze and actophotometer and antidepressant activity using despair swim test and tail suspension test.

Procedure for Antianxiety Activity

Elevated plus maze (EPM) model.

The apparatus comprises of two open arms (35x5cm) and two closed arms (30x5x15cm) that extend from a common central platform (5x5cm). The floor and walls of the closed arms are made of wood and painted black. The entire maze is elevated to a height of 50 cm above ground level. Rats weighing (150 – 200gms) were housed in a pair of 10 days prior to the test in the apparatus. During this time the rats were handled by the investigator on alternate days to reduce stress. 30 min and 60min after oral administration of the drug treatment, each rat was placed in the center of the maze facing one of the enclosed arms. During the five minutes session, number of entries into open arm and time spent in the open arm were noted[14,15]. The procedure was conducted preferably in a sound attenuated environment20.[8-20.]

Locomotor activity

The locomotor activity can be easily studied with the help of actophotometer, the rats were grouped and treated with drugs. Turn on the equipment (check & make sure that all the photocells are working for accurate recording) and place individually each rat in the activity cage for 10 minutes. Note the basal activity score of all the animals. Inject the drug diazepam (Dose: 5 mg/kg, ip; make a stock solution containing 0.5 mg/ml of the drug & inject 1 ml/100 g body wt. of mouse), and after 30 mins re-test each mouse for activity scores for 10 mins[16]. Note the difference in the activity, before and after chlorpromazine. Calculate percent decrease in motor activity.[21]

Procedure for Antidepressant Activity

Despair Swim Test Apparatus

For the determination of antidepressant activity, forced swim test (FST) protocol was employed. During the test, animals were individually placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with water up to a height of 10cm, at 25 ± 2°C. All animals were forced to swim for 5 min and the duration of immobility was observed and measured during the 5 min interval of the test. Immobility period was regarded as the time spent by the rats to float in water with no struggle and making only those movements necessary to keep their head above the water. To check the fitness level of each test animal, a pre-test was carried out 24 h before the FST by subjecting each test animal to a session of 15 min swimming.[22-23.]

Tail suspension test

The tail suspension test was performed based on the method prescribed17. The mice were suspended 58cm above the floor by means of an adhesive tape, placed approximately 1cm from the tip of the tail. The total duration of immobility was quantified during a test period of 5min. Mice were considered immobile when they were completely remaining motionless.[24]

Statistical analysis

The values were expressed as mean ± SEM data was analyzed using one-way ANOVA followed by T-test. Two sets of comparisons were made. i.e. Normal control Vs All treated groups. Differences between groups were considered significant at $P<0.001$ and $P < 0.05$ levels.

RESULT AND DISCUSSION

Antianxiety activity of *Desmostachya Bipinnata*

Elevated plus maze test.

Anxiolytic property of aqueous and alcohol solvent soluble fraction of the leaves of *D.Bipinnata* studied at a dose of 200 mg/Kg, using Elevated plus maze experiment.

In elevated plus-maze test (EPM), the ethanolic and aqueous extracts of *Desmostachya Bipinnata* leaves at a dose of 200 mg/kg p.o. significantly increased the number of entries and time spent into the open arm. The magnitude of the antianxiety effects 200mg/kg p.o. of alcoholic and aqueous extracts of *Desmostachya Bipinnata* was comparable to that of diazepam10 mg/kg i.p.

Table: 1 Data obtained from Elevated Plus Maze experiment.

S.No	Groups	Dose	% Preference	Open arm and enclosed arm			
				No. of entries in open arm(M±SEM)		Average time spent. (Sec ± SEM)	
				(O)	(C)	(O)	(C)
1.	Control	-	Open	1	2	34	268
			Closed	1	1	12	282
2.	standard	10	Open	2	1	42	258
			Open	2	1	11	284
			Open	1	1	24	274
			Open	1	1	35	264
3.	AQEDB	200	Open	3	2	42	126
			Open	2	1	32	145
			Open	1	1	28	142
4.	ALEDB	200	Open	3	2	47	130
			Open	1	1	29	151
			Open	2	1	33	147

From the experiment it was observed that mice taken aqueous and alcohol soluble fraction at dose of 200 mg/kg body weight, stayed more time in open arm of Elevated plus Maze apparatus in comparison to standard and negative control group. Moreover, they also spent less time in closed arm of Elevated plus Maze apparatus in comparison to standard and negative control group. The value obtained from these fractions were statistically significant ($p<0.05$).

Actophotometer Test:

Anxiolytic property of aqueous and alcohol solvent soluble fraction of the leaves of *D.Bipinnata* studied at a dose of 200 mg/Kg, using Actophotometer experiment.

The percentage of reduction in locomotor activity with diazepam (10 mg/kg i.p) after 1 hour is 91.0 % i.e. there is highly significant ($P<0.000$) decrease in locomotor activity compared to control, whereas dose of AQEDB and ALEDB (200mg/kg i.p) showed dose dependent decrease in locomotor activity that is 78.3% and75.8% respectively when compared to standard. The values are highly significant ($P<0.000$) The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

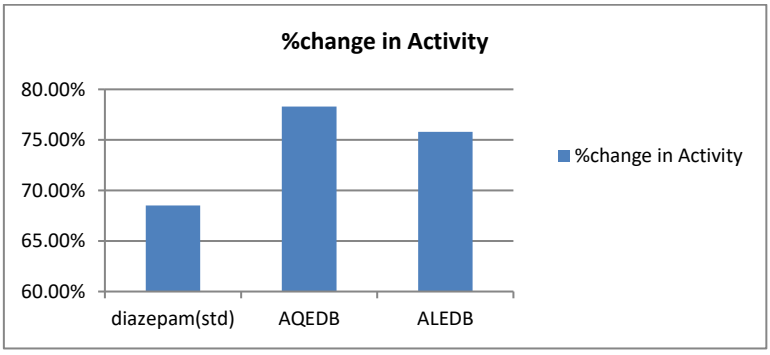

Fig No:1 --. Effect of extracts of *Desmostachya Bipinnata* on Locomotor activity.

Spontaneous locomotor activity is considered as an index of alertness and can be helpful to confirm the general depressive activity of any drug. The decrease in motor activity gives an indication of the level of excitability of the CNS and this decrease may be related to sedation resulting from depression of CNS. However, in the present study the AQEEU and ALEEU were found to have decreased effect on the locomomotor activity in actophotometer.

Antidepressant Activity of Desmostachya Bipinnata Forced Swim Test

Antidepressant activity of aqueous and alcohol solvent soluble fraction of the leaves of *D.Bipinnata* studied at a dose of 200 mg/Kg, using Forced Swim Test experiment.

The anti-depressant activity of AQEDB and ALEDB was assessed using Forced Swimming Test in Swiss albino rats It was observed that AQEDB and ALEDB at

a dose of 200mg/kg exhibited significant reduction in immobility time when compared to control in dose dependent manner. Similarly, the animals treated with diazepam (10mg/kg) as expected showed significant decrease in immobility time. The results are expressed as means ± S.E.M Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was assessed as $p < 0.05$.

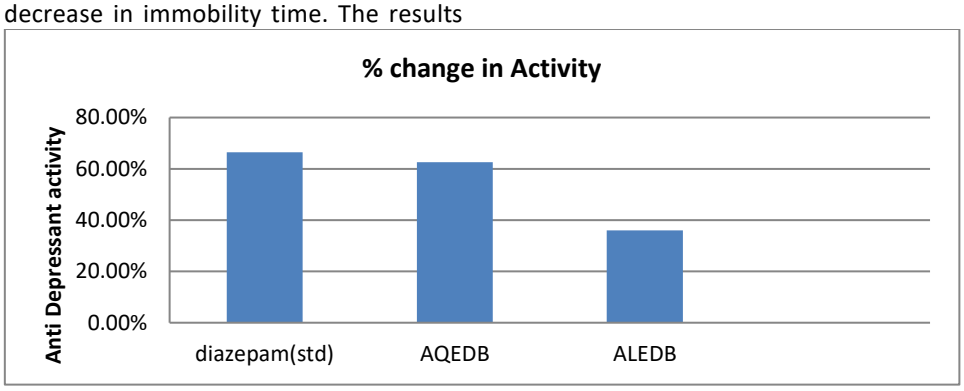

Fig.No.2: Effect of extracts of *Desmostachya Bipinnata* on Anti-depressant activity

Open Field Test

Antidepressant activity of aqueous and alcohol solvent soluble fraction of the leaves of *D.Bipinnata* studied at a dose of 200 mg/Kg, using Forced Swim Test experiment.In tail suspension test, the alcoholic and aqueous extracts of leaves of *Desmostachya Bipinnata* at a dose of 200 mg/kg i.p. significantly decreased the immobility time. The magnitude of the antidepressant effects of 200 mg/kg i.p. of alcoholic and aqueous leaves of *D.Bipinnata* was comparable to that of Diazepam 10 mg/kg i.p.

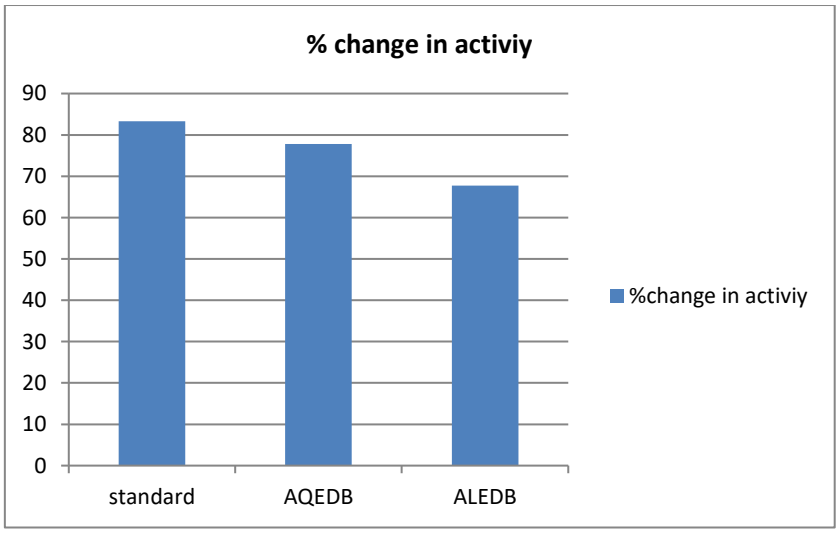

Fig.No.3- Effect of Ethanolic and Aqueous Extracts of *D.Bipinnata* Leaves on Open Field test

Open field behavioral model was used to study exploratory and locomotor activity in this investigation. Reported studies have shown that stress factors account for the decreases in mobility and functional responses against novel environment. The purpose of including this test was to assess the general activity of the animals after performing FST. The results observed in the open field test showed that i.p administration of aqueous and alcoholic extracts of *Desmostachya Bipinnata* (200 mg/kg) did not significantly increase the locomotor activity in unstressed groups of rats as compared with their control groups. However, aqueous, and alcoholic *Desmostachya Bipinnata* administered rats following the exposure to repeated restraint stress showed significant ($p<0.01$) increases in locomotor/ exploratory activity on an open field arena. It is therefore suggested that the extract has the ability to reverse or normalize the locomotor suppressant behavior in laboratory animals and hence may help to cope with immobility factor associated with depression in humans. In the present study that

administration of aqueous and alcoholic *Desmostachya Bipinnata* at the dose of 200 mg/kg significantly altered the behavioral deficits induced by injections of atypical neuroleptic, haloperidol, and increased brain serotonin metabolism in mice. The results are in general agreement with our previous studies in continuation to this plant and indicating its antidepressant-like activity in behavioral models of depression.

Conclusion

The results obtained in this study indicate that the n-hexane, ethyl acetate and methanol fractions of the leaves of Desmostacya Bipinnata have significant CNS Depressant and Anxiolytic activities in animal model systems. The medicinal values of the plant leaves may be related to their constituent phytochemicals. So, further detailed investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions and their efficacy need to be done. It will help in the development of novel and safe drugs for the treatment of different types of CNS disorders.

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