Research Article

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Evaluation of *Dregea volubilis* leaf extract for its potential against stress induced amnesia in experimental rats

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ABSTRACT

The world is focusing more upon the herbal medication for curing various ailments. The trend of validating the plants with traditional medicinal usage is increasing. The present investigation deals with the protective effect of Alcoholic extract of *Dregea volubilis* leaves (AEDV) against stress induced amnesia. In the present study the extract (250 and 500 mg/kg, p.o) was investigated for its Nootropic potential in normal and stress induced rats. The invitro antioxidant activity was carried out to correlate its protective affect against stress. Conditioned avoidance response using Cook's pole climbing apparatus was used in normal and stress induced rats to assess cognitive-improving activities. The invitro antioxidant activity was carried out by reducing power assay. Daily administration of *Dregea volubilis* at doses of 300 and 500 mg/kg, p.o enhanced cognition in dose dependent manner in normal rats. Fast retrieval was observed in extract treated stress induced rats, compared to that of stress control group. In Ferrous reducing power assay significant increase in absorbance was observed with extract in dose dependent manner and the results were comparable with that of standard, Ascorbic acid. The present study justifies scientific support for the protective effect of *Dregea volubilis* against stress induced amnesia & useful in combating the stress induced CNS disorders.

Key Words: *Dregea volubilis*, Oxidative Stress, Cognition, Antioxidant

INTRODUCTION

Stress is known to induce alterations in various physiological responses, leading to a pathological state. Stress causes disturbance in the body's normal physiological equilibrium and results in threatened homeostasis [1]. There is increasing evidence that severe stress affects cognitive functions and leads to the pathogenesis of various neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and aging [2, 3]

The plant *D.volubilis* is a large woody twinning shrub of the family Apocynaceae. It is found to be growing in high rainfall as well as in low rainfall regions. The leaves of D.volubilis are used as an application in boils and abscesses to promote suppuration. Root paste is applied to snake bites and given to women to cure headache after child birth. It is emetic, diaphoretic and diuretic. Traditional healers in Kerala use its leaves to treat inflammatory and painful conditions. The plant is being used very specifically in the indigenous systems of medicine such as Ayurveda, Siddha and Unani [4]. This plant is known as Madhumalathi, Hema Jeevanti (Sanskrit), Nakchhikni (Hindi), Titakunga (Bengali), Dudhipaalateega (Telugu), in Indian traditional medicine. The preliminary phytochemical screening of the roots revealed the presence of alkaloids, glycosides, flavanoids, resins, saponins, tanins & carbohydrates [5,6]. Till now there is no scientific work is reported on protective effect of *Dregea volubilis* against oxidative stress induced brain damage, hence we carried out this scientific study.

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MATERIALS AND METHODS:

Collection and extraction of Plant material

The leaves from plants were collected in the month of October-November from local forests of Rampachodavaram (India) and the plant was authenticated by taxonomist Dr. T.U.Raghuram. The leaves were washed, air dried under shade and coarsely powdered. The powdered material was extracted with 95% ethanol in soxhlet apparatus. Extract was concentrated by distilling of the solvent to obtain the crude extract. The percentage yield of alcoholic extract of *Dregea volubilis* leaves was found to be 9.9%w/w.

Experimental animals

Albino rats (80-120g) of either sex were used for the study. Animals were housed in colony cages at ambient temperature of 25±20 c, 12 h light/dark cycle and 50±5% relative humidity with free access to food

and water ad libitum. Food but not water was deprived overnight and during the experiment. All the experiments were carried out during the light period (9:00-16:00h). Each group consists of 10 animals and after learning each particular group was divided into two sub groups of 5 animals each. (One sub group without inducing stress & one sub group with inducing stress). The institutional animal ethical committee approved the study protocol.

EXPERIMENTAL DESIGN:

Stress procedure

The animals were subjected to chronic mild stress using following protocol [7]. The rats were forced to swim in a cylindrical vessel of height 60 cm and diameter 45 cm containing water at room temperature (28^0 C). Water depth was maintained at 40 cm. The swim stress was conducted for about 30min and 4 times a day about a week; the animals are placed on small platform (3 cm height&3.5cm diameter) fixed at center of chamber and surrounded by water 2 cm depth for 24 hrs, deprived of food and water during night, following 3hr access to restricted food and 2 hr access to an empty water bottle in alternate days about a week.

Nootropic activity

Conditioned avoidance response (CAR) using Cook's Pole Climbing

Apparatus

The Nootropic activity of AEDV leaves in normal and stress induced rats was evaluated by using the conditioned avoidance response (CAR) [8]. Rats were initially divided into 3 groups each containing ten animals. Groups II and III were administered orally with 250and 500mg/kg body weight of *Dregea volubilis* extract respectively, while animals in group I were served as control. After 60 minutes of drug administration, all the animals were subjected to a training schedule individually by placing inside the Perspex chamber of the apparatus. After an accustomed period of five minutes to the chamber, a buzzer was given followed by a shock through the grid floor. The rat had to jump on the pole to avoid foot shock. Jumping on the pole functionally terminates the shock and this was classified as an escape while such jumping prior to the onset of the shock was considered as avoidance. The session was terminated after completion of 30 trials with an interval of 20–30 seconds given for each trial. This procedure was repeated at 24 h intervals until all groups reach 95 to 99% avoidance. After attaining complete training of a particular group, the animals were divided into two sub groups containing 5 animals each. Then stress was induced to one of the sub group of a particular group for about week and another subgroup in each particular group were left normally. Drug administration is as usual during the period of stress. Again on 7th day retention of conditioned avoidance response was checked in both normal and stress induced groups. The training schedule was continued further with the daily doses of the extract and vehicle until they returned to normal level from stress induced amnesia.

Antioxidant activity

Reducing power assay

Different concentrations of the extract (100, 200, 300, 400 & 500µg/ml) and Ascorbic acid (100 & 200µg/ml) were prepared. Ascorbic acid is taken as the reference standard. 1ml of each concentration of both extract and standard were separately mixed with 1ml of 0.2M phosphate buffer (p^H 6.6) and 1ml of1% potassium ferricyanide. All the samples were incubated at 50°C for 20min.Then 1ml of 10% Trichloroacetic acid was added and centrifuged at 2000rpm for 10min. The upper layer (2.5ml) was then separated to mix with 2.5ml distill water and 0.5ml of freshly prepared ferric chloride. Finally absorbance was measured at 700nm [9].

RESULTS

Conditioned avoidance response (CAR) using Cook's Pole Climbing Apparatus

The CAR of rats administered with the AEDV leaves or vehicle increased gradually to 95-99% over six to ten days. The percentage avoidance was always higher in the extract treated groups compared to vehicle treated control group. The acquisition (time to achieve 95%CAR) for the extract treated groups (250&500mg/kg) was quicker (9&6 days respectively) when compared to control (10 days) and the results were found to be dose dependent. It is evident that, the reduction in CAR observed after stress is a clear indication of stress induced amnesia. However, continued treatment with AEDV produced better retention and recovery in a dose dependent manner than the vehicle treated animals in stress induced groups. There was a less fall in mean percentage of CAR and recovery in extract treated stress induced subgroups compared to vehicle treated stress control group. But in normal sub groups (which were not subjected to stress) there is no significant reduction in CAR. Results were given in fig 1, 2.

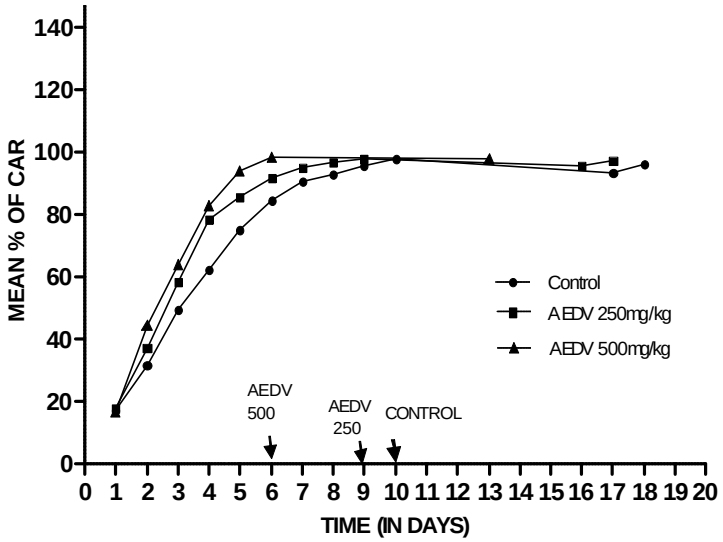


Fig.1: Effect of Alcoholic leaf extract of Dregea volubilis on Mean Percentage of Conditioned Response in normal rats

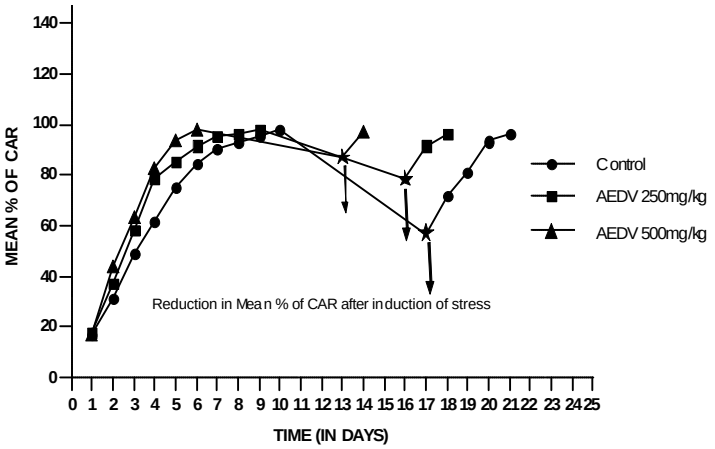
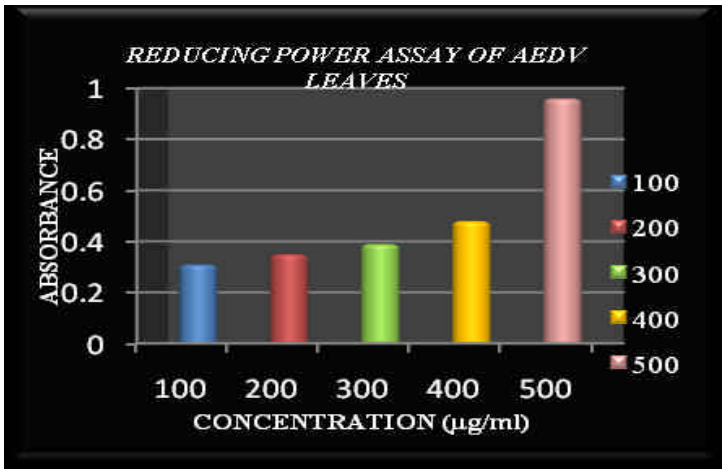
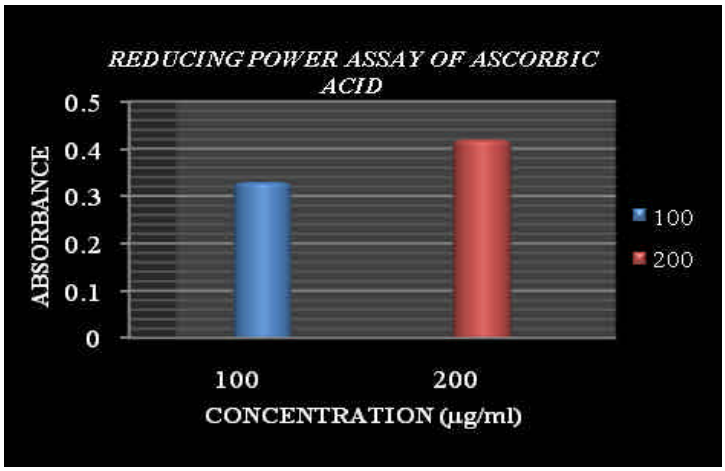


Fig.2: Effect of Alcoholic leaf extract of Dregea volubilis on Mean Percentage of Conditioned Response in stress induced rats

Antioxidant activity

Reducing power assay is based on the principle that substances which have reduction potential, react with potassium ferricyanide (Fe^{+3}) to form potassium ferricyanide (Fe^{+2}) which then reacts with ferric chloride to form ferrous complex that has an absorption maximum at 700nm. The reducing capacity of a compound may serve as a significant indicator of its potential Antioxidant activity [10]. The results were represented graphically in fig 3 & 4.





DISCUSSION:

The stress plays a major role in various (patho) physiological processes associated with neurodegenerative diseases & mental disorders [12]. The anti-oxidant and anti-stress activity were correlated with nootropic activity of extract since the role of stress and free radicals have been implicated in the loss of memory, concentration and also Alzheimer's disease [11, 12]

The Nootropic drugs facilitate intellectual performance, learning and memory [13]. However the neurological disease of such actions are not known and established. They are extensive evidences linking the central cholinergic system to memory [14]. Rats in normal groups of extract treated, exhibits more acquisition and retention compared to the vehicle treated control group in pole climbing indicates the nootropic activity. The nootropic activity of *Dregea volubilis* extract may be due to increase in acetylcholine and acetyl cholinesterase activity in rat brain. Since acetylcholine play a vital role in memory, increase in acetylcholine levels are responsible for nootropic action. In rats when challenged with stress, the amnesia was less in extract treated group showing better retention and recovery than stress control (only vehicle treated) group. The action may be due to its free radical scavenging mechanism apart from its cholinomimetic activity. The antioxidant activity of the extracts may be attributed to the presence of the phytochemicals like flavonoids. Furthermore, the antioxidant activity of the leaf extract provide mechanistic basis in relieving stress by way of combating oxidative damage. Therefore the protective effect of alcoholic leaf extract of *Dregea volubilis* against stress induced amnesia may be due to cholinomimetic and antioxidant activity.

CONCLUSION:

Phytochemical constituents like saponins, glycosides, alkaloids were already reported for their nootropic activity and these constituents were present in AEDV. Hence these chemical constituents can be accounted for the observed nootropic activity. Thus the present study demonstrates scientific support for the protectiver effect of alcoholic leaf extract of *Dregea volubilis* to combat stress induced amnesia and lends some credence to traditional claims of its therapeutic benefits in stress and stress-related disorders.

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