Reversal of Reserpine-Induced Orofacial Dyskinesia
And Catalepsy by Sida Cordifolia

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Abstract - Reserpine-induced catalepsy is an animal model used to mimic the behavioural symptoms of Parkinson’s disease (PD) in experimental animals. The present study was designed to investigate the effect of aqueous and hydro-ethanolic extracts of Sida cordifolia (AESC and EESC respectively), in reserpine-induced orofacial dyskinesia and catalepsy along with lipid peroxidation evaluated by the levels of thiobarbituric acid reactive substances (TBARS) in rat forebrain. Sida cordifolia is a well know Ayurvedic plant which has been administered anciently for nervous disorders such as hemiplegia, facial paralysis and PD. It also possesses significant in vitro and ex vivo antioxidant activity. Repeated administration of reserpine (1 mg/kg; s.c.) on alternate days (day 1, 3 and 5) for a period of 5 days significantly increased the vacuous chewing movements (VCM), tongue protrusions (TP), orofacial bursts (OB) and catalepsy along with increased forebrain TBARS levels in rats which was dose-dependently reversed by AESC (50, 100 and 250 mg/kg; p.o.) treatment. No significant effect on these behavioural parameters was observed following varying dose (50, 100 and 250 mg/kg; p.o.) treatment of EESC in reserpine treated rats. These findings suggest the involvement of antioxidant activity along with other underlying mechanisms for the ameliorative effect of AESC in reserpine-induced orofacial dyskinesia and catalepsy. It predicts the scope of AESC in the possible treatment of neuroleptic-induced orofacial dyskinesia and PD.

Key words - Reserpine; Parkinson’s disease; Sida cordifolia; oxidative stress; orofacial dyskinesia; catalepsy.

I. INTRODUCTION

Increased oxidative stress and free radical damage is a well-known feature of the age related brain disorders [1]. The free radical byproducts from catecholamine metabolism in the basal ganglia may cause neurotoxic effect seen in tardive dyskinesia and other motor related disorders [2]. The neuroleptic drugs cause secondary increase in turnover and metabolism of dopamine by blocking dopamine receptors, which may lead to increased formation of dopamine quinones as well as of hydrogen peroxide through the activity of monoamine oxidase [2]. Previous studies have shown that neuroleptic drugs induce oxidative stress and cell death which support this "free radical hypothesis" for generation of these dysfunctional motor symptoms [3]. According to some clinical studies, levels of lipid peroxidation byproducts in blood or cerebrospinal fluid of tardive dyskinesia patients are increased as compared to normal patients [4]. The increased lipid peroxidation in substantia nigra has also been reported in Parkinson’s disease (PD) [5].

PD is a progressive neurodegenerative disorder that primarily affects individuals between ages of 50 and 60. It currently affects nearly 1% of the population over 55 years of age. It is characterized clinically, by resting tremor, bradykinesia, hypokinesia, balance and gait disturbances. The pathologic hallmark of the disease, which is well known to contribute to these disabling symptoms in PD, is the progressive degeneration of dopaminergic neurons in the Substantia Nigra pars compacta and consequent loss of their projecting nerve fibers in the striatum that results in the profound deficit in striatal dopamine content [6]. The parkinsonian symptoms can be mimicked in the experimental animals by agents that block striatal dopamine D2 receptors like haloperidol or cause dopamine deficiency like reserpine and 6-OHDA [7].

Reserpine is known to be associated with the development of tardive dyskinesia [8] and behavioural symptoms of PD because of depletion of catecholamines [9]. Rats treated with this monoamine-depleting agent develop orofacial dyskinesia characterized by tongue protrusion (TP), orofacial bursts (OB), vacuous chewing movements (VCM) and cataleptic behaviour [10, 11]. This reserpine-induced animal model also showed the contributory role of increased levels of lipid peroxidation byproducts in producing these dysfunctional motor activities resembling PD features [12, 13]. Different experimental paradigms have confirmed the protective action of different antioxidant...
plants in reserpine-induced orofacial dyskinesia and catalepsy [12, 14].

Sida cordifolia, belonging to the family Malvaceae, is an important Ayurvedic medicinal plant, having significant in vitro and ex vivo antioxidant activity reported for its aqueous extract [15]. It is also reported in the ancient Ayurvedic literature that this plant can be administered for nervous disorders such as hemilegia, facial paralysis [16] and PD [17]. Franco et al. have reported the CNS pharmacological actions of 70% hydro-ethanolic extract of plant [18]. Sumanth and Mustafa have showed the antistress activity of hydro-ethanolic extract of this plant [19].

So the present study was designed to evaluate the effect of aqueous and 70% hydro-ethanolic extracts of Sida cordifolia (AESC and EESC respectively) on reserpine-induced orofacial dyskinetic (VCM, TP and OB) and cataleptic behaviors along with associated lipid peroxidation. The lipid peroxidation was evaluated by estimating the levels of thiobarbituric acid like reactive substances (TBARS) in rat forebrain.

II. MATERIALS AND METHODS

A. Animals
Male Sprague Dawley rats (5–7 week old) weighing 180-220 g, obtained from National Institute of Nutrition, Hyderabad, were used for the present study. The animals were housed in standard cages and maintained at an ambient temperature with natural day-and-night cycles (12:12 h light and dark cycles). All experiments were carried out between 09:00 and 16:00 hour. Animals were allowed a one-week habituation period to the animal room before testing. All procedures were conducted as per guidelines of the committee for the purpose of control and supervision of experimental animals. The protocol for the use of animals for this study was approved by the Institutional Animal Ethics committee, Dr. Hari Singh Gour Central University, Sagar, Madhya Pradesh, India.

B. Drugs and chemicals
Reserpine was provided as gift sample from Chemical Resources (Panchkula, India). Thiobarbituric acid, total protein test kit and all other chemicals were procured from local suppliers (Hi-Media Chemicals, Span Diagnostics Ltd, Spectrochem Pvt. Ltd. and Sisco Research Laboratory).

C. Preparation of plant extracts
The plant was collected during November, 2009 from Sagar, Madhya Pradesh, India. The plant was identified by Prof. T. R. Sahu, Taxonomist, Department of Botany, Dr. Hari Singh Gour Central University, Sagar, Madhya Pradesh, India and a voucher specimen has been deposited in the herbarium of the same department (Voucher Specimen no. Bot./Her./5234). The fresh whole plant was water washed, dried, finely powdered and sieved (400 µm). Powdered material was divided into two parts and one part was soaked in water in a ratio of 1:6 w/v at room temperature for 24 hours and filtered (45 µm) to obtain a clear filtrate. The marc was re-extracted and filtrates were reconstituted to get aqueous extract (9.6% w/w) of Sida cordifolia (AESC). Another part of powdered material was extracted with 70% hydro-ethanol using Soxhlet apparatus at 50 °C temperature for 72 hours followed by filtration to obtain hydro-ethanolic extract (10.2% w/w) of Sida cordifolia (EESC). Extracts were then freeze dried and stored at 4°C for pharmacological investigations.

D. Treatment schedule
The rats were randomly assigned into ten different groups (n = 6). Group 1 served as control group and was administered with 0.5% CMC solution (5 ml/kg; p.o.) along with a solution (1 ml/kg; s.c.) consisting of small quantity of glacial acetic acid in water as used in the other reserpine treated groups to dissolve reserpine. Group 2 served as reserpine treated negative control group and was administered with 0.5% CMC solution (5 ml/kg; p.o.) along with reserpine (1 mg/kg; s.c.) dissolved in small quantity of glacial acetic acid and then diluted in water. Group 3 and 4 served as per se groups of AESC and EESC respectively and received 100 mg/kg p.o. dose of respective treatment along with solution containing acetic acid in water as mentioned above (1 ml/kg; s.c.). Group 5 to 7 served as AESC (50, 100, 250 mg/kg; p.o) treated groups and Group 8 to 10 as EESC (50, 100, 250 mg/kg; p.o) treated groups. Groups 5 to 10 were co-administered with reserpine (1 mg/kg; s.c.) along with the respective treatment. The reserpine treatment was followed on alternate days (day 1, 3 and 5) for a period of 5 days in all reserpine treated groups. AESC and EESC was suspended in 0.5% CMC solutions and administered for 5 consecutive days. The different doses of extracts were selected based on acute toxicity studies done as per OECD guidelines in our previous unpublished study.

E. Evaluation of orofacial dyskinesia
After the injection of reserpine on the last day of treatment (day 5), rats were placed individually in a small (30x20x30 cm) plexiglas observation cage for a initial 10 minutes habituation period. Then all rats were observed for 5 minutes by an observer blinded to the treatment and number of occurrence of orofacial dyskinetic movements (VCM, TP and OB) were recorded as described by Naidu et al. [20]. VCM are referred to as single mouth openings in the vertical plane not directed toward physical material. Counting was
stopped whenever the rat began grooming, and restarted when grooming stopped.

F. Evaluation of catalepsy

Catalepsy was measured using the bar test in which, the rats were placed in half rearing position with both the front paws on a horizontal bar, 9 cm above and parallel to the base. The effect on catalepsy was observed after 1 hour of reserpine treatment on the last day (day 5). Rats were observed with a stopwatch to note the time of removal of one paw from the bar. The maximum cutoff time for observation was fixed at 180 s [21].

G. TBARS assay

After behavioural evaluation, animals were sacrificed, brains were removed, and forebrain was dissected out and weighted. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). It was subjected to centrifugation at 3000 rpm for 15 minutes to obtain the clear supernatant. The supernatant, 0.2 ml, of the homogenate was pipetted out in a test tube, followed by addition of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of thiobarbituric acid, and the volume was made up to 4 ml with distilled water. The test tubes were incubated at 95 °C, then cooled and added 1 ml of distilled water followed by addition of 5 ml of n-butanol-pyridine mixture (15:1 v/v). The tubes were centrifuged at 4000 g for 10 min. The absorbance of the developed pink colour was measured spectrophotometrically (Shimadzu UV spectrophotometer 1240) at 532 nm. A standard calibration curve was prepared using 1-10 nM 1, 1, 3, 3-tetra methoxy propane. The TBARS value was expressed in nM/mg of protein [22].

H. Protein estimation

The total protein was determined by the method of Lowry et al. with slight modification using Total Protein Modified Biuret, End Point Assay Test Kit [23].

I. Statistical analysis

All the results were expressed as mean ± SEM. All the data was analyzed using one-way analysis of variance (ANOVA) followed by Tukey test (Sigma Stat Software, 3.5). P-values <0.05 were considered as statistically significant for all comparisons.

III. RESULTS

A. Assessment of orofacial dyskinesia and catalepsy

Reserpine treatment resulted into significant (P<0.05) increase in VCM, TP, OB and catalepsy as compared to control group. Co-treatment with varying doses of AESC (50, 100 and 250 mg/kg) significantly (P<0.05) reversed the increase in reserpine-induced VCM, TP, OB and catalepsy, showing maximum effect at 100 mg/kg dose. Co-treatment with varying doses of EESC (50, 100 and 250 mg/kg) did not showed any significant (P>0.05) difference in VCM, TP, OB and catalepsy as compared to reserpine treated negative control group. AESC and EESC (100 mg/kg) per se treatment did not cause any significant (P>0.05) change in VCM, TP, OB and catalepsy as compared to control group (Fig. 1A, B, C and D).

Fig. 1 : Effect of Reserpine (Res), AESC per se (100 mg/kg), AESC (50, 100 and 250 mg/kg) plus Res, AFSC per se (100 mg/kg) and AFSC (50, 100 and 250 mg/kg) plus Res on (A): vacuous chewing movements (VCM), (B): tongue protrusions (TP), (C): orofacial bursts (OB) and (D): Catalepsy in rats on last day (day 5) of treatment. Data is represented as mean values ± S.E.M. *represents P<0.05 significant as compared to control group, #represents P<0.05 significant as compared to reserpine treated group.
B. **TBARS assay**

Reserpine treatment resulted in significant (P<0.05) increase in forebrain TBARS levels as compared to control animals. Co-treatment with varying doses of AESC and EESC (50, 100 and 250 mg/kg) significantly (P<0.05) prevented the increase in TBARS levels, showing maximum effect by AESC (100 mg/kg). AESC and EESC (100 mg/kg) per se treatment did not cause any significant (P>0.05) change in TBARS levels as compared to control group (Fig. 2).

![Fig. 2](image)

**Fig. 2**: Effect of Reserpine (Res), AESC per se (100 mg/kg), AESC (50, 100 and 250 mg/kg) plus Res, AFSC per se (100 mg/kg) and AFSC (50, 100 and 250 mg/kg) plus Res on thiobarbituric acid like reactive substances (TBARS) levels in rat forebrain. Data is represented as mean values ± S.E.M. * represents P<0.05 significant as compared to control group, # represents P<0.05 significant as compared to reserpine treated group.

**IV. DISCUSSION**

In the present study, reserpine-treated animals developed cataleptic behaviour along with orofacial dyskinesia, which was determined by an increase in VCM, TP and OB. The administration of varying doses of AESC showed protective effect against reserpine-induced behavioural changes. EESC, at varying doses, failed to reverse these reserpine-induced behavioural changes.

The reserpine causes the depletion of catecholamines in the forebrain, and thus producing these behavioural features of PD in rats. Previous literature indicates that imbalance in production and detoxification of free radicals may be associated with chronic neuroleptic use and it contributes to the initiation of catalepsy along with hyperkinetic movements in the orofacial regions [24]. The increased oxidative metabolism in forebrain after reserpine administration may be associated with decrease in antioxidant brain defense, as evidenced by increased levels of TBARS. These results are in accordance with the data from literature showing increased oxidative damage to forebrain after the administration of reserpine [12].

AESC dose-dependently protected reserpine-treated rats against the increase in TBARS levels in the forebrain. AESC might have prevented the oxidative damage caused by reserpine treatment and thus, reversed the behavioural changes due to reserpine. However, varying doses of EESC also protected the reserpine-treated rats against the increase in TBARS levels in the forebrain but failed to reverse the behavioural changes caused by reserpine.

These observations suggest the involvement of some other underlying mechanism in addition to antioxidant activity, for the protective effect of AESC in reserpine-induced behavioural changes. The antioxidant activity is common for both the AESC and EESC. So there is possibility for the presence of some important class of phyto-constituents in AESC which are responsible for the reversal of these reserpine-induced behavioural changes, through an additional underlying mechanism, along with the antioxidant activity. It may be involved through the reversal of reserpine-induced dopamine depletion by catecholaminergic pathways. Some important classes of phyto-constituents like sympathomimetic alkaloids and phytosterols have been reported in this plant which might be responsible for the above behavioural effects [25]. Further studies should be done for the screening and evaluation of the particular phyto-constituents present in AESC, which have shown the protective effect in this study.

The present study showed the reversal of reserpine-induced orofacial dyskinesia and catalepsy by AESC and thus, opens its scope as a possible potential candidate for the treatment and management of PD.

**ACKNOWLEDGMENT**

The authors are thankful to Council of Scientific and Industrial Research (CSIR), Government of India, for providing the financial assistance (Grant number 09/150/(0108)/2011/EMR-I) to Mr. Navneet Khurana for this project. The authors would also like to thank Chemical Resources, Panchkula, India for providing gift sample of reserpine.
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