ANTIOXIDANT EFFECTS OF ASPARAGUS RACEMOSUS WILD AND WITHANIA SOMNIFERA DUNAL IN RAT BRAIN

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Abstract

Asparagus racemosus Wild (shatawari) and Withania somnifera Dunal (ashwagandha) are rasayana commonly used in Indian traditional Ayurvedic medicinal system. Shatawari roots are used as tonic especially during pregnancy, gynecological disorders like menorrhagia and to increase lactation. Withania somnifera Dunal, is primarily used in ayurvedic preparations as powder, decoction, medicated wine etc., though primarily roots but seeds and leaves are also used for medicinal purposes. Present investigation was carried out with an aim to investigate the antioxidant properties of these plants using rat brain hippocampus as model system. Results of the present study demonstrate antioxidant effects of the root extracts of both Asparagus racemosus (Shatawari) and Withania somnifera (Ashwagandha) in rat hippocampus used as model system. These effects are evidenced by significant recovery of SOD and CAT enzyme level after drug treatment to the animals given 4hrs immobilization/ swim daily up to 30 days, in unpredictable manner. These enzymes are part of antioxidant defense of the body against free radicals and thus the significant increase after the drug treatment is indicative of free radical scavenging properties of both the drugs used in study. A significant increase in LDH activity after stress but significant decrease after drug treatment is indicative of reduced lipid peroxidation in the brain area studied.

Key Words : Asparagus racemosus, Withania somnifera, antioxidant

Introduction

Asparagus racemosus Wild (shatawari) and Withania somnifera Dunal (ashwagandha) are rasayana commonly used in Indian traditional Ayurvedic medicinal system. Shatawari roots are used as tonic especially during pregnancy, gynecological disorders like menorrhagia and to increase lactation (1). Its hormonal influences are best manifested in conjunction with female sex hormones (2). Presence of isolflavones in Shatawari are responsible for its estrogenic characteristics (3). It is also described as a rejuvenator and nervine tonic. Authors have examined the antioxidant effects of crude extract as well as purified polysaccharide fraction of A. racemosus (4-8). Withania somnifera Dunal, is primarily used in ayurvedic preparations as powder, decoction, medicated wine etc., though primarily roots but seeds and leaves are also used for medicinal purposes. The main pharmacologically active constituents are alkaloids and steroid lactones. Among alkaloids withaine, cuscohygraine, tropane, anahygraine, somniferrin, anaferine, withaminine, withaninine, while among lactones are withanoloids (9). Experimental evidences show that drug is useful in preventing senile dementia and Alzheimer's disease. Authors studying foot shock induced changes in the rat brain showed that ashwagandha also normalizes SOD and LPO activity and enhances CAT and GPX activity (10,11).

Present investigation was carried out with an aim to investigate the antioxidant properties of these plants using rat brain hippocampus as model system.

Materials and Methods

Animals: Adult Swiss albino rats were used for the present study (BW 165±5 gm). They were placed in animal room for seven days in polyutherine cages to acclimatize the laboratory conditions. All the rats were given food and water ad-libitum and were maintained at 12 : 12 hour light and dark cycle at 27+2°C. Prior to start of the experiment animals were divided into control and experimental groups.

Control group: Rat (n= 4) were kept in pathogen free environment in isolated room. Room was locked for 24 hours. Rats were provided enough food and water. Animal room is locked for 24 hrs. This exercise was necessary to avoid any stressful situation because of handling or noise.

Experimental group: Rats were divided into three experimental groups.

E1 (Stress) group : Rat (n=6) were given 6 hours unpredictable stress daily for 30 days.

E2 (Stress+ dose) group: Rat (n=6) was given 6 hours unpredictable stress and simultaneously treated with the methanolic extract of A .racemosus (100 mg/kg of BW).

E3 (Stress +dose) group : Rat (n=6) was treated with methanolic extract of W. somnifera (100 mg/kg BW).

Stress protocol: All the animals of GroupE1, E2 and E3 were subjected daily to restraint and swim stress alternatively in unpredictable manner up to 30 days. Animals were either forced to swim, or live in tightly fit container for 6 hours between 9:00 - 2:00 pm. Colonic/Rectal temperature was measured every day immediately before and after stress and other hyperactivities were also recorded for stress determination e.g. prostration, salivation etc. Stomach was dissected out to observe hemorrhagic spots as well. Beside this, daily record of food and water intake was also maintained for all the groups.

Drug preparation

Roots of A. recemosus and W. Somnifera were purchased from local supplier. Dried roots were purified using absorption method,

by keeping them in contact with brick powder. After purification, the roots were powdered. Finally, packed in filter paper and extract was prepared by continuous extraction method in soxhlet extractor using methanol as solvent. After vacuo-evaporation powder extract was dissolved in CMC.

Dose schedule

Rats (Group E2 and E3) were given daily drug extract dose of 100 mg/kg of BW dissolved in CMC before 1 hour of starting stress regimen. Dose was administered orally (using feeding tube). Treatment was continued for 30 days.

Biochemical analysis

Animals were sacrificed by decapitation. Only fore brain was selected for the study. Brain was quickly dissected out and placed in ice cold phosphate buffer saline. Tissue were palpated to remove the blood and then dissected on the ice chilled glass plate. The hippocampus was dissected out under stereo microscope. Tissue was pooled from 2 animals and was weighed, then chopped into small pieces and transferred to homogenizer tube. Cold 100mM phosphate buffer (pH 7.2) was added. Tissue was grounded in Teflon mechanical homogenizer. The homogenate was diluted 10 times and spun at 10,000 rpm for 15 minutes. Supernatant was used for enzymatic assay.

Enzyme assays

All the biochemical estimations were carried out according to established and standardized protocol. Ascorbic acid was assayed using Natelson (12). Superoxide dismutase (SOD) enzyme was measured using NBT method of Winterbourne et al (13). Catalase (CAT) was assayed colorimetrically by the method of Sinha (14). Malondialdehyde (MDA), an end product of lipid Peroxidation was assayed by Buege and Aust (15). Lactic dehydrogenase (LDH) was measured by method of weishaar (16).

Results

Superoxide dismutase (Fig.1): SOD level in stress group brain (hippocampus) significantly decrease ($P < 0.001$) as compared to control group. Treatment with A. racemosus extract as well as treatment with W. somnifera root extract showed significant ($P < 0.001$) recovery of the enzyme activity. CAT Level (Fig.2) also decrease significantly ($P < 0.001$) in stress group as compared to controls. Like SOD , CAT activity also showed significant recovery after treatment with A. racemosus root extract ($P < 0.001$) and W.somnifera root extract ($P < 0.01$). MDA level also increased significantly ($P < .0.001$) in stress group (Fig.3). After treatment with root extracts of A. racemosus ($P < 0.001$) and W.somnifera ($P < 0.01$) significant decrease in MDA level was observed. Ascorbic acid level (Fig.4) also decrease significantly ($P < 0.001$) in stress group. After drug treatment it shows significant recovery ($P < 0.001$). LDH level increased significantly ($P < 0.001$) after stress treatment (Fig.5). After treatment with root extracts of A. racemosus and W .somnifera both, a significant decrease ($P < 0.001$) in LDH activity was observed.

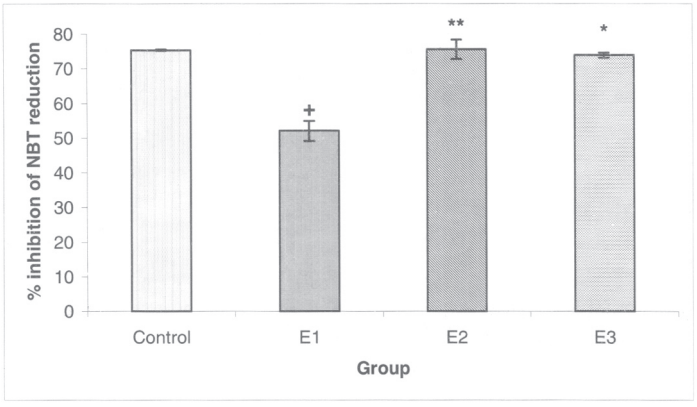

Fig-1 : Effect of stress and drug treatment on Super oxide dismutase activity (Mean ± SEM) in hippocampus.

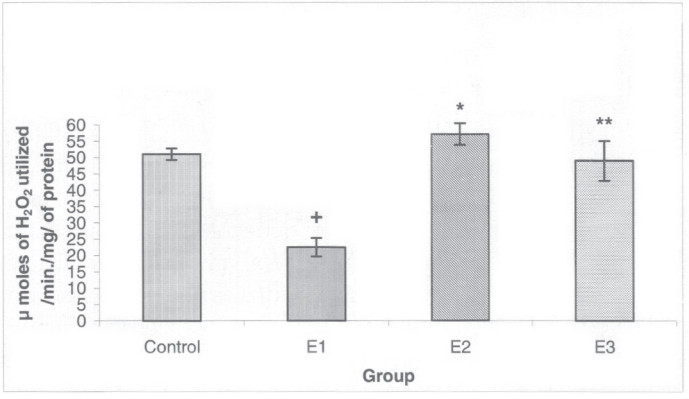

Fig.2 : Effect of stress and drug treatment on Catalase activity (Mean ±SEM) in hippocampus.

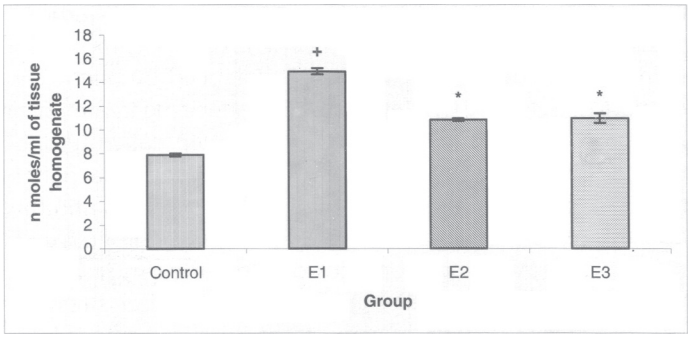

Fig-3 : Effect of stress and drug treatments on malonaldehyde level (mean ±SEM) in hippocampus.

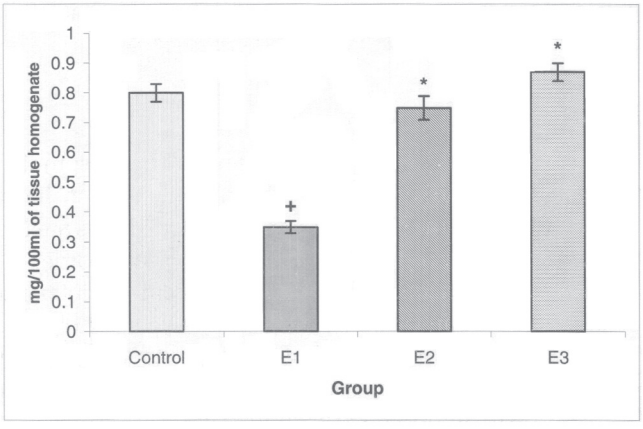

Fig-4 : Effect of stress and drug treatment on ascorbic acid level (Mean ± SEM) in hippocampus.

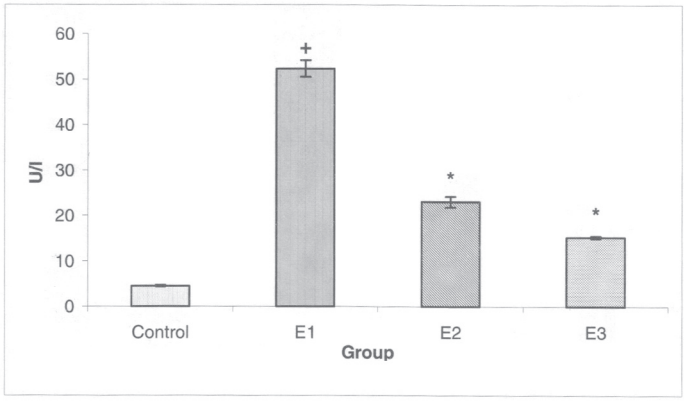

Fig-5 : Effect of stress and drug treatment on LDH (Mean ± SEM) in hippocampus.

Discussion

Results of the present study demonstrate antioxidant effects of the root extracts of both Asparagus racemosus (Shatawari) and Withania somnifera (Ashwagandha) in rat hippocampus used as model system. These effects are evidenced by significant recovery of SOD and CAT enzyme level after drug treatment to the animals given 4hrs immobilization/ swim daily up to 30 days, in unpredictable manner. These enzymes are part of antioxidant defense of the body against free radicals and thus the significant increase after the drug treatment is indicative of free radical scavenging properties of both the drugs used in study. A significant increase in LDH activity after stress but significant decrease after drug treatment is indicative of reduced lipid peroxidation in the brain area studied. Determination of MDA level also showed similar results and thus substantiate our observations. High MDA level is indicative of higher lipid peroxidation as observed in stress treated group. Significantly reduced MDA level after treatment with drug extract is clearly indicative of anti oxidant activities of these drugs. Ascorbic acid content is indicative of primary stress reaction and its lower level indicate high corticosterone secretion which has destructive effects on neuron cell bodies in hippocampus. In our study, lower concentration of ascorbic acid after stress treatment suggest detrimental effects on neuron cell bodies in hippocampus. Such effects were observed in cresyl violet preparation as acid phosphatase staining (published elsewhere). Thus higher ascorbic acid level observed after treatment with the extracts of both the drugs clearly demonstrate there corticosterone lowering capacity. Whether the endogenous elevation of glucocorticoids after stress is involved in decrease of antioxidant capacity of the nerve cells in brain. The results of the present study substantiate the above view. In fact, elevated levels of endogenous glucocorticoids during aging and chronic stress have been shown to induce nerve cell degeneration in hippocampus via increasing oxidative stress (17-21).

Application of herbal preparations as anti stress and cytoprotective agents has been attempted in past. Protection of hippocampal, cortex and striatum neurons after stress related degeneration has been reported in literature (11,22-25). Although on basis of these observations actual mechanism of the cell degeneration by stress regimen or protective effects of drug treatment can not be elucidated, however, study does suggest that A. racemosus as well as W. somnifera possess antioxidant properties however, our results indicates first time that the A. racemosus root extract shows more protective effects as compared to W. somnifera. Adaptogenic herbs have traditionally helped prevent the imbalances that can result from stress and thus may prevent or slow down the development and progression of the CNS disorders. In an Ayurvedic system of traditional medicine in India, Medya Rasayana is a group of herbal preparations known for their effects on nervous system. Few of them are also classified as adaptogen.

Results: Table 1-Effect of Asparagus racemosus and Withania somnifera on various biochemical parameters in control and experimental rats

Group	Treatment	Catalase μ Moles/ml/mg of protein	(MDA) nMoles/ml	SOD% Inhibition of NBT reduction	Ascorbic AcidMg/100 ml	LDH U/l
I	Control(Vehicle)	51.00 ± 1.73	7.90 ± 0.15	75.48 ± 0.27	0.80 ± 0.03	4.50 ± 0.17
II	Stress E1 (Swim) One Month	22.50 ± 2.89 +	14.94 ± 0.26+	52.09 ± 2.89 +	0.35 ± 0.02 +	52.35 ± 1.82 +
III	Asparagus racemosus E2 100 mg/ kg/day	57.25 ± 3.32*	10.85 ± 0.10*	75.59 ±2.80**	0.75 ± 0.04*	23.08 ± 1.17*
IV	Withania somnifera E3 100 mg/ kg/day	49.00 ± 6.06**	10.98 ± 0.40*	73.94 ± 0.76*	0.87 ± 0.03*	15.30 ± 0.23*

All values represents Mean ± SEM (n=4)
P values: + < 0.001; ++ <0.01; +++ < 0.05 When compared with control
Untreated animals. * < 0.001; ** < 0.01; *** < 0.05 When compared with stress animals .

Among them are Shankhpushpi (Covolvulus peuricaulis), Brahmi (Baccopa monniera), ashwagandha (Withania somnifera), Jyotishmati (Celestrus peniculatus), tulsi (Oscimun sanctum), shatawari (Asparagus racemosus) etc. They are said to improve mental disabilities, learning and memory. Although these drugs are claimed to improve nervous ailments, there is no experimental evidences available in literature. Whether they protect the cell, increase survivability, by enhancing synthesis of growth factors or scavenging free radicals or removing other toxins responsible for cell death. Earlier studies in various laboratories and in this laboratory has shown that most of the nervous system disorders from simple dementia to typical degenerative disorders are characterized by gradual cell loss in specific brain areas such as hippocampus, substantia nigra, cerebral cortex, striatum, putman etc. Pyramidal cell loss in CA1, CA3, CA4 areas of hippocampus (19,23,25-31) have been shown.

In modern therapeutic system, therapies for numerous central nervous system disorders are at present not available. Drugs available for treatments of anxiety, depression and mental health conditions are not satisfactory. Majority of currently available CNS drugs are of synthetic origin and most of them are derived from yet other synthetic molecule (Chlorpromazine and Reserpine, tricyclic and MAO inhibiting antidepressants, Benzodiazepines, Meprobamate, Pentylenetetrazol, Amphetamine, Methylphenidate, Barbiturates, Hydantoin, Oxazolidone, Succinimides, Acetyl urea as antileptics, Bromocriptine, Apomorphine, levodopa, Amantadine, Trihexiphenydyl, Procyclidine) Herbal remedies for such conditions have been known since time immemorial and efforts made during the past few decades reconfirm that several of herbs are indeed therapeutically useful for treatment of diverse CNS disorders.

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