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Research Paper

NEURONUTRIENT EFFICACY OF *WITHANIA SOMNIFERA* IN AGING BRAIN OF MICE

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Abstract

Withania somnifera is classified in Ayurveda an ancient Indian system of medicine, as a rasayana, a group of plant derived drugs which promote physical and mental health, augment resistance at the body against disease and diverse adverse environmental factors, revitalize the body in debilitated condition and increase longevity. We investigated the effect of *Withania somnifera* on aged mice and its effect on lipid peroxidation and antioxident enzyme in aging brain of mice. Treatment with *Withania somnifera* successfully attenuated GPx activity and inhibit lipid peroxidation in a dose depended manner. WS inhibited body LPO and protein oxidative modification induced by aging. The result indicates the therapeutic potential of *Withania somnifera* in brain aging.
Key words: Aging, Brain, *Withania somnifera*.

INTRODUCTION

Withania Somnifera (WS) commonly known as Ashwagandha, Indian Ginseng and winter cherry belonging to family Solanceae, is an important herb in Ayurvedic and indigenous medical system for centuries in India [1]. In general ashawagandha has been traditionally as a tonic and nootropic agent [2]. In ayurvedic medicine it has been used to increase longevity and vitality. It has been used as an antibacterial [3], antioxidant[4]-[6],adaptogen live tonic and anti inflammatory agent [7].The traditional use of *Withania somnifera* was to increase vital fluids, muscle fat, blood and lymph and cell production. It helps to counteract chronic fatigue, weakness, alehydration loose teeth, premature aging, and muscle tension [1].

Historically the plant has been used as an antioxident, adaptive and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. The phytochemical present in *Withania somnifera* are well known potent free radical scavenger and it has also been reported that the extract of *Withania Somnifera* tends to reverse the changes in lipid peroxidation [6],[8]-[9]and damage to cells. *Withania* contents active ingredients like steroidal saponin, alkaoids and steroidal lactones knwon as “withanolides”. Withaferin-A and withanolide D are the two main withanolides that contributes to most of biological action of *Withania* [10].

The herb was found to have a very good antioxidant activity which may in part explain the anti-stress, congestion facilitating, anti inflammatory and anti aging effects of this herb [11]. *Withania somnifera* extract and its constituent have mild to moderate hypnatic action and

antioxidative property in animals [12].As an antioxidant WS and active constituents sitoindosides VII-X and withaferin A(WA)have been proven to increase level of superoxide dismutase, catalase, and ascorbic acid ,while decreasing lipid peroxidation[13]-[17]. WS decrease refracotaroy depression in animal subjected to stress [18] and increase tumor sensitization to radiation and decrease radiation therapy effect [19], modulates the immune response, increasing the expression of T-helper 1cytokinase, CD4and CD8counts and natural killer cell activity [20].Protective role of Ashwagandha leaf extract on scopolamine induced changes in brain and brain derived cells[21]and hippocampal arc[22] also show its neuroptective properties.

There is strong scientific evidence that free radicals and the oxidative stress play an important role in ageing [23]. Free radical induced lipid peroxidation has gained much importance, because of its involvement in reversal pathological conditions such as ischemia, liver disorder, neural disorder, mental toxicity and pesticide toxicity. Lipid peroxidation (LPO) involves the formation of lipid radicals, oxidation of unsaturated lipids and the eventual destruction of membrane lipids producing a variety of breakdown products and deleterious effects. These deleterious processes can be prevented by reducing or destroying the formation of free radicals which are continuously formed in a biological system. In normal tissues endogenous enzymes are there that counteract the free radicals superoxide dismutase (SOD) and Catalase (CAT) being the most common ones .Various redox dependent gerontomodulatory approach include nutritional and pharmacological intervention by free radical scavengers(antioxidant theory). With the above mentioned properties of Ashwangha the present study was carried out to investigate the antioxidant effect of methanolic extract of Withania somnifera on brain in swiss albino mice. The present study has been carried out in order to evaluate the neuronutrative efficacy of Ashwagandha for promotion of healthy brain aging ,which could prove good nutraceuticals too. Ii is easily available to general public especially to the below poverty line without expending much money and energy.

MATERIAL AND METHOD

Experimental Plant: - In this experimental study fresh leaves of *Withania Somnifera* were collected from Bharatpur district of Rajasthan. The plant was identified by Taxonomist from Botany Department M.S.J. College Bharatpur. The collected plant materials were separately shade dried for one week and dried leaves were powdered with the help of grinder. The dried and powdered leaves (10gm) were extracted successively with methanol in a soxhlet extractor for 48 hours at a temperature 60ºC. The residual extract was filleted and then the solvent was evaporated to dryness at 40ºC by using a rotary evaporator. The residual extracts were stored in refrigerator 4ºC in small and sterile glass bottles. It was used in experiment after dissolved in distilled water. All chemical used in the study were of analytical reagent grade and obtained from Sisco Research laboratories , HIMEDIA and Central Drug House (India)

Experimental Animal :- Fifteen adults healthy male Swiss albino mice (*Mus Muscules*) weighing approximately 30-35gm (age 6-6.5 months) were obtained from PBRI Bhopal (M.P.) for experimental purpose. Animals were maintained under controlled condition of temperature 22 ºC ±3 ºC. All animals were given standard diet (Hindustan Lever Limited, India) and water *ad. libitum*. After an accumaliization of 7 days, mice were divided in to three groups of five each. The initial body weight of each was recorded. Group I received normal saline only served as vehicle control. Group II received 100mg/kg bodyweight methanolic extract of withania leaves P.P. only. Group III received 200mg/kg. methanolic extract of *Withania somnifera* leaves. P.P. only. Treatment was given orally and continued for 1 month. On the last day final body weight of each animal was recorded. Animals were then sacrificed under mild ether anesthesia. Brain from animal was removed and cleaned twice in phosphate buffered saline and the wet weight was noted and processed for biochemical estimations.

LPO was studied by the method of Ohkawa [24]. LPO was determined by thiobarbitarice acid (TBA) reaction with molondialdehyde (MDA), a product formed due to peroxidation of lipids. The LPO was expressed as nm of MDA formed per h per mg. Protein and SOD was determined

by the method of Paoletti *et.al.* (1986) [25] and Catalase was determined by the method of Goth. (1991)[26] .

Statistical Analysis :- The results were expressed as mean±S.E.M (standard error of mean) and percentage of changes. Level of significance between groups were set at P<0.05. The data was analyzed using the statistical package for social science program (S.P.S.S. 11). For comparison between different experimental groups, one way analysis of variation (ANOVA) was used followed by post hoc Tukey's test.

TABLE – 1 Body weight(gm) and brain weight(gm) of experimental groups before and after the treatment of *Withania somnifera* extract (Values are mean±S.E.M)

S.No.	Treatment Group	Body Weight(gm) Initial	Body Weight(gm) Final	Brain weight(gm) Initial	Brain weight(gm) Final
1.	Control	29.333 ± 3.0768	34.833 ± 2.9269	0.4672±0.122	0.4752±0.331
2.	Extract 100mg/Kg.	30.000 ±2.8983	33.845 ±3.4881†	0.4671±0.167	0.47872±0.133†
3.	Extract 200mg/Kg.	27.500 ±1.3784	31.667 ±2.5820†	0.4498±0.242	0.4634±0.167†‡

P<0.05: †as compared to control and ‡ as compared to Extract (100mg/Kg).

TABLE 2:- Effect of *Withania somnifera* extract on Oxidative Enzyme in Brain Tissue of mice (Values are mean±S.E.M n=5)

S.No.	Treatment	LPO (µ M MDA/mg wet tissue)	SOD (µ/gm wet. tissue)	CATALASE (µ/mg wet. tissue)
1.	Control	16.71 ± 1.87	41.08 ± 4.70	21.61 ± 4.28
2.	Extract (100mg/Kg.)	14.79 ± 1.07**	43.16 ±3.70*	23.73 ± 3.34*
3.	Extract (200mg/Kg)	12.75 ± 2.06**,#	47.61 ± 2.29*,##	24.84 ±4.16**,$

Statistical analysis was carried out using one way ANOVA followed by post hoc Tukey's test *P<0.05, ** P<0.001as compared to control vehicle, #P<0.05, ## P<0.001 as compared to extract (100mg/Kg), $ non significant as compared to extract (100mg/Kg)

RESULT AND DISCUSSION

There was significant improvement (Table:1) in the weight of brain treated with *Withania somnifera* (Group II and Group III) than control. It is concluded that oral administration of *Withania somnifera* extract for 30 days significantly improved the weight of Brain.

Accumulation of free radical damage as the cause of aging is supported by studies . These studies show that increased oxidatively induced protein and lipid damage accelerate aging in human and mice [27]. As Table-2, Showed that *Withania somnifera* decreased LPO significantly (P< 0.001) for both high and low doses. In contrast, it significantly increased the SOD (L<0.05 for low dose) and CAT (P<o.05 for low dose and P<0.001 for high dose) compared to the control values. These results clearly demonstrate that a drug induced inhibition in lipid peroxidative process, indicating protective effect of *Withania somnifera*. Similar to our finding protective effect with respect to LPO has been reported for two other plants *Rubia Cordifolia* [28] and *Bacapa Monnera* [29].

Hydrogen Peroxide is continuously generated in large concentrations by the mitochondrial inner membrane's of these radicals are destroyed by SOD, Superoxides anion (O2) radicals with water to form respiratory chain. Oxygen gets converted to superoxides anion radical (O2) when it acquires an extra electron. Most hydrogen peroxides which in turn responsible for the highly reactive hydroxyl (OH-) radicals that attack membrane fatty acids to initiate LPO and bring about necrotic changes in the tissue . Catalase appears to be the most effective defense agent against high concentrations of hydrogen peroxide. In the present study both SOD and CAT activities were increased by *Withania somnifera*, further supporting the defensive nature of the

withania somnifera against free radical damage and stress [30]. Thus *withania somnifera* may act as an exogenous antioxidant. However, further studies are elucidating the molecular mechanisms involved in order to support the clinical use of plant extract as a therapeutic agent.
Lipid peroxidation, a basic cellular deteriorative change, is one of the primary effect induced by oxidative stress aging due to presence of membrane rich in polysaturated highly oxidizable fatty acids[30]. Asawgandha extract was able to scavanges the oxidative damage as evidence by decreased lipid peroxidative process and increase oxidative status of the body. Several studies have suggested that these increases in oxidative stress vulnerability can be reduced through plant extract as dietary supplement that prevent brain atrophy [31]-[33].This would suggest that there might indeed be properties , in addition to antioxidant characteristics, that are promoted by phytochemical present in plant extract involved in increasing excitotoxic neuronal damage[34] .
Increase in oxidative stress would also contribute to progressive impairment of the antioxidant reserve of the brain. Many non enzymatic antioxidant molecule and anti oxidant enzyme (SOD,CAT, glutathione peroxidase and glutathione reductase) are the most common parameter used to evaluate oxidative damage. Reduced Glutathione plays a role in the protection of cells against oxidative stress. It can act as a non enzymatic antioxidant by direct interaction of the SH group with ROS.CAT and SOD are metalloprotein and accomplish their antioxidant function by enzymetically detoxifying the peroxides and superoxides anion.
Extract of *Withania somnifera* contain many active ingredients and these active ingredients are potent free radical scavengers. It has been reported that the root extract of *Withania somnifera* tend to reverse the change in lipid peroxidation and cell damage [35]. Although the major factor involved in these properties of Withania somnifera remain to be specified ,the finding of this study has suggested that plant extract reduced the effect of ageing .From the present study it is evident that *withaia somnifera* leaf extract is capable in preventing brain aging to some extent. Ageing itself cannot prevented however its complication can be mitigated and the present work has demonstrated the neuroprotective effect of *Withania somnifera* for prevention of age associated neurodegenration and promotion of healthy brain aging in aged mice. Thus it appears that these supplements could be beneficial for population below poverty line.

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