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# EVIDENCE FOR FREE RADICAL SCAVENGING ACTIVITY OF ASHWAGANDHA ROOT POWDER IN MICE

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**Abstract:** The effects of Ashwagandha root powder (0.7 and 1.4 g/kg body weight/day), administered for 15 and 30 days, was investigated on lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) activities in mice. While 15 days treatment with Ashwagandha root powder did not produce any significant change, 30 days treatment produced a significant decrease in LPO, and an increase in both SOD and CAT. Our findings indicate that Ashwagandha root powder possesses free radical scavenging activity, which may be responsible for its pharmacological effects.

**Key words:** Ashwagandha, superoxide dismutase, catalase, lipid peroxidation

## INTRODUCTION

Free radical induced lipid peroxidation has gained much importance, because of its involvement in several pathological conditions such as atherosclerosis, ischemia, liver disorder, neural disorder, metal toxicity and pesticide toxicity (1-3). 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. However, clinically other antioxidants such as vitamin E and vitamin C are also used. Among the herbal preparations only limited trials have been done and not much is known (2, 4). We studied the antioxidant activity of the root powder of a subtropical undershrub, Ashwagandha (Withania somnifera), that is well known for its medicinal value (5).

## METHOD

Fifteen adults healthy colony bred 'Swiss albino' male mice, weighing 30–35 g (approximate age 3 months) were maintained in controlled room temperature (27 ± 1°C) and light cycle (14 h light and 10 h dark), with food (obtained from Hindustan Lever Ltd., India) and water *ad libitum*. After an acclimatization of 7 days, mice were divided into three groups of five

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each. The initial body weight of each was recorded.

*Ashwagandha treatment* : Ashwagandha root, dried powder (with 1.75% Withanolides) supplied by Kisalaya Pharmaceuticals Pvt. Ltd., Indore, India was used to prepare suspensions of 20 mg/0.1 ml and 40 mg/0.1 ml concentrations using water : tween 80, (9 : 1), for the treatment.

Group I received equivalent amount of the suspending agent and served as control. Group II and Group III received 0.7 g and 1.4 g of Ashwagandha suspension per kg body weight/day respecively. Treatment was given orally by gastric intubation, every day between 10–11 h and continued for 30 days. On the last day, final body weight of each animal was recorded. Animals were then sacrificed, liver of each animal was removed, cleaned twice in phosphate buffered saline (pH 7.4) and immediately processed for biochemical estimations.

LPO was studied by the method of Ohkawa *et al* (6) followed earlier in our laboratory. In brief, liver was homogenised in 10% W/V ice cold phosphate buffer (0.1 m, pH 7.4) using Potter-Elvehjem teflon homogenizer. The homogenate was centrifuged at 2,000 g at 4°C for 30 min and supernatant was used for subsequent assay procedure. LPO was determined by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to peroxidation of lipids. The LPO was expressed as nM of MDA formed per h per mg protein. SOD activity was determined by the method of Marklund and Marklund (7) and CAT activity by the method of Aebi (8). The values were expressed as µmol of hydrogen peroxide decomposed/min/mg protein.

These experiments were repeated with another 15 albino mice. However, this time treatment was for 15 days only in order to evaluate the effectiveness of the short term drug therapy.

Data have been expressed as the mean ± standard error of the mean (SEM). For statistical evaluation, Students 't' test was used.

## RESULTS AND DISCUSSION

Ashwagandha suspension decreased LPO significantly ($P < 0.001$ for both high and low doses). In contrast, it significantly increased the SOD ($P<0.05$ for low dose and $P<0.001$ for high dose) and CAT ($P<0.05$ for low dose and $P<0.001$ for high dose) compared to the respective control values (Table I). These results clearly demonstrate a drug induced inhibition in lipid peroxidative process, indicating protective effect of the Ashwagandha root powder. Administration of this drug inhibited the formation of hepatic malondialdehyde

TABLE I : Values (mean ± SEM) of LPO (nM of MDA/h/mg protein), SOD (Units/mg protein) and CAT (µM of $H_2O_2$ decomposed/ min/mg protein) activities in control and Ashwagandha treated (0.7 and 1.4 g/kg body weight/day for 30 days) mice.

| *Groups* | *LPO* | *SOD* | *CAT* |
|---|---|---|---|
| Control | 0.48 ± 0.02 | 5.92 ± 0.18 | 55.19 ± 1.86 |
| Treated (0.7 g) | 0.35 ± 0.02*** | 7.56 ± 0.51* | 67.15 ± 2.21** |
| Treated (1.4 g) | 0.25 ± 0.01*** | 9.21 ± 0.65*** | 71.32 ± 1.5*** |

* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ compared to the respective control values.

significantly only when treatment was continued for 30 days suggesting that, the effectiveness of the drug depends on the duration of the treatment. Similar to our finding, protective effect with respect to LPO has also been reported for two other plants, *Rubia cordifolia* (2) and *Bacopa monniera* (4).

Hydorgen peroxide ($H_2O_2$) is continuously generated in large concentrations by the mitochondrial inner membrane's respiratory chain. Oxygen gets converted to superoxide anion radical ($O_2^-$) when it acquires an extra electron. Most of these radicals are destroyed by SOD. Superoxide anion ($O_2^-$) reacts with water to form $H_2O_2$ 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 (9). Catalase appears to be the most effective defensive agent against high concentrations of $H_2O_2$. In the present study, interestingly, both SOD and CAT activities were increased by Ashwagandha, further supporting the defensive nature of the drug against free radical damage.

Thus Ashwagandha may act as an exogenous antioxidant. Since Ashwagandha is known for its aphrodisiac, psychotropic, antitumor and cardiotonic effects, some of these pharmacological effects may be due to free radical scavenging activity of the Ashwagandha root powder.

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