Neuroprotective Effects of *Withania somnifera* Dunn. in Hippocampal Sub-regions of Female Albino Rat

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The neuroprotective effects of *W. somnifera* were studied on stressed adult female Swiss albino rats. Experimental rats were subjected to immobilization stress for 14 h and were treated with a root powder extract of *W. somnifera* available as Stresscom capsules (Dabur India Ltd). Control rats were maintained in completely, non stressed conditions. Thionin stained serial coronal sections (7 μm) of brain passing through the hippocampal region of stressed rats (E1 group) demonstrated 85% degenerating cells (dark cells and pyknotic cells) in the CA2 and CA3 sub-areas. Treatment with *W. somnifera* root powder extract significantly reduced (80%) the number of degenerating cells in both the areas. The study thus demonstrates the antistress neuroprotective effects of *W. somnifera*. Copyright © 2001 John Wiley & Sons, Ltd.

**Keywords:** *W. somnifera*; root powder; hippocampus; cell degeneration; immobilization stress; rat.

**INTRODUCTION**

*Withania somnifera* Dunn. (Family Solanaceae) commonly known as ‘Ashwagandha’ is used as ‘Medhya Rasayana’ for the treatment of mental diseases and anxiety states, in the traditional Indian medicinal system (Chatterjee and Prakesh, 1995). It is an erect shrub 0.5–2 m high and grows throughout the dry and sub-tropical parts of India. The drug reduces the levels of acetylcholine and catecholamine and increases the levels of serotonin and histamine in brain tissue (Handa, 1993). *W. somnifera* has also been shown to possess both depressant and anticonvulsant properties (Kulkarni and Verma, 1993; Kulkarni *et al.*, 1993; Kulkarni and George, 1996). Ziauddin *et al.*, (1996) studied its adaptogenic, antistress, anticonvulsant and cognitive dysfunction properties. The antioxidative effects of the plant extract have been demonstrated (Bhattacharya *et al.*, 1997; Panda and Kar, 1997). Cytotoxic effects of *W. somnifera* have also been demonstrated in cancer (Uma Devi, 1996).

The present study was undertaken to investigate the antistress and neuroprotective effects of *W. somnifera* root extract on neuron cell bodies in hippocampal sub-regions of the adult female rat brain.

**MATERIALS AND METHODS**

Ten adult female Swiss albino rats, body weight 60 ± 5 g, age 2 months, were maintained in the animal room at a controlled temperature (26° ± 2°C) and a light and dark cycle (12 h light and 12 h dark) for 7 days and were provided with food and water *ad libitum*. Rats were divided into two groups: control and experimental groups.

**Control group.** Rats were placed in the animal room (*n = 3 per cage*). It was locked for 24 h. The next morning the animal room was opened and the rats were killed immediately by decapitation.

**Experimental group.** Experimental animals were divided into five groups E1–E5. Animals in these groups were subjected to stress for specific periods and treated with the experimental drug.

**Stress protocol.** Rats were placed in tightly fitting ventilated plastic boxes (10 × 5 cm) for a specific stress period (14 h) every day. The animals were unable to move in these boxes and thus became stressed. Stress was given for 30 days. The stress period was selected on the basis of a pilot study to obtain the maximum percentage cell degeneration in the CA1-Dg region. Although habituation was observed in the general behaviour, cell degeneration was complete.

**Drug preparation.** An extract of *W. somnifera* Dunn. available as a commercial preparation Stresscom capsule (Dabur India Ltd), was used. The capsule consisted of a hydroalcoholic extract of Ashwagandha root powder. This extract was standardized for withanolids and withanolides. Soya lectins, bees wax and arachis oil was used as a base for medicament and packed in soft gelatin capsules. The capsule was dissolved in water and given orally, daily (20 mg/kg body weight) for 30 days. Treatment was performed everyday between 9 am and 10 am.

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Figure 1. (1) Photomicrograph of CA2 hippocampal region of the control animal (C) showing normal cell bodies (→) (×40). (2) Photomicrograph demonstrating CA2 region of stress (E₁) group totally dark cell (►) with few pyknotic nuclei (→) (×40). (3) Photomicrograph demonstrating CA2 region of dose (E₂) group. Cells showing normal cell morphology (×40). (4) Photomicrograph demonstrating CA2 region of dose + stress (E₃). Normal cells with few pyknotic nuclei (×40).

Figure 2. (1) Photomicrograph showing CA3 hippocampal sub-region of control (C). Normal cell bodies (→) present (×40). (2) Photomicrograph demonstrating CA3 region of stress (E₁) group showing dark cells (►) with pyknotic nuclei (⇒) (×40). (3) Photomicrograph showing CA3 region of dose + stress (E₃) group. All are normal cell bodies (→) (×40). (4) Photomicrograph showing CA3 of dose + stress (E₃) group demonstrating normal cell (→) (×40).
Treatment Schedule. Group E1: Rats were subjected to 14h stress every day for 30 days and killed on day 31.
Group E2: Normal rats were given the drug daily for 30 days.
Group E3: Rats were given the drug daily for 30 days and from day 31 onwards were subjected to stress for 30 days.
Group E4: Rats were subjected to stress for 30 days and from day 31 onwards given the drug for 30 days.
Group E5: Rats were given the drug and stress daily for 30 days.

Parameters of study. Daily body weight, food and water consumption was recorded. After completion of the experiments, the rats were killed by decapitation, and then the brain was dissected out. The ascorbic acid level ($n = 4$) was measured (Natelson, 1971), in the whole fore brain homogenate to confirm the stress effects.

The remaining brain tissue ($n = 6$) was rinsed in distilled water and fixed for 12 h in 10% neutral chilled formalin. After fixation the brains were dehydrated in alcohol series, embedded in paraffin wax and 7μm thick serial coronal sections were cut. Sections were stained with thionin stain (Clark and Sperry, 1945) for demonstration of nerve cells.

RESULTS

Hippocampal sub-regions CA1-Dg were studied in the control (C) and experimental (E1–E5) groups. Identification of the hippocampal sub-regions was based on a rat brain atlas by Paxinos and Watson (1986). Photomicrographs of C, E1, E2 and E3 groups are included only.

CA2 hippocampal sub-regions

In the control group (Fig. 1.1) the cell bodies were 5–6 μm in size, and multipolar in shape. All the cells in the region demonstrated a large and distinct nucleus, a centrally located nucleolus and darkly stained cytoplasm.

In group E1 (Fig. 1.2) the cells showed degenerating cells, (85%) darkly stained cells and pyknotic nuclei in CA2 (Fig. 3). Cell bodies were 3–5 μm in size and irregular in shape. A few apoptotic bodies were observed. The cells were compactly arranged in a tier of 3–5 cells.

In the E2 group (Fig. 1.3) the cells were large (5–6 μm), with a large centrally located nucleus. The cytoplasm was darkly stained. No degenerative characteristics were observed. In the E3 group (Fig. 1.4) the cell bodies were large, the nucleus and nucleolus were distinct in most of the cell bodies. The number of dark cell bodies, pyknotic nuclei or apoptotic bodies were reduced significantly. In the E4 and E5 groups darkly stained cells, pyknotic nuclei and vacuolated spaces were observed, similar to the E1 group.
CA3 hippocampal sub region

In the control group (C, Fig. 2.1), the cells were large (7–8 μm) and multipolar in shape. All the cells demonstrated a large, distinct nucleus, centrally located nucleolus nissl material in the cytoplasm was intensely stained. Cell bodies were arranged compactly in 4–6 tiers of cells.

In group E1 (Fig. 2.2) the thionin stained preparation showed degenerating cells, darkly stained cells and pyknotic nuclei (85%). Pyknotic cell bodies were comparatively small (4–5 μm) and irregular in shape. Cell bodies with condensed cytoplasm, apoptotic bodies and vacuolar spaces were also observed.

In group E2 (Fig. 2.3) the pattern of cell distribution was similar to the controls. The cells were large with a distinct nucleus and nucleolus. All the cells were arranged compactly in the layer. Vacuolar spaces, pyknotic nuclei etc. were not observed. In group E3 (Fig. 2.4) the cell bodies were large with a distinct and centrally placed nucleus. The number of vacuolar spaces, pyknotic nuclei, cells with condensed or shrunken cytoplasm were drastically reduced. In the E2 and E3 groups darkly stained cells, pyknotic nuclei and vacuolated spaces were observed, similar to the E1 group.

No significant change in body weight was observed in the rats of the control or experimental group in the present study.

The study of ascorbic acid level in the fore brain homogenate of the control and experimental groups showed a significant reduction in ascorbic acid level after stress commensurate with the drug treatment (Fig. 4).

DISCUSSION

The results of the present study demonstrates that Aswagandha (W. Somnifera Dunn.) produces neuroprotective effects and reduces the stress induced changes in neuron cell bodies in the CA2 and CA3 sub-area of the hippocampus. Stress caused a significant degeneration in the cell bodies of these areas showing as dark cells, pyknotic cells, cells with condensed nuclei and cytoplasm. Vacuolar spaces in the cell cytoplasm were observed in thionin stained serial sections. In sections demonstrating histochemical localization of Acapase, passing through the region. After treatment with Aswagandha root powder extract, a significant reduction in the number of these degenerated cells was also observed. Dark cells, cells with shrunken nucleus and cytoplasm, and vacuolar spaces were significantly reduced in both CA2 and CA3. Interestingly animals of group E3 (drug treatment for at least 30 days given prior to subjecting to stress) showed more cytoprotective effects than the post or simultaneous treatment.

Although the mechanism of degeneration and cytoprotective effects of the drug can not be explained on basis of these experiments it is possible that an increase in corticosteroid level during stress might be associated with the cell degeneration. Several authors have reported the neurotoxicological effects of corticosterone on hippocampal cells (Sapolasky et al. 1985, 1991; Gould et al., 1990). A significant decrease in the ascorbic acid level in the brain homogenate of the stressed group and restoration to the normal level after treatment with the herbal drug substantiate our observation.

The cytoprotective properties of W. somnifera and several other plants have been studied earlier. Al-Harbi et al. (1997) showed anti-ulcer and gastric cytoprotective effects of Commifora mol mol. Zhu et al. (1997) showed such effects using Cyperus rotundus in rats. Dhuley and Naik (1997) demonstrated the protective effects of a Rhinax herbal formulation on CCl4 induced liver injury. Uma Devi et al. (1993) demonstrated cytoprotective andradiosensitization effects of W. somnifera.

The present study is the first to report the cytoprotective antistress effects of W. somnifera on hippocampal cell bodies.

REFERENCES


