

Evaluation of Nootropic Potential of Herbal Extract in Stressed Rats

K.M. GEETHA*, S.R.B. PRIYADHARSHINI and V. MURUGAN

Department of Pharmacology, Dayananda Sagar College of Pharmacy

Kumaraswamy Layout, Bangalore-560 078, India

E-mail: geethakm@yahoo.com

An ethanolic extract containing a combination of the roots of *Withania somnifera* and leaves of *Ocimum sanctum* was investigated for its nootropic activity in stressed rats. Cold swimming for 5 h induced stress. Learning and memory was assessed using the brightness discrimination apparatus. Stress induced impairment of learning and memory in rats was reversed by the combined extract when administered orally for 7 d at 100 mg/kg b.w. However the combined extract was found to be ineffective on a single acute administration.

Key Words: Cold swimming stress, Learning and memory, Combined extract.

INTRODUCTION

Stress represents a reaction of the body to a stimulus that tends to alter its normal physiological equilibrium or homeostasis and has been defined as a nonspecific response of the body to any demand imposed on it¹. Cure of cognitive disorders such as dementia, amnesia, attention deficit and Alzheimer's disease is still a nightmare in the field of medicine. Nootropic agents such as piracetam, aniracetam and choline esterase inhibitors like donepezil are being used to improve memory, mood and behaviour, but the resulting side effects associated with these agents have made their use limited. Stress is known to cause memory impairment. Cortisol is produced in the body during stress and there are receptors for cortisol in the hippocampus, the region of the brain responsible for memory. Long-term exposure to these hormones can cause atrophy of the hippocampus, leading to memory impairment.

Withania somnifera Dunal (Ashwagandha) is widely used in Ayurvedic medicine, the traditional medical system of India. It is an ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism) and as a general tonic to increase energy and to improve overall health and longevity. The plant is used to cope with depression, anxiety and stress induced conditions through its powerful antioxidant and adaptogenic properties². *Ocimum sanctum* (Family: Lamiaceae)

is commonly known as Holy basil. The plant is reported to possess adaptogenic properties when tested against a battery of tests in mice and rats³. It increased physical endurance in swimming mice, prevented stress induced ulcers and protected mice and rats against carbon tetrachloride induced hepatotoxicity. The plant is reported to augment learning and memory in mice^{4,5}. Since the nootropic activity of the two individual extracts are established to some extent, the present investigation was carried out to study the nootropic activity of the combined extract of *Withania somnifera* and the leaves of *Ocimum sanctum* in stressed rats, since stress is known to impair learning and memory.

EXPERIMENTAL

The roots of *Withania somnifera* and leaves of *Ocimum sanctum* were dried and powdered separately. The powders were individually subjected to ethanolic extraction in soxhlet apparatus. Each extract was dried on a steam bath to constant weight. The two extracts were combined in the ratio of 1:1.

The pharmacological investigations were conducted on albino rats (180-220 g) of both sex. The animals had free access to standard pellets and water. A recommended dose of 100 mg/kg b.w was given for 7 d orally.

Rats weighing between 200 to 220 g were divided into three groups of six animals each. Group **1** received saline for 7 d and served as normal control, Group **2** was subjected to cold swimming stress (5 h in cold water) and served as stress control group and Group **3** received the combined extract at 100 mg/kg orally for 7 d. Group **2** and **3** were subjected to cold swimming stress by allowing the animals to swim individually in cold water for 5 h continuously.

Learning and memory was assessed using the "brightness discrimination apparatus" as per the following procedure:

The apparatus consists of the following parts (i) an entrance compartment in which the rat is placed, (ii) a discrimination chamber into which the rat is released by opening the door, (iii) the discrimination chamber is divided into two alley-ways having wire grills on the floor to make it possible to shock the animal, (iv) at the end of each alley way is a window for presenting a visual stimulus. Thus a bright area of any colour may be present at the end of one passage way and a dark or different coloured area at the end of the other, (v) doors in the end of the alley permit the animals to escape into the compartments, (vi) two lights against the respective windows any or both of which may be lighted up slits allow different colour slides to be placed in position and (vii) electrical unit which controls lights and selects grills for shocks and regulates its intensity. It operates at 220 V AC only. The removable lid covering the tops of the entrance and discrimination compartments may be used as and when necessary.

35 Rats weighing between 180-220 g of both sexes were trained for a period of 40 d. Preliminary habituation of the animal is necessary before the training. A rat is placed in the apparatus for a period of 0.5 h for 5 d before regular training period begins. It was assumed that the bright (lamp A) area is the positive or the correct stimulus object. The white filter was placed in the slit and lamp A was lighted. The grill of the other negative alley (B) was therefore charged and the corresponding door was closed. The grill selector control was set at B and intensity control is set at a previously determined value to give the desired shock. A hungry rat was released into the discrimination chamber from the entrance compartment as it crosses the grill and was thus induced to retrace its path. If the rat runs down the positive alley, no shock was received and it found the door into the food compartment. Some food was placed in the compartment to reward the animal.

After a large number of such trials in which the rat was punished by shocks upon entering the negative alley the rat learnt to run consistently towards the positive stimulus, even though other factors, such as relative position of the positive and negative alleys cues from smell, *etc.* are systematically varied by the experimenter.

After the training period, the animals were divided into 3 groups of six animals each consisting of the normal control, stress control and the extract treated. Thereafter the animals were assessed for learning and memory by subjecting each animal to 25 trials. The number of errors committed by each animal was noted and the results were statistically reported.

RESULTS AND DISCUSSION

The results are summarized in Tables 1 and 2. The results showed that the errors committed by rats treated with 100 mg/kg b.w of the combined extract were few compared to the stress controlled group indicating that the loss of learning and memory induced by stress was significantly reversed by the combined extract. The antistress activity of the extract could be correlated with the nootropic activity since the role of stress has been implicated in loss of memory and concentration in Alzheimer's disease⁶.

TABLE-1
EFFECT OF COMBINED EXTRACT ON LEARNING AND
MEMORY IN RATS

Group	Mean no. of errors \pm SEM of 6 animals		
	Normal control	Stress control	Combined extract
OBDL	9.83 \pm 0.91	16.83 \pm 0.54	12.83 \pm 0.61
RBDL	11.66 \pm 0.49	20.83 \pm 0.55	14.50 \pm 0.55

OBDL = Original brightness discrimination learning.

RBDL = Reversal brightness discrimination learning.

TABLE-2
STATISTICAL ANALYSIS OF THE EFFECT OF THE COMBINED
EXTRACT ON LEARNING AND MEMORY IN RATS

Group	Calculated 'F'	Table 'F'	Remarks
OBDL	70.85	4.82	Significant
RBDL	37.49	4.82	Significant

OBDL = Original brightness discrimination learning.

RBDL = Reversal brightness discrimination learning.

Agents that have selective facilitatory effect on intellectual performance and learning and memory are classed as nootropic agents. Many adaptogen species ameliorate learning and memory problems. Saponin compounds are thought to be responsible for the nootropic properties of *Withania somnifera*. In *Ocimum sanctum* memory and learning enhancement is thought to be due to phenyl propanoids and other flavonoids derivatives.

Hence, the combined extract could be beneficial in the treatment of cognitive disorders such as Dementia and Alzheimer's disease. More detailed experimentation parameters of learning and memory processes are necessary to evaluate the synergistic mechanism of action.

ACKNOWLEDGEMENTS

The authors wish to place on record their thanks to Government College of Pharmacy, Bangalore for providing all facilities to pursue the research work. Thanks are also due to Dayananda Sagar College of Pharmacy for their encouragement and support.

REFERENCES

1. H. Selye, *J. Neuropsych. Clin. Neurosci.*, **10**, 230 (1998).
2. Monograph, Thorne Research, *Alt. Med. Rev.*, **9**, 211 (2004).
3. K.P. Bhargava and N. Singh, *Indian J. Med. Res.*, **73**, 443 (1981).
4. R. Schliebs, A. Liebmann, S.K. Bhattacharya, A.K. Ghosal, V.R. Bigl, *Neurochem. Int.*, **30**, 181 (1997).
5. H. Joshi and M. Parle, *Indian J. Exp. Biol.*, **44**, 133 (2006).
6. T. Esch and G.B. Stefano, *Neuroendocrinol. Lett.*, **23**, 199 (2002).

(Received: 12 April 2007; Accepted: 25 February 2008)

AJC-6379