Memory Enhancing Effect of Ethanolic Extract of *Stevia rebaudiana* (Bert.)

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**Abstract**

Suspension of ethanolic extract of leaves of *Stevia rebaudiana* bert. in gum acacia were administered orally to evaluate the memory enhancing activity in the aged rats. Memory enhancing effect was evaluated by using Morris water maze and elevated plus maze. Mean escape latency time (ELT) calculated each day during acquisition trial was used as an index of acquisition and mean time spent in target quadrant in search of missing platform provided an index of retrieval. Significant reduction in transfer latency (TL) value of retention indicated improvement of memory. Significant memory enhancing effect of ethanolic extract *Stevia rebaudiana* was observed in aged rats at 200 mg/kg p.o. dose.

**Key words:** *Stevia rebaudiana*, Memory, Alzheimer disease,

**Introduction**

Alzheimer disease is age related neurodegenerative disorder, characterized by the extracellular deposition of β-amyloid fibrils within the brain and the subsequent association and phenotypic activation of microglial cells associated with amyloid plaque[1-2]. The activated microglia mounts a complex local pro-inflammatory response with secretion of acute phase proteins including α-antichymotrypsin, α-antitrypsin, and serum amyloid P, C-reactive protein and complement components[3-4]. Importantly, activation of microglia results in the synthesis and secretion of proinflammatory cytokines, interleukins-1β, IL-1β, IL-6 and tumor necrosis factor-α (TNF-α) and chemokine macrophage chemotactic protein, which leads to oxidative stress[5]. Reducing oxidative stress by antioxidants, protecting inflammatory lesions using anti-inflammatory drugs are some positive approaches to management of AD[6-8]. The nature provides a new opportunity to regain one’s full mental capacity. A number of herbs traditionally employed in the Indian system of medicine have yielded positive results. The current study was aimed to investigate the effects of *Stevia rebaudiana* in rats.

*Stevia rebaudiana* (Bertoni) is a perennial shrub of the Asteraceae family native to the certain regions of South America (Paraguay and Brazil). It is often referred to as the sweet herb of Paraguay[9]. It is single sweetner which has antidiabetic activity and also used for the treatment of number of ailments like hypertension and hyperlipidemia, having anti-inflammatory and antioxidant property[10]. The plant is reported to contain stevioside and rebaudioside which are 250 times sweeter than sucrose. Other compounds present in trace amounts are steviolbioside A and dulcoside[11-12].
The aim of the present study was to investigate the effects of oral administration of Stevia extract on learning and memory.

**Materials and Methods**

**Collection and Processing of Plant Material**

The leaves of *S. rebaudiana* were collected in March 2009 from herbal garden of, Department of Pharmacy Bhimtal, District Nainital, Uttarakhand India. Further taxonomic identification was conducted by Dr. K N Pandey, Head, Department of Botany, Kumaun University, UK, India. A voucher specimen was deposited in the herbarium of our laboratory under the number (Pharm/0102/09)

The air-dried leaves of *S. rebaudiana* (50 g) were powdered and then extracted with 500 ml of ethanol by using soxhlet apparatus. The crude extract was filtered and evaporated under reduced pressure to give a viscous dark mass with a percentage yield of 4.5% (w/w). This crude extract was suspended in 1% gum acacia in normal saline (vehicle).

Preliminary phytochemical investigation of the extract showed the presence of glycosides, terpenoids, flavonoids & sterols

**Animals**

All the experiments were carried out using Wister rats of either sex procured from IVRI, Bareilly, U.P. India. Young (4-6 months old) rats weighing about 40g and aged (24-28 months old) rats weighing about 180g were used in the present study. The animals were housed, 12 hr. light and 12 hr. dark cycle in the departmental animal house with free access to water and standard diet. All experiments were performed as per the norms of the ethical committee and the studies were approved and clearance obtained by the ‘Institutional Review Board’.

**Morris Water Maze**

Morris water maze (Morris, 1984) was employed to evaluate learning and memory. It consisted of a circular water tank (diameter 150 cm. and height 45 cm.) and was filled with water up to 30 cm. (at 25°C). The tank was divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A platform (10 cm²) of 29 cm. height was located in the center of one of these four quadrants. The position of the platform and clues were kept constant throughout the training session. In the present study, the target quadrant was Q₄. Each animal was subjected to four consecutive trials on each day with an interval of 5 min, during which they were allowed to remain on the platform for 20 sec. In case the animal was unable to locate the hidden platform with in 120 sec. It was gently guided by hand to the platform and was allowed to remain there for 20 sec. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition. Rats were subjected to acquisition trial for four consecutive days. On the 5th day, the platform was removed and time spent by animal in each quadrants was noted. The time spent by the animal in target quadrant and (Q₄) in search of missing platform was noted as an index of retrieval.

**Acquisition Trial**

Each rat was subjected to four trials on each day (after 16 day of drug treatment). A rest interval of 5 min was allowed in between each trial. Four trials per day were repeated for four consecutive days. Starting position on each day to conduct four-acquisition trial was changed as described below and Q₄ was maintained as target quadrant in all acquisition trial.

<table>
<thead>
<tr>
<th>Day</th>
<th>Q₁</th>
<th>Q₂</th>
<th>Q₃</th>
<th>Q₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day I</td>
<td>Q₂</td>
<td>Q₃</td>
<td>Q₄</td>
<td>Q₁</td>
</tr>
<tr>
<td>Day III</td>
<td>Q₃</td>
<td>Q₄</td>
<td>Q₁</td>
<td>Q₂</td>
</tr>
<tr>
<td>Day IV</td>
<td>Q₄</td>
<td>Q₁</td>
<td>Q₂</td>
<td>Q₃</td>
</tr>
</tbody>
</table>

Mean escape latency time (ELT) calculated each day during acquisition trial was used as an index of acquisition.

**Retrieval Trial**

On day 5th, the platform was removed. Each rat was placed in water maze and allowed to explore the maze for 120 sec. Each rat was subjected to four such trials and each trial was started from different quadrant. Mean time spent in target
quadrant i.e. Q4 in search of missing platform provided an index of retrieval. Care was taken that relative location of water maze with respect to other subject in laboratory serving as visual clues were not disturbed during the total duration of the study.

**Elevated Plus Maze**
Plus maze consisted of two open (50 x 10 cm) and two enclosed (50x10x40 cm) arms, connected by a central platform (5 x 5cm). The apparatus was elevated to a height of 25 cm above the floor. A fine line was drawn in the middle of the floor of each enclosed arm. On the day first (i.e. 16th day of drug treatment) each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency time (in seconds) was recorded first day (training session). The rats was allowed to explore the maze for 2 min and returned to home case. Retention of this learn task (memory) was examined 24 hr after the first day trial (i.e. 16th day, 24 after last dose).

**Quantitative Estimation of Serum Glucose**
Serum glucose was estimated spectrophotometrically at 505 nm by glucose oxidase/peroxidase method13 using a commercially available kit (Span Diagnostic Ltd, Surat, INDIA). 1500μL working glucose reagent was added to 20μL of serum, 20μL of standard solution of glucose (100mg/dL) and 20μL of purified water to prepare test, standard and blank sample respectively. All test tubes were incubated at room temperature for 30 min. To each test tube 1500μL of purified water was added. The absorbance of test and standard was measured against blank 505 nm spectrophotometrically (Elico, 164). Concentration of glucose (mg/dl) = O.D. test / O.D. std. x 100

**Statistical Analysis**
All results were expressed as mean ± SEM. Data was analyzed by using one way ANOVA followed by Tukey’s test and Bonferroni test. p<0.05 was considered to be statically significant

**Results**

**Effect on Escape Latency Time (ELT) and Time Spent in Target Quadrant (Using Morris Water Maze)**
Stevia extract (50 mg/kg, 100 mg/kg, 200 mg/kg, p.o.) showed dose dependent reduction on ELT in aged animals during acquisition trial conducted on day 1 to day 4. Stevia extract (200 mg/kg, p.o.) significantly (p<0.05) prevented aged induced increase ELT (Fig 1) when compared with respective control group. Stevia extract (200 mg/kg, p.o.) also attenuated significantly (p<0.05) decrease in time spent in target quadrant (Q4) in aged animals, in search of missing platform during retrieval trial conducted on day 5 (Fig. 2) when compared with respective control group.

![Fig-1](image1.png)
*Fig-1. Effect of Stevia extract (50, 100, 200 mg/kg, administered orally daily for 16 days in aged rates) on ELT (acquisition trails conducted on day 1 to day 4) using morris water maze.*

![Fig-2](image2.png)
*Fig-2. Effect of Stevia extract (50, 100, 200 mg/kg, administered orally daily for 16 days in aged rates) on retrieval trails (conducted on day 5) using morris water maze.*
Effect on Transfer Latency (TL) (Using Elevated Plus Maze)

Stevia extract (50 mg/kg, 100 mg/kg, 200 mg/kg, p.o.) showed dose dependent reduction on TL in aged animals. Stevia extract (200 mg/kg, p.o.) significantly (p<0.05) decrease TL time in aged animals (Fig 3) when compared with respective control group.

![Fig-3](image_url)

**Fig-3.** Effect of Stevia extract (50, 100, 200 mg/kg, administered orally daily for 16 days in aged rates) on TL time using elevated plus maze.

Effect on Blood glucose levels

Stevia extract (50 mg/kg, 100 mg/kg, 200 mg/kg, p.o.) does not showed any significant effect on blood glucose level (Fig 4).

![Fig-4](image_url)

**Fig-4.** Effect of Stevia extract (50, 100, 200 mg/kg, administered orally daily for 16 days in aged rates) on blood glucose level.

Discussion

Alzheimer’s disease is a progressive and fatal neurodegenerative disorder manifested by cognitive and memory deterioration, progressive impairment of routine activities of living and a variety of neuropsychiatric symptoms and behavioral disturbances. Oxygen free radicals are implicated in the process of age related decline in cognitive performance and may be responsible for the development of Alzheimer’s disease in elderly patients. There is a theory based on the concept that most change during aging arise from free radicals reactions and the formation of lipid peroxides in tissue, leading to age related damage. In case of brain damage caused by reactive oxygen species (ROS) induced through oxidative stress during aging, it is evident that nervous system in the brain are injured oxidatively and hence cognitive deficit may be induced by dysfunction of neurotransmission. Thus it is purposed that one of the pathogenesis of dementia is ROS generated by oxidative stress. The effect of antioxidants and antioxidant rich extract from natural product such as vitamin E, vitamin C, coca, apple juice, melatonin on the cognitive defect has been widely investigated. Thus it has been believed that antioxidants improve cognitive impairment through protection of neurons against oxidative stress. There is a strong association between free radical accumulation and the evolution of inflammation and inflammatory related responses. Proinflammatory cytokines, particularly IL-6 and TNF-α, which are major mediators in the induction of acute phase response, appear throughout the literature to play an important role in the pathology of cerebrovascular disease. It has been also observed that elderly patients suffering from Alzheimer’s disease showed reduction in symptoms upon chronic use of anti-inflammatory drugs.

It has been reported that Stevia rebaudiana have anti-inflammatory and antioxidant activity. These anti-inflammatory and antioxidant effects may reduce brain damage and improve the neuronal functions of brain. It is reported that reduce blood sugar also impairment of memory long term treatment of Stevia rebaudiana at the given doses not a have any significant affect on blood glucose level.

In conclusion we observed that in the present study Stevia rebaudiana improve memory in
aged rats may be due to the anti-inflammatory and antioxidant activity.

References
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