Abstract
Dementia is one of the age-related mental problems, and a characteristic symptom of Alzheimer’s disease. Nootropic agents and cholinesterase inhibitors like donepezil® are clinically used in situations where there is organic disorder in learning abilities and for improving memory, mood and behavior, but the resulting side-effects associated with these agents have made their utility limited. Ayurveda emphasizes use of herbs, nutraceuticals or life-style changes for controlling age related neurodegenerative disorders. The present study was undertaken to assess the potential of an ayurvedic rasayana (rejuvenator) drug *Zingiber officinale* Roscoe as a memory enhancer. Elevated plus maze and passive avoidance paradigm were employed to evaluate learning and memory parameters. *Z. officinale* extract (50 and 100 mg/kg, p.o.) were administered for 8 successive days to both young and aged mice. The dose of 100 mg/kg of *Z. officinale* extract significantly improved learning and memory in young mice and also reversed the amnesia induced by diazepam (1 mg/kg, i.p.), and scopolamine (0.4 mg/kg, i.p.). Furthermore, it also reversed aging induced amnesia due to natural aging of mice. *Z. officinale* significantly increased whole brain acetyl cholinesterase inhibition activity. Hence, *Z. officinale* might prove to be a useful memory restorative agent in the treatment of dementia seen in the elderly. The underlying mechanism of its action may be attributed to its antioxidant and acetyl cholinesterase inhibition property.

Key words: *Zingiber officinale*; Amnesia; Learning; Memory.

Introduction
Alzheimer’s disease is a neurodegenerative disorder associated with a decline in cognitive abilities; patients also frequently have non-cognitive symptoms, such as depression, apathy and psychosis that impair daily living (Jewart et al., 2005). It is the most common form of onset of adult dementia and attention deficit disorders (Robert...
Katzman et al., 1998). Centrally acting anti-muscarinic drugs (like scopolamine) impaired learning and memory of rats (Higashida et al., 1987) and human beings (Sitaram et al., 1978). Benzodiazepine receptor agonists such as diazepam and alprazolam have been shown to produce anterograde amnesia in rodents (Preston et al., 1989; Singh et al., 1998) and human beings (Lister et al., 1985). Nootropic agents such as Piracetam (Schever et al., 1999) and cholinesterase inhibitors like Donepezil® are used primarily for improving memory, mood and behavior. However, the resulting side-effects associated with these agents have limited their use (Blazer et al., 1983; Rogers et al., 1998).

Ayurveda, the Indian system of medicine, is gaining greater attention and popularity in many parts of the world. The disease preventive and health promotive approach of ayurveda, which takes into consideration the whole body, mind and spirit while dealing with the maintenance of health promotions, now enjoys increasing acceptability. The ancient ayurvedic physicians understood the delicate cellular mechanisms of the body and the deterioration of the functional efficiency of the body tissues. These ayurvedic scholars had thus developed certain dietary and therapeutic measures to delay ageing and rejuvenating whole functional dynamics of the body organs. This revitalization and rejuvenation is known as the ‘Rasyana chikitsa’ (rejuvenation therapy) (Govindarajan et al., 2005). Rasayana drugs act inside the human body by modulating the neuro-endocrino-immune systems and have been found to be a rich source of antioxidants (Brahma et al., 2003). According to ayurveda, Alzheimer’s disease is an imbalance of vata, pitta and kapha. Medhya (intellectual promoting) herbs such as, Convolvulus microphyllus (C. pluricaulis), Centella asiatica, Bacopa monnieri, Acorus calamus and Celastrus paniculatus are beneficial in cognitive disorders (Sharma, 1987; Govindadasa, 1884; Lolamba Raj, 1947). Zingiber officinale Roscoe is commonly known as ginger and used as a ‘rasayana’ drug in the traditional system of medicine since time immemorial. The dried rhizomes of ginger are implicated in the treatment of cardiac diseases, piles, colic, asthma, diseases of kapha, vata and pitta (Yoganarasimhan, 2000). It is reported to possess antioxidant (Masuda et al., 2004), anti-migraine activity (Mustafa et al., 1990). Used in nausea and vomiting during pregnancy (Borelli et al., 2005) and in osteoarthritis (Fajardo et al., 2005). It also reported to possess anti-obesity (Han et al., 2005); anti-hypertensive (Ghayur et al., 2005), analgesic, anti-inflammatory (Young et al., 2005), anti-atherosclerotic (Verma et al., 2004), anti-carcinogenic (Surh et al., 1999) activity, enhances learning on Morrison water maze (Topic et al., 2002) and inhibits β-amyloid peptide-accumulation, thus useful in delaying the onset and progression of neurodegenerative disorders (Grazanna et al., 2004).

Animals

Swiss mice of either sex weighing around 18 g (younger ones, aged 8 weeks) and 25 g (older ones, aged 28 weeks) were used in the present study. Animals were procured from disease free animal house of CCS Haryana Agriculture University, Hisar (Haryana, India). They were acclimatized to the laboratory conditions for 5 days before behavioral studies. The animals had free access to food and water and maintained under 12:12 h light and dark cycles. All experiments were carried out during day time from 0900 to 1400 h. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and
care of animals was taken as per guidelines of CPCSEA, Dept. of Animal Welfare, Govt. of India.

**Drugs and chemicals**

Scopolamine hydrobromide (Sigma Aldrich, USA), piracetam (Nootropil® UCB India Pvt. Ltd., Vapi, Gujarat), diazepam (Calmose® Ranbaxy, India) and phenytoin (Dilantin® suspension, Parke Davis) were diluted in normal saline. Volume of oral and i.p. administration was 1 ml/100 g.

**Preparation of ginger extract**

One kilogram of dried ginger was purchased from the local herbal market and was identified and authenticated at Department of Pharmacognosy, M. S. Ramaiah College of Pharmacy, Bangalore. The dried rhizomes were crushed to a coarse powder in a disintegrator and fine powder (60#) was collected by sifting. The coarse fibrous powder was extracted with ethyl alcohol (95%) at 65-70 ºC. The ethanol extract was evaporated under reduced pressure using rotavap vapor evaporator. The extract was suspended in demineralised water (5-6 times), homogenized at 5000-6000 rpm and slurry obtained was dried at 80 ºC (Rajpal, 2002). The yield of the dried extract was 3.6% w/w. Alcohol soluble extractive value: 80.2%, Volatile matters: 15-20%, Moisture content: 1.3%, Residue on ignition: 13%, Total plate count: <560 cfu/g, Yeast and moulds: < 60 cfu/g, *E.coli*: negative, *Salmonella / shigella*: negative. The ginger extract was suspended in a mixture of Tween 80: Distilled Water in a ratio of 2: 8. The suspension was orally administered to animals. The volume of administration was 1 ml/100 g, body weight of mice.

**Selection of the dose**

Ginger extract at different doses (10-500 mg/kg) was administered orally to normal mice. During the first four hours after the drug administration, the animals were observed for gross behavioral changes if any for 7 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, mortality were observed and doses selected were 50 mg/kg and 100 mg/kg, b.w. /day/mouse.

**Behavioral paradigms**

a) **Elevated plus Maze**

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm x 5 cm) and two covered arms (16 cm x 5 cm x 12 cm). The arms extended from a central platform (5 cm x 5 cm), and maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by mouse to move into one of the covered arm with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arm within 90 sec., it
was gently pushed into one of the two covered arms and the TL was assigned as 90 sec. The mouse was allowed to explore the maze for 10 sec and then returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day (Parle et al., 2004; Parle et al., 2005; Itoh et al., 1990).

Group I: Represented Control group for young mice (n=6). 10 ml/kg Distilled water (DW), p.o., was administered for 8 days. TL was noted after 45 min of administration on 8th day and after 24 h i.e. on 9th day. Group II & XIV: Piracetam, 200 mg/kg, i.p. was injected to both young and aged mice respectively. TL was noted after 45 min of injection and after 24 hours. Group III: Diazepam, 1 mg/kg, i. p., was administered to young mice and TL was noted after 45 min of injection on 8th day and after 24 h i.e. on 9th day. Group IV: Scopolamine (0.4 mg/kg, i.p.) was administered to young mice and TL was noted after 45 min of injection on 8th day and after 24 h i.e. on 9th day. Group V & VI: GE, 50 mg/kg & 100 mg/kg, was administered orally to young mice for 8 days. The last dose was given 45 min before subjecting the animals to elevated plus maze test. TL was noted on 8th day and again after 24 h. Group VII: GE (100 mg/kg, p.o.) was administered to young mice for 8 days. After 60min of administration of the last dose on 8th day, diazepam (1 mg/kg, i.p.) was administered. TL was noted after 45 min of administration of diazepam and after 24 h that is on the 9th day. Group VIII: GE (100 mg/kg, p.o.) was administered to young mice for 8 days. After 45min of administration of the last dose on 8th day, scopolamine hydrobromide (0.4 mg/kg, i.p.) was administered. TL was noted after 45 min of administration of diazepam and after 24 h that is on the 9th day. Group IX: Piracetam (200 mg/kg, i.p.) was administered to young mice for 8 days. After 60min of administration of the last dose on 8th day, diazepam (1 mg/kg, i.p.) was administered. TL was noted after 45 min of administration of diazepam and after 24 h that is on the 9th day. Group X: Piracetam (200 mg/kg, i.p.) was administered to young mice for 8 days. After 45min of administration of the last dose on 8th day, scopolamine hydrobromide (0.4 mg/kg, i.p.) was administered. TL was noted after 45 min of administration of diazepam and after 24 h that is on the 9th day. Group XI: Served as Control group for aged mice. 10 ml/kg DW, p.o., was administered for 8 days. TL was noted after 45 min of administration on 8th day and after 24 h i.e. on 9th day. Group XII & XIII: GE, 50 mg/kg and 100 mg/kg, were administered orally to aged mice for 8 days respectively. The last dose was given 45 min before subjecting the animals to elevated plus maze test. TL was noted on 8th day and again after 24 h.

b) Passive shock avoidance paradigm

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a box (27 X 27 X 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 X 7 X 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20V AC) was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped down and placed on the wooden platform set in the center of the
grid floor. When the mouse stepped down and placed all its paws on the grid floor, shocks were delivered for 15 sec and the step-down latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range (2-15 sec) during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. When the animals stepped down before 60 sec, electric shocks were delivered for 15 sec. During the second test, animals were removed from shock free zone if they did not step down for a period of 60 sec. Retention was tested after 24 h in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded, with an upper cut-off time of 300 sec (Parle et al., 2003).

**Group I**: Control group for young mice (n = 6). Distilled water (1 ml/100 g) was administered p.o. for 8 days. After 90 min of administration on 8th day, SDL was recorded. Retention was examined after 24 h. Group II and III (n = 5 each): GE (50 and 100 mg/kg respectively) orally for 8 days. SDL was recorded after 90 min of administration on 8th day and after 24 h. Group IV: Scopolamine hydrobromide (0.4 mg/kg) was administered i.p. to young mice after training on the 8th day and SDL was recorded at 45 min after injection. Retention was examined after 24 h. Group V: GE (100 mg/kg, p.o.) was administered to young mice for 8 days. After 45 min of administration of the last dose on 8th day, scopolamine (0.4 mg/kg, i.p.) was administered. SDL was recorded after 90 min of administration on 8th day and after 24 h. Group VI: Control group for aged mice (n=6). Distilled water (1 ml/100 g) was administered p.o. for 8 days. After 90 min of administration on 8th day, SDL was recorded. Retention was examined after 24 h. Group VII & VIII: GE (50 and 100 mg/kg) orally for 8 days. SDL was recorded after 90 min of administration on 8th day and after 24 h.

**Estimation of brain acetyl cholinesterase (AChE) activity**

Swiss mice of either sex weighing around 25 g were used. Group I (n=6), served as control and treated with distilled water. Group II (n=5), were treated with phenytoin (12 mg/kg, p.o.), Group III (n=5) with piracetam (200 mg/kg, i.p.), Group IV and Group V (n=5) were treated with GE (50 mg/kg and 100 mg/kg, p.o.) respectively for 8 days. On the 9th day the animals were euthanized by cervical dislocation carefully to avoid any injuries to the tissue. The whole brain AChE activity was measured using the Ellman method (Ellman et al., 1961). This was measured on the basis of the formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The sample was first treated with 5, 5'-dithionitrobenzoic acid (DTNB) and the optical density (OD) of the yellow color compound formed during the reaction at 412 nm every minute for a period of three minutes was measured. Protein estimation was done using Folin’s method. AChE activity was calculated using the following formula:

\[
R = \frac{\Delta \text{O.D.} \times \text{Volume of Assay (3 ml)}}{E \times \text{mg of protein}}
\]
Where \( R = \) rate of enzyme activity in ‘n’ mole of acetylcholine iodide hydrolyzed / minute / mg protein

\[ \delta \text{ O.D.} = \text{Change in absorbance / minute} \]

\[ E = \text{Extinction coefficient} = 13600 / \text{M / cm} \]

**Statistical analysis**

All the results were expressed as mean (±SEM). The data from elevated plus maze and passive avoidance tasks were analyzed using ANOVA followed by Student’s (Unpaired) ‘t’ test. Kruskal Wallis one-way ANOVA followed by multiple range tests was used for the analysis of non-normally distributed data of whole brain AChE activity. \( P < 0.05 \) was considered significant.

**Results**

Aged mice showed higher transfer latency (TL) values on first day and on second day (after 24 h) as compared to young mice, indicating impairment in learning and memory (i.e. ageing-induced amnesia). Piracetam (200 mg/kg, i.p.) pretreatment for 8 days decreased transfer latency on 8th day and after 24 h, i.e. on 9th day as compared to distilled water-treated group, indicating improvement in both learning and memory. Scopolamine (0.4 mg/kg) and diazepam (1 mg/kg) increased TL significantly (\( P < 0.05 \)) in young mice on first and second day as compared to control, indicating impairment of memory (Table-1).

GE (50 mg/kg, p.o.) decreased the TL on 8th day and 9th day in young and aged mice (\( P < 0.05 \)) when compared to control groups. Higher dose of GE (100 mg/kg, p.o.) more significantly enhanced the learning and memory of aged animals rather than the young mice as reflected by marked decrease in TL on 8th day and 9th day when subjected to elevated plus maze tests (Table 1). The higher dose of GE pretreatment for 8 days successively protected young mice (\( P < 0.05 \)) against scopolamine, diazepam and ageing-induced amnesia. GE (100 mg/kg, p.o.) profoundly increased step-down latency (SDL) significantly as compared to control group, indicating improvement in memory of young mice. Scopolamine (0.4 mg/kg, i.p.) decreased SDL on second day after training, indicating impairment of memory. GE (100 mg/kg, p.o.) administered orally for 8 days significantly reversed amnesia induced by scopolamine and natural aging (Table 2). The whole brain AChE activity with phenytoin (12 mg/kg, p.o.) demonstrated significant rise in AChE activity as compared to control and piracetam (200 mg/kg, i.p.) treated groups. GE (50 and 100 mg/kg, p.o.) significantly (\( P < 0.05 \)) lowered AChE activity (Table 3).
### Table 1: Effect of *Z. officinale* on transfer latencies of young and aged mice on elevated plus maze

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose mg/Kg</th>
<th>Transfer latency 1st day (score±SEM)</th>
<th>Transfer latency 2nd day (score±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (Young)</td>
<td>Distilled water</td>
<td>21.63±0.3</td>
<td>19.34±1.28</td>
</tr>
<tr>
<td>II</td>
<td>Piracetam 200</td>
<td></td>
<td>18.04±3.04*</td>
<td>16.46±3.01*</td>
</tr>
<tr>
<td>III</td>
<td>Diazepam 1</td>
<td></td>
<td>28.02±3.05*</td>
<td>31.66±3.63*</td>
</tr>
<tr>
<td>IV</td>
<td>Scopolamine 0.4</td>
<td></td>
<td>46.4±3.86*</td>
<td>38.61±2.41*</td>
</tr>
<tr>
<td>V</td>
<td>GE 50</td>
<td></td>
<td>17.22±1.76*</td>
<td>16.52±0.52*</td>
</tr>
<tr>
<td>VI</td>
<td>GE 100</td>
<td></td>
<td>12.34±4.15*</td>
<td>10.13±2.36*</td>
</tr>
<tr>
<td>VII</td>
<td>GE + Diazepam 100</td>
<td></td>
<td>15.3±3.21*</td>
<td>9.64±2.11*</td>
</tr>
<tr>
<td>VIII</td>
<td>GE + Scopolamine 0.4</td>
<td></td>
<td>17.54±1.81*</td>
<td>11.46±1.04*</td>
</tr>
<tr>
<td>IX</td>
<td>Piracetam + Diazepam 100</td>
<td>1</td>
<td>21.43±1.92</td>
<td>15.41±3.0*</td>
</tr>
<tr>
<td>X</td>
<td>Piracetam + Scopolamine 100</td>
<td>0.4</td>
<td>21.10±2.79*</td>
<td>19.42±1.91*</td>
</tr>
<tr>
<td>XI</td>
<td>Control (Aged)</td>
<td>Distilled water</td>
<td>36.97±1.4*</td>
<td>32.11±1.81*</td>
</tr>
<tr>
<td>XII</td>
<td>GE 50</td>
<td></td>
<td>18.20±3.42 b</td>
<td>10.71±2.29 b</td>
</tr>
<tr>
<td>XIII</td>
<td>GE 100</td>
<td></td>
<td>16.43±2.32 b</td>
<td>9.12±2.78 b</td>
</tr>
<tr>
<td>XIV</td>
<td>Piracetam 200</td>
<td></td>
<td>18.40±3.1 b</td>
<td>12.3±1.9 b</td>
</tr>
</tbody>
</table>

Each group consists of 5 animals each except the control groups (n=6)

Values are Mean ± SEM, ANOVA followed unpaired ‘t’ test

* P<0.05 compared to control (young group)

# P<0.05 compared to diazepam treated group

a P<0.05 compared to scopolamine treated group

b P<0.05 compared to control (aged group)

### Discussion

The present study indicates that *Z. officinale* is a potential anti-cholinesterase agent. It also possesses nootropic activity in view of its facilitatory effect on retention of acquired learning. Central cholinergic system plays an important role in learning and memory. Phenytoin is known to reduce hippocampal ACh concentration and cause cognitive impairment (Sudha et al., 2001).
Table 2: Effect of *Z. officinale* on step-down-latency (SDL) using passive-avoidance apparatus

<table>
<thead>
<tr>
<th>Group</th>
<th>Mice</th>
<th>Treatment</th>
<th>Dose (mg/Kg)</th>
<th>SDL after 24 h (Score/sec±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Young</td>
<td>Control (DW)</td>
<td>10</td>
<td>112.1±3.2</td>
</tr>
<tr>
<td>II</td>
<td>Young</td>
<td>GE</td>
<td>50</td>
<td>191±2.36*</td>
</tr>
<tr>
<td>III</td>
<td>Young</td>
<td>GE</td>
<td>100</td>
<td>284.2±4.62*</td>
</tr>
<tr>
<td>IV</td>
<td>Young</td>
<td>Scopolamine</td>
<td>0.4</td>
<td>16.2±2.19*</td>
</tr>
<tr>
<td>V</td>
<td>Young</td>
<td>GE + Scopolamine</td>
<td>100</td>
<td>253.6±3.21*^a^</td>
</tr>
<tr>
<td>VI</td>
<td>Aged</td>
<td>Control (DW)</td>
<td>10</td>
<td>42.46±6.31</td>
</tr>
<tr>
<td>VII</td>
<td>Aged</td>
<td>GE</td>
<td>50</td>
<td>62.51±4.31^b^</td>
</tr>
<tr>
<td>VIII</td>
<td>Aged</td>
<td>GE</td>
<td>100</td>
<td>98.19±1.96^b^</td>
</tr>
</tbody>
</table>

Values are each Mean ± SEM, ANOVA followed unpaired ‘t’ test
* Indicates p<0.05 compared to control (for young and aged mice)
^a p<0.05 compared to scopolamine treated group alone
^b p<0.05 compared to control (aged mice alone)

In the present study, phenytoin *per se* (12 mg/kg, p.o.) significantly elevated brain AChE activity. Piracetam (200 mg/kg, i.p.) and GE (100 and 200 mg/kg, p.o.), on the other hand, significantly (P<0.05) lowered this activity indicating the counteracting action of the two drugs on the cholinergic system. They also reversed the scopolamine-induced impairment in learning and memory, when assessed on passive avoidance paradigm.

Table 3: Effect of *Z. officinale* and piracetam on AChE activity in aged mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>AChE (µ moles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>118.45±6.20</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>12</td>
<td>192.2±1.84*</td>
</tr>
<tr>
<td>Piracetam</td>
<td>200</td>
<td>90.55±8.68*</td>
</tr>
<tr>
<td>GE</td>
<td>50</td>
<td>93.27±8.52*</td>
</tr>
<tr>
<td>GE</td>
<td>100</td>
<td>86.71±8.10*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM,
AChE- whole brain AChE activity
* P<0.05 Vs control (multiple range test)
Immunohistochemical studies suggested the existence of chronic inflammation in certain regions of the brain in Alzheimer’s disease patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer’s disease. This hypothesis is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in Alzheimer’s disease patients (Rao et al., 2002). Moreover, it has also been observed that elderly patients suffering from Alzheimer’s disease showed reduction in symptoms of Alzheimer’s disease upon chronic use of anti-inflammatory drugs. Indomethacin, a non-steroidal anti-inflammatory drug, exhibited a memory protective effect against electro-convulsive shock induced retrograde amnesia and also against amyloid deposits in the brain (Stephen et al., 2003). Anti-inflammatory action (Young et al., 2005) of *Z. officinale* might also be contributing to the observed memory-enhancing activity. Oxygen free radicals, the harmful by-products of oxidative metabolism are known to cause organic damage to the living system, which may be responsible for the development of Alzheimer’s disease in the elderly (Parihar et al., 2004). *Z. officinale* is reported to possess antioxidant activity (Masuda et al., 2004). Thus, a combination of acetyl cholinesterase inhibition, anti-inflammatory, antioxidant and neuroprotective role (Grazanna et al., 2004) of *Z. officinale* could all be leading to the net memory enhancing effect. Hence *Z. officinale* may be useful as a nootropic agent in the treatment of various cognitive disorders. Further investigations using more experimental paradigms are required for further confirmation of nootropic potential of dried rhizome of *Z. officinale* in the treatment of various cognitive disorders.

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