Evaluation of relationship between the Centella asiatica (Linn) fresh leaf extract induced spatial learning and memory enhancement and increased body weight gain in neonatal and adult rats

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Citation

Abstract
Centella asiatica (CeA) is a plant which grows in damp places in India and other Asian Countries. In Ayurvedic system of medicine, an alternative system of medicine practised mainly in India, leaves of CeA are used along with some other herbs as medhya drugs for memory enhancement and as promoter of strength and digestive power. In the present study, we have tried to establish the relationship between the two of the properties of this plant. That is, spatial learning and memory enhancing property and its effect on overall body weight growth in neonatal and adult rats. The present study was conducted in neonatal (7 day old) and adult (2.5 month old) rats. Both the groups of rats were fed with 2, 4 and 6ml/kg body of fresh leaf extract of CeA for 2, 4 and 6 weeks. During the treatment period the body weight of rats were noted at the end of every week. The body weight gained by the rats was compared with the spatial learning and memory enhancing effects of CeA on these rats reported earlier. The results showed a dose and duration dependent significance increase in the body weight gain in rats treated with CeA. This increase in the body weight was seen only in those groups were there was significant increase in spatial learning performance and enhanced memory retention power. This indicates that, there is a relationship between the spatial learning and memory enhancing effect of CeA and the body weight gain in rats.

INTRODUCTION
Ayurveda, an alternative system of medicine practiced widely in India, uses a number of plants for treatment of variety of diseases. “Medhya rasayana” are a group of medicines in Ayurveda, known to act on the nervous system. These drugs contain mainly extracts from plants like Centella asiatica, Acorus calamus, Jatamansi, Clitoria ternatea, Baccopa monnieri, Withania somnifera, Celastrus panniculatus, Guduchi and areca (1,2,3,4). The medhya rasayana have been claimed to improve mental ability (5). In addition, Centella asiatica is also used as promoter of strength and digestive power along with some other plants (6).

Figure 1
Figure 1: A photograph showing the fresh leaves of Centella asiatica (Linn)

Centella asiatica (Figure 1) is a creeping herb growing in wet places throughout India and other South Asian countries. Whole plant of Centella asiatica or its leaves in the fresh or
Centella asiatica is shown to be very useful in improving learning and memory (12, 13). In addition, the plant is also used in mentally retarded children to improve general mental ability and in people suffering from cognitive disorders (14, 15). In addition to the clinical studies (12, 13), spatial learning and memory enhancing effect of Centella asiatica fresh leaf extract has also been proved in neonatal and adult rats (13, 14). The plant is also reported to increase the digestive power and overall body growth (16). However, there are no reports on the direct effect of CeA on body weight and the relationship between the spatial learning and memory enhancing effects of CeA and body weight.

Thus, this study was designed to find the effect of different doses of CeA fresh leaf extract for different durations on the body weight in neonatal and adult rats. And correlate the body weight with the enhanced spatial learning and memory retention power reported earlier in these rats. In the present study, we have aimed to conduct the experiment in the same way as explained in the classic texts of Ayurveda (17), i.e. without going for extraction, but using the fresh leaf extract.

METHODS

RATS

The experiment was conducted in two major groups. In the first group, neonatal rat pups (7 days old) of both sexes and in the second group, adult rats (2.5 month old) of both sexes were used. Rats of both the groups were bred and maintained in our central research animal facility. They were fed with food and water ad libitum and maintained in 12:12 hours dark and light cycle. The room temperature was kept constant at 25°C throughout the experimental period. All the experiments were carried out with prior approval from the institutional animal ethical committee and only minimum required number of rats were used and handled in humane way.

EXPERIMENTAL GROUPS

Rats of each major group were assigned into three minor groups of two, four and six week treatment. Rats in each of these groups were divided into 2ml/kg (CeA 2ml), 4ml/kg (CeA 4ml) and 6ml/kg (CeA 6ml) body weight dose groups (n=8 for each dose). Each rat in the given dosage group was fed through gastric intubation with given amount of fresh leaf extract of Centella asiatica daily for two, four or six weeks. Along with these experimental groups, normal control group (NC) and saline (vehicle) control groups (SC) (n=8 in both groups) were also maintained.

EXTRACTION AND ADMINISTRATION OF CENTELLA ASIATICA FRESH LEAF EXTRACT

The plant, Centella asiatica was identified and entered in the registry of the department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India. A voucher specimen number “525PP” has been given to this plant by the department of Pharmacognosy. For the present experiment, we have cultivated the plant in a uniform soil condition in order to maintain same source of plant throughout the experiment. Fresh, 15-20 days mature leaves of CeA were collected in the morning. Fresh leaf juice was extracted from these leaves after washing, air drying and homogenizing in a glass vessel and finally filtered through a sterile gauge cloth. Leaves are extracted maximally so that from a given weight of leaves (5.0g), a known volume of juice was extracted (1.63ml ± 0.15ml, n=6). Since soil water was maintained uniform we could extract same volume of juice from given weight of leaves on different days. Further, we have established that, the dry weight of a given volume (1ml) of juice prepared on different days is same (0.079g ± 0.01g, n=6). The fresh leaf extract so obtained was fed to the rats as such, through a gastric tube, a capillary tube attached to a 1ml hypodermic syringe. Since volume of extract to be fed to individual rat is very little, its dose was blended with appropriate volume of saline for convenient feeding. Control rats remained undisturbed in their home cage, and saline control rats were fed with a volume of saline equivalent to volume of extract that their age matched experimental rats received on each day.

Since standard extraction procedures, which involve boiling in water, ethyl alcohol or other organic solvents, may alter the structure of bioactive principles, we have avoided standard extraction protocols. Though there may be a minor variation in daily preparations, it will be minimal as leaves of equal maturation are collected from the same place on all days. This minor daily variation will be compensated by long period (2, 4 and 6 weeks) of treatment. It has been shown in a recent literature that Centella asiatica plant extract obtained from ethanol extraction, is different from water extraction in its biological activity (18).

RECORDING THE BODY WEIGHT

The initial body weight of the rats of all the groups was...
recorded before the beginning of the treatment with CeA fresh leaf extract. There after the body weight of the rats of all the groups was recorded at the end of every week. The net body weight gain was calculated for each rat at the end of 2 weeks, 4 weeks and 6 weeks after the beginning of administration of CeA fresh leaf extract using the following formula.

\[
\text{Net body weight gain} = \text{Weight of the animal at the end of treatment period (2, 4 and 6 weeks)} - \text{Initial weight}
\]

Further, the weight gained by the rats treated with different doses (2, 4 and 6ml) of fresh leaf extract of CeA for different durations (2, 4 and 6 weeks) was compared with the dose and duration matched normal and saline control rats. Results of the body weight gain are then compared with the results of the behavioral experiments of the different groups reported earlier (13, 14).

**DATA ANALYSIS**

Data was analyzed using analysis of variance (ANOVA) followed by Bonferroni’s post test using Graph Pad in Stat (GPIS) software, version 1.13.

**RESULTS**

The rats treated with Centella asiatica remained healthy and active throughout the treatment period. There was no significant difference in the body weight gain between the control and saline treated rats, suggesting that daily handling of the rats (handling stress and vehicle) itself did not affect the body weight. Since there was no significant difference in the body weight gain between control and vehicle group, only comparisons between control and experimental groups were detailed bellow and in all figures.

**BODY WEIGHT GAIN IN NEONATAL GROUP OF RATS**

**RESULTS OF 6 WEEKS TREATMENT GROUP (FIGURE 2)**

The rats belonging to all the three (2, 4 and 6ml) dose groups showed a significant increase in the body weight gain. [66.57 ± 5.72 g. in normal control vs 86.0 ± 6.4 g. in CeA 2ml group (29.17% increase), P< 0.05; 90.12 ± 13.89 g. in CeA 4ml group (35.37 % increase), P< 0.01 and 97.25 ± 8.48 g. in CeA 6ml (46.08% increase), P< 0.001].

**RESULTS OF 4 WEEKS TREATMENT GROUP (FIGURE 3)**

The rats belonging to all the three (2, 4 and 6ml) dose groups showed a significant increase in the body weight gain. [55.25 ± 6.38 g. in normal control vs 66.55 ± 4.55 g. in CeA 2ml group (20.45% increase), P< 0.05; 72.58 ± 5.63 g. in CeA 4ml group (31.36 % increase), P< 0.001 and 85.57 ± 2.56 g. in CeA 6ml (54.87% increase), P< 0.001].

**RESULTS OF 6 WEEKS TREATMENT GROUP (FIGURE 2)**

Figure 2: Shows the body weight gain in neonatal rats treated with 2, 4 and 6ml/day/kg body weight of CeA fresh leaf extract for six weeks and age matched control (NC) and saline treated (SC) rats. Each bar represents mean ±SD. F-value: 16.08. Note the significant increase in the body weight gain in 2, 4 and 6ml/kg CeA treated rats compared to control rats. NC vs. CeA 2ml: # P< 0.05; CeA 4ml: ** P< 0.01; CeA 6ml: ??? P< 0.001 (One way ANOVA, Bonferroni’s test).
Evaluation of relationship between the *Centella asiatica* (Linn) fresh leaf extract induced spatial learning and memory enhancement and increased body weight gain in neonatal and adult rats

**Figure 3**
Figure 3: Shows the body weight gain in neonatal rats treated with 2, 4 and 6ml/day/kg body weight of CeA fresh leaf extract for four weeks and age matched control (NC) and saline treated (SC) rats. Each bar represents mean +SD. F-value: 33.86. Note the significant increase in the body weight gain in 2, 4 and 6ml/kg CeA treated rats compared to control rats. NC vs. CeA 2ml: # P< 0.05; CeA 4ml: *** P< 0.001; CeA 6ml: ??? P< 0.001 (One way ANOVA, Bonferroni’s test).

**RESULTS OF 2 WEEKS TREATMENT GROUP (FIGURE 4)**
There was no significant change in the body weight in the group treated with 2ml CeA fresh leaf extract. Only the rats belonging to 4 and 6ml dose groups showed a significant increase in the body weight gain. [13.34 ± 2.68 g. in normal control vs 33.21 ± 1.66 g. in CeA 4ml group (148.95% increase), P< 0.001 and 37.9 ± 1.96 g. in CeA 6ml (184.1% increase), P< 0.001].

**Figure 4**
Figure 4: Shows the body weight gain in neonatal rats treated with 2, 4 and 6ml/day/kg body weight of CeA fresh leaf extract for two weeks and age matched control (NC) and saline treated (SC) rats. Each bar represents mean +SD. F-value: 62.88. Note the significant increase in the body weight gain in 4 and 6ml/kg CeA treated rats compared to control rats. NC vs. CeA 4ml: *** P< 0.001; CeA 6ml: ??? P< 0.001(One way ANOVA, Bonferroni’s test).

**BODY WEIGHT GAIN IN ADULT GROUP OF RATS**
**RESULTS OF 6 WEEKS TREATMENT GROUP (FIGURE 5)**
The rats belonging to only 6ml dose group showed a significant increase in the body weight gain [54.14 ± 20.51g. in normal control vs 78.66 ± 15.99 g. in CeA 6ml (45.28% increase), P< 0.01]. The lower dose groups (2 and 4ml) did not show any significance difference in the body weight gain compared to the normal control rats.
Figure 5

Figure 5: Shows the body weight gain in adult rats treated with 2, 4 and 6ml/day/kg body weight of CeA fresh leaf extract for six weeks and age matched control (NC) and saline treated (SC) rats. Each bar represents mean ±SD. F-value: 5.12. Note the significant increase in the body weight gain in 6ml/kg CeA treated rats compared to control rats. NC vs. CeA 6ml: ?? P< 0.01 (One way ANOVA, Bonferroni’s test).

RESULTS OF 4 WEEKS TREATMENT GROUP

There was no significant change in the body weight gain in rats treated with CeA for 4 weeks in any of the dose (2, 4 and 6ml/kg) groups (data not illustrated).

Results of 2 weeks treatment group:

There was no significant change in the body weight gain in rats treated with CeA for 2 weeks in any of the dose (2, 4 and 6ml/kg) groups (data not illustrated).

DISCUSSION

In the neonatal group, treatment with higher dose (6ml/day/kg body weight) of Centella asiatica fresh leaf extract for 2, 4 and 6 weeks showed 184.1%, 54.87% and 46.08% increase in the body weight gain respectively. Similarly, a moderate dose (4ml/day/kg body weight) of Centella asiatica fresh leaf extract was also able to increase the body weight gain by 148.95%, 31.36% and 35.37% when treated for 2, 4 and 6 weeks respectively. However, the lower dose (2ml/day/kg body weight) of Centella asiatica fresh leaf extract induced a significant increase in the body weight gain by 20.45% and 29.17% only when administered for a longer duration i.e, 4 and 6 weeks respectively.

Whereas, in the adult group, treatment with only higher dose (6ml/day/kg body weight) of Centella asiatica fresh leaf extract for longer duration (6 weeks) showed 45.28% increase in the body weight gain.

As reported earlier (13, 14), Centella asiatica treated rats showed enhanced learning and memory compared to that of control rats in both neonatal and adult groups. Interestingly, significant improvement in the learning ability and memory retention power was seen in the groups which showed significant increase in the body weight gain. That is, in neonatal group, under 6 weeks treatment group during spatial learning (T- Maze) tests showed better spatial learning performance when treated with 2, 4 and 6ml/day/kg body weight of fresh leaf extract of CeA. Similarly, during passive avoidance memory retention tests also rats treated with 2, 4 and 6ml/day/kg body weight of fresh leaf extract of CeA showed better performance. Under 4 weeks treatment group, during spatial learning (T- Maze) tests, rats treated with 2, 4 and 6ml/day/kg body weight of fresh leaf extract of CeA showed better spatial learning performance. Similarly, during passive avoidance memory retention tests also rats treated with 2, 4 and 6ml/day/kg body weight of fresh leaf extract of CeA showed improved memory retention power. However, lower dose group (2ml/day/kg body weight) showed improved spatial learning performance in only 4 and 6 weeks groups (Table 1). Similar result was observed during the retention period of passive avoidance test in these rats indicating improved memory retention power (Table 2) (13). In the adult group, the rats treated with higher dose (6ml) of CeA performed significantly better during spatial learning T-Maze tests These rats also showed enhanced memory retention power during the retention period of passive avoidance test (Table 3) (14).
**Figure 6**

Table 1: Results of spatial learning (T-maze) tests of the neonatal rats treated with 2, 4, and 6ml/kg body weight of CeA for 2, 4 and 6 weeks and age matched control and saline treated rats. Each value represents mean ± SD. n, number rats; CeA, ; NC vs. CeA 2ml: # P<0.05; ## P<0.01; ### P<0.001; NC vs. CeA 4ml: * P<0.05; ** P<0.01, *** P<0.001; NC vs. CeA 6ml: ? P<0.05; ?? P<0.01, ?? P<0.001. (One way ANOVA, Bonferroni’s test) [Table reproduced from Rao et al. Neuroanatomy. 2005; 4: 18-23 (13).]

<table>
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<td>Rev. alt. test</td>
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<td>% Bias</td>
<td>No. of alterations % correct response</td>
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<td>62.1 ± 4.02</td>
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<tr>
<td>CeA 6ml</td>
<td>13.0 ± 2.88</td>
<td>54.0 ± 16.44</td>
<td>61.3 ± 4.02</td>
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**RESULTS OF PASSIVE AVOIDANCE TESTS OF NEONATAL GROUP OF RATS**

**Figure 7**

Table 2: Results of passive avoidance memory retention tests of the neonatal rats treated with 2, 4, and 6ml/day/kg body weight of CeA for 2, 4 and 6 weeks and age matched control and saline treated rats. Each value represents mean ± SD. n, number rats; CeA, ; NC vs. CeA 2ml: ## P<0.01, ### P<0.001; NC vs. CeA 4ml: * P<0.05, ** P<0.01, *** P<0.001; NC vs. CeA 6ml: ?? P<0.001. (One way ANOVA, Bonferroni’s test) [Values reproduced from Rao et al. Neuroanatomy. 2005; 4: 18-23 (13).]

<table>
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<th>Groups</th>
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<th>% of correct response</th>
<th>% of correct response</th>
<th>Total time in small compartment (sec)</th>
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<tr>
<td>Normal control (NC)</td>
<td>14.5 ± 2.94</td>
<td>64.8 ± 7.94</td>
<td>59.57 ± 7.94</td>
<td>7.79 ± 7.94</td>
<td>111.66 ± 35.12</td>
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<tr>
<td>Salline control (SC)</td>
<td>14.7 ± 2.94</td>
<td>64.8 ± 7.94</td>
<td>59.57 ± 7.94</td>
<td>7.79 ± 7.94</td>
<td>111.66 ± 35.12</td>
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<tr>
<td>CeA 2ml</td>
<td>13.65 ± 2.94</td>
<td>61.11 ± 7.94</td>
<td>58.89 ± 7.94</td>
<td>7.59 ± 7.94</td>
<td>112.33 ± 35.12</td>
</tr>
<tr>
<td>CeA 4ml</td>
<td>14.27 ± 2.94</td>
<td>60.29 ± 7.94</td>
<td>58.89 ± 7.94</td>
<td>7.59 ± 7.94</td>
<td>112.33 ± 35.12</td>
</tr>
<tr>
<td>CeA 6ml</td>
<td>21.85 ± 2.94</td>
<td>43.73 ± 7.94</td>
<td>94.64 ± 7.94</td>
<td>7.1 ± 7.94</td>
<td>95.77 ± 35.12</td>
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Results of Spatial learning T-maze and passive avoidance memory retention tests of adult 6 weeks group of rats.
Table 3: Results of spatial learning (T-maze) and passive avoidance memory retention tests of the adult rats treated with 2, 4, and 6ml/kg body weight of CeA for 6 weeks and age matched control and saline treated rats. Each value represents mean ± SD. n, number rats; CeA, ; NC vs. CeA 6ml: ??? P<0.001. (One way ANOVA, Bonferroni's test) [Table reproduced from Rao et al. Neurosciences 2007;12:236-41 ()].

Though the spatial learning and memory enhancing effect of CeA has been reported before (13, 14), there is no report on its effect directly on the body weight. The increase in the body weight gain may be because of the role of CeA in increasing physical strength and digestive power and its antiemaciatic effect (1, 3, 16). CeA enhances the digestive power, may be through its anti protozoal activity against entamoeba histolytica (17). In addition, there are reports of administration of aqueous extract CeA restoring the body weight loss, due to radio therapy (18). A number of modern scientists have proved that this plant is useful in a number of disorders including wound healing (19, 20). In addition, extract of CeA was shown to stimulate extra-cellular matrix accumulation around experimental wounds in rats (21).

Healing of wounds, involves the activity of an intricate network of blood cells, tissue types, cytokines, and growth factors. This results in increased cellular activity, which causes an intensified metabolic demand for nutrients. Several nutritional factors and vitamins are required for wound repair. Adequate dietary protein is absolutely essential for proper wound healing, and tissue levels of the amino acids arginine and glutamine may influence wound repair and immune function (22). Such properties of Centella asiatica may also be responsible for the increased body weight. In addition to its positive effect on body weight, CeA has also shown its positive effect on increasing learning ability, memory retention power (13, 14), and dendritic arborization of hippocampal CA3 and amygdaloid neurons (13, 14) of these rats. This relationship between the body weight gain and the improved behavior and structural changes in the hippocampal neurons of the rats supports the view that physical wellbeing and mental wellbeing are related to each other.

To conclude, we state that the oral administration of fresh leaf extract of Centella asiatica enhances the body weight gain in neonatal and adult rats and there is a relationship between the increased body weight and spatial learning and memory enhancement induced by fresh leaf extract of CeA reported earlier (13, 14).

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