STUDIES ON NOOTROPIC ACTIVITY OF RUTIN AND NARINGIN ON SCOPOLAMINE INDUCED AMNESIA IN RATS USING MORRIS WATER MAZE TASK.

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ABSTRACT

Nootropics are the new type of compounds affecting learning and memory but are pharmacologically neither CNS stimulant nor depressant. Several nootropics from allopathic system are under clinical trials. In the present study, we have used the Morris water maze to know the effect of Rutin and Naringin on spatial memory. The major advantage of the water maze task over the radial arm maze task is that the rats do not need to be water or food deprived, they are quite motivated to escape from the water. Standard test of learning in which the animal repeatedly searches for a platform hidden beneath the surface of opaque water in round pool. This test is especially sensitive to hippocampal damage and reflects attention, memory and learning strategy. Thus in the present study we have evaluated the nootropic activity of bioflavonoids mainly Naringin and Rutin as they had been reported potential antioxidant activity. Rutin had offered better degree of nootropic activity than Naringin. Both the doses 10mg/kg and 5mg/kg have shown slight different degree of nootropic activity.

Keywords: Nootropic activity, Rutin, Naringin, Scopolamine, Morris water maze task.

INTRODUCTION

Bioflavonoids are now used in many investigations and have reported in having antiviral, antibacterial and antifungal activities. Especially Rutin and Naringin are the phytoconstituents used in the management of venous edema that most of these biological effects are related to their antioxidant activity. Rutin may help to strengthen capillaries, protect against some toxins and have anti-inflammatory effects, as well as some anti-cancer
effects. Naringin found in grapefruit, giving bitter flavor and may help to prevent cancer and heart failure.

MATERIALS AND METHODS

Materials
Albino rats (wt: 90g-130g)
Morris water maze apparatus
Scopolamine (dose-2mg/kg body wt)
Milk (1.5 l)

Drugs studied
Rutin (5mg/kg, 10mg/kg)
Naringin (5mg/kg, 10mg/kg)
Rutin, Naringin was diluted by using vehicle Sodium CMC 0.1%.

Wistar rats weighing between 90-130 g purchased from M/S Ghosh Enterprises, Kolkata were used. They were housed in colony cages in groups of 4-5 in the departmental animal house, at an ambient temperature with a 12 h light/ dark cycle. The animals had free access of standard pellet chow and tap water. The animals were acclimatized to the laboratory environment for at least a week before experimentation. The present investigation confirms to the guidelines laid by the Institutional Animals Ethics Committee (Reg.no.516/01/A/CPCSEA). Rutin and Naringin obtained from Sigma Aldrich ltd, Bangalore and all other reagents used were of analytical grade.

Method used in the present study

Constitution of the Morris water maze
A water tight pool consisting of 120 cm in diameter, 40 cm deep and filled with water up to 30 cm height. The water is made opaque by adding milk (1.5l). A 10x15 cm rectangular escape platform is constructed of a water-resistant material and covered with the material that allows the animal to remain on the top when it is submerged. The platform is made heavy enough to remain upright when submerged or may be attached to the bottom of the pool. The platform is 28 cm in height so that it is submerged 2 cm below the level of water surface. The water temperature is maintained at \(-26^\circ\text{C}\).
**Procedure**

In the present study, we have used the Morris water maze to know the effect of Rutin and Naringin on spatial memory. The major advantage of the water maze task over the radial arm maze task is that the rats do not need to be water or food deprived, they are quite motivated to escape from the water. The task is also free from the errors of omission or abortive choices i.e., rat makes an attempt to find the platform on every trial.

**Animals: rats**

Rats are generally used in research employing behavioral tasks because a considerable amount is known about their brain anatomy and chemistry. Experimental studies have clearly shown that rats can be used to investigate the structural / functional relationships between selected brain regions and learning or memory.

**Evaluation**

**Basic protocol followed to test spatial memory**

The water maze task has been most extensively to investigate specific aspects of spatial memory. This task was based up on the premise that animals have evolved an optimal strategy to explore their environment and escape from the water with a minimum amount of effort i.e. swimming the shortest distance possible. The time it takes a rat to find a hidden platform in a water pool after previous exposure to the set up, using only available external cues was determined as a measure of spatial memory.

**Experimental groups used in the present study**

Rats were treated with Rutin and Naringin by using both oral and intraperitoneal routes. Each group comprises of 6 rats.

**A) Rats treated Orally**

Group 1: Control Oral  
Group 2: Rutin Oral (Scopolamine 2mg/kg + Rutin 10 mg/kg)  
Group 3: Naringin Oral (Scopolamine 2mg/kg + Naringin 10 mg/kg)

**B) Rats treated Intraperitoneally**

Group 4: Control I.P  
Group 5: Rutin I.P (Scopolamine 2mg/kg + Rutin 5 mg/kg)  
Group 6: Naringin I.P (Scopolamine 2mg/kg + Naringin 5 mg/kg)
RESULTS AND DISCUSSIONS

- As the results obtained from Group I- Group VI are compared with each other, whether showing significant improvement in basal performance or not. The results were tabled and given separately below.
- where as the results from Group II and Group III (Rutin 10 mg/kg and Naringin 10 mg/kg) has shown significant improvement in basal performance, in comparison to Group I (Control -Scopolamine oral), that was evidenced from 4hr and 5hr trial (P<0.05).
- Data from Group V and Group VI (Rutin 5 mg/kg and Naringin 5 mg/kg) has also shown significant improvement in basal performance, in comparison to Group IV (Control Scopolamine IP), that was evidenced from 3hr and 4hr trial (P<0.05).
- After induction of amnesia with scopolamine (2 mg/kg) in all the groups, rats from group II and group III (Rutin 10 mg/kg and Naringin 10 mg/kg), were able to retain the memory significantly in comparison to group I (Control -Scopolamine oral).
- Results from group V and group VI (Rutin 5 mg/kg and Naringin 5 mg/kg) have also indicated, that rats were able to retain the memory significantly after scopolamine oral treatment, in comparision to group IV (Control -Scopolamine I.P.).
- In this present work, Rutin had offered better degree of nootropic activity than Naringin.
- Both the doses 10mg/kg and 5mg/kg have shown slight different degree of nootropic activity.

Statistical Analysis

All the values were expressed as mean ± S.D. The data were analysed using 1 way repeated measure ANOVA. A level of P<0.05 was considered as statistically significant. Tukey’s test was performed to find the significant difference at P<0.05.

- In the present study rutin and naringin were evaluated for their effect on scopolamine induced impaired performance of spatial memory. Laboratory studies have shown that animals with hippocampal lesions are often severally impaired when the task requires spatial discriminations. There was significant improvement in the memory of rats tested as above with both rutin and naringin.
- Rutin and naringin given oral as well as IP route, oral bioavailability is less. Our results also indicate that, both rutin and naringin given in IP route shown maximum efficiency.
- Rutin shown better activity when compare to naringin. The literature also indicates that rutin had more antioxidant activity when compared to naringin and was correlated to their
structure. In fact rutin showed higher scavenger efficiency than naringin in DPPH, and TBA test. This effect may be attributable to the catechol structure of ring B, the 2, 3 double bond in conjugation with a 4-oxo function and the presence of both 7, 5 –hydroxyl groups.

Table: 1: Consolidated table to compare the latent period in oral groups for spatial memory. (Mean time in sec)

<table>
<thead>
<tr>
<th>Time in hrs</th>
<th>Control (Oral)</th>
<th>Rutin (Oral)</th>
<th>Naringin (Oral)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>8.34 ± 0.06</td>
<td>8.5 ± 0.137</td>
<td>8.6 ± 0.088</td>
</tr>
<tr>
<td>30 min</td>
<td>30.31 ± 0.10</td>
<td>28.2±0.114</td>
<td>28.2 ± 0.153</td>
</tr>
<tr>
<td>1 hr</td>
<td>28.55 ± 0.20</td>
<td>25.1±0.236</td>
<td>25.5 ± 0.125</td>
</tr>
<tr>
<td>2 hr</td>
<td>25.45 ± 0.04</td>
<td>21.0±0.197</td>
<td>20.5 ± 0.089</td>
</tr>
<tr>
<td>3 hr</td>
<td>22.57±0.08</td>
<td>15.3±0.235</td>
<td>18.5 ± 0.145</td>
</tr>
<tr>
<td>4 hr</td>
<td>20.69±0.07</td>
<td><strong>8.6±0.161</strong></td>
<td>15.4 ± 0.090</td>
</tr>
<tr>
<td>5 hr</td>
<td>18.19 ± 0.14</td>
<td>8.6±0.249</td>
<td><strong>8.5 ± 0.099</strong>*</td>
</tr>
<tr>
<td>6 hr</td>
<td>15.15 ± 0.08</td>
<td>8.6±0.156</td>
<td>8.5 ± 0.156</td>
</tr>
<tr>
<td>8 hr</td>
<td><strong>11.30 ± 0.10</strong></td>
<td>8.5±0.309</td>
<td>8.5 ± 0.117</td>
</tr>
<tr>
<td>12 hr</td>
<td>8.06 ± 0.06</td>
<td>8.3±0.251</td>
<td>7.6 ± 0.182</td>
</tr>
</tbody>
</table>

Table: 2: Consolidated table to compare the latent period in I.P. groups for spatial memory. (Mean time in sec)

<table>
<thead>
<tr>
<th>Time in hrs</th>
<th>Control (IP)</th>
<th>Rutin (IP)</th>
<th>Naringin (IP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>8.56 ± 0.10</td>
<td>8.7 ± 0.105</td>
<td>8.4 ± 0.09</td>
</tr>
<tr>
<td>30 min</td>
<td>30.22 ± 0.13</td>
<td>25.1 ± 0.133</td>
<td>25.4 ± 0.09</td>
</tr>
<tr>
<td>1 hr</td>
<td>25.63 ± 0.11</td>
<td>20.7 ± 0.277</td>
<td>21.6 ± 0.08</td>
</tr>
<tr>
<td>2 hr</td>
<td>22.45 ± 0.13</td>
<td>15.4 ± 0.174</td>
<td>18.6 ± 0.11</td>
</tr>
<tr>
<td>3 hr</td>
<td>20.37 ± 0.10</td>
<td><strong>8.8 ± 0.149</strong></td>
<td>15.6 ± 0.11</td>
</tr>
<tr>
<td>4 hr</td>
<td>15.55 ± 0.11</td>
<td>8.1 ± 0.337</td>
<td><strong>8.4 ± 0.07</strong>*</td>
</tr>
<tr>
<td>5 hr</td>
<td><strong>12.55 ± 0.15</strong></td>
<td>8.3 ± 0.118</td>
<td>8.5 ± 0.11</td>
</tr>
<tr>
<td>6 hr</td>
<td>9.30 ± 0.06</td>
<td>8.4 ± 0.211</td>
<td>8.3 ± 0.11</td>
</tr>
<tr>
<td>8 hr</td>
<td>8.52 ± 0.11</td>
<td>7.9 ± 0.195</td>
<td>8.4 ± 0.19</td>
</tr>
<tr>
<td>12 hr</td>
<td>7.49 ± 0.15</td>
<td>7.9 ± 0.240</td>
<td>8.1 ± 0.11</td>
</tr>
</tbody>
</table>
CONCLUSIONS

- Rutin at a dose of 10 mg/kg, 5 mg/kg improved both basal as well as scopolamine impaired performance significantly with respect to spatial memory processes, when compared to control groups.
- Naringin at a dose of 10 mg/kg, 5 mg/kg improved both basal as well as scopolamine impaired performance significantly with respect to spatial memory processes, when compared to control groups.
- Both the drugs Rutin and Naringin have shown different degree of nootropic activity.
• The results obtained suggesting a role for bioflavonoids in the cognitive and memory function.

ACKNOWLEDGEMENTS
One of the authors, Rajesh Konathala is grateful to Prof. A. Annapurna for providing laboratory facilities at Department of Pharmacology, AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India.

REFERENCES