Rutin (a bioflavonoid antioxidant) attenuates age-related memory deficits in mice

Author(s): Kamal Kishore and Manjeet Singh

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Kamal Kishore* and Manjeet Singh#

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#Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, India.

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Abstract

Ageing and age-related neurodegenerative disorders, which are associated with free radical generation, may cause impairment of learning and memory in animals and human beings. Rutin, a bioflavonoid, is a potent free radical scavenger. Present study was designed to investigate the effect of rutin (30mg and 40mg/Kg i.p.) in 6, 9 and 12 months old mice and on sodium nitrite (75mg/kg i.p.) induced amnesia in mice. Rutin at a dose of 40mg, significantly decreased the escape latency time (ELT) during the acquisition trials for 4 consecutive days, and increased the time spent (TS) in target quadrant (TQ) in search of missing platform, during the retrieval trials on 5th day. Rutin at a dose of 40mg, also significantly attenuated the increased ELT during the acquisition trials, and stimulated the mice to spent higher time in TQ for searching the hidden platform during the retrieval trials on 5th day in aged as well as sodium nitrite treated mice. On the basis of these observations, it is concluded that rutin at a higher dose i.e. 40mg, improves learning and memory, and attenuates the age-related memory deficits and experimental amnesia possibly by its potent free radical scavenger or antioxidant property, atleast in mice.

Introduction

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder and the most common cause of dementia of ageing. Clinically, AD is a syndrome that first presents with memory impairment, which is followed by increasing cognitive and eventually global deficits [1]. It is well established that ageing and age-associated neurodegenerative disorders lead to an impaired behaviour [2-3], progressive deterioration of memory [4,5], thoughts and even the gradual loss of brain cells [6-10]. Several reports suggested that aged animals exhibit impairment in memory tasks relative to their younger counterparts [11]. Gagnon and Winocur [12] found that aged rats take longer time to acquire the

delayed matching-to-sample task and also showed poor memory performance than younger rats. Blokland et al. [13] reported that aged rats take longer time than younger ones to find the hidden platform in a water maze procedure. These reports indicate that aged animals are unable to use spatial cues. In the past few years, involvement of free radicals is increasingly being implicated in neurodegenerative diseases like AD. It has been demonstrated that β-amyloid augments the production of superoxide anion in endothelial cells [14], H2O2 in nerve cells [15], and NO from microglial cells [16]. Oxidative stress is reported to induce aggregation of soluble β-amyloid into insoluble plaques [17]. Moreover, products of lipid peroxida-tion are elevated in cortical regions of brain with AD [18]. These findings point towards the possible beneficial effect of the free radical scavengers in AD. Antioxidants, Vitamin E and idebe-none, have been shown to prevent cell death caused by β-amyloid protein and also prevent the progression of schizophre-nia and other neurological abnormalities [19,20].

Rutin belonging to a group of plant compounds called bioflavonoids is a powerful antioxidant that fights free radicals [21-24]. Free radicals are said to be responsible for as much as 90% of all the human diseases, such as cancer, atherosclerosis, strokes, and senility due to aging. Moreover, recent research on Bacopa monniera [25], Ginkgo biloba [26], ginseng [27] and melatonin [5] has shown that the potent antioxidant properties, possibly due to bioflavonoids which may also play a significant role in attenuation of amnesia or improving learning and memory in animals and human beings. The above findings, therefore, propose the possible involvement of free radicals in various age-related diseases, amnesia or impairment of learning and memory. Joseph et al., [28] revealed that antioxidants or free radical scavengers play an important role in age-related problems and may delay or inhibit the progression of such neurode-generative disorders. These observations therefore formed the basis to investigate the role of rutin in cognition, using mice in water maze test [29].

Material and Methods

Swiss albino mice (22-30 gm) of either sex, purchased from Indian Veterinary Research Institute (IVRI) Izatnagar-243022, kept in an animal house with 12 hours light and dark cycle, and had free access to water and standard diet (Kisan feed India Ltd., Mumbai). All the animals used in the present study were naïve to water maze. The experiments were conducted in a semi-sound proof laboratory between 10.00 AM to 5.30 PM.

Apparatus

ELT and TS of each animal were measured by employing the water maze test. The test allows the evaluation of spatial memory. Water provides a uniform intramaze environment, thus eliminating any olfactory interference. Food and water deprivation were not required in this test as required in other models. Water maze apparatus consists of a circular pool, made of a galvanized iron sheet having a diameter of 150 cm. and a height of 45 cm. The pool was filled with water upto a height of 30cm. Water was made opaque with commercially available white color and maintained at 25°. The pool was hypothetically divided into four equal quadrants with the help of two threads, fixed at

right angle to each other, on the rim of the pool. Platform ($11cm^2$) of 29 cm. height, was placed in the center of one of these four quadrants i.e. target quadrant. The platform was submerged 1 cm. below the water surface.

Procedure

Acquisitional Trials

Each mouse was placed in water maze for four consecutive days, with a five minutes interval between the trials from the midpoint of peripheral wall of each quadrant with its face towards the wall. The mice were given a 120 second swims. After locating the hidden platform the mice were allowed to remain on it for 10 sec. before being returned to home cage. Mice that could not locate the hidden platform within 120 sec. were placed on it by hand and scored as 120 Sec. The time taken by the mice to locate the hidden platform was noted down and assigned as escape latency time (ELT). Mean of the four-escape latency time was calculated for each day. The mean was used as index of acquisition or learning.

Retrieval Trails

On the 5th day, platform was removed and time spent (TS) by the animal in each quadrant was noted down. The TS by the animal in target quadrant searching for missing platform is taken as an index of retrieval of memory. Utmost care was taken not to change the relative location of water maze with respect to any object serving as a visual clue in the laboratory.

Experimental amnesia

Experimental amnesia was induced by administration of sodium nitrite (75mg/kg i.p.) in mice [30].

Experimental Protocol

Fifteen groups (n=5) of mice were employed in the present study

Control groups (normal mice)

In mice (groups I-II), normal saline (10ml/kg i.p.) was injected 30 min before the first acquisition trial for 4 consecutive days and 30 min before the first retrieval trial on 5th day.

Saline-treated groups (aged mice)

Mice (groups III, IV and V) having age of 6, 9 and 12 months were treated with normal saline (10ml/kg i.p.) 30 min before the first acquisition trial for 4 consecutive days and 30 min before the first retrieval trial on the 5th day.

Sodium nitrite-treated groups (normal mice)

Mice (group VI) were treated with sodium nitrite (75mg/kg i.p.) 30 min before the first acquisition trial for 4 consecutive days and distilled water (10ml/kg i.p.) was administered 30 min before the first retrieval trial on the 5th day. Mice (groups VII) were treated with distilled water (10ml/kg i.p.) 30 min before the first acquisition trial for 4 consecutive days and sodium nitrite (75mg/kg i.p.) was administered 30 min before the first retrieval trial on the 5th day.

Rutin-treated groups (normal mice)

Mice (groups VIII-IX) were treated with rutin (30mg and 40mg/kg i.p.) respectively, 30 min before the first acquisition trial for 4 consecutive days and distilled water (10ml/kg i.p.) was administered 30 min before the first retrieval trial on the 5th day. Mice (groups X-XI) were treated with distilled water (10ml/kg i.p.) 30 min before the first acquisition trial for 4 consecutive days and rutin (30mg and 40mg/kg i.p.) respectively, was administered 30 min before the first retrieval trial on the 5th day.

Rutin-treated groups (12 months old mice)

Mice (group XII) were treated with rutin (40mg/kg i.p.) 30 min before the first acquisition trial for 4 consecutive days and distilled water (10ml/kg i.p.) was administered 30 min before the first retrieval trial on the 5th day. Mice (group XIII) were treated with distilled water (10ml/kg i.p.) 30 min before the first acquisi-

tion trial for 4 consecutive days and rutin (40mg/kg i.p.) was administered 30 min before the first retrieval trial on the 5th day.

Sodium nitrite + Rutin-treated groups (normal mice)

Mice (group XIV) were treated with sodium nitrite (75mg/kg i.p.) and after 5 min rutin (40mg/kg i.p.) was injected 30 min before the first acquisition trial for 4 consecutive days and distilled water (10ml/kg i.p.) was administered 30 min before the first retrieval trial on the 5th day. Mice (group XV) were treated with distilled water (10ml/Kg i.p.) 30 min before the first acquisition trial for 4 consecutive days. Sodium nitrite (75mg/kg i.p.) and after 5min rutin (40mg/kg i.p.) was administered 30 min before the first retrieval trial on the 5th day.

Drugs and Solutions

All the drug solutions were freshly prepared prior to use. Rutin (Mercury Laboratories Ltd. India) and sodium nitrite (s.d. fine Chemicals Ltd. India) were dissolved in water at 40OC and at ordinary temperature, respectively.

Statistical analysis

All the results were statistically interpreted using one-way analysis of variance (ANOVA) followed by Dunnett's test. A value of $P<0.05$ was considered statistically significant.

Results

Control group (normal mice)

Escape latency time (ELT) was significantly decreased during consecutive learning trials on day 2,3,4 as compared to the means of day 1(Fig. 1). The time spent by the mice in the target quadrant (Q2) in search of missing platform was significantly higher as compared to the time spent in the other quadrants Q1, Q3, Q4 during retrieval trials (Fig. 2).

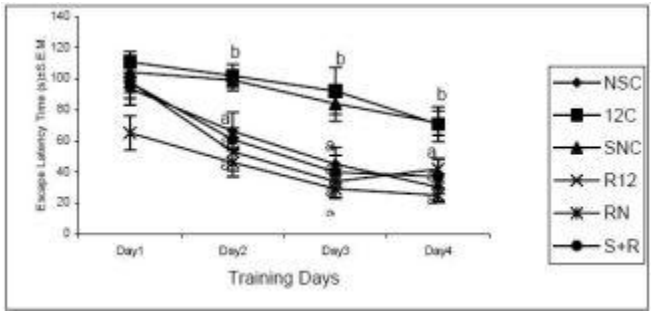

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Fig. 1: Effects of rutin on learning

NSC, 12C, SNC, RN, R12 and S+R, represents, administration of normal saline (10ml/kg i.p.) in normal mice, distilled water (10ml/kg i.p.) in 12 month old mice, sodium nitrite (75mg/kg i.p.) in normal mice, rutin (40mg/kg i.p.) in normal mice, rutin (40mg/kg i.p.) in 12 month old mice, and sodium nitrite (75mg/kg i.p.)+rutin (40mg/kg i.p.) in normal mice, respectively,30 min before the first acquisition trial for 4 consecutive days. Each value represents mean±S.E.M. (n=5). a=$P<0.05$ Vs ELT on day 1. b=$P<0.05$ Vs ELT of control group for the same day.

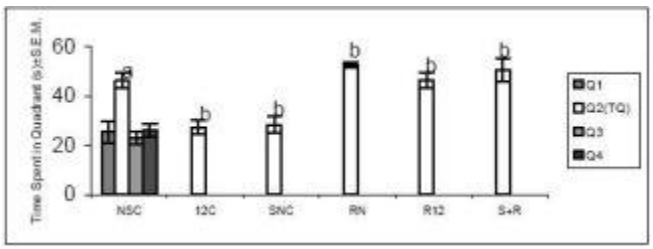

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Fig. 2: Effects of rutin on retrieval of learning

NSC, 12C, SNC, RN, R12 and S+R, represents, administration of normal saline (10ml/kg i.p.) in normal mice, distilled water (10ml/kg i.p.) in 12 month old mice, sodium nitrite (75mg/kg i.p.) in normal mice, rutin (40mg/kg i.p.) in normal mice, rutin (40mg/kg i.p.) in 12 month old mice, and sodium nitrite (75mg/kg i.p.)+rutin (40mg/kg i.p.) in normal mice, respectively,30 min before the first retrieval trial on the 5th day. Each value represents mean±S.E.M. (n=5). a=P<0.05 Vs TS in other quadrants i.e. Q1, Q3 and Q4. b=P<0.05 Vs TS in TQ i.e. Q2 in control group.

Aged mice (6,9 and 12 months old)

Escape latency time (ELT) was increased during consecutive learning trials on day 2,3,4 as compared to the means of day 1(Fig. 1). The time spent by the mice in the target quadrant (Q2) in search of missing platform was decreased, compared to the time spent in the other quadrants Q1, Q3, Q4 during retrieval trials but the results were more significant in 12 months old mice (Fig. 2).

Effects of sodium nitrite in normal mice

Escape latency time (ELT) was increased during consecutive learning trials on day 2,3,4 as compared to the means of day 1(Fig. 1). The time spent by the mice in the target quadrant (Q2) in search of missing platform was decreased, compared to the time spent in the other quadrants Q1, Q3, Q4 during retrieval trials (Fig. 2).

Effect of rutin on learning and memory of normal mice

Anterograde (before learning trials) administration of rutin (30mg/kg i.p.) significantly decreased the ELT during the learn-ing trials for 4 consecutive days (Fig. 1) and increased the time spent (TS) in target quadrant for searching the missing platform during the retrieval trial on the 5th day. On the other hand, retrograde (after learning trials) administration of rutin (30mg/kg i.p.) did not produce any significant effect on higher time spent in target quadrant for the search of missing platform during the retrieval trials, as compare to the control group. But rutin (40mg/kg i.p.) significantly increased higher time spent in TQ by the mice (Fig. 2).

Effects of rutin on aged amnesia (12 months old mice)

Anterograde administration of rutin (40mg/kg i.p.), 30 min before the learning trials for 4 consecutive days, significantly prevented age-related increased ELT during the learning trials (Fig. 1). Rutin also reversed the age-related attenuation of higher TS in target quadrant by the mice to search the missing platform dur-ing the retrieval trials on the 5th day (Fig. 2).

Effects of rutin on sodium nitrite-induced amnesia

Anterograde administration of sodium nitrite (75mg/kg i.p.) and after 5min of it rutin (40mg/kg i.p.) was administered 25 min before the learning trials for 4 consecutive days significantly prevented sodium nitrite-induced attenuation of decrease in ELT during the learning trials (Fig. 1). Rutin also reversed the at-tenuation caused by sodium nitrite of higher time spent in target quadrant to search the missing platform during the retrieval trials on the 5th day (Fig. 2). Retrograde administration of sodium nitrite (75mg/kg i.p.) and after 5min of it rutin (40mg/kg i.p.), significant reversed the decreased TS by the mice in the target quadrant in search of missing platform during the retrieval trials (Fig. 2).

Discussion

It is well known that ageing and age-associated neurodegenera-tive disorders such as Alzheimer's, Huntington chorea, Parkin-son disease etc. cause abnormal behaviour, thoughts and even gradual loss of brain cells or apoptosis. These disorders provoke progressive deterioration of learning and memory. Several anecdotal reports have shown that younger performs better than their older counterparts [11-13]. Vitamin E [19,20], idevenone, Bacopa monniera [25], Ginkgo biloba [26], Ginseng [27], bioflavonoids and numerous others potent free radical scavengers or antioxidants attenuate amnesia or improve cognition in animals and human beings. These studies revealed that age-related impairment of memory might be due to free radical generation.

In the present study, rutin, a bioflavonoid and a potent antioxi-dant or free radical scavenger, is found to improve learning and memory of normal mice with no cognitive deficits in the doses of 30mg and 40mg. This indicates that rutin may have potential to improve learning and to retain the learned task of normal beings. This observation is further supported by several recent scientific outcomes on Bacosides or Bacopa monniera [25], Ginkgo biloba [26], Ginseng [27] and others sources of bioflavonoids antioxidants in the improvement of learning and memory of animals and human beings. Rutin also reverses natural, age-related as well as experimentally-induced amnesia in mice. It is a well known fact that free radicals are involved in age-related impairment of memory. Schindler et al., [31] postulated that sodium nitrite- stimulated hypoxic amnesia, is identical to old age amnesia. Piracetam and related antioxidants agents have been reported to attenuate memory deficits induced by sodium nitrite [32]. Furthermore, bacosides the major constituents of well known ayurvedic medicine, Brahmi used in neurodegenerative disorders, have potent antioxidant properties, and attenuate sodium nitrite-induced amnesia of mice [25].

Conclusions

On the basis of our present findings, it may be concluded that rutin improves learning and memory of normal, aged and ex-perimentally-induced amnesic mice, possibly by its potent antioxidant action. The drug may be considered as a potent candidate for age-related memory impairment due to neurodegenera-tion and neurosychopharmacological disorders, associated with free radical generation. However, further experimental verification is required to justify our statement.

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Correspondence:
Dr. Kamal Kishore
236, Green Park
Bisalpur Road
Bareilly 243006, India
e-mail: Kamal bareilly (at) Yahoo co.in Phone: 0091-581 2527900