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ORIGINAL ARTICLE

Naringin and Rutin Alleviates Episodic Memory Deficits in Two Differentially Challenged Object Recognition Tasks

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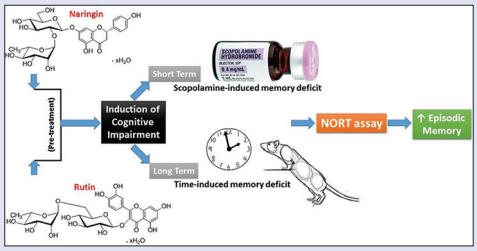
ABSTRACT

Background: Cognitive decline or dementia is a debilitating problem of neurological disorders such as Alzheimer's and Parkinson's disease, including special conditions like chemobrain. Dietary flavonoids proved to be efficacious in delaying the incidence of neurodegenerative diseases. Two such flavonoids, naringin (NAR) and rutin (RUT) were reported to have neuroprotective potential with beneficial effects on spatial and emotional memories in particular. However, the efficacy of these flavonoids is poorly understood on episodic memory, which comprises an important form of autobiographical memory. **Objective:** This study objective is to evaluate NAR and RUT to reverse time-delay-induced long-term and scopolamine-induced short-term episodic memory deficits in Wistar rats. **Materials and Methods:** We have evaluated both short-term and long-term episodic memory forms using novel object recognition task. Open field paradigm was used to assess locomotor activity for any confounding influence on memory assessment. Donepezil was used as positive control and was effective in both models at 1 mg/kg, *i.p.* **Results:** Animals treated with NAR and RUT at 50 and 100 mg/kg, *p.o.* spent significantly more time exploring novel object compared to familiar one, whereas control animals spent almost equal time with both objects in choice trial. NAR and RUT dose-dependently increased recognition and discriminative indices in time-induced long-term as well as scopolamine-induced short-term episodic memory deficit models without interfering with the locomotor activity. **Conclusion:** We conclude that, NAR and RUT averted both short- and long-term episodic memory deficits in Wistar rats, which may be potential interventions for neurodegenerative diseases as well as chemobrain condition.

Key words: Alzheimer's disease, chemobrain, cognition, episodic memory, flavonoids, neurodegenerative disease, novel object recognition task

SUMMARY

- Incidence of Alzheimer's disease is increasing globally and the current therapy is only symptomatic. Curative treatment is a major lacuna. NAR and RUT are natural flavonoids proven for their pleiotropic pharmacological effects with potential neuroprotective benefits. The study evaluated these flavonoids for their potential to improve the most common form of episodic memory (memory of autobiographical events in relation to time, places etc.) in two differential animal models assessing short-term and long-term memory, respectively. We also found that NAR and RUT were able to reverse both short-term and long-term memory deficits dose dependently in female Wistar rats.



Abbreviations used: AD: Alzheimer's disease, AChE: Acetylcholinesterase, COX: Cyclooxygenase, DI: Discriminative index, ITI: Inter trial interval, NAR: Naringin, RUT: Rutin, NORT: Novel object recognition task, NOS: Nitric oxide synthase, QOL: Quality of life, RI: Recognition index, WFI: Water for injection.

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INTRODUCTION

Neurodegenerative diseases are often associated with cognitive, motor and behavioral dysfunction.[1] According to WHO, by 2040, neurodegenerative disorders could be the leading cause of mortality, surpassing cancer.[2] The most common neurodegenerative disorder is the Alzheimer's disease (AD) characterized by progressive cognitive deterioration and personality changes leading to complete need for care and is found to be the most common cause of dementia in geriatric population.[3] About 5.2 million people of all age groups in the US have AD in 2013, and it is projected to nearly triple, that is, 13.8 million by 2050.[4]

Although the etiology of AD remains unclear, restoration of cholinergic transmission in the brain using cholinesterase inhibitors remains the mainstay of treatment, which is only supportive or symptomatic rather than curative or disease-modifying.[5] Satisfactory outcomes were not accomplished through pharmacological treatment by conventional drugs despite advances in understanding the pathology of the disease.[6] Hence, complementary and alternative medicines become a potential source of new drugs for the prevention of neurodegenerative diseases.[2] Earlier reports revealed that dietary intake of rich polyphenolic vegetables and fruits proved to delay the onset of dementia associated with AD.[7,8] Animal behavioral data support the fact that, flavonoids can improve various components of cognitive processes through neuronal differentiation, long-term potentiation and also by enhancing the synaptic plasticity.[9-12]

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Naringin (NAR), a citrus flavanone glycoside found in grape seeds and constitutes naringenin its aglycone part. NAR possess anti-oxidant, anti-inflammatory, anti-apoptotic and cardio-protective effects *in vivo*.[13,14] It was found that inflammatory mediators play a vital role in the pathogenesis of neurodegenerative anomalies.[15] Earlier works confi med that NAR prevented the lipopolysaccharide-induced pro-inflammatory cytokines and gene expression of cyclooxygenase and nitric oxide synthase (NOS) *in vitro*[13,16] as well as *in vivo*.[17] Moreover, NAR was reported to prevent the behavioral changes and cognitive defic ts in kainic acid-induced epilepsy[18] and 3-nitropropionic acid-induced Huntington's disease animal models.[19] NAR also attenuated colchicine[20] and D-galactose[21] induced learning and memory defic ts in rats.

Another flavonoid used in the present work, that is, rutin (RUT) is a flavonol glycoside (with aglycone flavanol, Quercetin) has diverse benefic al effects viz., antioxidant, anticancer, antiplatelet, antithrombotic, vasoprotective, cardioprotective and hepatoprotective effects with potential neuroprotective activities.[22-26] Researchers reported that RUT inhibited microglial activation and pro-inflammatory cytokines[27] and is effective against trimethyltin induced spatial memory defic ts through amelioration of neuronal damage in hippocampal CA3 subregion, crucial for acquisition learning in rodents.[28] RUT also prevented scopolamine-induced cognitive defic ts in inhibitory avoidance test in zebrafish.[29]

NAR and RUT have been reportedly known for their anti-inflammatory effects in many experimental studies through inhibition of NO production.[30-32] They showed potential neuroprotective effects against ischemic reperfusion induced cerebral injury by ameliorating oxidative damage, mitochondrial dysfunction, and neurological impairments.[33,34] Moreover, researchers found that NAR and RUT alleviated AD-type neurodegeneration and the associated cognitive impairment of spatial and working memories induced by intracerebroventricularly injected streptozotocin in rats.[35,36]

Increasing evidence shows that cholinergic neurotransmission is an essential prerequisite for the formation of recognition memories.[37] Scopolamine-induced cognitive impairment is considered as an important research tool to explore the mechanisms underlying cognitive changes observed in AD patients.[38] Due to an increased enzymatic activity of acetylcholinesterase, there will be an increased breakdown of acetylcholine in AD patients, which lead to hindered cholinergic transmission and thereby the affected memory function.[6,39]

NAR and RUT were found to be effective in enhancing the cognitive function in behavioral tasks assessing spatial, working and emotional memories in animal models, however episodic memory defic ts are often observed clinically in neurodegenerative diseases, especially in AD associated dementia[40,41] and also in chemotherapy-induced cognitive dysfunction (chemobrain) in cancer survivors, especially in breast cancer survivors.[42] Hence, it is important to evaluate the efficacy of NAR and RUT for their potential to prevent short- and long-term episodic memory defic ts, so as to contribute to the increasing knowledge about these plant-derived products and their use as medicines in treating neurodegenerative diseases.

We observed paucity for the studies assessing nootropic potential of NAR and RUT on episodic memory, hence in the current study, we focused on the efficacy of these flavonoids for episodic memory retrieval using novel object recognition task (NORT). Initially, we have evaluated both the flavonoids to reverse the time delay induced natural defic ts of long-term episodic memory. Th s was followed by assessment in a drug-induced, that is, scopolamine-induced short-term episodic memory defic ts.

MATERIALS AND METHODS

Animals

A 12-week-old, healthy ($n = 72$) female Wistar rats weighing 150–200 g were used in the present study. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) (Approval No. IAEC/KMC/17/2013) and the experiments were performed in compliance with the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA) guidelines, India. Animals were maintained (with temperature, 25°C ± 2°C and relative humidity, 55% ± 5%) at Central Animal Research Facility of Manipal University, Manipal as per the directions specifi d by the CPCSEA guidelines. A 12/12 h of light and dark cycle was maintained, and potable water was given *ad libitum* throughout the study period.

Chemicals and apparatus

Pharmacological agents, donepezil (Matrix Laboratories Ltd., Secunderabad, TS, India), scopolamine hydrobromide (Sigma-Aldrich Co. LLC, St. Louis, MO, USA), NAR (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan), RUT (HiMedia Laboratories, Mumbai, MH, India) and water for injection (Aculife Healthcare Pvt. Ltd., Ahmedabad, GJ, India) were used in this study.

Apparatus consisted of six square boxes (arenas) of 48 cm (length × breadth × height) which are made with plywood. Inner portions of arenas were covered with black laminate. All the six boxes were assigned for six individual animals in each group through number allocation. These boxes were also used as open fi ld boxes for assessing the locomotor activity along with the use of ANY-maze video tracking system (Stoelting Co., Wood Dale, IL, USA). ANY-maze can be used to track the animal behavior in an open fi ld to assess the locomotor activity by video tracking system. The system can generate the open fi ld test parameters once we program for it. Behavioral observation of rats in arenas was monitored and recorded using a camera (model: Quickcam Pro9000, Logitech International SA, Lausanne, Switzerland) mounted above the behavioral observation arenas. Other essential accessories such as timer alarms and handheld stopwatches were procured for accurate scoring and behavioral observation. Th s equipment was used to assess time induced as well as scopolamine-induced episodic memory defic ts in NORT. A proficie t observer who was blind to the treatment groups manually scored the behavioral parameter, that is, exploration/investigation time.

Formulations and treatments

Animals were treated with either NAR and RUT at two dose levels i.e. 50 and 100 mg/kg, *p.o.,* that were formulated as suspensions using 0.25% w/v sodium carboxy methyl cellulose (CMC) for 15 days in both the animal models prior to experimentation and also during the trials, 1 h prior to familiarization and/or choice trial. All the rats received a constant volume (2 ml/kg) of either vehicle or test compound/drug (donepezil/scopolamine) depending on the type of model being used. Rats were injected intraperitoneally with donepezil (1 mg/kg, *i.p.*), 30 min prior to familiarization trial and choice trials in time induced memory defic ts model. In case of scopolamine-induced short-term memory defic ts model, donepezil was given at 1 mg/kg, *i.p.*, 30 min prior to familiarization trial and scopolamine was given at a dose of 0.5 mg/kg, *i.p.*, 20 min prior to the familiarization trial. All the doses and the predose interval periods were selected based on the past laboratory experience and from the previous studies.[33,43,44]

Experimental groups

Two separate sets of animals were used for assessing short-term and long-term episodic memories. A total of six treatment groups

were used in each model for assessing either time delay induced or scopolamine-induced episodic memory defic ts in NORT. Vehicle group (Group 1) was treated orally with 0.25%w/v sodium carboxymethyl cellulose (CMC). Groups 2 and 3 animals were treated with NAR (50 and 100 mg/kg, *p.o.*), respectively whereas in Groups 4 and 5, rats were treated with RUT at (50 and 100 mg/kg, *p.o.*), respectively. Group 6 was positive control and received intraperitoneal donepezil at a dose of 1 mg/kg. In case of scopolamine-induced short-term episodic memory defic ts model, all the six groups of animals were treated with scopolamine at a dose of 0.5 mg/kg, *i.p.* along with their respective treatments as mentioned above.

Change in body weight

To know the effect of test flavonoids, NAR and RUT, body weight recordings were made on alternate days throughout the study period in time induced episodic memory defic ts study. The percentage increase in body weight (%IBW) was noted at the end of the study with respect to day 1 and compared among the treatment groups. Th s %IBW was noted for one set of animals (time induced defic ts model) as the treatment for both sets of animal models was similar.

Open field test

Activation or suppression of locomotor activity will have confounding influences on any neuro-behavioral paradigm assessing cognitive functions. In the present study, to know whether NAR and RUT have influencing effects on locomotion, we have tested locomotion following the 15 days flavonoid treatment (prior to object recognition task). All the rats from diverse treatment groups in time-induced episodic memory defic ts study were assessed for locomotion using an open fi ld paradigm. Open fi ld test is done using same square arenas (48 cm × 48 cm × 48 cm) that are used for object recognition tasks. The procedures followed were according to the earlier reports with slight modifi ations.[45,46] Briefly, rats from different treatment groups were individually placed in open fi ld boxes (following the acclimatization period). Using the system software, an imaginary 15 cm circular zone was created in the center of the arena. The locomotor activity, that is, distance (cm) travelled, mean velocity (cm/s) and time spent in the center zone during a 15 min test duration were assessed using the ANY-maze software tracking system (Version 4.82 m, Stoelting Co., Wood Dale, IL, USA). The comparison was made for the parameters monitored among different treatment groups.

Novel object recognition task

Episodic memory can be assessed typically by using NORT in rodents. Procedures were conducted as per the previous reports with some modifi ations.[47,48] Briefly, the experiment was conducted in a soundproof isolated room having a light intensity of 30–40 lux over a period of 2–3 days. The experiment was carried out between 09:00 am to 04:00 pm and basically consisted of three phases, habituation, familiarization, and choice trial phases as illustrated in Figure 1. On day 1, animals were habituated to the behavioral observation arenas for a period of 20 min. On day 2, after treating the animals with their respective treatments and following the postdose intervals, they were subjected to familiarization trial. In scopolamine-induced short-term memory defic t model, choice trial was conducted following an inter-trial interval (ITI) of 3 min (as scopolamine produces short-term memory defic ts), whereas in case of time-induced long-term memory defic ts, the choice trial was conducted after an ITI of 24 h, that is, on day 3, as this time delay produces long-term episodic memory defic ts. In familiarization trial, animals were allowed to explore a pair of similar objects in experimental arenas. The objects used were made of glass or plastic material, which were cylindrical in shape with dimensions of 18 cm × 5 cm. Exploration of animals was defi ed as sniffing, touching the objects with nose and/or any other investigative behavior directed toward object within 1 cm distance. The choice trial was performed by replacing one of the familiar objects with a novel object and cumulative exploration time of each animal toward familiar or novel object was noted for 3 min trial duration.

The investigatory behavior that is, exploration of rats directed towards objects was recorded during trials using a camera mounted above the behavioral observation arenas connected to a computer system. Recognition index (RI) and discriminative index (DI), which refl cts the animal's memory for the objects were determined from the exploration time in choice trial according to the earlier reports.[49] RI refl cts the animal's memory for the familiar object relative to that of the novel object, whereas DI indicates the degree of discrimination showed by animals among the objects.

Hematological profiling

Following the behavioral assessment in time induced episodic memory defic ts study, blood sampling was carried out using dipotassium ethylenediaminetetracetic acid (10% w/v) as an anticoagulant. Complete blood profile was performed using automated veterinary blood cell counter (model: PCE-210 VET, ERMA Inc., Tokyo, Japan) and the mean values of major parameters like, red blood cell (RBC), white blood cell (WBC), hemoglobin, platelet counts of different treatment groups were compared and analyzed.

Statistical analysis

Data are represented as mean ± standard error of the mean of time in sec spent by the animals exploring either familiar or novel objects and also as recognition and discriminative indices. Statistical analysis was carried out using Prism 6.03 trial version (GraphPad Software Inc., La Jolla, CA, USA). Exploration time was compared by paired *t*-test within the group and between the objects. Comparison of means for RI and DI

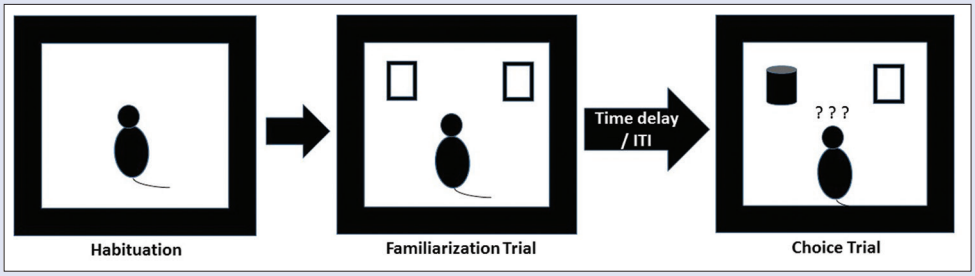

Figure 1: Illustration showing methodology of novel object recognition task for assessing episodic memory. Following a habituation period, animals were subjected to familiarization trial with two similar objects. After certain inter trial interval, choice trial was conducted using one familiar and one novel object

were analyzed by using Kruskal–Wallis test followed by Dunn's *post-hoc* test. A value of $P < 0.05$ was considered as statistically signifi ant. Locomotion was expressed as distance traveled in cm, time spent in center zone and mean velocity. %IBW, hematological parameters as well as locomotion were analyzed by using one-way ANOVA followed by Dunnett's *post-hoc* test.

RESULTS

Change in body weight

Th oughout the experimental groups, we observed a gradual increase in body weight over 15 days study duration. The average %IBW for any of the treatment group was approximately 12–15%. With respect to the normal control animals, treatment with NAR and RUT at both tested doses, that is, 50 and 100 mg/kg, *p.o.* for 15 days did not result in any signifi ant change in %IBW. The %IBW among different treatment groups is shown in Figure 2.

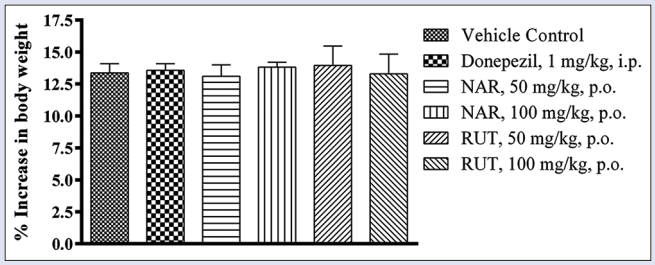

Figure 2: The effect of naringin and rutin on percentage increase in body weight at the end of the study as compared to day 1, that is, treatment start day ($n = 6$)

Open field test (locomotion assessment)

Like %IBW measure, locomotion assessment was carried out in one set of animals (time induced defic ts model) as the treatment was same. It was found that no signifi ant difference in the locomotor activity measures, that is, distance traveled, time spent in the center zone and mean velocity was observed among the treatment groups. Hence, it was confi med that NAR and RUT have no confounding influence on locomotion on the improving potential for episodic memory. Th s is indicated by almost equal amount of distance traveled, time in the center zone and the mean velocity among the treatment groups [Figure 3].

Time-induced long-term episodic memory deficits in novel object recognition task

In familiarization trial, all the animals spent nearly equal time exploring two similar objects, which indicate that there was no preference or discrimination toward any of the objects used. Vehicle treated animals spent almost equal time with both familiar and novel objects in the choice trial, indicating their inability to remember familiar object after an ITI of 24 h. Both the flavonoids, NAR and RUT dose-dependently reversed the time induced episodic memory defic ts at 50 and 100 mg/kg, *p.o.* which is evident from the signifi ant increase in novel object exploration time compared with familiar object. Also, we found that there was a signifi ant improvement in recognition and discriminative indices for the groups treated with NAR and RUT as compared to vehicle control.

It was found that low dose NAR (50 mg/kg, *p.o.*) treated animals spent signifi antly more time with novel object compared to familiar one in choice trial with a comparative improvement of recognition and discriminative indices over vehicle control; however this improvement was not statistically different. Positive control, donepezil produced

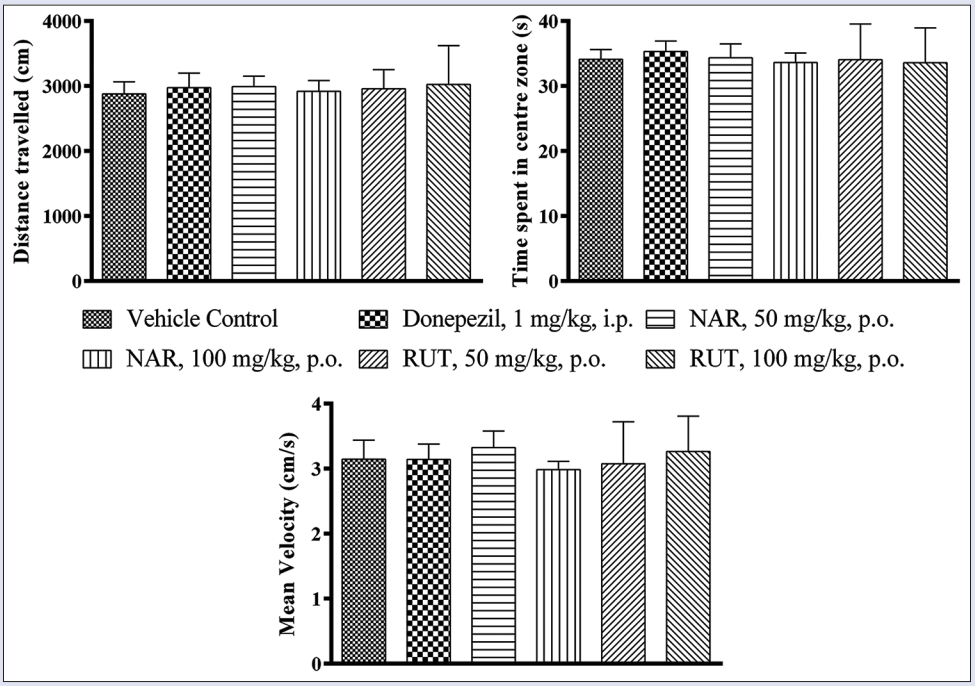

Figure 3: The influence of flavonoids, naringin and rutin on locomotor activity in open field test conducted in rats. Data are expressed as mean ± standard error of the mean of distance travelled, time spent in the center zone and the mean velocity of animals in open field test. No significant changes were observed in any of the parameters among the various treatment groups ($n = 6$)

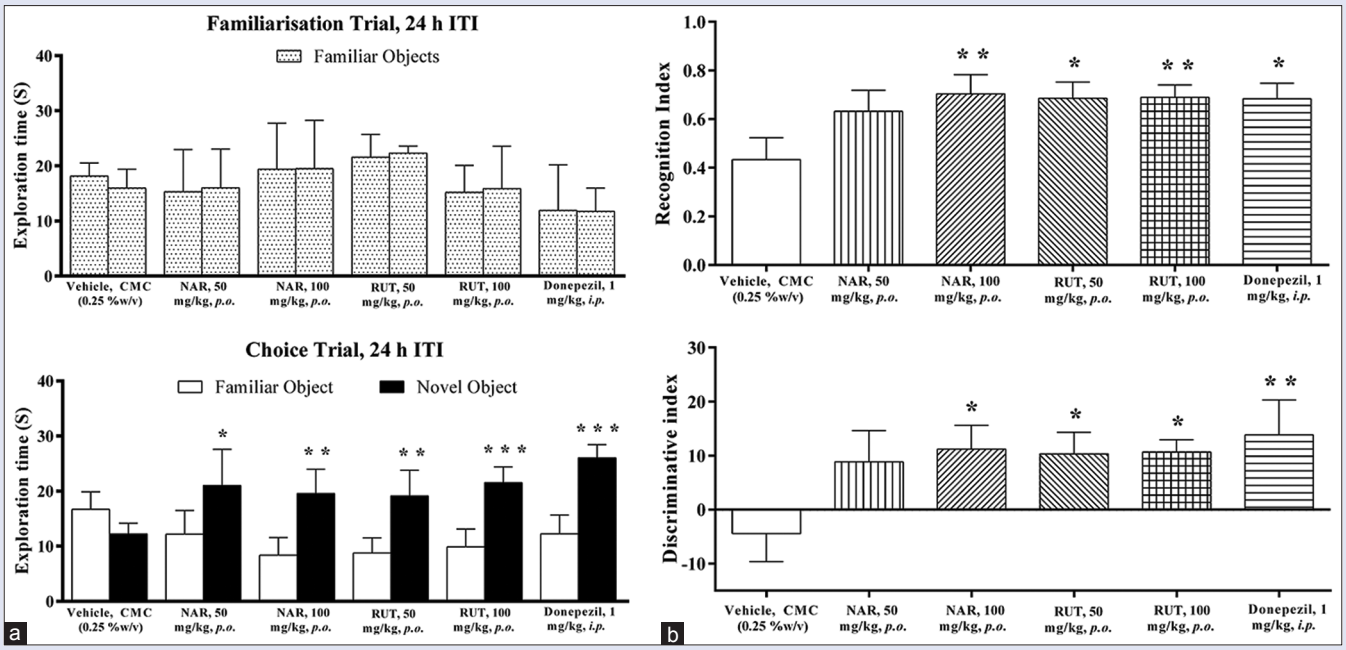

Figure 4: (a) Effect of naringin and rutin on exploration time of familiar or novel objects in time-induced memory deficits in familiarization and choice trials in novel object recognition task. Data represents mean ± standard error of the mean of duration of exploration (s), $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus familiar object using 24 h inter trial interval. (b) Effect of naringin and rutin on discriminative and recognition indices in time induced memory deficits in novel object recognition task data represents mean ± standard error of the mean of recognition and discriminative indices, $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle control using 24 h inter trial interval

signifi ant improvement of time induced memory defic ts at a tested dose of 1 mg/kg, *i.p.* which was indicated by signifi ant increase in time spent exploring novel object compared to familiar object in choice trial and also by signifi ant increase in DI and RI compared to vehicle group [Figure 4a and b].

Scopolamine-induced short-term episodic memory deficits in novel object recognition task

In scopolamine-induced short-term impairment of episodic memory, locomotion of the animals treated with scopolamine was comparatively increased due to the central cholinergic (M1 receptor) blockade and the resulting short-term memory defic ts. Also, it was observed that the duration of the exploration time was comparatively more when compared to that of time induced defic ts model. All the rats explored a pair of similar objects for the almost equal amount of time in familiarization trial. Animals of vehicle group treated with scopolamine could not able to discriminate between novel and familiar objects as they spent almost equal time with both the objects in the choice trial. However, pretreatment with NAR and RUT at 50 and 100 mg/kg for about 15 days signifi antly reversed the scopolamine-induced short-term episodic memory defic ts in a dose-dependent manner, which was evident from signifi ant difference in duration of exploration time directed toward either familiar or novel object.

It was also observed that discriminative and recognition indices were signifi antly improved for the groups treated with NAR and RUT when compared to the vehicle group. Although no signifi ant increase in RI and DI were observed for NAR and RUT low dose (50 mg/kg) groups, rats of these groups spent signifi antly more time exploring the novel object compared to that of familiar one in choice trial. Animals treated with donepezil at a dose of 1 mg/kg, *i.p.,* spent signifi antly more time with novel object compared to familiar object, hence prevented the scopolamine-induced short-term episodic memory defic ts [Figure 5a and b].

Hematological profiling

We found that NAR and RUT at tested doses of 50 and 100 mg/kg, *p.o.* for 15 days have improved RBC and hemoglobin levels as compared to vehicle control. However, the improvements observed were not statistically signifi ant. Hence, these improving effects of these flavonoids on blood profile, particularly on RBC and hemoglobin may underlie as one of the reason for the procognitive like effects on episodic memory in object recognition task. Other parameters such as WBC, total granulocyte, lymphocyte, monocyte, and platelet counts were unaffected. The hematological data for one set of animals (time-induced defic ts model) is represented in Table 1.

DISCUSSION

Prefrontal cortex plays a pivotal role in encoding and retrieval of episodic memory.[50,51] Moreover, the anatomical structures of the medial temporal lobe that are crucial for the formation of episodic memory comprise amygdala, brainstem, and hippocampus. Declarative or explicit memory is one of the two major types of memory, consisting of episodic, and semantic memories.[52] The memory of autobiographical events in relation to times, places is considered as episodic memory. Defic ts of episodic memory are one of the primary cognitive complications that are observed in presenile or senile dementia associated with AD that can lead to impairment of QOL.

Finding the effective treatments requires better understanding of the physiological mechanisms underlying the disease pathogenesis.[53] Earlier studies confi med the potential efficacy of flavonoids in formation of memory through activation of gene expression and neuronal signaling in the brain that result in changes of synaptic plasticity and neurogenesis, which can eventually influence learning and memory processes.[12] Flavonoids found to improve blood flow and possess vascular protective effects in humans.[54] These vascular effects are vital as the improved

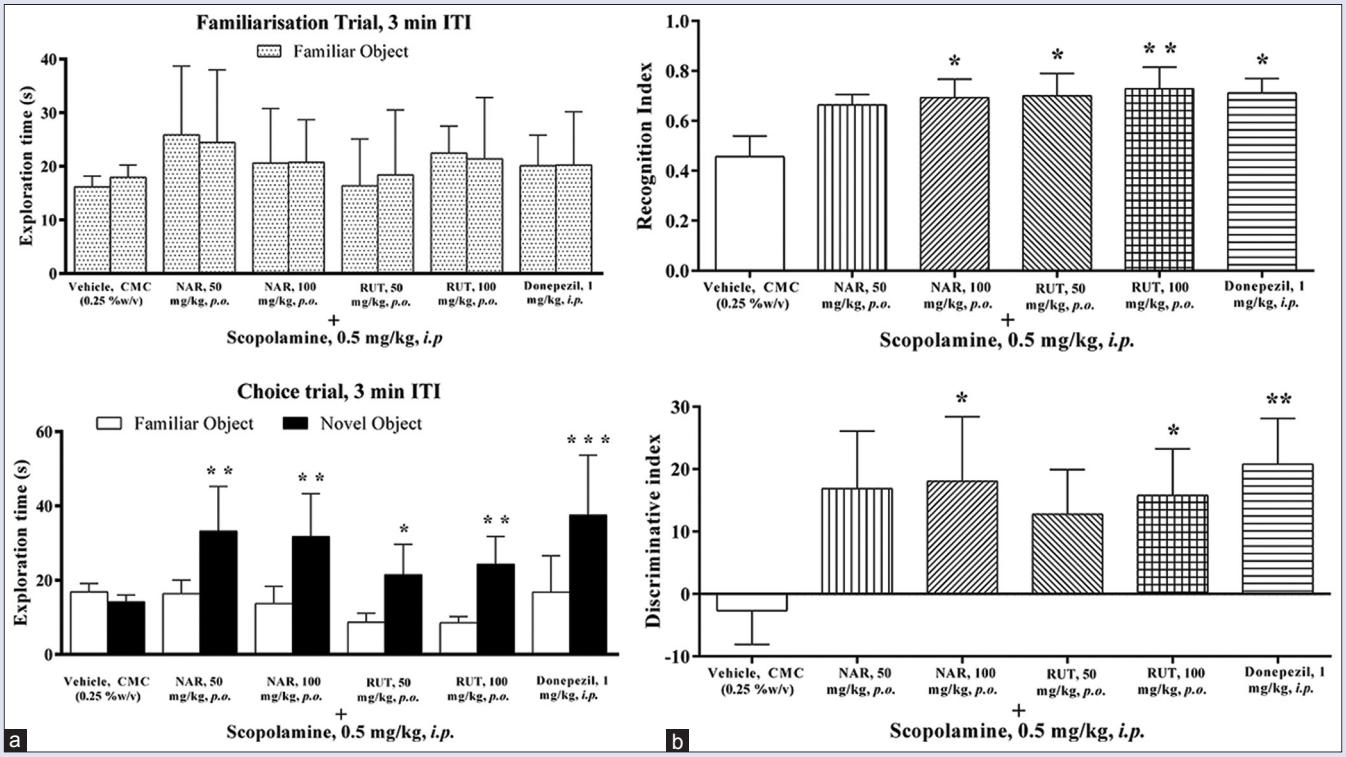

Figure 5: (a) Effect of naringin and rutin on exploration time of familiar or novel objects in scopolamine-induced memory deficits in familiarization and choice trials in novel object recognition task, Data represents mean ± standard error of the mean of duration of exploration (s) $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus familiar object using 3 min inter trial interval. (b) Effect of naringin and rutin on discriminative and recognition indices in scopolamine-induced memory deficits in novel object recognition task, data represent mean ± standard error of the mean of recognition and discriminative indices, $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$ versus vehicle control using 3 min inter trial interval

Table 1: Effect of NAR and RUT on hematological parameters

Treatment	Dose (mg/kg)	RBC (×10⁶ cells/µl)	WBC (×10³cells/µl)	Hb % (g/dl)	Granulocytes (×10³ cells/µl)	Lymphocytes (×10³cells/µl)	Monocytes (×10³ cells/µl)	Platelets (×10³ cells/µl)
Vehicle control	CMC (0.25% w/v)	7.58 ± 0.24	8.01 ± 0.39	12.36 ± 0.28	1.18 ± 0.09	5.92 ± 0.27	1.11 ± 0.03	564 ± 52
Naringin	50	7.83 ± 0.45	7.11 ± 0.38	13.24 ± 0.99	1.17 ± 0.10	4.98 ± 0.92	0.98 ± 0.04	586 ± 32
	100	8.24 ± 0.44	7.21 ± 0.47	13.69 ± 0.96	1.18 ± 0.09	5.09 ± 0.67	0.83 ± 0.04	564± 69
Rutin	50	7.97 ± 0.83	7.56 ± 0.68	13.44 ± 0.87	1.16 ± 0.13	5.98 ± 0.76	1.08 ± 0.07	581 ± 48
	100	8.54 ± 0.4	7.17 ± 0.42	13.84 ± 0.99	1.17 ± 0.09	5.77 ± 0.86	0.96 ± 0.02	609 ± 34
Donepezil	1	7.23 ± 0.38	7.87 ± 0.32	12.61 ± 0.2	1.20 ± 0.11	5.91 ± 0.57	1.09 ± 0.09	581 ± 47

Data represents mean ± SEM of major blood parameters, n=6. Although some improvements in RBC and hemoglobin were observed, the changes were not statistically signifi ant

cerebrovascular function is known to enhance neurogenesis in the hippocampus based on an *in vitro* study.[55] New hippocampal cells near to blood vessels can multiply rapidly in the presence of vascular growth factors and can influence cognitive function.[56]

The pharmacologically active aglycone moieties of NAR and RUT, that is, naringenin and quercetin respectively showed potential anticholinesterase activity in various cognitive animal models for assessing memory patterns in rodents.[57,58] Several studies at preclinical stage have focused mainly on the effect of NAR and RUT on spatial acquisition learning in water maze task, emotional memory in inhibitory avoidance task. However, it is essentially important to assess episodic memory and the influence of NAR and RUT on episodic memory as these defic ts were known to occur frequently in AD patients and were proved clinically. Down regulation or reduction in cholinergic transmission is linked to the most common form of dementia associated with AD. Scopolamine is a muscarinic receptor blocker, which blocks central M1 receptors that are crucial for memory formation through cholinergic neurotransmission and can lead to memory defic ts in humans following its administration.

Scopolamine-induced cognitive defic ts through the impairment of the cholinergic neurotransmission is one of the widely used methods to investigate the underlying mechanisms for cognitive changes of different forms of memory viz., episodic, working, and spatial memory noticed in AD patients. Memory defic ts can also be produced naturally by an

increased time delay, that is, by increasing the time between the sample and test phases, which has a natural origin.

We have used NORT paradigm to assess the short-term as well as long-term episodic memory defic ts. In time delay model, vehicle treated animals forgot the familiar object after a trial delay of 24 h, as they showed no discrimination between the objects in the choice trial. We found that NAR and RUT were effective dose-dependently, against this time induced long-term episodic memory impairment in Wistar rats at both the tested doses, i.e. 50 and 100 mg/kg, *p.o.*, as there was a signifi ant improvement in the recognition and discriminative indices compared to vehicle control. Donepezil at a dose of 1 mg/kg, *i.p.* prevented the episodic recall defic ts, which showed the validity of this model for assessing episodic memory.

In scopolamine-induced cognitive defic ts model, vehicle group spent almost equal time investigating both novel and familiar objects in choice trial, which indicates that, scopolamine at a dose of 0.5 mg/kg, *i.p.* produced short-term episodic memory defic ts at an ITI of 3 min. These defic ts were signifi antly reversed by prior administration of either donepezil at 1 mg/kg, *i.p.* or flavonoids, that is, NAR and RUT at a dose of 50 and 100 mg/kg, *p.o.* Hence, these flavonoids were able to reverse the episodic memory defic ts, which were induced either by increased time delay or by scopolamine administration in a dose-dependent passion. Among the flavonoids tested, RUT showed superior activity in both short-term and long-term episodic memory models.

Further, we assessed the effect of the tested flavonoids on locomotion using open fi ld test. The locomotion of the animals was not influenced by the treatment with NAR and RUT at any of the tested doses, which would otherwise influence the cognitive functional measurements. No signifi ant changes were observed in view of distance moved, time spent in the central zone and mean velocity. Hence it was proved that the tested flavonoids, NAR and RUT have produced procognitive like effects without influencing the locomotor activity and reversed the time delay induced long-term as well as scopolamine-induced short-term episodic memory defic ts supporting their potential use in neurodegenerative diseases, especially for the treatment of AD. No signifi ant difference in the %IBW was noted among the treatment groups, while moderate improvement in RBC and Hemoglobin was observed for the groups treated with flavonoids at the tested doses compared to vehicle control.

Numerous mechanisms and signaling pathways may underlie the enhanced cognitive function by these flavonoids. It was found that the biological activity of flavonoids within CNS is not only attributed to their antioxidant effects,[59] but also probably through their potential to enhance surviving neuronal function by protecting vulnerable neurons, stimulating neuronal regeneration and by inducing neurogenesis.[12,59,60] Moreover, flavonoids were found to enhance the extracellular signal-regulated protein kinase cAMP response element-binding protein and PI3 kinase-mTOR pathways that can lead to alterations in synaptic plasticity and were also able to influence neurogenesis by activation of PI3-kinase-Akt-eNOS pathway.[61] Further, flavonoids were known to inhibit oxidative stress-induced neuronal injury by preventing the caspase-3 action, which is responsible for their neuroprotective effects.[62] Collectively, multiple mechanisms of flavonoids underlie the cognitive improving effects of NAR and RUT in rodent animal models, including the episodic memory which was assessed in the present study. Based on the current fi dings, studies are underway to evaluate NAR and RUT for their potential to alleviate cancer-therapy related cognitive complications such as chemobrain.

CONCLUSION

NAR and RUT exhibited procognitive like effects dose-dependently by reversing the time induced long-term episodic memory defic ts in NORT. Further, scopolamine-induced short-term episodic memory defic ts were also found to be alleviated by prior treatment with the flavonoids at both the tested doses in a dose-dependent passion. Although, the mechanisms underlying the memory enhancement is beyond the scope of the present work, we conclude that NAR and RUT improved episodic memory impairments in Wistar rats without affecting the locomotor activity. The procognitive effects observed might have involved improved cholinergic neurotransmission by inhibition of acetylcholinesterase. In addition, potential ability of the flavonoids to interact with intracellular signaling pathways to enhance vascular function, synaptic plasticity, neurogenesis and their classical antioxidant effects also contribute to their nootropic effect in reversing the short-term as well as long-term episodic memory defic ts in recognizing the objects.

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Conflicts of interest

There are no confli ts of interest.

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