



# Neurobehavioral and neurochemical evaluation of rutin in $\beta$ -amyloid oligomer-induced Alzheimer's disease in Swiss mice

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## Abstract

**Background:** Alzheimer's disease (AD) is one of the major neurodegenerative disorders responsible for more than 60-80% of dementia cases globally. Satisfactory outcomes were not accomplished through pharmacological treatment by conventional drugs. Complementary and alternative medicines are projected as a source of new drugs for the prevention of neurodegenerative diseases. Rutin (RUT) a natural flavonoid was shown to prevent a few cases of memory deficits; it can be a preventive strategy against the development of AD.

**Aim:** The present study was designed to investigate the neurobehavioral and neurochemical effects of rutin in  $A\beta_{1-42}$  induced AD in male Swiss albino mice.

**Methods:** AD was induced by intracerebroventricular (*i.c.v.*) injection of  $A\beta_{1-42}$  oligomer (4  $\mu$ g/4  $\mu$ l) into the lateral ventricles of mice brain. The test compounds *i.e.*, RUT (50 and 100 mg/kg of body weight) and reference drug *i.e.*, donepezil (DP, 2 mg/kg) were administered orally from the 10<sup>th</sup> to 28<sup>th</sup> days. The AD-associated neurobehavioral changes were assessed by the novel object recognition test (NORT). Further, neurochemical biomarkers *i.e.*, brain acetylcholinesterase (AChE), thiobarbituric acid reactive substances (TBARS), and reduced glutathione (GSH) levels were estimated from brain hippocampal, cortex, and cerebellar samples.

**Results:** The administration of RUT was shown to ameliorate the  $A\beta_{1-42}$  induced neurobehavioral and neurochemical changes. These results were similar to the donepezil-treated group.

**Conclusion:** RUT ameliorates the  $A\beta_{1-42}$  induced AD pathology due to its potential antioxidant, anti-inflammatory, and neurotransmitter regulatory actions.



**Keywords:** Acetylcholinesterase, flavonoid, intracerebroventricular, neurotoxin, reduced glutathione, thiobarbituric acid reactive substances.

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## Introduction

AD is one of the major neurodegenerative disorders responsible for more than 60-80 % of dementia cases globally (Alzheimer 1907). Clinically, some of the drugs commonly used for Alzheimer's disease management are cholinesterase inhibitors such as donepezil, rivastigmine, and galantamine. However, these drugs also produced a loss of appetite, and increased frequency of bowel movements nausea, and vomiting (Nguyen et al., 2021). Similarly, glutamate regulators like memantine, used for mild-to-severe AD, also produced side effects such as headache, constipation, confusion, and dizziness (Olajide et al., 2021). In addition, FDA also approved the orexin receptor antagonist *i.e.*, suvorexant, and anti-amyloid antibody molecule *i.e.*, aducanumab for mild-to-moderate AD and dementia. Nevertheless, it also produced common side effects like swelling in the brain and headaches (Cummings 2021).

Over the last decade, complementary and alternative medicines are projected as an option to manage AD-associated neurodegenerative diseases (Ip et al. 2012). There was a growing interest in the potential of phytochemicals to improve learning, memory, and general cognitive ability (Ramassamy 2006). Rutin (RUT) was a flavonol glycoside also known as quercetin-3-rutinoside or vitamin P or sophorin comprising of the flavonol quercetin and the disaccharide rutinose (Calabro et al., 2005). RUT is primarily plant-derived and from various parts of the plant, including citrus fruits like lemon, lime, orange, and grapefruit. Further, asparagus; buckwheat; the flowers and fruits of the Pagoda tree; and berries like mulberry and common rue also possess significant quantity of RUT compound

(Ruta graveolens). One of the best dietary sources of rutin is buckwheat (Kodahet *al.*, 2008; Yang *et al.* 2008). RUT has been reported to increase the scavenging activity of free radicals (Horvathova et al., 2003). It also possesses diverse therapeutic potential like anti-hypertensive (Kaur and Muthuraman, 2016), anti-cancer, anti-thrombotic, anti-platelet, cardio-protective (Muthuraman et al., 2015; Singh et al., 2015), vasoprotective and hepatoprotective effects (Casa *et al.*, 2000) with a potential neuroprotective action (Budzynska *et al.* 2019; Spencer *et al.* 2019). Studies reported that RUT inhibited pro-inflammatory cytokines and microglial activation and is effective against trimethyltin-induced spatial memory deficits through amelioration of neuronal damage in the hippocampal CA3 subregion, which is crucial for acquisition learning in rodents (Koda et al., 2008). Further, RUT-derived natural products *i.e.*, quercetin also showed ameliorative potential against pentylene tetrazole-associated neurotoxicity and cognitive impairments (Narahari and Arunachalam 2018). Moreover, quercetin and rutin administration is also known to treat scopolamine-induced memory dysfunction (Richetti *et al.*, 2011). Therefore, the present study was designed to investigate the role of RUT in amyloid  $\beta_{1-42}$  oligomer-induced AD in male Swiss albino mice.

## Materials and methods

### Animal

The disease-free Swiss albino mice (12 months old; 20-35 g) were used in this research work. Animals were maintained in the central animal house, AIMST University with a standard laboratory diet (Soon Soon Oilmills Sdn Bhd, Pinang, Malaysia). The animal was allowed to access the free water *ad libitum*. The 12 hours



of natural light and dark cycles were maintained. The macro-environmental temperature and humidity of animal houses were made at 25 °C and 50%. The experimental protocol was approved by AIMST University Animal Ethics (AUAEC/FOM 2020/02 – Amendment No. 1). The caring of animals was done as per the guidelines of AUAEC.

### Chemicals

Amyloid ( $A\beta_{1-42}$ ; Biotek Abadi, Cayman Chemicals, USA), rutin (iHerb, Perris, CA, USA), ketamine hydrochloride injection (Dechra Pharmaceuticals PLC, United Kingdom), xylazine Injection (XYLAMAX<sup>®</sup>, Bimeda Canada), 5,5'-dithiobis-(2-nitrobenzoic acid), acetylthiocholine iodide, thiobarbituric acid, 1,1,3,3-tetra methoxy propane, reduced glutathione, Foiln-Ciocalteu reagent, and bovine serum albumin were purchased from Merck & Co., Inc., Japan.

### Preparation of $A\beta_{1-42}$ oligomer

$A\beta_{1-42}$  oligomer solution was freshly prepared before intracerebroventricular (*i.c.v.*) injection. Briefly,  $A\beta_{1-42}$  protein was dissolved in filtered phosphate-buffered saline (PBS: 1  $\mu\text{g}/\mu\text{l}$ ). It consists of 10 mM sodium-dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ), disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ), and 100 mM of sodium chloride (NaCl) were dissolved in glass-distilled deionized water (pH = 7.5).  $A\beta_{1-42}$  solution was then incubated at 37 °C for over 3 days before use (Li *et al.* 2010).

### Induction of AD mice model

AD was induced in mice by intracerebroventricular (*i.c.v.*) injection of  $A\beta_{1-42}$  under anesthetic conditions by a mixture of ketamine (75 mg/kg) and xylazine (5 mg/kg). According to the procedure established by Paxinos and Franklin (2004), a total of (4  $\mu\text{g}/4 \mu\text{l}$ ) of  $A\beta_{1-42}$  oligomer solution was injected into the lateral ventricles of mice brain (2  $\mu\text{l}$  on each side) at stereotaxic coordinates

(anteroposterior- 0.2 mm; mediolateral - 1.0 mm; dorsoventral - 2.5 mm) taken from the atlas of the mouse brain (Park *et al.* 2012). The animals in the normal group received 0.9% NaCl injections and those in the other groups were injected with an  $A\beta_{1-42}$  solution.

### Experimental protocol

Five groups of male adult Swiss albino mice (n = 8) were employed in this study. Group-I served as a normal control group. Group II served as the AD group. AD was induced by *i.c.v.* injection of  $A\beta_{1-42}$  (4  $\mu\text{g}/4 \mu\text{l}$ ) into the lateral ventricles of mice brain (2  $\mu\text{l}$  bilaterally). Group III and IV served as test compound treatment groups *i.e.*, RUT doses 50 and 100 mg/kg; oral administration from 10<sup>th</sup> to 28<sup>th</sup> days. Group V served as a reference drug treatment group *i.e.*, donepezil (DP, 2 mg/kg) oral administration for 10<sup>th</sup> to 28<sup>th</sup> days. Thereafter, the behavioral training for the novel object recognition test (NORT) was given from the 19<sup>th</sup> to the 21<sup>st</sup> day and the NORT test was carried out on the 22<sup>nd</sup> day. On the 28<sup>th</sup> day, the animal was sacrificed and brain tissue samples were collected for neurochemical estimation. The molecular mechanisms were confirmed by estimation of brain acetylcholinesterase (AChE), thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) activities in the hippocampus, cortex, and cerebellum tissue of mice brain.

### Assessment of novel object recognition test (NORT)

The NORT was modified from a previously described method by Yuede *et al.* (2009). The apparatus consists of an evenly illuminated sound-proof solid plastic box (45 cm × 45 cm × 45 cm) (See **Figure 1**). The mouse behavior was recorded with a video camera. The procedure consists of 4 phases: pre-habituation, habituation, training, and testing. On the 1<sup>st</sup> day of the experiment, animals were brought to the testing room 30 min before the

3350



experiment to familiarize themselves with the experimental environment. Mice were then allowed to freely explore the box in the absence of objects for 5 min. On the 2<sup>nd</sup> and 3<sup>rd</sup> days, mice were habituated to the empty box for 20 min per day. On the 4<sup>th</sup> day, each mouse was given a training trial followed by a testing trial (See **Figure 2**). During the training trial, two identical objects were placed at two opposite positions (A and A1) within the box at the same distance from the nearest corner. The mice were freely allowed to explore the identical objects for 10 min and then they were returned to their home cages. One hour later, the animals were placed back in the same box, where one of the two familiar objects was replaced by a novel object (N), to start a 10 min testing phase. All objects used in the study were different in shapes and colors but almost identical in size. The objects were fixed on the floor of the box to avoid displacement of the object. To exclude the existence of olfactory

cues, the entire box and objects were thoroughly cleaned with 70% vol/vol ethanol after each trial. Object exploration time was defined as the time length when an animal directs its nose within a 2-3 cm distance to the object, or sniffing or pawing the object. Sitting or standing on the object without active vibrissae sweeping or sniffing does not count as exploration time. The exploration time was analyzed manually using 2 stopwatches. In the training session, the object exploration time for the two similar objects A and A1 were recorded individually, from which the location preference (LP) for objects A and A1 were calculated. In the testing phase, the object exploration time for the novel object N and object exploration time for one of the familiar objects was recorded. In this experiment, the familiar object A was kept and A1 was replaced with a novel object. From the object exploration time, the recognition index (RI) was calculated using the following formula:

$$\text{Location preference (LP)} = \frac{\text{Time exploring one of the identical objects}}{\text{Time exploring the identical object pairs}} \times 100\%$$

*Recognition index (RI)*

$$= \frac{\text{Time exploring the novel object}}{(\text{Time exploring novel object} + \text{Time exploring familiar object})} \times 100\%$$

Location preference was used as an environmental control, which should be 50%, to rule out the influence of the location of the object. Animals with a total exploration time of lower than 20 seconds during the testing session were excluded from the analysis.

3351



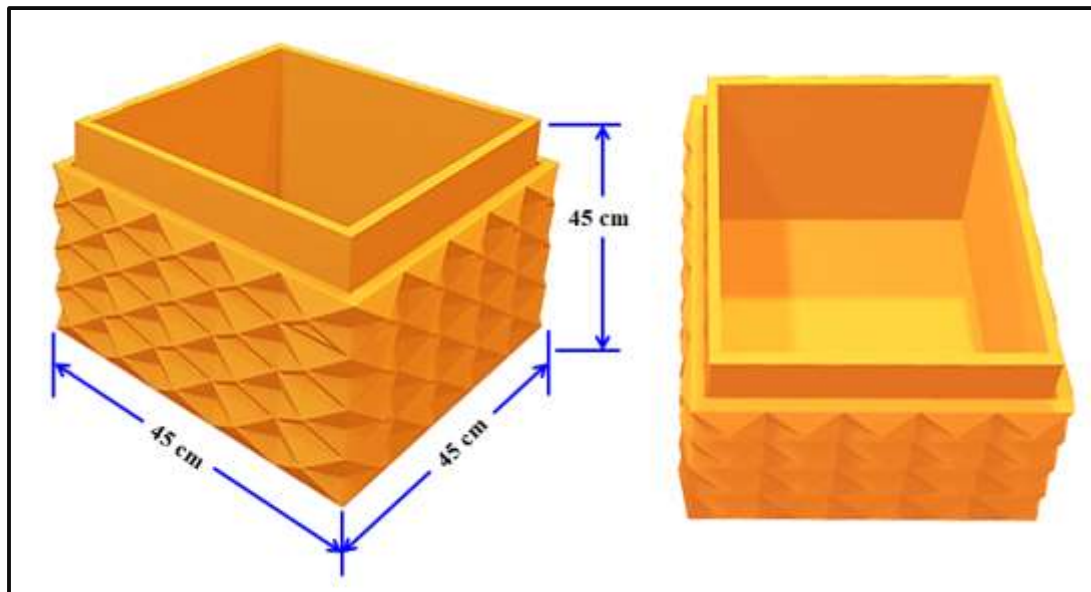


Figure 1: Image of NORT apparatus.

3352

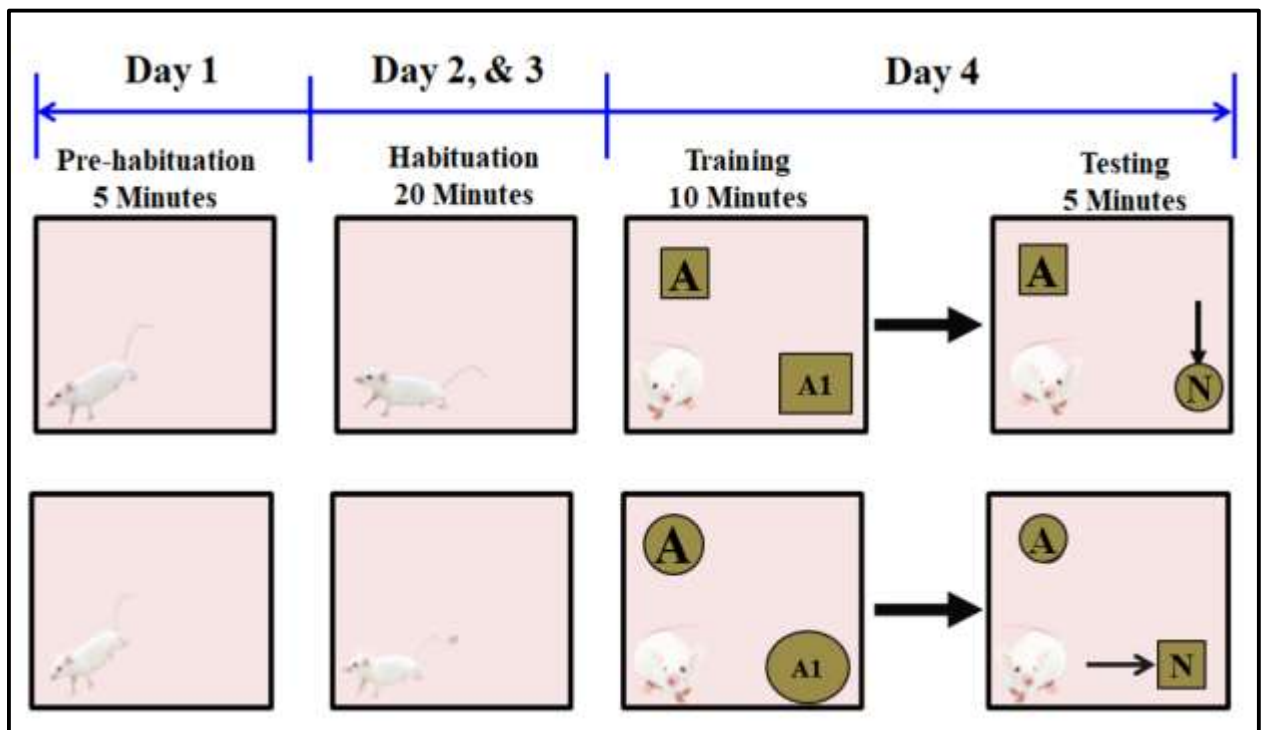


Figure 2: Illustration of RUT in AD-associated changes in NORT test. *Abbreviation:* A, location of one of the identical objects; A1, location of another identical object; and N, location of the novel object.

### Biochemical estimations

On the 28<sup>th</sup> day, all the animals were anesthetized with diethyl ether. Thereafter,

animals were sacrificed and brain tissues were collected for the estimation of tissue biomarker changes like AChE, TBARS, and GSH.

#### **Estimation of AChE as an indication of cholinergic neurochemical alteration in CNS**

AChE levels of brain tissues were estimated by the method described by Ellman *et al.* (1961). Briefly, 500  $\mu$ l of brain supernatant was mixed with 0.25 ml of Ellman's reagent

$$R = \frac{\delta \text{ O.D. } \times \text{ Volume of the assay (3 ml)}}{\epsilon \times \text{ mg of protein}}$$

In the above formula, 'R' represented the rate of enzyme activity in 'n' mole of acetylthiocholine iodide hydrolyzed/minute/mg of protein; ' $\delta$  O.D.' represented the changes in absorbance/minutes; and ' $\epsilon$ ' represented the extinction coefficient *i.e.*, 13600/M/cm.

#### **Estimation of TBARS as an indication of lipid peroxidation**

TBARS level of brain tissue was estimated as described by Ohkawa *et al.* (1979). Briefly, 0.2 ml of brain tissue supernatant of homogenate was mixed with 0.2 ml of 8.1 % sodium; 0.2 ml of 8.1 % sodium dodecyl sulphate; 1.5 ml of 30 % acetic acid; and 1.5 ml of 0.8 % of thiobarbituric acid (TBA) in a test tube. The total volume of 4 ml was made up of distilled water. Further, test tubes were incubated at 90 °C for 1 hour. After that, 1 ml of

$$\text{MDA} \left( \frac{\text{nmol}}{\text{ml}} \right) = \frac{\delta \text{ O.D. sample}}{\epsilon \times \text{ PL}} \times \text{ DF}$$

In the above formula, ' $\delta$  O.D.' represented the changes in absorbance/minutes; ' $\epsilon$ ' represented the extinction coefficient *i.e.*,  $1.56 \times 10^5$ /m/cm; DF represented the dilution factor and PL represented the path length. The calculated level of MDA (nmol/ml) value was further integrated with mg of protein. The net value of MDA was expressed as nmol of MDA per mg of protein.

5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB (0.001 M)] and it was allowed to develop a yellow colour chromogen. Corresponding to changes in absorbance (O.D., optical density) the changes in yellow colour chromogen were quantified by using a spectrophotometer (DU 640B Spectrophotometer, Beckman Coulter Inc., CA, USA) at 420 nm. The O.D. values were used for further calculation of the AChE activity level by using the following formula.

distilled water was added and centrifuged at 4000 revolutions per minute (rpm) for 10 minutes. It developed the pink colour chromogen. In the lipid peroxidation process, the active lipid peroxidation byproducts *i.e.*, malondialdehyde (MDA) readily reacted with TBA and formed the intermediate MDA-TBA<sub>2</sub> adduct and water. This adduct has shown a pink colour. Using a spectrophotometer (DU 640B Spectrophotometer, Beckman Coulter Inc., CA, USA) at 532 nm, the changes in absorbance of the pink colour chromogen were recorded. Standard curve was prepared with absorbance value (y-axis) versus reference standard concentration (x-axis) *i.e.*, 1,1,3,3-tetra methoxy propane (TMP) 0-100 nanomole per milliliter (nmol/ml). Using the following formula, the level of TBARS was quantified

#### **Estimation of GSH as an indication of oxidative stress**

The GSH level of brain tissue was estimated by the method described by Ellman (1959). Briefly, the brain tissue supernatant was mixed with 10% w/v of trichloroacetic acid (1:1 ratio) to make the protein precipitations. After that, at 4 °C the test tube samples were centrifuged at 1000 rpm for 10 min. About 0.5 ml of clear aliquot was mixed with 2 ml of 0.3 M disodium hydrogen phosphate. Thereafter, 0.25 ml of 0.001 M freshly prepared 5,5'-dithiobis (2-

3353



nitrobenzoic acid) (DTNB) solution was added. In 1% w/v sodium citrate solution the DTNB was dissolved and it developed the yellow colour chromogen. GSH readily reacted with DTNB and produced the yellow colour chromogen product *i.e.*, 5-mercapto-2-nitrobenzoic acid (TNB). Using a spectrophotometer (DU 640B Spectrophotometer, Beckman Coulter Inc., CA,

$$GSH = \frac{\delta O.D. \text{ Standard} - (y - \text{intercept})}{\text{Slope}} \times DF \times 2$$

In the formula, ' $\delta$  O.D.' represented the changes in absorbance of the standard;  $y$ -intercept represented the  $y$ -intercept of linear curve value from the standard plot; slope represented the value obtained from the standard plot; DF represented the dilution factor, and number 2 represented the conversion one oxidized glutathione to two reduced glutathione. The calculated level of GSH ( $\mu\text{mol/ml}$ ) value was further integrated with mg of protein. The net value of GSH was expressed as  $\mu\text{mol}$  of GSH per mg of protein.

### Statistical analysis

All the results were expressed as mean  $\pm$  standard deviation (SD). The behavioral data were statistically analyzed using two-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test and data of tissue biomarkers *i.e.*, AChE, TBARS, and GSH levels were analyzed using one-way ANOVA followed by Tukey's Multiple Range test using Graph pad prism version-5.0 software. The value of  $p < 0.05$  was considered to be statistically significant.

## Results

### Effect of RUT in AD-induced behavioural changes

In the present study the *i.c.v.* injection of  $A\beta_{1-42}$  oligomer into the lateral ventricle of mice brain as shown significant ( $p < 0.05$ )

USA) at 412 nm the changes in the absorbance of yellow colour chromogen were recorded. The standard curve was prepared with absorbance value ( $y$ -axis) versus reference standard concentration ( $x$ -axis) *i.e.*, GSH 10-100 micromole per milliliter ( $\mu\text{mol/ml}$ ). The GSH level was quantified by using the following formula.

impairment in memory and cognitive function in NORT test when compared to the normal animal group. The administration of RUT (50 & 100 mg/kg; *p.o.*) significantly ameliorated the above neurobehavioral changes in a dose-dependent manner compared to the AD group. The effect of high dose RUT showed a similar effect to the comparison reference drug *i.e.*, DP (2 mg/kg; *p.o.*) treated group. The details are described in the following section.

### Effect of RUT in DR in NORT

The administration of  $A\beta_{1-42}$  (4  $\mu\text{g}/4 \mu\text{l}$ ; *i.c.v.*) induced AD mice showed significant ( $p < 0.05$ ) impairment of cognitive dysfunction in the NORT test when compared to the normal control group. Besides, in the training session the two similar objects (A and A1), showed no significant differences in object exploration among all the groups. However, in the testing session with two different objects, one familiar object and one novel object (N) showed a significant recognition ability for the novel object than the familiar object in the normal group. But  $A\beta_{1-42}$  treated group did not show any differences in the recognition ability when compared to the normal group. Meanwhile, the administration of RUT (50 and 100 mg/kg; *p.o.*) showed a high novel object recognition response. Among these, high dose RUT (100 mg/kg; *p.o.*) has shown a more significant novel object recognition ability indicating a good cognitive ability when compared to the AD group. These ameliorative effects were shown to have a similar effect to the reference drug



*i.e.*, DP (2 mg/kg; *p.o.*) treated group. The results were tabulated in **Table 1**.

**Table 1.** Effect of RUT in LP and RI responses in NORT assessment.

Groups	Training Session		Testing Session	
	LP-A	LP-A1	LP-A	RI
Normal	50.5 ± 2.4	49.4 ± 1.4	31.8 ± 1.3	68.1 ± 1.8
AD	51.2 ± 2.1	48.9 ± 1.9	51.4 ± 1.6 <sup>a</sup>	48.9 ± 1.9 <sup>a</sup>
AD + RUT (50)	49.7 ± 1.7	50.9 ± 2.2	45.3 ± 2.4 <sup>a</sup>	54.6 ± 1.3 <sup>a</sup>
AD + RUT (100)	50.2 ± 2.6	49.7 ± 2.5	33.2 ± 1.6 <sup>b</sup>	66.7 ± 1.6 <sup>b</sup>
AD + DP (2)	51.8 ± 2.3	48.7 ± 1.6	33.6 ± 1.2 <sup>b</sup>	66.3 ± 1.7 <sup>b</sup>

**Table 1.** Effect of RUT in LP and RI responses in NORT assessment. Digits in parenthesis indicate a dose of mg/kg. Data were expressed as mean ± SD, n = 8 mice per group. <sup>a</sup>*p* < 0.05 Vs normal group. <sup>b</sup>*p* < 0.05 AD group. *Abbreviation:* AD, Alzheimer's disease; DP, donepezil; RUT, rutin; LP-A, location preference for familiar object A; LP-A1, location preference for familiar object A1; and RI, recognition index.

#### Effect of RUT in $A\beta$ oligomer induced AD tissue biomarker changes

The administration of  $A\beta_{1-42}$ -oligomer (4  $\mu$ g/4  $\mu$ l; *i.c.v.*) showed significant (*p* < 0.05) alteration of brain tissue biomarkers *i.e.*, an increase in AChE and TBARS activity and a decrease in GSH activity in all the brain regions such as the hippocampus, cerebral cortex, and cerebellum when compared to the normal

control group. The administration of RUT (50 and 100 mg/kg; *p.o.*) significantly attenuated the  $A\beta_{1-42}$ -oligomer-induced changes of tissue biomarkers when compared to the AD group in a dose-dependent manner. These ameliorative effects of RUT were shown a similar effect to reference drug *i.e.*, DP (2 mg/kg; *p.o.*) treated group. The results were indicated in **Table 2-4**.

3355

**Table 2.** Effect of RUT in the changes of AChE levels in brain tissue biomarkers.

Groups	Hippocampus	Cortex	Cerebellum
Normal	21.5 ± 1.5	20.7 ± 2.3	20.2 ± 1.3
AD	41.1 ± 1.3 <sup>a</sup>	42.5 ± 1.2 <sup>a</sup>	36.4 ± 1.5 <sup>a</sup>
AD + RUT (50)	30.2 ± 0.9 <sup>b</sup>	30.6 ± 1.9 <sup>b</sup>	27.1 ± 0.9 <sup>b</sup>
AD + RUT (100)	24.5 ± 1.2 <sup>b</sup>	21.9 ± 2.9 <sup>b</sup>	21.6 ± 2.1 <sup>b</sup>
AD + DP (1)	22.3 ± 1.3 <sup>b</sup>	22.9 ± 1.4 <sup>b</sup>	21.3 ± 1.4 <sup>b</sup>

Digits in parenthesis indicate dose mg/kg, and the value of AChE level was expressed as  $\mu$ mole/mg of protein/min. Data were expressed as mean ± SD, n = 8 mice per group. <sup>a</sup>*p* < 0.5 Vs normal group. <sup>b</sup>*p* < 0.5 Vs AD group. *Abbreviation:* AD, Alzheimer's disease; DP, donepezil; and RUT, rutin.

**Table 3.** Effect of RUT in the changes of TBARS levels in brain tissue biomarkers.

Groups	Hippocampus	Cortex	Cerebellum
Normal	4.2 ± 0.11	4.2 ± 0.13	3.5 ± 0.13
AD	7.5 ± 0.09 <sup>a</sup>	7.9 ± 0.23 <sup>a</sup>	5.9 ± 0.08 <sup>a</sup>



AD + RUT (50)	5.1 ± 0.07 <sup>b</sup>	5.2 ± 0.09 <sup>b</sup>	4.2 ± 0.07 <sup>b</sup>
AD + RUT (100)	4.7 ± 0.03 <sup>b</sup>	4.8 ± 0.12 <sup>b</sup>	3.9 ± 0.12 <sup>b</sup>
AD + DP (1)	4.2 ± 0.03 <sup>b</sup>	4.2 ± 0.08 <sup>b</sup>	3.6 ± 0.14 <sup>b</sup>

Digits in parenthesis indicate dose mg/kg, and the value of TBARS level was expressed as nmol/mg of protein. Data were expressed as mean ± SD, n = 8 mice per group. <sup>a</sup>*p* < 0.5 Vs normal group. <sup>b</sup>*p* < 0.5 Vs AD group. *Abbreviation*: AD, Alzheimer's disease; DP, donepezil; and RUT, rutin.

**Table 4.** Effect of RUT in the changes of GSH levels in brain tissue biomarkers.

Groups	Hippocampus	Cortex	Cerebellum
Normal	81.6 ± 1.4	88.1 ± 1.3	74.6 ± 2.1
AD	52.9 ± 0.5 <sup>a</sup>	58.5 ± 1.6 <sup>a</sup>	54.2 ± 0.8 <sup>a</sup>
AD + RUT (50)	67.4 ± 1.8 <sup>b</sup>	65.4 ± 1.5 <sup>b</sup>	64.9 ± 1.9 <sup>b</sup>
AD + RUT (100)	73.1 ± 1.7 <sup>b</sup>	77.6 ± 1.4 <sup>b</sup>	69.8 ± 1.4 <sup>b</sup>
AD + DP (1)	79.7 ± 1.5 <sup>b</sup>	86.4 ± 1.3 <sup>b</sup>	73.1 ± 1.2 <sup>b</sup>

Digits in parenthesis indicate dose mg/kg, and the value of GSH level was expressed as  $\mu$ mole/mg of protein. Data were expressed as mean ± SD, n = 8 mice per group. <sup>a</sup>*p* < 0.5 Vs normal group. <sup>b</sup>*p* < 0.5 Vs AD group. *Abbreviation*: AD, Alzheimer's disease; DP, donepezil; and RUT, rutin.

## Discussion

The administration of  $A\beta_{1-42}$ -oligomer (4  $\mu$ g/4  $\mu$ l; *i.c.v.*) was shown to significant (*p* < 0.05) induction of AD with the reflection of changes in neurobehavioral and neurochemicals. Further, it also altered the tissue biomarkers *i.e.*, an increase in AChE and TBARS activity; and a decrease in GSH activity in the hippocampus, cerebral cortex, and cerebellum of mice brain samples. However, the administration of RUT (50 and 100 mg/kg; *p.o.*) and DP (2 mg/kg; *p.o.*) significantly ameliorated the  $A\beta_{1-42}$ -oligomer-associated AD progression.

Data analysis of the present study, RUT showed a potential ameliorative effect against the  $A\beta_{1-42}$  oligomer-associated AD. Various experimental reports evidenced that  $A\beta_{1-42}$  oligomer is enhancing the  $\beta$ -amyloid and tau protein accumulation. Moreover, it also enhances the senile plaque formation in neuronal tissue and neurodegeneration leads to progress the mild to severe AD (Zheng et al., 2013; Picone et al., 2020). Furthermore, the accumulation of  $A\beta_{1-42}$  can also produce neurotoxic effects, leading to the appearance of

oxidant stress and promoting microglial activation (Yan et al., 1996; Park et al., 2021). The accumulation of free radicals and inflammatory mediators leads to the neurodegenerative process (Saeed et al., 2020). The present result findings of RUT are similar to the previous research findings.

The NORT test results are evidence that RUT ameliorated the  $A\beta_{1-42}$  oligomer-induced cognitive dysfunctions. Similar results are supported by other research reports (Bevins and Besheer 2006; Lv et al., 2021). The present study revealed that RUT has a higher significant recognition ability to novel objects in NORT with similar action of reference drug *i.e.*, donepezil treatment. Similar behavioral changes are observed in another research lab report stating that  $A\beta_{1-42}$  oligomer associated deposition of senile plaques in the brain regions (hippocampus and cortex) with cognitive dysfunction and neuronal death (Li *et al.* 2010). Further, the *i.c.v.* injection of  $A\beta_{1-42}$  oligomer is enhancing the cerebrospinal fluid (CSF) leading to alter Spatial learning (Simon and Iliff 2016; Haughey et al., 2002).

3356



Moreover, the administration of  $A\beta_{1-42}$  oligomer induced potential alteration of brain biomarkers in mice brain tissues (hippocampus, cortex, and cerebellum) *i.e.*, rising of AChE, TBARS levels, and decrease in GSH levels. It is the hallmark of  $A\beta_{1-42}$  oligomer-induced neurodegeneration, oxidative stress, and metabolic alteration in mice brains. These biomarker alterations are known to produce with the administration of  $A\beta_{1-42}$  oligomer in experimental animals (Rehman et al. 2021). Experimentally, the inhibitor of acetylcholinesterase activity *i.e.*, donepezil is known to produce neuroprotective, antioxidant, and anti-inflammatory actions in brain tissue (Thamban Chandrika et al., 2020). Similarly, RUT also produced the ameliorative effect against the  $A\beta_{1-42}$  oligomer-induced neurotoxicity. RUT possesses the potential antioxidant properties for neuroprotective actions (Yang et al., 2008). Moreover, RUT is known to possess the potential modulatory action against acetylcholinesterase activity (Adefegha et al., 2018). The present study also demonstrated that RUT was shown similar antioxidant and anti-acetylcholinesterase activity against the  $A\beta_{1-42}$  toxicity for the management of AD.

### Conclusion

The administration of RUT was shown to ameliorate the  $A\beta_{1-42}$  oligomer-associated neurotoxicity due to its potential anti-oxidant, anti-lipidperoxidative, and regulation of cholinergic neurotransmitters. Therefore, RUT can be a novel natural medicine for the management of neurodegenerative disorders like AD and dementia.

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