Original Article

Quercetin nanoparticles attenuates scopolamine induced spatial memory deficits and pathological damages in rats

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ABSTRACT

Quercetin is a well-known flavonoid, has low bioavailability. Quercetin nanoparticles (NQC) enhance its bioavailability. NQC were not explored for their potential therapeutic activities in Alzheimer's disease (AD). Hence, the present study was performed to evaluate the protective effect of NQC in comparison to free quercetin against scopolamine induced spatial memory impairments.

NQC prepared by anti solvent precipitation method. Quercetin, NQC (30 mg/kg p.o.) and rivastigmine (2 mg/kg i.p.) as a reference drug were administered for 8 consecutive days. At the end of the treatment period memory impairments were induced by a single injection of scopolamine (20 mg/kg; i.p.). Conditioned avoidance and rectangular-maze tests were conducted 30 min thereafter then rats were sacrificed and brain homogenates were used for the estimation of glutathione (GSH), catalase and malondialdehyde (MDA) contents together with acetyl cholinesterase (AchE) activity. In addition, histopathologic studies were also performed.

The size of NQC was observed below 300 nm. NQC significantly reduced the transfer latency and conditioned avoidance response compared to scopolamine treated group (p < 0.05). Pretreatment with NQC showed a significant (p < 0.05) decrease in MDA, AchE levels and increase in brain catalase and GSH levels to be similar to that observed in the rivastigmine group.

In all the behavioral, biochemical and histological experiments, the rats treated with NQC showed additional distinguished results compared to quercetin group indicating that a preventive strategy against the progression of AD. This approach of quercetin nanoparticles provides the potential therapeutic application in human neurodegenerative disease in future.

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1. Introduction

Alzheimer's disease (AD) is a chronic neurodegenerative disorder with the progressive decline in memory [1]. AD is characterized by cerebral oxidative stress accompanied by loss of cholinergic neurons in the basal forebrain and hippocampus [2,3]. Central cholinergic neuronal activity plays an important role in learning and memory [4]. Multiple neurotransmitters and neuronal pathways are involved in the process of memory formation [5]. Functional deficits in the cholinergic system are associated with cognitive impairments observed in AD [6].

Scopolamine a muscarinic cholinergic receptor antagonist has profound amnesic effects in experimental animals. Scopolamine induced amnesia has been widely adopted the experimental animal model to screen for drugs with potential therapeutic values in dementia [7] it interferes with acetylcholine transmission in the central nervous system, leading to cholinergic dysfunction and memory impairments in rats [8,9].

Acetyl cholinesterase inhibitors, such as rivastigmine, galantamine and donepezil, are the most effective and approved pharmacotherapeutic agents for cognitive dysfunction [10]. Rivastigmine is a pseudo irreversible inhibitor of both acetylcholinesterase and butyrylcholinesterase [9]. Despite intensive advancement in research, available drugs are not ideal for clinical use due to their undesirable side effects [11,12] thus, it is necessary to search for alternative or adjuvant anti amnestic therapies. Today, it is best known that medicinal plants attracted attention due to their use in the treatment of cognitive disorders [13].

Quercetin is a well-known flavonoid in the human diet, present in vegetables, herbs, edible fruits and other related products eg. Red wine [14], Ginko Biloba [15] and onions [16]. Quercetin is one of the prominent dietary antioxidants it shows biological

effects that include protection against certain forms of cancer [17], inflammation [18] and cardiovascular diseases [19]. Despite these medicinal benefits, quercetin has low bioavailability (less than 17% in rats and even 1% in human) [20,21] due to its poor aqueous solubility, as a result, the clinical application of this drug greatly restricted. Therefore, it becomes necessary to develop a system which could increase the solubility of quercetin.

Nanoparticles are particularly suitable for drug delivery for water insoluble compounds such as quercetin. According to Noyes-Whitney equation [22] a decrease in particle size will lead to an increase in effective surface area which results in enhanced bioavailability. In the present study, quercetin nanoparticles (NQC) prepared by anti solvent precipitation method using syringe pump to enhance its bioavailability in therapeutic application. The aim of our present study was to evaluate the protective effect of NQC in comparison to free quercetin against scopolamine induced spatial memory impairments.

2. Materials and methods

2.1. Materials

Rivastigmine used as a reference drug obtained from Vasudha Pharma Chemicals Ltd, Hyderabad, India. Scopolamine hydrobromide used as a dementia inducing agent obtained from Boehringer Ingelheim, Quercetin, 5,5-dithio-bis2-nitro benzoic acid, (Ellman's reagent), acetylthiocholine iodide, were purchased from Sigma–Aldrich (USA), the absolute ethanol (99.5–99.8%) was obtained from Merck, Mumbai, India. All other reagents used were also of analytical grade.

2.2. Preparation of NQC [23]

Quercetin was dissolved in the solvent (ethanol) at a concentration of 5 mg/ml. The syringe was filled with the prepared solution and secured onto a syringe pump. The drug solution was quickly injected at a fixed flow rate (8 ml/min) into the anti-solvent (deionized water) of definite volume under magnetic stirring (1000 rpm). Ethanol to water volume ratios used was 1:25. The quercetin nanoparticles were filtered and vacuum dried.

2.3. Particle morphology

The particle size and morphology of samples was observed using a Scanning Electron Microscope (SEM) Zeiss EVO 18-EDX special edition machine compatible with EDX machine. The powder samples were spread on a SEM stub and sputtered with gold before the SEM observations. The particle size and texture of nanoparticles can be analyzed by using image magnification software compatible with SEM and helps in determining the presence and formation of NQC. Five SEM pictures were used to find the average range of particle diameter.

2.4. Animals

The study was performed on male Albino Wistar rats (150–250 g). All animals were procured from Mahaveera enterprises, Hyderabad. The animals were maintained under a controlled 12 h light/dark cycle. Handling and experimentation were conducted in accordance with the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi and the experimental protocol was approved by Institutional Animal Ethical Committee (IAEC), Kakatiya University Warangal.

2.5. Treatments

In both behavioral tasks such as conditioned avoidance test and rectangular maze, rats were randomly allocated into five groups (6 rats each) as follows: group I received saline and served as control while group II received scopolamine (20 mg/kg, i.p.). Groups III–V received rivastigmine (2 mg/kg, i.p.), Quercetin (30 mg/kg, p.o.), NQC (30 mg/kg, p.o.). The animals were trained for 7 days. During which they do not receive any drug. The completely trained animals were chosen for the study. These animals were dosed once in a day with the respective drugs for 8 days along with daily training trial. Scopolamine was administered as a single dose 30 min after the last administration in groups II–V.

2.6. Behavioral experiments

2.6.1. Conditioned avoidance test

The test was carried out using a shuttle box as described by Hinrichs et al. [24]. In this experiment, the rat is placed in a two-compartment shuttle box and presented with a conditioned stimulus such as a light, followed after a short delay by an aversive unconditioned stimulus foot-shock. After injection of scopolamine, each rat was placed in the shuttle box and allowed to adapt for 3 min. Following adaptation, the conditioned stimulus was presented for 20 s prior to the unconditioned stimulus. If the rat crossed to the next compartment during 20 s of conditioned stimulus the electric shock was avoided otherwise, failure of avoidance was recorded.

2.6.2. Rectangular maze test

Rectangular maze is used for studying learning, memory in animals. The maze consists of completely enclosed rectangular box divided into chamber A, in which the rat is placed and has a sliding door that is opened to allow the rat to enter the maze; chamber C. The maze, animal has to explore and reward chamber B, at the other end of maze in which the reward is kept. Well-trained animals were taken for the experiment. Transfer latency (Time taken in seconds by the animal to reach reward chamber from chamber A) was recorded. For each animal, four readings were taken and the average is taken as learning score (transfer latency) for that animal. Lower scores of the assessment indicate efficient learning while higher scores indicate poor learning in animals [25].

2.7. Brain homogenate preparation

Immediately after performing the behavioral tests, rats were sacrificed by decapitation. The brains were removed; a (10% w/v) homogenate was prepared in ice-cold 50 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 min and aliquots of supernatant were separated and used for biochemical estimation.

2.8. Histopathologic examination of brain tissues

The histopathologic assessment was performed on the brains of different groups. Brains were removed from the skull and post-fixed overnight in paraformaldehyde. Coronal sections of 5 μm thickness were cut using microtome and the sections were stained with hematoxylin and eosin and examined microscopically.

2.9. Determination of biochemical markers in brain homogenate

The method for the assessment of MDA content in the brain homogenates was based on that of Ruiz-Larrea et al. [26] the supernatant was read spectrophotometrically at 532 nm and MDA brain content was expressed as nmol/mg tissue. GSH level

in the brain was determined quantitatively by performing the method as described by Ellman and Bulaj et al. [27,28]. The optical density of the produced colored product was determined at 412 nm using the spectrophotometer. Calculation of GSH content was based on a standard glutathione curve and expressed as μmol/mg tissue. Catalase activity was estimated by the method of Beer and Seizer [29] based on the ability of catalase to oxidize hydrogen peroxide. The change in absorbance was recorded at 240 nm using the spectrophotometer. The results were expressed as nano moles of H_2O_2 decomposed per minute per mg protein. Ellman method [27] was used to estimate brain AChE activity. AChE activity was determined spectrophotometrically at 412 nm. The activity of AChE was expressed in μmole/min/mg of protein.

2.10. Statistical analysis

The mean ± SEM, were calculated for all data. Significant difference between means was evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison tests. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. NQC characterization by SEM

SEM micrographs of the quercetin and NQC are shown in Fig. 1a and b. It is observed that the quercetin powder (Fig. 1a) exhibited particles lacking uniformity in size and were much larger than the NQC (Fig. 1b). On the other hand, NQC prepared by syringe pump exhibited particles uniformity in size, less crystallinity, and absence of larger particles (Fig. 1b). As depicted in the image, the particles possessed uniform shape. The size of all particles was found to be less than 300 nm.

3.2. Effect of NQC on scopolamine-induced memory impairment in the conditioned avoidance test

Administration of scopolamine (20 mg/kg) as a single i.p. injection resulted in a significant increase in conditioned avoidance response ($p < 0.05$) as compared to the control group. Pretreatment with rivastigmine or quercetin or NQC significantly reduced the conditioned avoidance response compared to scopolamine treated group ($p < 0.05$) and control group ($p < 0.05$), respectively (Fig. 2).
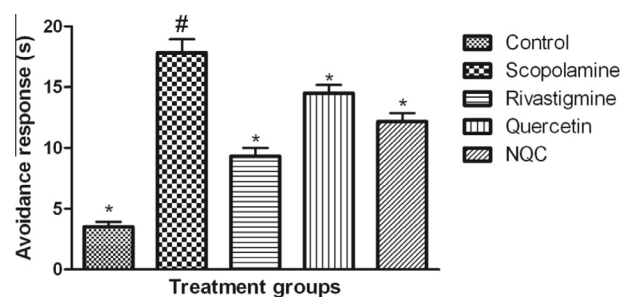
Fig. 2. Effect of NQC on avoidance response in conditioned avoidance test against scopolamine induced amnesia. Each bar with vertical line represents the mean of 6 rats ± S.E.M. Statistical analysis was carried out using One-Way ANOVA followed by Bonferroni multiple comparisons test; $^{\#}p < 0.05$ vs. control, $^{*}p < 0.05$ vs. scopolamine.

3.3. Effect of NQC on scopolamine-induced memory impairment in the rectangular maze test

Scopolamine-induced dementia significantly increased the transfer latency ($p < 0.05$) as compared to the normal group. However, Pretreatment with rivastigmine or quercetin or NQC significantly reduced the transfer latency compared to scopolamine treated group ($p < 0.05$) and control group ($p < 0.05$), respectively (Fig. 3).

3.4. Effect of NQC on lipid peroxidation

Table 1 depicts effect of different treatments on LPO, which measured as MDA levels in brain homogenate. The MDA content expressed as nmol/mg tissue was significantly higher in scopolamine-treated rats (3.579 ± 0.1762) as compared to the control group (0.6853 ± 0.2096). Pre treatment with NQC significantly reduced MDA levels ($p < 0.05$) when compared scopolamine group and restored MDA brain levels to be similar to rivastigmine group (1.151 ± 0.1703). Quercetin did not show the significant reduction in MDA levels (2.927 ± 0.1245) when compared scopolamine group.

3.5. Effect of NQC on GSH activity

Animals subjected to scopolamine treatment showed a significant reduction in GSH (1.167 ± 0.3380) levels as compared to control group (4.933 ± 0.4723). NQC pretreatment showed a significant increase in brain GSH levels ($p < 0.05$) when compared to scopolamine group. NQC increased brain GSH levels to a similar level as the rivastigmine group. Quercetin did not show the
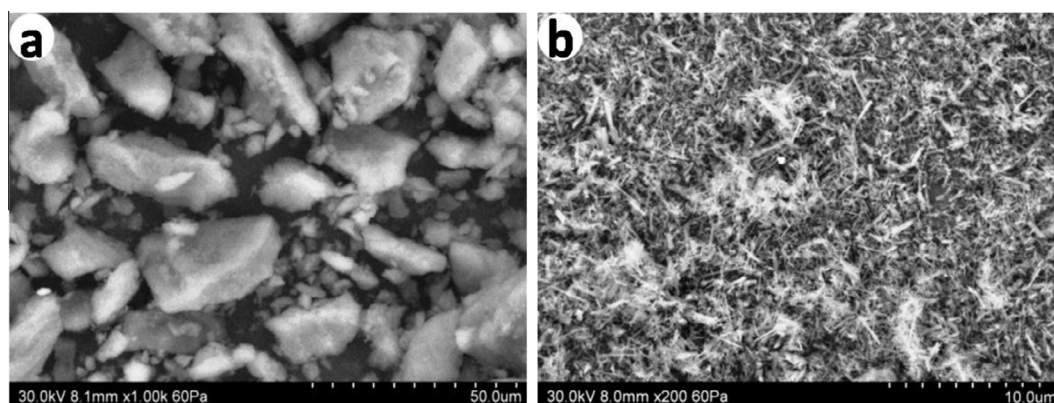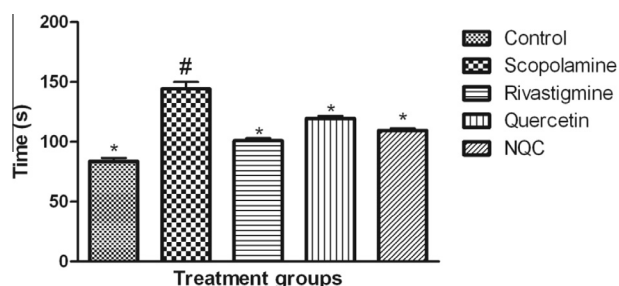
Fig. 1. SEM photographs of a) Quercetin and b) NQC.

Fig. 3. Effect of NQC on latency time in rectangular maze test against scopolamine induced amnesia. Each bar with vertical line represents the mean of 6 rats ± S.E.M. Statistical analysis was carried out using One-Way ANOVA followed by Bonferroni multiple comparisons test; #p < 0.05 vs. control, *p < 0.05 vs. scopolamine.

Table 1
Effect of NQC on scopolamine induced oxidative stress parameters in rat brain.

Treatment groups	MDA (nmol/mg tissue)	GSH (μmol/mg tissue)	Catalase (nmol/mg tissue)
I Control	0.6853 ± 0.2096*	4.933 ± 0.4723*	5.124 ± 0.3174*
II Scopolamine	3.579 ± 0.1762#	1.167 ± 0.3380#	1.574 ± 0.1736#
III Rivastigmine	1.151 ± 0.1703*	4.402 ± 0.7910*	4.581 ± 0.1834*
IV Quercetin	2.927 ± 0.1245	2.960 ± 0.2749	2.340 ± 0.1120
V NQC	1.988 ± 0.2214*	4.378 ± 0.5163*	3.682 ± 0.2676*

The results expressed as mean ± SEM, n = 6. Statistical analysis was carried out by One-way ANOVA followed by Bonferroni multiple comparison test.
* $p < 0.05$ versus scopolamine group.
# $p < 0.05$ versus control group.

significant increase in GSH levels (2.960 ± 0.2749) when compared scopolamine group (Table 1).

3.6. Effect of NQC on catalase activity

Catalase levels were significantly reduced in scopolamine-treated groups (1.574 ± 0.1736) compared to the control group (5.124 ± 0.3174). NQC pretreatment showed a significant increase in brain catalase levels ($p < 0.05$) when compared to scopolamine group. Quercetin did not show the significant increase in catalase levels (2.340 ± 0.1120) when compared scopolamine group (Table 1).

3.7. Brain cholinesterase activity

Scopolamine administration significantly increased the brain AchE activity ($p < 0.05$) as compared to the control group. Rivastigmine or NQC treatment significantly reduced the ($p < 0.05$) scopolamine-induced elevation in AchE activity as compared to scopolamine control group. Quercetin did not show the significant reduction in brain AchE activity when compared scopolamine group (Table 2).

Table 2
Effect of NQC on brain cholinesterase activity of scopolamine-treated rats.

Treatment groups	AchE activity (μmol/min/mg pr)
I Control	0.03261 ± 0.001123*
II Scopolamine	0.06585 ± 0.001683#
III Rivastigmine	0.0416 ± 0.003533*
IV Quercetin	0.05722 ± 0.003927
V NQC	0.04946 ± 0.0008207*

The results expressed as mean ± SEM, n = 6. Statistical analysis was carried out by One-way ANOVA followed by Bonferroni multiple comparison test.
* $p < 0.05$ versus scopolamine group.
# $p < 0.05$ versus control group.

3.8. Histopathological studies

Histological examination of hematoxylin and eosin stained brain sections revealed serious damaging effects of scopolamine on brain tissues compared to normal brain sections. A total number of degenerating neurons with pyknotic and condensed nuclear morphology were evaluated. Here, the data presented are the average of ten fields/section and shown in the graph. A. In the control group, normal architecture of brain observed. B. In scopolamine administered group abnormal cellular morphology with changes like gliosis were observed. C. Normal architecture similar to the control group was observed in rivastigmine pretreated group. D. Protected cellular morphology was observed in quercetin treated group. E. Morphological abnormalities were significantly reduced in NQC group (Fig. 4).

4. Discussion

In the field of medical science and drug delivery nanotechnology has opened several new possibilities which improve site-specific action of drugs [30,31]. Nanoparticles have increasingly been used for a variety of neurological disorders [32]. Many approaches have been developed to enhance the bioavailability of poorly water soluble compounds including particle size reduction or generation of amorphous particle state and also the physical modification of drug which increases the surface area and solubility of the drug particles [33,34]. Nanoparticles in the size of approximately 10–1000 nm have a higher potential to circulate in the blood for a longer period of time and also have the advantage of high biocompatibility and increased bioavailability [35].

In the present study, the particle size of quercetin made smaller and uniform by antisolvent precipitation method. The size range of NQC was observed below 300 nm using scanning electron microscope which makes the delivery system very much suitable for the drug delivery to target organs.

Previous studies revealed that Quercetin has antioxidant activity [36,37], but to our knowledge NQC were not explored for their potential therapeutic activities against scopolamine- induced spatial memory impairments. Scopolamine, a nonselective muscarinic antagonist produces memory deficits that are similar to those found in age related senile central nervous system dysfunction [5]. It became a widely used standard drug for inducing cognitive deficits in animal model besides its use as a treatment for central nervous system dysfunction such as motion sickness and opioid addiction [38].

In the present experiments, i.e. rectangular maze test and conditioned avoidance test, it is clearly seen that there was an increase in the transfer latency in scopolamine treated group, which demonstrates memory impairment. Quercetin and NQC administration to scopolamine received animals reversed the increase in latency time. NQC treated group has shown the more protective effect against scopolamine-induced memory loss when compared to quercetin treated group, which indicates improved therapeutic efficacy of NQC.

Cholinergic neurotransmission plays an important role in learning and memory process [39,40], acetylcholinesterase is the enzyme responsible for termination of cholinergic transmission by hydrolysis of acetylcholine [41]. Therefore, acetylcholinesterase inhibitory activity allows more amount of acetylcholine to retain in the brain which enhances cognitive function. The cholinergic hypothesis and evidence of the involvement of this system in the etiology of AD play an important role in the identification of novel cholinergic interventions as treatment for this disease.

Flavonoids have also reported to act as acetylcholinesterase inhibitors [42]. Interestingly, NQC group has shown the more
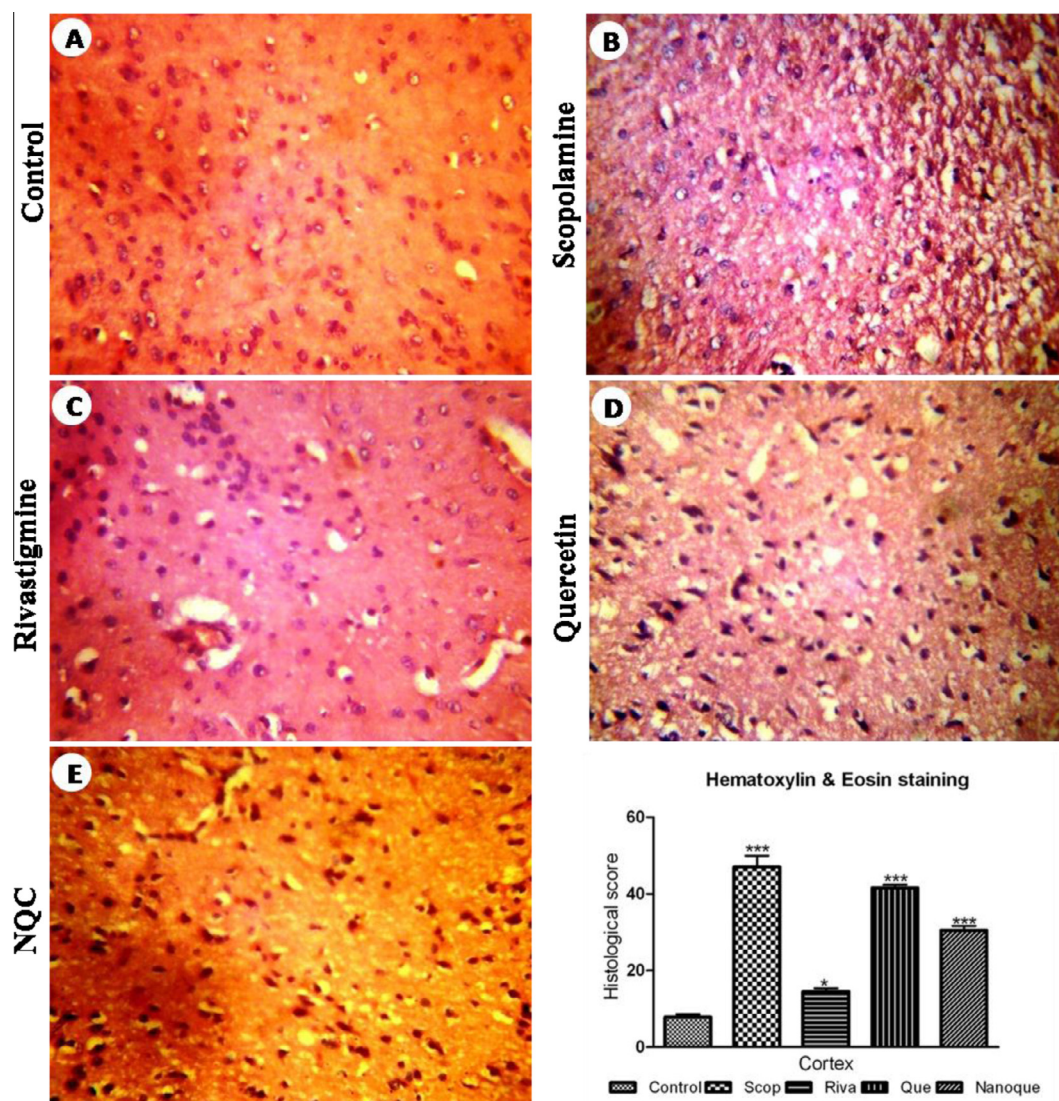
Fig. 4. Representative photomicrographs of Hematoxylin and Eosin (H&E) stained rat brains from each studied group. These Figures (A), (B), (C), (D), (E) are control, scopolamine, rivastigmine, quercetin and NQC, respectively, representing the histological sections of the brain tissue showing damaging effects of scopolamine and protective cellular morphology against scopolamine.

prominent inhibitory effect on acetylcholinesterase enzyme when compared to quercetin group.

Several studies have shown that oxidative stress is associated with the pathophysiology of many neurological disorders and brain dysfunction [43]. Therefore, neuronal cell death by oxidative stress and progression of neurodegenerative disorders can be attenuated by the supplementation of antioxidants and free radical scavengers [44]. Administration of scopolamine is associated with increased brain lipid peroxides and reduced brain antioxidant levels [45]. GSH is an essential tri peptide, an antioxidant found in all animal cells. It protects cells from singlet oxygen, superoxide radical and hydroxyl radical damage. MDA is an end product of lipid peroxidation, a measure of free radical generation [10].

In the present study, Scopolamine administration resulted in increased brain lipid peroxidation parallel to reduced catalase and GSH activities. Pretreatment with NQC showed a significant decrease in MDA and increase in brain catalase and GSH contents to be similar to that observed in the rivastigmine group when compared to quercetin group.

Recently, many studies reported that antioxidation is one mechanism of action of the flavonoids, these antioxidant abilities results in effective protection of neurons against neurotoxins, suppression of neuronal inflammation and enhanced neuronal function [46,47]. Previous findings suggest that quercetin perhaps possess memory enhancing ability through modulation of signal cascades, in addition to its antioxidant properties [48].

5. Conclusion

In conclusion, the formulated nanoparticles exhibited nanometer range in size. According to our results, scopolamine-induced behavioral, biochemical and histological changes were attenuated by pretreatment of rats with NQC indicating that a preventive strategy against the progression of AD. In all the experimental models NQC has shown more prominent results compared to quercetin group suggesting that increased efficacy is due to prolonged residence time in systemic circulation and increased bioavailability. This approach of delivering a nontoxic herb origin antioxidant, quercetin in the form of nanoparticles offers the potential clinical application in human neurodegenerative disease in future.

Conflict of interest

Acknowledgments

References

[1] K. Blennow, M.J. de Leon, H. Zetterberg, Alzheimer's disease, Lancet 368 (2006) 387–403.

[2] J.L. Cummings, Alzheimer's disease, N. Engl. J. Med. 351 (2004) 56–67.

[3] A.D. Korczyn, V. Vakhapova, The prevention of the dementia epidemic, J. Neurol. Sci. 257 (2007) 2–4.

[4] A. Blokland, Acetylcholine: a neurotransmitter for learning and memory?, Brain Res Rev. 21 (1996) 285–300.

[5] A. Kanaka Latha, A. Butchi Raju, Y. Venu Gopal, V. Prabhakar Reddy, P. Suresh, Antiamnesic activity of Syzygium cumini against scopolamine induced spatial memory impairments in rats, Brain Dev. 34 (2012) 844–851.

[6] S.K. Richetti, M. Blank, K.M. Capiotti, A.L. Piato, M.R. Bogo, M.R. Vianna, Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish, Behav. Brain Res. 217 (2011) 10–15.

[7] D.A. El-Sherbiny, A.E. Khalifa, A.S. Attia, Eel-D. Eldenshary, Hypericum perforatum extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine, Pharmacol. Biochem. Behav. 76 (2003) 525–533.

[8] I. Misane, S.O. Ogren, Selective 5-HT(1A) antagonists WAY 100635 and NAD-299 attenuate the impairment of passive avoidance caused by scopolamine in the rat, Neuropsychopharmacology 28 (2003) 253–264.

[9] J.H. Oh, B.J. Choi, M.S. Chang, S.K. Park, Nelumbo nucifera semen extract improves memory in rats with scopolamine-induced amnesia through the induction of choline acetyltransferase expression, Neurosci. Lett. 461 (2009) 41–44.

[10] S.A. El-Marasy, S.M. El-Shenawy, A.S. El-Khatib, O.A. El-Shabrawy, S.A. Kenawy, Effect of Nigella sativa and wheat germ oils on scopolamine-induced memory impairment in rats, Bull. Faculty Pharm. Cairo Univ. 50 (2012) 81–88.

[11] L.G. Howes, Cardiovascular effects of drugs used to treat Alzheimer's disease, Drug Saf. 37 (2014) 391–395.

[12] F.M. Garlich, K. Balakrishnan, S.K. Shah, M.A. Howland, J. Fong, L.S. Nelson, Prolonged altered mental status and bradycardia following pediatric donepezil ingestion, Clin. Toxicol. (Phila) 52 (2014) 291–294.

[13] T.P. Ng, P.C. Chiam, T. Lee, H.C. Chua, L. Lim, E.H. Kua, Curry consumption and cognitive function in the elderly, Am. J. Epidemiol. 164 (2006) 898–906.

[14] Z. Kerem, B.A. Bravdo, O. Shoseyov, Y. Tugendhaft, Rapid liquid chromatography–ultraviolet determination of organic acids and phenolic compounds in red wine and must, J. Chromatogr. 1052 (2004) 211–215.

[15] D.G. Watson, E.J. Oliveira, Solid-phase extraction and gas chromatography–mass spectrometry determination of kaempferol and quercetin in human urine after consumption of Ginkgo biloba tablets, J. Chromatogr. 723 (1999) 203–210.

[16] M.G.L. Hertog, P.C.H. Hollman, D.P. Venema, Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits, J. Agric. Food Chem. 40 (1992) 1591–1598.

[17] C. Kanadaswami, L.T. Lee, P.P. Lee, J.J. Hwang, F.C. Ke, Y.T. Huang, The antitumor activities of flavonoids, In Vivo 19 (2005) 895–909.

[18] M. Comalada, D. Camuesco, S. Sierra, I. Ballester, J. Xaus, J. Galvez, In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway, Eur. J. Immunol. 35 (2005) 584–592.

[19] A.W. Boots, G.R. Haenen, A. Bast, Health effects of quercetin: from antioxidant to nutraceutical, Eur. J. Pharmacol. 585 (2008) 325–337.

[20] M.M. Chan, D. Fong, K.J. Soprano, W.F. Holmes, Inhibition of growth and sensitization to cisplatin-mediated killing of ovarian cancer cells by polyphenolic chemopreventive agents, J. Cell. Physiol. 194 (2003) 63–70.

[21] K.A. Khaled, Y.M. El-Sayed, B.M. Al-Hadiya, Disposition of the flavonoid quercetin in rats after single intravenous and oral doses, Drug Dev. Ind. Pharm. 29 (2003) 397–403.

[22] A.A. Noyes, W.R. Whitney, The rate of solution of solid substances in their own solutions, J. Am. Chem. Soc. 19 (1897) 930–934.

[23] Mitali Kakran, Sahoo Nanda Gopal, Lin Li, Zaher Judeh, Fabrication of quercetin nanoparticles by anti-solvent precipitation method for enhanced dissolution, Powder Technol. 223 (2012) 59–64.

[24] J.V. Hinrichs, S.P. Mewaldt, M.M. Ghoneim, J.L. Berie, Diazepam and learning: assessment of acquisition deficits, Pharmacol. Biochem. Behav. 17 (1) (1982) 165–170.

[25] M. Parle, N. Singh, Animal models for testing memory, Asia Pac. J. Pharmacol. 16 (2004) 101–120.

[26] M.B. Ruiz-Larrea, A.M. Leal, M. Liza, M. Lacort, H. de Groot, Antioxidant effects of estradiol and 2-hydroxyestradiol on iron induced lipid peroxidation of rat liver microsomes, Steroids 59 (1994) 383–388.

[27] G.L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys. 82 (1959) 70–77.

[28] G. Bulaj, T. Kortemme, D.P. Goldenberg, Ionization-reactivity relationships for cysteine thiols in polypeptides, Biochemistry 37 (1998) 8965–8972.

[29] R.F. Beer, T.W. Seizer, A spectrophotometric method for measuring breakdown of hydrogen peroxide by catalase, J. Biol. Chem. 115 (1951) 130–140.

[30] M. Jahanshahi, Z. Zhang, A. Lyddiatt, Subtractive chromatography for purification and recovery of nano-bioproducts, IEE Proc. Nanobiotechnol. 15 (2005) 121–126.

[31] K.S. Soppimath, T.M. Aminabhavi, A.R. Kulkarni, W.E. Rudzinski, Biodegradable polymeric nanoparticles as drug delivery devices, J. Control. Release 70 (2001) 1–20.

[32] K.K. Jain, Role of nanotechnology in developing new therapies for diseases of the nervous system, Nanomedicine 1 (2006) 9–12.

[33] H. Chen, C. Khemtong, X. Yang, X. Chang, J. Gao, Nanonization strategies for poorly water soluble drugs, Drug Discov. Today 16 (2011) 354–360.

[34] E. Merisko-Liversidge, G.G. Liversidge, E.R. Cooper, A formulation approach for poorly water soluble compounds, Eur. J. Pharm. Sci. 18 (2003) 113–120.

[35] A. Muhlen, C. Schwarz, W. Mehnert, Solid lipid nanoparticles (SLN) for controlled drug delivery-drug release and release mechanism, Eur. J. Pharm. Biopharm. 45 (1998) 149–155.

[36] K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships, J. Nutr. Biochem. 13 (2002) 572–584.

[37] M.E. Inal, A. Kahraman, The protective effect of flavonol quercetin against ultraviolet A induced oxidative stress in rats, Toxicology 154 (2000) 21–29.

[38] X.H. Xiang, H.L. Wang, W.R. Wu, H.S. Wang, Y. Guo, D.Y. Cao, Ethological analysis of scopolamine treatment or pretreatment in morphine dependent rats, Physiol. Behav. 88 (2006) 183–189.

[39] J.F. Flood, D.W. Landry, M.E. Jarvik, Cholinergic receptor interactions and their effects on long-term memory processing, Brain Res. 215 (1981) 177–185.

[40] N.M. Weinberger, Food for thought: honeybee for aging, memory, and acetylcholine, Sci. STKE 336 (2006) e23.

[41] C.G. Ballard, N.H. Greig, A.L. Guillozet-Bongaarts, Cholinesterases: roles in the brain during health and disease, Curr. Alzheimer Res. 2 (2005) 307–318.

[42] T. Ahmed, A.H. Gilani, Inhibitory effect of curcuminoids on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia may explain medicinal use of turmeric in Alzheimer's disease, Pharmacol. Biochem. Behav. 91 (2009) 554–559.

[43] V.P. Reddy, X. Zhu, G. Perry, M.A. Smith, Oxidative stress in diabetes and Alzheimer's disease, J. Alzheimers Dis. 16 (2009) 763–774.

[44] H. Schroeter, R.J. Williams, R. Matin, L. Iversen, C.A. Rice-Evans, Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low density lipoprotein, Free Radic. Biol. Med. 29 (2000) 1222–1233.

[45] H.F. Zaki, M.A. Abd-El-Fattah, S. Amina, A.S. Attia, Naringenin protects against scopolamine-induced dementia in rats, Bull. Faculty Pharm. Cairo Univ. 52 (2014) 15–25.

[46] K. Vafeiadou, D. Vauzour, J.P. Spencer, Neuro inflammation and its modulation by flavonoids, Endocr. Metab. Immune Disord. Drug Targets 7 (3) (2007) 211–224.

[47] C.P. Ward, K. Redd, B.M. Williams, J.R. Caler, Y. Luo, J.G. McCoy, Ginkgo biloba extract: cognitive enhancer or anti stress buffer, Pharmacol. Biochem. Behav. 72 (2002) 913–922.

[48] C. Prouillet, J.C. Maziere, C. Maziere, A. Wattel, M. Brazier, S. Kamel, Stimulatory effect of naturally occurring flavonols quercetin and kaempferol on alkaline phosphatase activity in MG-63 human osteoblasts through ERK and estrogen receptor pathway, Biochem. Pharmacol. 67 (2004) 1307–1313.