Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2015, 6(3):8-13

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Effect on the learning and memory activity of quercetin on colchicine induced amnesia in mice

Aswin R. Deshmukh[1]*, Shital D. Pande[2], Anita S. Wagh[3], Swapnil G. Patil[3] and Nishant Jain[4]

[1]J. L. Chaturvedi College of Pharmacy, Nagpur
[2]Dr. Vedprakash Patil Pharmacy College, Aurangabad
[3]School of Pharmacy, S R T M University, Nanded
[4]Institute of Pharmaceutical Sciences, GGV Central University, Bilaspur, Chhattisgarh

---

ABSTRACT

The aim of present study is to investigate the therapeutic potential of quercetin in prevention of senile dementia of the Alzheimers type using intra-cerebroventricular administered Colchicine using Morris water maze model. Adult Swiss albino Wistar mice were treated with different dose of quercetin (Dose 5, 10, 20 and 40mg/kg) and the acquisition, retention and retrieval of spatial recognition memory was determined, by using Morris water maze models (interoceptive behavioral models). Prior administration of quercetin 40mg/kg with colchicine significantly decreased escape latency as compared to colchicine group. The use of quercetin warrants evaluation for the treatment of neurological disorder, which is associated with free radical generation and cognitive impairment such as Alzheimer's disease.

Keywords: Quercetin, Colchicine, Morris water maze, Nootropic activity

---

INTRODUCTION

Alzheimer's disease (AD) is a progressive and complex neurodegenerative disease, characterized by progressive decline in memory, language and other cognitive functions. It is associated with impairment of the basal forebrain cholinergic system, especially in the elderly [1].Senile dementia of Alzheimer type (SDAT) has been shown to be associated with microtubule dysfunction and characterized by the appearance of specific cytoskeletal cellular abnormalities, including neurofibrillary bodies and senile plaques [2]. Of particular interest are the SDAT-associated changes in the cholinergic markers because of the possible association between these alterations and deficits in cognitive abilities [3]. It is now well established that free radical generation and subsequent oxidative damage occur prior to cytopathology and play a key role in the pathogenesis of AD [4]. Conversely, oxidative damage has been implicated as a prime candidate mediating behavioral impairment and memory deficits in age-related neurodegenerative disorders [5]. Indian systems of medicine emphasize use of nutraceuticals, herbs or life style changes for controlling age related neurodegenerative disorders.

Indian system of medicine contains number of plants claimed to promote learning, memory and intelligence. Plants like *Bacopa monaneria, Azadirechta indica, Glycyrrhiza glabra, Argyria specoisus, Butea frondasa, Vitis vinifera,*

Ginseng, Rubia cordifolia, Emblica officinalis, Molinga olifera, and Trigonella foenum has been investigated for their cognitive function on brain. These plants are grouped as Rejuvenators, means the drugs which counter the degenerative changes associated with ageing. Additionally some of these plants act specifically in augmenting the cognitive functions of the brain called as Nootropic agent.

In flavonoid family, widely distributed Quercetin (3, 5, 7, 3V, 4V-pentahydroxyflavone), a safe and dietary flavonoid, is found in onions, apples, and other fruits and vegetables. It has been reported that quercetin prevents oxidant injury and cell death by several mechanisms including scavenging oxygen radicals, protecting against lipid peroxidation, and chelating metal ions [6].

Quercetin, the most abundant of the flavonoid consists of 3 rings and 5 hydroxyl groups Quercetin is a member of the class of flavonoid called flavonol and forms the backbone for many other flavonoids including the citrus flavonoids like rutin, tangeritin, hesperidins and Naringenin. Moreover, several experimental investigations showed the potential of quercetin against cognitive deficit in various animal models [7].

According to a study, a potent antioxidant (quercetin) in apples and in vegetables appear to protect brain cells against oxidative stress, a tissue damaging process associated with Alzheimer and other neurodegenerative disorders [8].Quercetin seems to protect the brain functions by inhibiting the formation if fibrillated amyloid–beta, the senile plaque found in Alzheimer's brain [9]. An experiment was performed to demonstrate the possible effects of quercetin on cognitive performance of young and aged, ethanol intoxicated mice (animal model), where chronic quercetin treatment had shown the reversal of cognitive deficits. Quercetin, through its COMT and MAO enzymes inhibiting properties, might potentiate the anticatabolic effect of L-dopa plus carbidopa treatment. The results from the study strongly suggest that quercetin could serve as an effective adjunct to L-dopa therapy in Parkinson disease [10].Quercetin also is a powerful antioxidant that may protect brain cells from damage.

Quercetin, a naturally occurring flavanoid found effective in improving cognitive dysfunction in rat model of chronic cerebral ischemia produced through bilateral occlusion of the carotid arteries [7]. A study in which effect of quercetin on D-galactose-induced aged mice was evaluated using the Morris water maze (MWM) test [11]. Quercetin also showed protective effect against intracerebral streptozotocin induced reduction in cerebral blood flow and impairment of memory in mice [12]. Quercetin also proved to be effective against colchicine-induced memory impairment and oxidative damage in rats. In this study chronic treatment with significantly improved the cognition in Morris water maze and passive avoidance task. It also significantly attenuated elevated lipid peroxidation and restored the depleted reduced glutathione, acetyl cholinesterase activity and nitrite activity [7].

Realizing the fact, this research was carried out to evaluate the effect on the learning and memory activity of quercetin on colchicine induced amnesia in mice.

MATERIALS AND METHODS

Animals

Male Swiss albino mice weighing 20–25g will be used. The animals will be housed under standard laboratory conditions, maintained on natural 12-h light and dark cycle and having free access to food and water. Animals were acclimatized to the laboratory conditions prior to experimentation.

All the experiments were carried out between 09:00 and 15:00 h. Experimental protocol was approved by Institutional animal Ethical committee.

Drugs and solution preparation

Colchicine and quercetin (Sigma Aldrich) solutions were made fresh at the beginning of each experiment. Colchicine was prepared in ACSF such that a 15 µg dose was delivered in a volume of 2µl injection for intracerebroventricular administration. Artificial cerebrospinal fluid (acsf)

The acsf was prepared by dissolving various salts in double distilled water or (water for injection IP). Acsf have composition: 0.2M NaCl, 0.02 M NaH2Co3, 2 Mm KCl, 0.5 mM KH2PO4, 1.2 mM CaCl2, 1.8 mM MgCl2, 5 mM Na2SO4, and 5.8 mM D- glucose.

For oral administration, Quercetin was suspended in 0.5% sodium carboxy methylcellulose. For experimental protocol dose of quercetin was standardized by various doses of quercetin were given and effective dose of quercetin was selected Animals were randomly divided into seven groups of six animals each. The first group, sham operated group, received vehicle for quercetin orally and acsf (2µl i. c.v.). The second group received colchicine (15 µg/ 2 µl, i. c.v.). The second received equivalent volume of vehicle for quercetin throughout the study period. The third and fourth groups, received quercetin only in doses of 5 and 40 mg/kg once daily for a period of 21 days. The fifth and sixth group, received quercetin in doses of 5 and 40 mg/kg once daily beginning 4 days before colchicine injection for a period of 21 days. The eighth group received equivalent volume of vehicle for quercetin once daily beginning 4 days before colchicine injection along with daily i. c. v treatment of (Serotoninergic, Histaminergic and Nicotininergic) modulators for a period of 21 days. The ninth group, received quercetin in doses of 5 and 40 mg/kg once daily beginning 4 days before colchicine injection along with daily i. c. v treatment of (Serotoninergic, Histaminergic and Nicotininergic) modulators for a period of 21 days. The doses of quercetin and modulators were selected on the basis of previous studies conducted in laboratory and those reported in literature.

Morris water maze

The Morris water maze task has often been used in the validation of rodent models for neurocognitive disorders and the evaluation of new therapeutic agents ([13]. Different types of memory can be assessed in MWM. The common one used in this study includes reference memory acquistion ([14].

In present study the acquisition and retention of a spatial navigation task was examined using a Morris water maze. Animals were trained to swim to reach a platform in a circular pool, painted black (120cm diameter × 51cm) located in a test room. The pool was filled with water (26±2 ◦C) to a depth of 30 cm. A round platform of 10 cm diameter was placed in the water dipped 1cm below water. The escape latency (time taken to find the hidden platform) was noted in each experiment ([15].

Acquisition test

Acquisition test involves training of mice to find the hidden platform placed at a quadrant. For 4 consecutive days, Mice were given 2 trials /day by changing the platform in each trial to different quadrant (EN, WS). Each trial started when mice, held facing the pool was immersed in the water the starting point to release animal was exactly opposite from the quadrant where platform was situated, inter trial interval was 10 min. The mice was given a maximum time of 60 s (cut-off time) to find the platform If animal failed to locate the platform within 60 sec., animal was manually guided to the platform and placed on it. Irrespective of whether the mice finds the platform or was placed on it was allowed to stay on it for 10 s. The mice was then removed and placed to a cage under infrared bulb fixed above the floor of the cages.

Retrieval test (probe test)

Retrieval test (probe test) is performed to check the ability of animal to retain and recall the previously learned information. After 4 days of acquisition trial, a retrieval trial was performed at time interval of 24 hours during which the platform was removed from the pool and the trained animal was allowed to swim freely for 30 seconds. In this probe trial the spatial accuracy of the animal was determined, represented by time spent by mice in searching the platform in the target quadrant where the platform was previously placed during the acquisition test.

Locomotor activity

Locomotor activity was assessed in Actophotometer for period of 5 min. Actophotometer (Inco, Ambala), which has a lid covered square chamber (30cm × 30cm × 25cm), and equipped with six pairs of light sources and sensors these are connected to a digital counter to record the number of interruption. Locomotor activity was expressed in terms of total number of counts of light beam interruptions in 5 min. [16].

To validate the MWM 6 female mice were trained in pool and escape latencies were noted further to include the effect of any surgery on MWM performance mice either i.c.v. cannula implantation were treated with a CSF (2µl) and trained in MWM.

Dose dependant study

To study the influence of quercetin in AD like condition induced mice, different group were treat with quercetin at various doses (5,10,20 and 40 mg/kg) for the period of 21 days and colchicne was injected icv four days after start of

Quercetin treatment. Animals were subjected to MWM 60 min after the Quercetin treatment p.o. on days 18, 19, 20 and 21. The control group were treated with colchicine (15µg/mice) n=6.

Combination study

To study the modulator effect of various neurotransmitters on Quercetin mediated learning and memory in colchicine injected mice. Doses of various agonist and antagonist icv was administered 10 min prior to (Quercetin 5 and 40 mg/kg) and there after 60 min. animals were subjected to MWM on day 18, 19, 20 and 21of quercetin treatment. Control group was treated with Quercetin p.o. in colchicine injected mice. Animal were given acsf (2µl/mice), n=6 10 min prior to Quercetin and thereafter 60 min. animals were subjected to MWM.

Statistical analysis

The escape latency in sec. of all 2 acquisition trials were averaged for individual animals and the mean of averaged escape latencies of all animals in each group were taken. The results were represented as mean escape latency (sec.) ± S.E.M. The behavioral assessment data was analyzed by a repeated measures of two-way analysis of variance (ANOVA) (Graph pad prism 5.0) with drug-treated groups as between and sessions as the within-subjects factors. The interaction drug treatment × session considered to test for drug effect on retention. A difference between group means was considered significant when P value is less than 0.05.

For time retrieval, time spent in target quadrant (sec) by animals in each group was average. For probe trial data was analyzed by one way ANOVA followed by tukeys test

For locomotor activity no counts were observed in 5min. The result were represented as no of counts in 5 min, the values are represented ± SEM. Data were analyzed by two way ANOVA (n= 6), followed by Bonferroni posttests.

RESULTS AND DISCUSSION

Table No.1 Effect of colchicine in Morris water maze

Sr. no.	Treatment icv	Mean escape latency (Sec ± SEM)			
		Day 1	Day 2	Day 3	Day 4
1	Acsf	56 ± 1.21	45 ± 2.03	13 ±1.58	14 ± 1.19
2	Colchicine	57±2.66	55± 3.80*	56±3.16#	53±3.72#

SEM Standard error of mean
Number of animals in each group= 6
**P < 0.05, #P< 0.001*

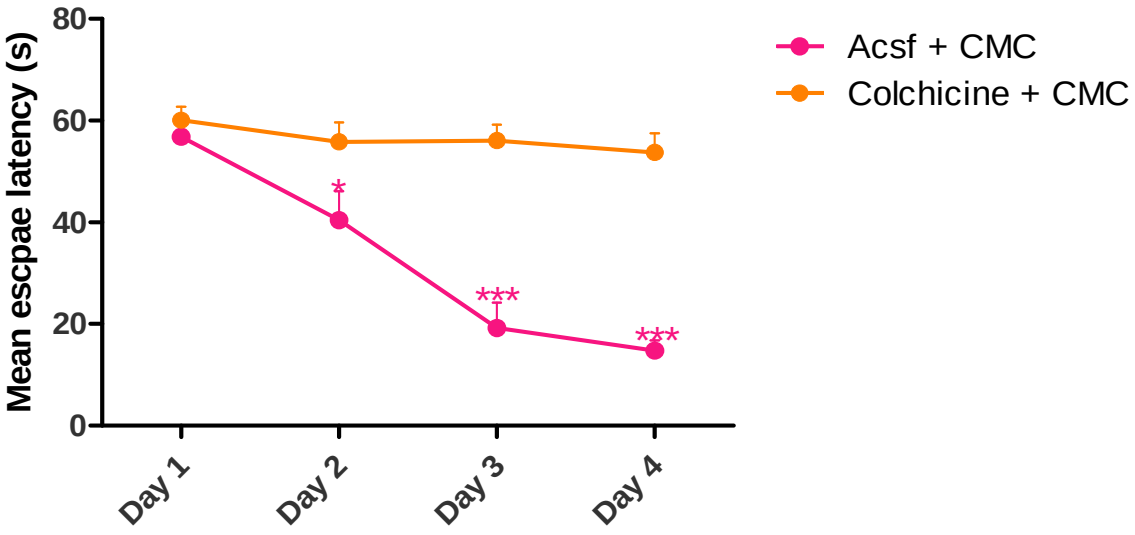

Fig.1-Effect of Colchicne on mean escape latency

Colchicine at 15µg/mice, icv significantly increased escape latency in acquisition training as compared to acsf-treated control group. Application of two-way ANOVA showed interaction between variables viz, Colchicine treatment and acquisition days [F (3, 40) = 11.05, P< 0.0001]. Application of posthoc Bonferroni multiple comparison tests revealed that colchicine significantly increased escape latency on all four days of acquisition training as compared to a CSF treated control group. Colchicine significantly increased escape latency (sec) on first day to 57±2.66 to 56 ± 1.21 (P<0.0001), on second day from 55±3.80 to 45 ±2.03 (P<0.0001), on third day from 56 ± 3.16 to 13 ± 1.58 (P<0.0001) and on fourth day from 53 ± 3.72 to 14 ± 1.19 (P<0.0001). Two way ANOVA revealed a main effect of treatment [F (1, 40) = 82.84 P<0.0001] and acquisition days [F (3, 40) = 17.79 P<0.0001].

For the dose of Quercetin by using Morris water maze

Prior administration of Quercetin 40mg/kg with colchicine significantly decreased escape latency as compared to colchicine group. Application of two ANOVA showed significant interaction between variables viz; colchicines treatments and acquisition days [F (12, 100) = 5.30, P< 0.0001]. Post hoc Bonferroni multiple comparison tests revealed that prior administration of Quercetin significantly decreased escape latency two way ANOVA revealed a main effect of treatment [F (4,100)= 36.87 P<0.0001]. And acquisition days [F (3,100) = 88.24 P<0.0001].

Table No.2 Effect of quercetin on escape latency

Sr. no.	Treatment (icv)	Mean escape latency (sec ± SEM)			
		Day 1	Day 2	Day 3	Day 4
1	Colchicine	57±2.66	55± 3.80	56±3.16	53±3.72
2	Quercetin 5mg	60 ± 1.10	54 ± 3.52	44 ± 3.56	30 ± 1.94
3	Quercetin 10mg	60 ± 4.6	51 ± 4.2	44 ± 4.66	24 ± 6.09
4	Quercetin 20mg	60 ± 1.10	47 ± 3.52	32 ± 3.56	17 ± 1.94
5	Quercetin 40mg	58 ± 4.6	33 ± 4.2	17 ± 4.66	9 ± 6.09

SEM: Standard error of mean
Number of animals in each group= 6

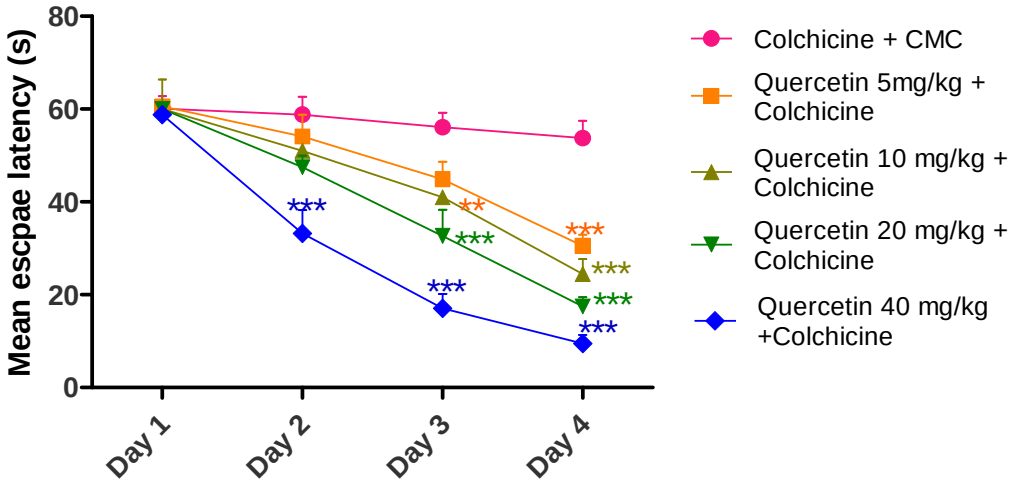

Fig.2-Effect of quercetin on mean escape latency

Chronic administration of Quercetin starting prior to the central injection of Colchicine was effective in improving poor memory performance. Quercetin shows nootropic effect in dose dependant manner. The first step followed was standardisation for the dose of Quercetin. Animals were treated with different dose of Quercetin (Dose 5, 10, 20 and 40mg/kg). group treated with 40mg/kg p.o. Quercetin showed significant decrease in escape latency to reach the platform on all successive days 19, 20, 21 but the animal treated with 10, 20mg/kg showed decrease in latency only on day 20 and 21, on the other hand animal treated with 5mg/kg do not show significant decrease in latency time on day 19 and 20 but showed significant difference on day 21 only. On this basis we selected 5mg/kg as subacute dose and 40mg/kg as an effective dose for Quercetin.

CONCLUSION

From the above results the amelioration of colchicine induced memory impairment could be due to enhancement of nicotinergic transmission or it can modulate the Histaminergic and Serotoninergic neurotransmission which indirectly modulate the cholinergic neurotransmission. Chronic administration of quercetin may enhance memory by acting directly or indirectly through one of this neurotransmission system. The use of quercetin warrants evaluation for the treatment of neurological disorder, which is associated with free radical generation and cognitive impairment such as Alzheimers Disease.

Acknowledgement
Authors are thankful to the Principal of J L Chaturvedi College of pharmacy, Nagpur for his support to make available the facilities for experiment.

REFERENCES

[1] Auld D., Kornecook T , Bastianetto S , *Prog Neurobio*, **2002** , 168, 209.
[2] Iqbal K, Grunde-Iqball, Zaidi T, *Lancet*, **1986,** 11, 421.
[3] Whitehouse P, Price D, Struble R, Clark A, Coyle J and Delong M, **1982**: *Science* 215:1237–1239.
[4] Markesbery W, Biol. Med. **1999**, 23,134.
[5] Pratico D, *Biochem Pharmacol*, **2002**, 63, 563.
[6] Naraynana K. Reddy M , Chaturvedi M , Krishna D, *Indian J Pharmacol*, **2001**, 33, 2..
[7] Kumar A, Sehgal N , Pattipati S, Naidu, Satyanarayana S , Padi V , Goyal R, *Phytother res*, **2008**, 22 1563.
[8] Heo H, Kim D, Choi S, *J Food Sci*, **2004**, 69 (9), 357.
[9] Tzeng S, Ko W, Ko F, Teng C, *Thromb Res*, **1991**, 64, 91.
[10] Singh A, Naidu P, Kulkarni S, *Free Radics Res.* **2003**, 37, 1245.
[11] Liu J, Yu H, Ning X, *Sci China C Life Sci*. **2006**, 9(6), 583.
[12] Tota S , Himani A , Kamata P , Nath C , Kashif H, *Behav Brain Res.* **2010**, 209,73.
[13] D'Hooge, De Deyn P.: *Brain Research Reviews 36*, **2001,** *60–90.*
[14] Markowska, A.; Long, J; Johnson, C; Olton, D.: *Behavioral Neuroscience, Vol 107(4), Aug* **1993**, *627-632.*
[15] Vorhees C, Williams M **2006**: *Vol. 1 No.2 Nature protocol.*
[16] 16. Umathe S, Bhutada P, Dixit P, Jain N **2008**: *Neuropept 42: 345–353.*