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Memory enhancement and reduction in lipid peroxidation by dietary antioxidants in brain

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Abstract: Reactive oxygen species produced during metabolic pathways cause progressive damage over a lifetime. Free radicals have harmful effects on biological systems, induce damage to important biomolecules, accelerate disease progression and shorten lifespan, whereas antioxidant therapy is supposed to attenuate these effects. The present study aimed to assess neuroprotective effects of dietary antioxidants, particularly bioflavonoids against oxidative damage produced by oxygen derived free radicals. For this purpose rats weighing 100-150 g were orally given naringenin (Nar), quercetin (Que) and curcumin (Cur) at a dose of 50 mg/kg, 50 mg/kg and 200 mg/kg body weight respectively once daily for 2 weeks. Learning and working memory performance was evaluated at 14th, 15th and 16th day of treatment by elevated plus maze test, Morris water maze test and passive avoidance test. After behavioral assessment rats were sacrificed to remove their brain samples for further biochemical estimation. Malondialdehyde (MDA) levels were estimated in whole brain, as a marker of oxidative stress. It was found that Que, Nar and Cur significantly enhanced cognitive functions of animals. Treatment of dietary antioxidants also significantly reduced MDA levels in whole brain. In comparison to Cur and Que, Nar showed greater memory improving and inhibitory effects on lipid peroxidation due to its potential antioxidant effects. The memory enhancing and neuroprotective effect of Nar might be credited to its free radical scavenging and antioxidative properties. Therefore, it is suggested that dietary antioxidants such as bioflavonoids may provide a possible way to treat oxidative damage and associated diseases.

Keywords: Flavonoids, antioxidant, learning and memory, oxidative stress, lipid peroxidation.
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INTRODUCTION

Oxidative stress is an imbalance between generation and removal of oxygen derived free radicals results in multiple oxidative alterations in a biological system[1-3]. Oxidative stress is a normal biological phenomenon caused by exogenous and endogenous factors results in generation of free radical mediated oxidative damage. Free radical generation can result in oxidative stress and various disease progressions including cancer, neurodegeneration, cardiovascular disease and diabetes[4]. Moreover, oxidative modification of biological molecules such as protein, lipids and nucleic acid may further stimulate cell proliferation and ultimately result in cell death[5]. Number of ways are involved in the production of oxidative stress and many of them can strongly relate to well known pathologies[6]. Low availability or inactivity of antioxidant enzymes are among the main factors that are involved in oxidative stress mechanism[7]. Electron transport chain constantly produces reactive oxygen species (ROS). Some enzymes like aldehyde oxidase, xanthine oxidase and cytochrome P450 monoxygenase also promote free radical generation. Exogenous factors like temperature variation, ultraviolet radiation and radioactivity are also the leading cause of free radical generation. Moreover, impaired endogenous antioxidant enzyme activity such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) or reduced metabolism of antioxidant substances including reduced glutathione (GSH) may further contribute in progression of damaging episodes of oxidative stress[8]. Primarily whenever free radical generation increases in body due to any environmental factor like hypoxia, drug metabolism or metal ions can results in diverse pathological conditions[9]. Additionally deficiency or impairment of antioxidant enzyme activity due to ROS attack could exacerbate or heightened pathological state of that disease. This reduced activity of antioxidant enzyme could be due to specific environmental conditions[8]. Regardless, whether disease progression is caused by primary or secondary oxidative stress, exogenous antioxidant therapy may provide a promising beneficial approach alone or in combination with specific drugs against deleterious health effects caused by oxidative stress[7,10,11].

Flavonoids are a group of compounds that are generally recommended as a potential candidate for treatment against oxidative stress due to their significant role in maintaining good health[12,13]. Flavonoids are polyphenolic compounds found in a variety of seeds, vegetables, nuts and fruits and exhibit various valuable biological fucntions such as anti-inflammatory, antioxidant and tumor reducing properties[14,15]. Flavonoids contain hydroxyl (-OH) groups in their structure that are responsible for their free radical scavenging properties[16]. A number of -OH substitutions in flavonoids structure are directly linked to the flavonoids antioxidant capacity[17]. More -OH group exhibit more effective antioxidant activity[18]. Hydroxyl group in flavonoid structure has a capability to transfer electrons or hydrogens and can scavenge

free radicals including superoxide anions and hydroxyl radicals[19,20]. Electron donating capability of flavonoids is also due to β-ring catechol group in their strucrure that can help in stabilizing a free radical species [21].

Naringenin (Nar) is an aglycone form of naringin, a dietary antioxidant and potent bioflavonoid widely distributed in natural products such as citrus fruits, grape fruits, cherries and cocoa[22]. Nar possesses a plethora of pharmalogical effects including anti-inflammatory, antitumor and hepatoprotective properties[16, 23, 24]. The antioxidant nature of Nar is due to existence of 4`-hydroxyl group in its structure which is responsible for electron donating properties and hence protecting biological membranes from free radical attack[25]. Quercetin (Que) (3, 3`, 4`, 5, 7-pentahydroxyflavanone) is a potent and most abundant bioflavonoid found in apples, onion and broccoli. Que has shown more than 60% of the average flavonoid ingestion. Que works as a strong antioxidant and free radical scavenger [26]. Pharmacological effects of Que includes prevention of platelet aggregation, protection of low density lipoprotein oxidation and induction of apoptosis in tumor cells[27]. Preclinical and clinical studies have confirmed that numerous underlying mechanisms that are devastating to health can be prevented by Que[17]. Curcumin (Cur) is a polyphenolic compound isolated from a common spice turmeric. Cur is well documented for its medicinal properties including anticancer, anti-inflammatory, tumor reducing, antiviral, inhibition of arachidonic acid metabolism and antimutagenic[28]. Cur stabilizes ROS including superoxide anion, hydroxyl radical and nitric oxide[29].

Despite the fact that antioxidant properties of Que, Nar and Cur are well explained, but to our knowledge, no comparative investigation so far has been carried out on the memory enhancing effect of these dietary antioxidants. Taking the above into account, the current study was planned to elucidate the memory enhancing and lipid peroxidation reducing effects of Que, Nar and Cur in rats by using different behavioral test and biochemical estimations.

MATERIALS AND METHODS

Animals

Thirty locally bred young adult male Albino-Wistar rats (weight 100-150g) were used in the present study. Rats were kept individually in specifically designed plastic cages in an environmentally controlled room (22 ± 2 °C), 12:12 h light/dark cycle (lights on at 7:00 am) with *ad libitum* access to standard rodent diet (cubes) and tap water. Rats were allowed to acclimatize for 1 week before starting experimental and behavioral protocols to abolish the psychological suffering of environment. The experimental protocols carried out in this study were approved by the Animal Care Committee and Institutional Ethics.

Drugs:

Que, Nar and Cur was purchased from Sigma Aldrich Chemical Co. (St. Louis, USA) while all other reagents used in experiments were of the highest purity available. All drugs were freshly prepared daily before administration.

Experimental protocol

Rats were randomly assigned to five groups of six rats in each: saline, vehicle, Nar, Cur and Que. Both Nar and Cur was suspended in neutral oil while Que was prepared in physiological saline. Rats in group III received oral administration of Nar (50mg/kg), group IV received Cur (200mg/kg) and group V rats received Que (50mg/kg) daily for two weeks. The selection of dose was based on the previous studies[2, 30-32]. While rats in saline (I) and vehicle (II) group that served as control, received same volume of physiological saline and neutral oil respectively. Behavioral assessments were carried out at day 14th, 15th and 16th including Morris water maze (MWM) test, elevated plus maze (EPM) test and passive avoidance test (PAT). The treatment was ongoing during the behavioral assessment. After behavioral assessment rats were decapitated on day 16th and brain samples were collected from the skull within 30 sec and washed thoroughly with ice cold saline to eliminate blood and instantly kept at −70 °C for further neurochemical and biochemical estimations. Each experiment was conducted in a balance design with proper conditions and responses were evaluated in a fixed schedule to avoid time and order effect.

Behavioral protocol

Assessment of Memory

Learning and memory functions of rats was monitored by MWM, EPM and PAT. In MWM escape latency was monitored as described previously[33,34]. Significant decrease in escape latency is an indication of improved cognitive functions. EPM is used to evaluate working memory of rats by monitoring transfer latency[35]. Decreased transfer latency is observed in cognitive improving effects. PAT is extensively used to monitor fear memory in rats. In PAT step through latency was monitored[33]. Increased step through latency can strongly relate to enhanced cognitive functions.Short term memory (STM) was monitored 1h after training session in all memory task.

Biochemical analysis

Estimation of malondialdehyde (MDA)

Lipid peroxidation (LPO) was estimated in terms of MDA and was done as previously described by Chow and Tappel[36] along with modifications[33]. LPO data was expressed in µmol of MDA/g of brain.

Statistical analysis

The results are presented as mean ± SD (n = 6). The statistical significant differences were assessed by Tukey's test following one-way ANOVA using SPSS version 20.0. Pearson's correlation test via SPSS was done to findout correlation between escape latency and MDA content. Significance level was set at $p \leq 0.05$.

RESULTS

Morris water maze test

To determine the effects of supplementation of Nar, Cur and Que on working memory, MWM test was performed. One-way ANOVA analysis showed significant effect of flavonoids supplementation [F (4, 25) = 48.256, $p < 0.01$] on escape latency. Post-hoc comparisions by Tukey's test evaluated that escape latency of Nar (8.64 ± 1.76 sec) and Cur (15.95 ± 1.93 sec) supplemented rats was significantly decreased as compared to vehicle (21.92 ± 2.51 sec) ($p < 0.01$) and observed escape latency of Que treated rats (13.11± 1.17 sec) was also significantly decreased as compared to control (17.42 ± 0.79 sec) ($p < 0.01$).

Fig. 1 shows that Que, Nar and Cur treated rats exhibited a significant 40.21%, 52.96%, and 51.20% decline in escape latency in the test session in comparision to control rats, respectively. Supplementation of Nar, Cur and Que showed memory enhancing effects by a remarkable decrease in escape latency.

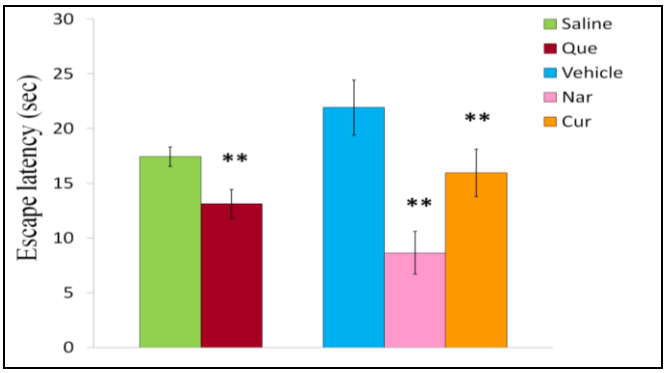

Figure:1 Effects of supplementation of dietary antioxidant such as flavonoids, quercetin, naringenin and curcumin on working memory in Morris water maze was monitored by escape latency (sec). Values are mean + standard error showed by error bars (n=6). Data of Morris water maze test was analysed by Tukey's test following one-way ANOVA. Significant difference is presented as **p < 0.01.

Elevated plus maze test

Decreased transfer latency is an indication of memory improving effects. One-way ANOVA analysis showed significant effect of flavonoids supplementation [F (4, 25) = 75.557, $p < 0.01$] on transfer latency. Post-hoc comparisions by Tukey's test evaluated that flavonoids treatment significantly enhanced cognitive functions showed by significant ($p < 0.01$) decreased transfer latency in Nar treated rats (15.00 ± 2.45 sec) and Cur (15.56 ± 3.64 sec) as compared to control rats (31.88 ± 2.91 sec). This decrease in transfer latency was also significantly observed in Que rats (11.00 ± 2.08 sec) as compared to control rats (18.40 ± 1.75 sec) ($p < 0.01$) in fig 3. This decrease was 40.21%, 52.96% and 51.20% in Que, Nar and Cur treated rats, respectively than rats in control group.

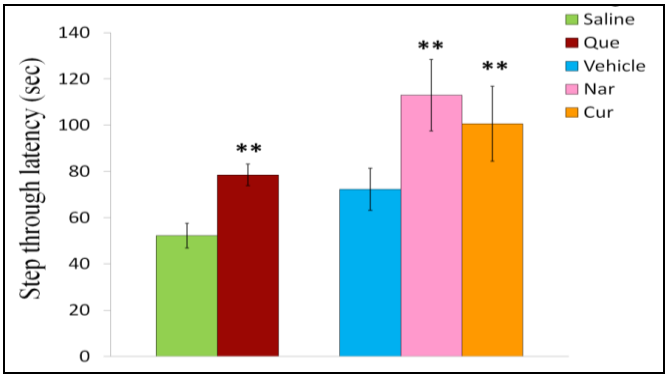

Figure 2: Effects of supplementation of quercetin, naringenin and curcumin on avoidance memory performance was monitored by step through latency (sec) in passive avoidance task. Values are mean + standard error showed

by error bars (n=6). Data of passive avoidance test was analysed by Tukey's test following one-way ANOVA. Significant difference is presented as **p < 0.01.

Passive avoidance test

PAT was performed to evaluate memory performance of rats. One-way ANOVA analysis showed significant effect of flavonoids supplementation [F (4, 25) = 32.667, $p < 0.01$] on step through latency. Post-hoc comparisions by Tukey's test evaluated that step through latency after 60 min of training session of Nar (113.02 ± 13.81 sec) and Cur (100.56 ± 14.51 sec) supplemented rats was significantly increased as compared to vehicle (72.28 ± 9.10 sec) ($p < 0.01$) group rats. In Que (78.48 ± 4.20 sec) treated rats step through latency was significantly increased as compared to control group (52.26 ± 4.81 sec) ($p < 0.01$) in fig 2. The increased in step through latency was 56.35 %, 39.12 % , 50.17 % in Nar, Cur and Que rats respectively, as compared to that of controls. Present study indicated that supplementation of flavonoid showed a significant memory enhancement as apparent from increased step through latency.

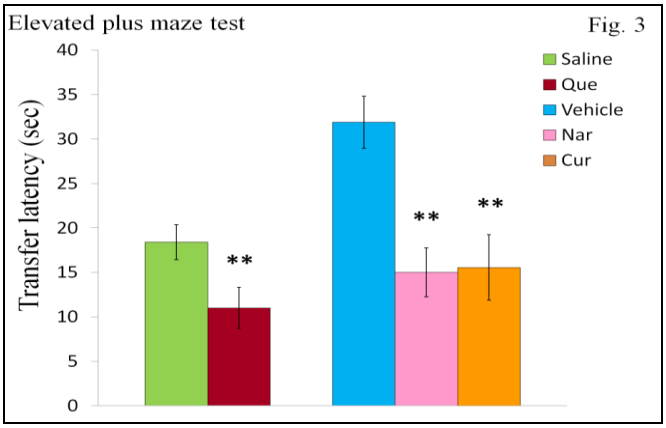

Figure 3: Effects of administration of quercetin, naringenin and curcumin on cognitive functions was evaluated by elevated plus maze test by monitoring transfer latency (sec). Values are mean + standard error showed by error bars (n=6). Data of elevated plus maze test was analysed by Tukey's test following one-way ANOVA. Significant difference is presented as **p < 0.01.

Effect of supplementation of flavonoids on brain MDA levels

Brain MDA levels

LPO by-product, MDA was measured in whole brain of rats. One-way ANOVA analysis revealed significant effect of flavonoids supplementation [F (4, 25) = 31.846, $p < 0.01$] on MDA levels. Post-hoc comparisions by Tukey's test evaluated that rats treated with Nar (70.22 ± 8.88 µmole/g) exhibited a significant decrease ($p < 0.01$) in brain MDA as compared to their control rats (127.23 ± 4.59 µmole/g). Non-significant decrease was observed in Cur (119.86 ± 4.04 µmole/g) treated rats compared to control (127.23 ± 4.59 µmole/g). Que supplementation also showed inhibitory effects on brain LPO as there is a significant ($p < 0.01$) reduction in brain MDA content (112.15 ± 4.23 µmole/g) as compared to control group rats (136.85 ± 18.10 µmole/g) in fig 4. This decrease was significant in Nar and Que treated rats (44.80 %) and (17.5 %) ($p < 0.01$) respectively.

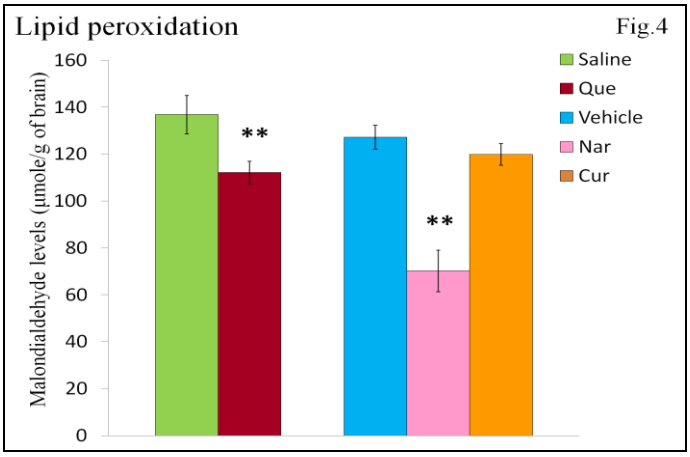

Figure 4: Effects of quercetin, naringenin and curcumin on brain lipid peroxidation was investigated in terms of malondialdehyde levels. Values are mean + standard error showed by error bars (n=6). Data for MDA levels was analyzed by Tukey's test following one-way ANOVA. Significant difference is presented as **$p < 0.01$.

Correlation analysis between LPO and escape latency

Pearson correlation analysis between escape latency and oxidative stress (MDA) was evaluated. Decreased in escape latency is an indication of improved cognitive functions. In the present study decreased escape latency was positively correlates with reduction of lipid peroxidation in brain and hereafter with cognitive enhancement.

The ability of Nar, Cur and Que to enhance cognitive functions in rats could be attributed to their observed lipid peroxidation inhibitory effects and acting as potent antioxidant which prevents brain from oxidative damage and hence improving memory performance. It was observed that there is a positive correlation between brain MDA content and observed escape latency in Que treated (r = 0.581, $p < 0.05$**,** fig. 5a), Nar treated (r= 0.950, $p < 0.01$, fig. 5b) and Cur treated (r=0.682 , $p < 0.05$**,** fig. 5c) rats. Current results suggest a close relation between lipid peroxidation and cognitive dysfunctions.

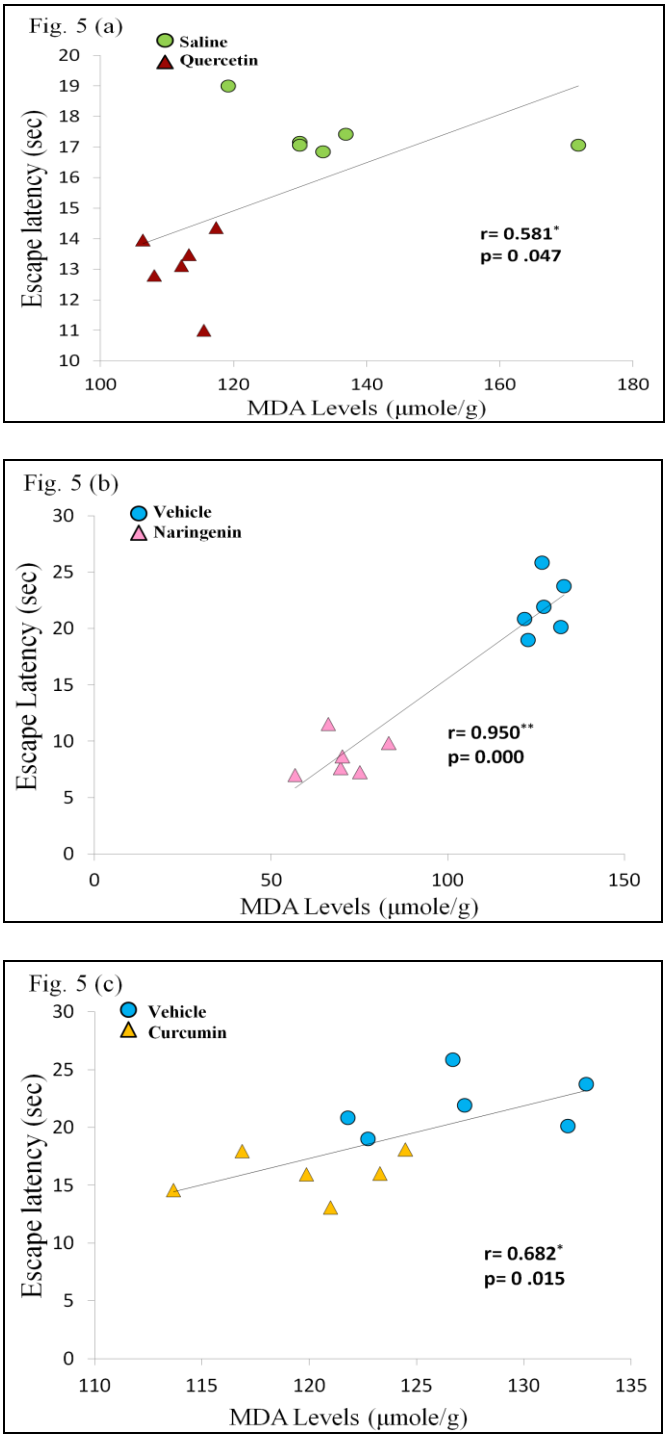

Figure 5: The Pearson correlation test was performed to find out the possible relation between escape latency and oxidative stress marker malondialdehyde. Results showed the presence of a significant positive correlation of escape latency (a) in quercetin (*p < 0.05), (b) naringenin (**p < 0.01) and curcumin (*p < 0.05) treated rats with malondialdehyde levels.

DISCUSSION

The aim of current study was to assess protective effects of Nar, Cur and Que on cognitive abilities of rats. An imbalance of proxidant/ antioxidant status of cell is responsible for the generation of ROS[7]. These species are injurious to health and cause oxidative damage to crucial biomolecules[37]. Increased production of ROS creates an alarming state of oxidative stress [18]. Keeping an appropriate balance between generation and removal of ROS is essential to avoid these deleterious health effects [13]. Endogenous antioxidant enzymes are considered as body's primary defense, prevent important biomolecules from oxidative damage by elimination of harmful free radicals from the cell [7, 38]. However, antioxidant enzymes themselves can also be at a target of ROS attack. Reports have shown that endogenous antioxidant enzymes are also highly susceptible to oxidative attack[8]. These presumptions demonstrate the usefulness of exogenous antioxidant therapy against oxidative stress[39]. Brain is vulnerable to damage caused by oxidative stress as it has excessive oxygen utilization, rich in polyunsaturated fatty acids and low activity of antioxidant defense system compared to other tissues [40]. Keeping in mind it is anticipated that oxidative stress may cause intense neuronal damage and cognitive dysfunction in animals and humans[33, 41, 42]. Thus, these previously reported facts draw attention to protective effects of dietary antioxidants particularly flavonoids and to identify the possibility that these protective agents might exert an influence on brain. It has been found that flavonoids can activate natural antioxidant defense system of brain that further activates cellular defense and prevent neuronal cell death[43]. Thus, current study was designed to findout the usefulness of dietary antioxidants against oxidative stress induced cognitive dysfunction. It has been reported that flavonoids are involved in neuroprotection and some of the behavioral features of neurocognition[2, 5, 26]. Consistent with the previous findings, supplementation of bioflavonoids Nar, Cur and Que in current study significantly improved cognitive abilities of rats. Escape latency is the time taken by the rats to locate a escape platform in MWM, was declined significantly in flavonoids supplemented rats as compared to control rats. Nar, Cur and Que treated rats located the hidden platform sooner after training as compared to saline and vehicle administered rats. In EPM working memory of flavonoids treated rats was also significantly enhanced as compared to saline and vehicle treated rats as indicated by reduced transfer latency by test rats. Present data provide evidences that naturally occurring flavonoids such as Nar,

Cur and Que improved working memory and learning capabilities, observed in all three learning and memory performance task that are, MWM, EPM, and PAT.

The physiological state of lipid is thought to be a reflection of membrane stability and measuring MDA levels is considered as an index of LPO[44]. Free radicals are constantly produced during normal physiological event and can easily initiate LPO processes and ultimately results in release of lipid peroxides[29]. In current study, oral intake of Nar, Cur and Que significantly decreased MDA levels in test rats compared to saline and vehicle group. Protective effects of Nar, Cur and Que in the present could be linked to the inhibition of LPO as evident from decreased MDA levels in test rats. Neuronal degeneration due to oxidative stress can produce difficulties in learning and memory abilities[45]. Hence, we can assume that Nar, Cur and Que may exert part of their observed memory enhancing and neuroprotective effects due to their antioxidant properties, based on strong connection between oxidative stress and cognitive dysfunctions. In the same context, several investigations showed a close association between memory impairment and elevated brain lipid peroxides[33, 46]. This was apparent in present study by elevated brain LPO in control rats whereas in test rats the observed memory enhancing effect of flavonoids was paralleled by decreased brain LPO levels. To further strengthen our observation, MDA levels as index of LPO, was correlated with escape latency. A positive correlation was found between escape latency and brain MDA levels in Nar, Cur and Que treated rats. Flavonoids supplementation enhances cognitive functions by reducing LPO in brain. Flavonoids protect neurons from free radical induced neurodegeneration through their antioxidant nature. The mechanism contributing to their effectiveness involves metal chelation, antioxidative and quenching of free radicals and also their capability to cross the blood brain barrier[17, 47, 48]. Taken together, present findings revealed that flavonoids provide neuroprotection against oxidative stress, protect neurons from damaging effects of ROS and oxidative stress[49]. In comparison to Que and Cur results of present study suggests that Nar is more potent antioxidant and may be useful as a possible therapeutic agent to protect from oxidative stress induced cognitive dysfunctions and improved learning and memory abilities. Thus, it is possible that Nar might generate a protective environment in brain because of its greater antioxidant activities. A marked decrease in LPO, as measured by brain MDA content was found in Nar treated rats. This decrease in LPO is accredited to scavenge free radicals and antioxidative properties of Nar[12].

The prevalence of Alzheimer's disease (AD) and dementia has increased in last few years. As none of the available medication appear to be able to stop the progression of disease. There is an immense medical need for the evolution of novel therapeutic agents that not only prevent but also target the underlying pathological mechanisms associated with AD[50]. Progress in understanding the injurious effects of oxidative damage and valuable protective effects of dietary antioxidant will expedite the development of new therapeutic management against ROS induced cognitive dysfunction and other neurodegenerative disorders. Knowledge of oxidative stress as a causal agent of disorder might considerably change therapeutic approaches[8]. Over and above that awareness of valuable effects of dietary antioxidants, particularly flavonoids might also suggest some significant variations in human lifestyle and preventive measures against oxidative stress by improving dietary habits.

In conclusion, improved cognitive functions and reduced LPO levels in whole brain of test rats show that dietary antioxidants are valuable protective candidates in enhancing memory functions and prevention against injurious effects of oxygen derived free radicals. Here it is suggested that, these dietary antioxidants may be a useful therapeutic agent in preventing oxidative stress induced neurodegenerative diseases like AD. Moreover, among the tested bioflavonoids, Naringenin may be strongly considered as a potential agent for developing promising therapeutic strategy against oxidative stress and associated diseases.

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REFERENCES

1. Kovacic P and Cooksy A. Iminium metabolite mechanism for nicotine toxicity and addiction: Oxidative stress and electron transfer. *Med. Hypotheses.* 2005; 64: 104-111.

2. Khan M, Khan MM, Khan A, Ahmed ME, Ishrat T, Tabassum R, Vaibhav K, Ahmad A and Islam F. Naringenin ameliorates Alzheimer's disease (AD)-type neurodegeneration with cognitive impairment (AD-TNDCI) caused by the intracerebroventricular-streptozotocin in rat model. *Neurochem. Int.* 2012; 61: 1081-1093.
3. Zhu X, Lee HG, Casadesus G, Avila J, Drew K, Perry G and Smith MA. Oxidative imbalance in Alzheimer's disease. *Mol. Neurobiol.* 2005; 31: 205-217.
4. Lonkar P and Dedon PC. Reactive species and DNA damage in chronic inflammation: reconciling chemical mechanisms and biological fates. *Int. J. Cancer*. 2011; 128: 1999-2009.
5. Heo HJ, Kim D, Shin SC, Kim MJ, Kim BG and Shin DH. Effect of Antioxidant Flavanone, Naringenin, from Citrus junos on Neuroprotection. *J. Agric. Food Chem.* 2004; 52: 1520-1525.
6. Butterfield DA, Drake J, Pocernich C and Castegna A. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid â-peptide. *Trends. Mol. Med.* 2001; 12: 7548-7554.
7. Boots AW, Haenen GR and Bast A. Health effects of quercetin: From antioxidant to nutraceutical. *Eur. J. Pharmacol*. 2008; 585: 325-337.
8. Gospodaryov D and Lushchak V. In: Oxidative Stress: Cause and Consequence of Diseases, Oxidative Stress and Diseases, First Edition, Editors: Lushchak V, Ukraine. 2012; pp 13-39.
9. Nishimura FCY, Almeida AC, Ratti BA, Ueda-Nakamura T, Nakamura CV, Ximenes VF, and Silva SO. Antioxidant Effects of Quercetin and Naringenin Are Associated with Impaired Neutrophil Microbicidal Activity. *Evid. Based. Complement. Alternat. Med*. 2013; 2013: 1-7.
10. Dryden GW Jr, Deaciuc I, Arteel G and McClain CJ. Clinical implications of oxidative stress and antioxidant therapy. *Curr. Gastroenterol. Rep*. 2005; 7: 308-316.
11. Vertuani S, Angusti A and Manfredini S. The antioxidants and pro-antioxidants network: an overview. *Curr. Pharm*. 2004; 10: 1677-1694.
12. Raza S, Khan MM, Ahmad A, Ashafaq, Islam MF, Wagner AP, Safhi MM and Islam F Neuroprotective effect of naringenin is mediated through suppression of nf-jb signaling pathway in experimental stroke. *Neuroscience*. 2013; 230: 157-171.
13. Tiwari AK. Antioxidants: new-generation therapeutic base for treatment of polygenic disorders. *Curr. Sci.* 2004; 86: 1092-1102.
14. Lu J, Zheng Y, Luo L, Wu D, Sun D and Feng Y. Quercetin reverses d-galactose induced neurotoxicity in mouse brain. *Behav. Brain. Res*. 2006; 171: 251-260.
15. Jeong YJ, Choi YJ, Kwon HM, Kang SW, Park HS, Lee M and Kang YH. Differential inhibition of oxidized LDL-induced apoptosis in human endothelia cells treated with different flavonoids. *Br. J. Nutr.* 2005; 93: 581-591.
16. Renugadevi J and Prabu SM. Naringenin protects against cadmium-induced oxidative renal dysfunction in rats. *Toxicology.* 2009; 256: 128-134.
17. Sriraksa N, Wattanathorn J, Muchimapura S, Tiamkao S, Brown K and Chaisiwamongko K. Cognitive-Enhancing Effect of Quercetin in a Rat Model of Parkinson's Disease Induced by 6-Hydroxydopamine. *Evid. Based. Complement. Alternat. Med.* 2012; 2012: 1-9.
18. Hendriks JJ, de Vries HE, van der Pol SM, van den Berg TK, van Tol EA and Dijkstra CD. Flavonoids inhibit myelin phagocytosis by macrophages; a structure-activity relationship study. *Biochem. Pharmacol.* 2003; 65: 877-885.
19. Heijnen CG, Haenen GR, van Acker FA, van der Vijgh WJ and Bast A. Flavonoids as peroxynitrites scavengers: the role of the hydroxyl groups. *Toxicol. In. Vitro*. 2001; 15: 3-6.
20. Haenen GR and Bast A. The use of vitamin supplements in self-medication. *Therapie.* 2002; 57: 119-122.
21. Bors W, Michel C and Stettmaier K. Structure-activity relationships governing antioxidant capacities of plant polyphenols. *Methods. Enzymol.* 2001; 335: 166-180.
22. Mershiba S, Dassprakash MV and Saraswathy SD. Protective effect of naringenin on hepatic and renal dysfunction and oxidative stress in arsenic intoxicated rats. *Mol. Biol. Rep.* 2013; 40: 3681-3691.
23. Vafeiadou K, Vauzour D, Lee HY, Rodriguez-Mateos A and Williams RJ. The citrus flavanone naringenin inhibits inflammatory signalling in glial cells and protects against neuroinflammatory injury. *Arch. Biochem. Biophys.* 2009; 484: 100-109.
24. Spencer JP. Food for thought: the role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. Proc. Nutr. Soc. 2008; 67: 238-252.
25. Amic D, Amic DD, Beslo D and Trinajstic N. Structure radical scavenging activity and relationships of flavonoids. *Clin. Chim. Acta*. 2003; 76: 55-61.
26. Ashrafpour M, Parsaei S and Sepehri H. Quercetin improved spatial memory dysfunctions in rat model of intracerebroventricular streptozotocin-induced sporadic Alzheimer's disease. *Natl. J. Physiol. Pharm. Pharmacol*. 2015; 5: 411-415.
27. Mardla V, Kobzar G and Samel N. Potentiation of antiaggregating effect of prostaglandins by alpha-tocopherol and quercetin. *Platelets.* 2004; 15: 319-324.
28. Chainani-Wu N. Safety and anti-inflammatory activity of curcumin: a component of tumeric (Curcuma longa). *J. Altern. Complement. Med.* 2003; 1: 161-168.
29. Ak T and Gulcin I. Antioxidant and radical scavenging properties of curcumin *Chem. Biol. Interact.* 2008; 174: 27-37.
30. Rahigude A, Bhutada P, Kaulaskar S, Aswar M and Otari K. Participation of antioxidant and cholinergic system in protective effect of naringenin against type-2 diabetes-induced memory dysfunction in rats. *Neuroscience*. 2012; 226: 62-72.
31. Ishisaka A, Ichikawa S, Sakakibara H, Piskula MK, Nakamura T, Kato Y, Ito M, Miyamoto K, Tsuji A, Kawai Y and Terao J. Accumulation of orally administered quercetin in brain tissue and its antioxidative effects in rats. *Free Radic Biol Med.* 2011; 51: 1329-1336.
32. Gupta YK, Briyal S and Sharma M. Protective effect of curcumin against kainic acid induced seizures and oxidative stress in rats. *Indian. J. Physiol. Pharmacol.* 2009; 53: 39-46.
33. Haider S, Liaquat L, Shahzad S, Sadir S, Madiha S, Batool Z, Tabassum S, Saleem S, Naqvi F, and Perveen T. A high dose of short term exogenous D-galactose administration in young male rats produces symptoms simulating the natural aging process. *Life. Sci.* 2015; 124: 110-119.
34. Morris RG. Spatial localization does not depend on the presence of local cues. *Learn. Motiv.* 1981; 12: 239-260.
35. Dhingra D, Parle M and Kulkarni SK. β-Alanine protects mice from memory deficits induced by ageing, scopolamine, diazepam and ethanol. *Indian. J. Pharm. Sci.* 2006; 68: 216-221.
36. Chow CK and Tappel AL. An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. *Lipids.* 1972; 7: 518-524.
37. Jain A and Flora SJS. Dose related effects of nicotine on oxidative injury in young, adult and old rats. *J. Environ. Biol.* 2012; 33: 233-238.
38. Jain A, Dwivedi N, Bhargava R and Flora SJS. Silymarin and naringenin protects nicotine induced oxidative stress in young rats. *Oxid. Antioxid. Med. Sci.* 2012; 1: 41-49.
39. Hollman PC and Katan MB. Health effects and bioavailability of dietary flavonols. *Free. Radic. Res.* 1999; 31: 75-80.
40. Floyd RA and Hensley K. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol. Aging.* 2002; 23: 795-807.
41. Dufour E and Larsson NG. Understanding aging: revealing order out of chaos. *Biochim. Biophys. Acta*. 2004; 1658: 122-132.
42. Haddadi M, Jahromi SR, Sagar BK, Patil RK, Shivanandappa T and Ramesh SR. Brain aging, memory impairment and oxidative stress: a study in Drosophila melanogaster. *Behav. Brain. Res.* 2014; 259: 60-69.
43. Bournival J, Plouffe M, Renaud J, Provencher C and Martinoli MG. Quercetin and sesamin protect dopaminergic cells from MPP+-induced neuroinflammation in a microglial (N9)-neuronal (PC12) coculture system. *Oxi. Med. Cell. Longev*. 2012; 2012: 921941.
44. Gawel S, Wardas M, Niedworok E and Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiad. Lek.* 2004; 57: 453-455.
45. Fukui K, Omoi NO, Hayasaka T, Shinnkai T, Suzuki S, K. Abe K and Urano S. Cognitive impairment of rats caused by oxidative stress and aging, and its prevention by vitamin E. *Ann. N. Y. Acad. Sci*. 2002; 959: 275-284.
46. Jeong E, Ma CJ, Lee KY, Kim SH, Sung SH and Kim YC. KD-501, a standardized extract of Scrophularia buergeriana has both cognitive-enhancing and antioxidant activities in mice given scopolamine. *J. Ethnopharmacol*. 2009; 121: 98-105.
47. Baluchnejadmojarad T and Roghani M. Effect of naringenin on intracerebroventricular streptozotocin-induced cognitive deficits in rat: a behavioral analysis. *Pharmacology*. 2006; 78: 193-197.
48. Youdim KA, Qaiser MZ, Begley DJ, Rice-Evans CA, and Abbott NJ. Flavonoid permeability across an in situ model of the blood-brain barrier. *Free. Radic .Biol. Med.* 2004; 36: 592-604.
49. Kanaze FI, Bounartzi MI, Georgarakis M and Niopas I. Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. *Eur. J. Clin. Nutr.* 2007; 61: 472-477.
50. Zaki HF, Abd-El-Fattah MA and Attia AS. Naringenin protects against scopolamine-induced dementia in rats. *Bull. Fac. Pharm.* 2014; 1: 15-