

EVALUATION OF NEUROPROTECTIVE EFFECT OF QUERCETIN AND COENZYME Q10 IN
ETHANOL INDUCED NEUROTOXICITY IN MICE*R. Sashindran¹, M. Balasundaram¹, R. Jegathambigai² and P. Kumar³¹Department of Biochemistry, AIMST University, Semeling, Bedong-08100, Kedah²Asia Metropolitan University, Selongor, Kuala Lumpur.³Management and Science University, Shah Alam, Selangor.

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ABSTRACT: Ethanol is a principle ingredient of alcoholic beverages with potential neuronal-genotoxicity, and associated neuronal oxidative DNA damage, neurodegeneration in the CNS and neurocognitive deficits is well documented. Chronic consumption of alcohol is associated with disturbances of mnemonic functions and behavioural deficit. DNA-damaging molecules such as reactive oxygen species (ROS), lipidperoxidation product malondialdehyde (MDA) and acetaldehyde are potent genotoxic agents. Combined application of Quercetin and Coenzyme Q10 ameliorated the neurotoxicity by significantly reducing the potential biomarkers of oxidative stress, augmenting neurotransmitter, and cellular DNA and ATP contents. These results suggest that combined application of Quercetin and Coenzyme Q10 will be beneficial in prevention of neurodegeneration and cognitive deficits associated with alcoholism and these therapeutic interventions could have a clinical implication associated with alcoholism. The combined neuroprotective treatment of Quercetin and Coenzyme Q10 has been proven to ameliorate the neurotoxicity induced by ethanol.

Key words: Quercetin, Coenzyme Q10, neurodegeneration, genotoxic agents & biomarkers

INTRODUCTION

Neurotoxicity is the most prominent cause of brain damage, it alters the normal activity of the neuronal system and eventually kills the neuronal cells (National Institute of Neurological Disorders and Stroke, 2007). Acute alcohol intoxication disrupts memory acquisition in humans and laboratory animals. It has been known for three decades that ethanol, the most widely abused drug in the world, has deleterious effects on the developing human brain, but progress has been slow in developing animal models for studying this problem, and the underlying mechanisms have remained elusive. Chronic alcohol consumption can induce alterations in the function and morphology of most if not all brain systems and structures (Fabio Fadda *et al.*, 2008). The hippocampus of the brain is mainly affected with loss of neurons and signs of inflammation are also found here (Mayeux R *et al.*, 2010). Memory loss, behavioral change, visual problems has been a shown hallmark in neurotoxicity. Amyloid Beta plaques and neurofibrillary tangles which can be found in the hippocampus of the brain are major pathology findings in this age related disease. It causes neuronal cell damages, where neurons loss their ability which leads to neurodegenerative disorder (Querfurth HW *et al.*, 2010).

Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) is an important dietary flavonoid present in several fruits and vegetables, it acts as an antioxidant, anti-inflammatory, and anticancer properties (De Biasi S, *et al.*, 2011). It's known to be an antioxidant agent and it possess free radical scavenging properties and neuroprotection from oxidative injury by their ability to modulate intracellular signals promoting cellular survival Quercetin which has potent antioxidant effects, combined with free radical species to form considerably less reactive phenoxy radicals. Quercetin is one of the most abundant dietary flavonoids and its derivatives constitute about 99% of the flavonoids in apple peel and it is also one of the major constituents in foods consumed in the United States (He and Liu *et al.*, 2008).

In the past decade, the antioxidant activity of flavonoids has been given much attention, since many flavonoids such as quercetin, luteolin and catechins may be better antioxidants than the antioxidant nutrients vitamin C, vitamin E and β -carotene (3). Oral administration of quercetin was also able to improve learning and memory ability (Wattanathorn *et al.*, 2007).

Coenzyme Q10 (ubiquinone) is an antioxidant which naturally produced in human body. It plays an important role in the production of ATP in mitochondria, it is found in most of the organs but it exists at highest concentration in the brain, kidney, heart and liver. It can be synthesized endogenously in the body. The level of CoQ10 as a person ages and it may lead to potential neurodegenerative disorders (Matthews RT *et al.*, 1998).

There are now multiple lines of evidence that strongly support mitochondrial dysfunction and oxidative stress as major contributors to pathogenesis of neurodegenerative diseases (Beal MF *et al.*, 1999). Besides the common causes, oxidative stress is also known to be a factor in Alzheimer disease individual. Oxidative stress is a condition where imbalance of pro oxidant and anti-oxidant homeostasis which eventually generate toxic Reactive Oxygen Species. This free radical's compound damages the cellular structure. A reduction in complex IV activity has been demonstrated in mitochondria from the hippocampus and platelets of neurotoxic patients, as well as in animal models and cybrid cells in reviewed (B. Halliwell *et al.*, 2006). Previous study have shown that AD brains also show evidence of ROS mediated-injury; there is an increase in levels of malondyaldehyde and 4-hydroxynonenal in brain and cerebrospinal fluid of neurotoxic patients compared to controls (M. A. Lovell *et al.*, 1995). This oxidative stress can cause damage to cellular components, such as membranes, DNA, and proteins. Moreover, oxidative stress can induce cell death processes through several mechanisms, including the release of apoptosis-inducing factors (Bredensen 1996.a)

The present study determined the combined effect of Quercetin and Coenzyme Q10 in treating ethanol induced Neurotoxicity. Biochemical analysis of the Hippocampus were conducted to evaluate the oxidative properties which was found to be the potential hallmark of neurodegenerative disorder.

MATERIALS AND METHODS

Adult male Swiss Albino mice were obtained from USM, Penang and were placed in the AIMST Central Animal House under standard laboratory condition ($25\pm 2^\circ\text{C}$, 12 hours light and dark cycles). All the mice were fed with normal food and water ad libitum and were observed for seven days, prior to the treatment period. All the animals were handled according to the standard handling procedure which has been approved by the AIMST university human and animal ethical committee (AUHAEC). A total of 30 mice were randomly divided into five groups. Each group contained six mice and received the following treatment for 30 days. Group 1 (normal) mice were given normal saline water. Group 2 mice were treated with alcohol (9.875mg/kg body weight via orally). Group 3 rats were treated with alcohol and Quercetin (50mg/kg body weight via orally). Group 4 rats were treated with alcohol and Coenzyme Q10 (50mg/kg body weight via orally). Group 5 rats were treated with alcohol and a combination of Quercetin and Coenzyme Q10. The dosage of were determine based referred literature (Balasubramaniya *et al.*, 2006). The general behavioural changes were observed for 30 min daily. Upon completion of 30 days treatment, the mice were sacrificed by decapitation under sodium pentobarbital (40mg/kg body weight, i.p injection). Brain tissues were excised immediately, the hippocampus region was isolated (Glowinski and Iverson method, 1966) and immersed in ice cold saline, then homogenized using PBS for biochemical studies.

Xanthine Oxidase assay

The hippocampus were homogenate samples were obtained from (control, alcohol, quercetin, coenzyme Q10, combination of quercetin +coenzymeQ10) groups were diluted with distilled water (50 μl). Add 50 μl reaction mix (46 μl assay buffer+ 2 μl substrate mix+ 2 μl enzyme mix) to all the wells except for the negative control wells. The negative control (background control) were prepared by adding 48 μl assay buffer and 2 μl enzyme mix and diluted with 50 μl distilled water. The absorbance of the wells was measured using micro plate Elisa reader.

TAC assay

Diluted uric acid standards (20 μL) and samples (brain striatal tissue samples) were added to 96 well micro titer plate. Then 180 μL of the reaction buffer (1X) were added and mixed thoroughly. Initial absorbance of the solution was obtained by reading the plate at 490 nm using a micro plate reader. Then the reaction was initiated by adding 50 μL of copper ion(1X) reagent into each well and incubated for 5 minutes with orbital shaker, followed by addition of 50 μL of stop solution (1X) into each well to stop the reaction. The absorbance of the plate at 490nm was determined by using micro plate reader.

PARP detection

Strip wells were washed two times with a mixture of PBS (1X) and 0.1% Triton X-100 (200 μ l/well) followed by two washes with PBS (1X). All the liquid was removed following each wash by tapping strip wells onto paper towels. Then 50 μ l per well of diluted Strep-HRP were added and incubated for 60 minutes at room temperature. After incubation strip wells were washed two times again with mixture of PBS (1X) and 0.1% Triton X-100 (200 μ l/well) followed by two washes with PBS (1X), then the liquid was removed following each wash by tapping strip wells onto paper towels. Finally 50 μ l per well of pre-warmed TACS-Sapphire™ colorimetric substrate was added and incubated in the dark for 15 minutes at room temperature. Finally the reaction was stopped by the addition of 50 μ l per well of 0.2 M HCl/well to stop the reactions. At the end the absorbance was read at 450 nm in a microplate reader (Bio Rad microplate reader, Model 680).

RESULTS

Poly (ADP-ribose) polymerase (PARP) assay

Results of PARP activity in the hippocampus shows a significant ($p < 0.05$) increase in PARP activity in ethanol treated group. The mean value of the control group was found to be 92.2 ± 5.2 mol/mg protein, while it was found 168.4 ± 8.92 mol/mg protein. Treatment with quercetin significantly ($p < 0.05$) reduced the changes, the activity of PARP decreased, the mean value was found to be 134.7 ± 10.3 mol/mg. Treatment with Coenzyme Q10 also significantly ($p < 0.05$) reduced these changes, the mean value was found to be 104.8 ± 9.3 mol/mg. Combination of Quercetin and Coenzyme Q10 treatment shows a significant ($p < 0.05$) change, the mean value was decreased, it was found to be 97.6 ± 7.6 mol/mg. (Table-1).

Table 1: Effect of Quercetin and Coenzyme Q10 on PARP activity in hippocampus

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	Ethanol-treated	Ethanol+ Quercetin	Ethanol+CoQ10	Ethanol+Quercetin+ CoQ10
PARP-1 (pmol of NAD ⁺ consumed/mg of protein)	92.2 ± 5.2	$168.4 \pm 8.92^\dagger$	$134.7 \pm 10.3^*$	$104.8 \pm 9.3^*$	$97.6 \pm 7.6\$$

The PARP activity was determined following universal colorimetric PARP assay method. The values are expressed in mean \pm s d (n=3). \$ values are significantly different ($p < 0.05$) from ethanol treated group

Xanthine Oxidase Assay (XO) assay

XO activities of the hippocampus were determined and results can be seen from figure 2 There was significant ($p < 0.05$) increase in ethanol induced group, the mean value was found to be 0.956 ± 0.0028 , the mean value for control group was found to be 0.315 ± 0.0008 . Treatment with Quercetin showed a reduction in the significant ($p < 0.05$), the mean value was found to be 0.473 ± 0.0026 , treatment with Coenzyme Q10 also reduced these changes, the mean value was found to be 0.453 ± 0.0032 . The combination Quercetin and Coenzyme Q10 treatment showed a significant ($p < 0.05$) reduction, the mean value was found to be 0.367 ± 0.0035 (Table-2).

Table 2: Effect of Quercetin and Coenzyme Q10 on XO activity in hippocampus

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
	Control	Ethanol-treated	Ethanol+ Quercetin	Ethanol+CoQ10	Ethanol+Quercetin+ CoQ10
XO μ moles formazan/mg protein/hr	0.315 ± 0.0008	$0.956 \pm 0.0028^\dagger$	$0.473 \pm 0.0026^*$	$0.453 \pm 0.0032^*$	$0.367 \pm 0.0035\$$

The XO level was determined following XO assay Abnova method. And the values are expressed in mean \pm s d (n=3). \$ values are significantly different ($p < 0.05$) from ethanol treated group

Total Antioxidant Capacity (TAC)

The TAC activities of the hippocampus were determined and can be seen in figure 3. Results show that there is a significant ($p<0.05$) decrease of TAC activity in the ethanol treated group, the mean value for the control group was 3.49 ± 0.13 , while the ethanol treated group was 1.75 ± 0.06 . Treatment with Quercetin and Coenzyme Q10 showed a significant ($p<0.05$) increase, the mean value was found to be 3.08 ± 0.24 and 3.27 ± 0.28 respectively. The combination treatment of both Quercetin and Coenzyme 10 shows a significant ($p<0.05$) increase, the mean value was found to be 3.33 ± 0.28 . (Table-3).

Table 3: Effect of Quercetin and Coenzyme Q10 on TAC activity in hippocampus

Parameters	Group 1 Control	Group 2 Ethanol-treated	Group 3 Ethanol+ Quercetin	Group 4 Ethanol+CoQ10	Group 5 Ethanol+Quercetin+CoQ10
Total antioxidant status (nM/mg)	3.49 ± 0.13	$1.75\pm 0.06^{\dagger}$	$3.08\pm 0.24^*$	$3.27\pm 0.28^*$	$2.86\pm 0.21^{\$}$

The TAC was determined following TAC assay kit (oxiselect™, cell biolabs, Inc.). Values are expressed in mean \pm s.d (n=3). \$ values are significantly different ($p<0.05$) from ethanol treated group

DISCUSSION

Based on the results, excessive consumption of ethanol induces neurotoxicity and eventually activates the free radicals. Ethanol induced oxidative stress and an ultimate membrane-molecular damage is accompanied by elevated levels of biomarkers in serum which are indicative of cellular leakage and loss of functional integrity of the cell (Sien-Sing Yang *et al.*, 2005). Substantial documental evidence has suggested that administration of micro-quantities of iron chelating antioxidants such as L-carnitine, α -lipoic acid, resveratrol, quercetin and coenzyme Q10 exerts free radical scavenging and antioxidant sparing effects in heavy alcohol drinking (Scalbert *et al.*, 2005;). Among these, quercetin can impact mitochondrial biogenesis by modulating enzymes and transcription factors in the inflammatory signaling cascade (P. Yao *et al.*, 2007). In addition, there are in vitro (Van Hoorn *et al.*, 2002) and in vivo (Mo *et al.*, 2007) evidences that quercetin, a flavonol found in apples, onions, and other plant foods, is an inhibitor of xanthine oxidase (XO), one source of production of reactive oxygen species (ROS) during exercise. Coenzyme Q10 is an endogenous antioxidant that scavenges free radicals directly, inhibits biomolecule oxidation and affects antioxidants in vivo (Modi *et al.*, 2006). Although its structural characteristic allows it to diffuse into the membrane phospholipids bilayer, where it serves as an electron transfer intermediate in the mitochondrial respiratory chain, its reduced form is a powerful antioxidant. The suppression of oxidative damage in the brains of CoQ10-fed animals may be explained in a number of possible ways; one possibility is that the ratio of reduced to oxidized CoQ10 might be favorably altered by CoQ10 supplementation, resulting in an antioxidant effect. It is also possible that systemic CoQ10 is able to achieve antioxidant effects by an indirect mechanism for example by, restoration of other brain antioxidants (Wadsworth *et al.*, 2010). Increase in the level of xanthine oxidase, nitric oxide synthase and the DNA repair enzyme PARP-1 is due to the increased oxidative stress induced by ethanol and an ultimate membrane damage and DNA oxidation (Pricilla P Cherian *et al.*, 2008). In the present study increased levels of oxidative stress biomarkers suggested that an extensive liver injury and systemic toxicity was caused by ethanol due to increased lipid peroxidation indicating membrane and DNA damage. It has been documented that, ethanol causes structural and functional damage to the cell membrane and increased membrane permeability leading to the leakage of hepatic enzymes into the circulation. Interested in the possibility that PARP-1, known to be activated by oxidative stress and DNA strand breaks, could have direct upstream actions on ethanol-induced neurodegeneration. Treatment with quercetin and/or coenzyme Q10 resulted in a significant decrease in the levels of PARP-1 and Xanthine oxidase content and increase the TAC activity when compared with ethanol treated mice. The free radical scavenging efficiency of quercetin may be associated with the presence of two hydroxyl groups in the ring of its molecule (Mubeen Ahmad Ansaria *et al.*, 2009).

CONCLUSION

The results justifies that the combination treatment of Quercetin and Coenzyme Q10 reactivates the antioxidant properties which eventually reduces the oxidative stress in ethanol induced neurotoxicity in mice.

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