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ORIGINAL ARTICLE

Quercetin treatment against NaF induced oxidative stress related neuronal and learning changes in developing rats

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Abstract The present study evaluates the protective role of quercetin against sodium fluoride (NaF) induced neurotoxicity in developing rat brain using biochemical, behavioural and histopathological parameters. Timed pregnant Wistar rats were chosen for the study and they were distributed into three groups (group 1: control, group 2: fluoride and group 3: fluoride- quercetin). The study duration is for 53 days (i.e. Gestational and post gestational period), where in the control group received normal tap water; Fluoride group received 20 ppm fluoride and the third group received both 20 ppm of Fluoride and 20 mg of quercetin through oral gavage. Behavioural studies were done using postnatal pups of 21 and 30 days age. The brains of postnatal pups of 14, 21 and 30 days age were collected and used for biochemical and histopathological analysis. The present study indicates the reversal of NaF induced alterations, such as decreased body and brain weights, increased lipid peroxidation, epinephrine levels as well as decreased superoxide dismutase, catalase, glutathione, acetylcholine and norepinephrine levels. Quercetin administration has reversed learning inability and morphological changes in neuron structure. Thus, quercetin exerts neuroprotective property in developing rat brain by ameliorating the NaF induced oxidative stress, alterations in behavioural, neurotransmitters and histopathological alterations.

1. Introduction

Fluoride induces fluorosis which is characterized by dental and skeletal damages. It is also associated with early damage of soft tissues including brain. The physiological changes have been alarming the research fraternity and it is important to identify possible preventive, curative and therapeutic possibilities to handle the condition (Wenjing et al., 2011). Fluoride enters into the body through food, drinking water, tooth-

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pastes, fluoride rinses and fluoride containing compounds (Mullenix et al., 1995; Nurgul et al., 2014). The concentration of fluoride permitted in drinking water as per the WHO is 0.7–1.2 ppm (Kumar and Takalkar, 2010). The high concentrations of fluoride in groundwater in some parts of India have raised an alarm regarding the possible damage or toxicity due to chronic fluoride exposure on humans and animals. Fluoride also affects non-skeletal organs such as the liver, kidney, heart and brain (Nabavi et al., 2012c,d). Chronic exposure of fluoride could induce the loss of structure and function of skeletal muscles, brain and spinal cord (Olusegum et al., 2013; Vani and Reddy, 2000). Fluoride is capable of crossing blood brain barrier and placental barrier and causing low birth weights (Diouf et al., 2012; Gouri et al., 2011). Fluoride passes through blood brain barrier and leads to neurodegeneration in the brain, causing learning disabilities, memory deficits, and alters the activities of enzymes in the brain (Madhusudhan and Piler, 2009; Shivarajashankara et al., 2002a,b). In the developing stage, fluoride enters weanling pups through mother's milk and leads to neuron alteration, morphological changes and neurotransmitters abnormalities in the foetal brain (Varner et al., 1998). Accumulation of fluoride in foetal stage leads to alteration of growth, cell differentiation and subcellular organization in the brain (Yaning et al., 2005). In endemic fluoride areas children lose their intelligence quotient (Wang et al., 2004).

Recent studies reported the potential benefits of natural products such as Curcumin, Silymarin, and Resveratrol against sodium fluoride induced oxidative stress in soft tissues of rats (Nabavi et al., 2011, 2012a–d, Yousef et al., 2010). Micronutrients such as vitamin C, D, E and calcium, treatments did not show expected amelioration. Quercetin is a ubiquitous flavonoid taken as a part of diet and it is extensively available in broccoli, apple, onions, tomatoes, red wine, potatoes, soya beans, tea and coffee (Renugadevi and Milton, 2010). Quercetin exists in the form of glycosides i.e. quercetin glycosides (quercetin glucoside, quercetin galactoside, or quercetin arabinoside) in fruits and vegetables get absorbed passively in small intestine in quercetin aglycone form and quercetin aglycone is also available as a dietary supplement, but it is limited to 1 g/day (Costa et al., 2016). Quercetin aglycone undergoes biotransformation by involving phase II enzymes to yield glucuronidated, sulfated, and methylated metabolites. The phase II enzymes are UGT (uridine 5′-dipho sphoglucuronosyltransferase), SULT (sulfotransferase), and COMT (catechol-O-methyltransferase). Quercetin has the ability to cross the blood brain barrier but the amount is very less i.e. in the order of picomolar to nanomolar are found in brain tissue (Costa et al., 2016; Sharma et al., 2015).

Quercetin is a powerful antioxidant, due to the presence of two pharmacophores that is, the catechol group in the B ring and the OH group at position 3 within the molecule which scavenges the free radicals (Nabavi et al., 2015; Sharma et al., 2015). Concentration of 5–50 μM quercetin works as an excellent ROS scavenger in *In vitro* condition (Saw et al., 2014). Zhang et al. (1999) showed quercetin neuroprotection against hydroxydopamine induced damage in both *In vitro* (PC12 cell lines) and *In vivo* by decreasing the expression of iNOS, NO levels and also restricted neuron loss in brain and the quercetin pre-treatment has given more neuroprotection. Till date there are no reports on quercetin's neuroprotective property against sodium fluoride induced toxicity in developing rat brain. The present study reports the beneficial role of quercetin against sodium fluoride induced neurotoxicity in developing rat brain through oxidative, neurotransmitters, histopathological and behavioural analysis.

2. Materials and methods

2.1. Chemicals

Quercetin, epinephrine norepinephrine and sodium fluoride were purchased from Sigma–Aldrich chemicals. All others chemicals used for biochemical analysis and histopathological analysis were of analytical grade from Himedia.

2.2. Animals

Pregnant Wister albino rats were obtained from NCLAS, National Institute of Nutrition, Hyderabad, India. The pregnant rats were maintained under standard laboratory conditions in single rat polypropylene cages, exposed with 12 h light/dark cycle at room temperature 24 ± 2 °C. The animals were provided standard diet and drinking water with ad libitum. The laboratory conditions were maintained as per the guidelines given by the CPCSEA (Committee for the Purpose of Control and Supervision on Experimental Animals) at Osmania University, Hyderabad, India. Timed pregnant rats aged (160–180 days) were randomly divided into three groups (n = 6/gp). The control group received normal tap water; the experimental group received sodium fluoride 20 ppm/kg bw (Banala and Karnati, 2015) through drinking water and protective group received quercetin 20 mg/kg bw (Nabavi et al., 2012c,d) by gavage and 20 ppm Sodium fluoride through drinking water (Table 1). The experimental animals were maintained for 53 days. Postnatal pups of 14, 21 and 30 days of age were selected for experimental studies. The brains were dissected from of 14, 21 and 30 days old pups and stored at 20 °C. The brains were used for biochemical and histopathological studies.

2.3. Methods

Body and brain weight: The body and brain weights of postnatal pups of 1, 7, 14, 21 and 30 days age old from each respective groups were noted and analysed.

2.4. Oxidative stress markers

2.4.1. Lipid peroxidation (LPO)

Lipid peroxidation in cerebral cortex of brain tissue was measured by modified method of Garcia et al. (2005). 1 mL of 10% homogenate was added to 1 mL of 20% TCA and heated at 70 °C for 10 min, and cooled at room temperature and

Table 1 Experimental groups.

Groups	Dose
Control	Tap water
Experimental	Fluoride water (20 ppm)
Protective	Fluoride–quercetin (20 mg/kg bw)