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CURCUMIN PROTECTION AGAINST OXIDATIVE STRESS INDUCED NEURAL DAMAGE IN DEVELOPING BRAIN OF RAT WITH FLUORIDE EXPOSURE

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Abstract: Curcumin is a pivotal spice and ayurveda medication mostly used in India and other Asian countries. The curcumin is been part of people for centuries as a detoxificant, pain reliever, anti-inflammatory agent and it has also demonstrated to have anti-cancer properties. Fluoride is becoming an irreversible environmental pollutant causing various alignments in both humans and animals on chronic exposure. The ameliorative studies in developing rats were studied in presence of Curcumin against sodium fluoride induced oxidative stress. Curcumin (10mg/kg body wt.) was orally administered to NaF (20 ppm fluoride) induced rats for 53 days. The behavioural study such as Rota rod was conducted at 21st and 30th day. The brains of 1, 7, 14, 21 and 30th day old pups were collected and analysed to check the levels of oxidation markers such as SOD, catalase and LPO. The levels of SOD and Catalase increased and whereas LPO were decreased with treatment of Curcumin. The results suggest that the Curcumin ameliorated the NaF induced alterations and proved to an excellent protective agent.

Key word: Sodium fluoride; Oxidative stress; Curcumin

INTRODUCTION

Fluoride is an electronegative element whose accumulation affects both hard and smooth tissues like bone (Rich *et al.,* 1964), muscles, intestine, liver, kidney, brain etc. (Vani and Reddy, 2000; Reddy *et al.,* 2011). Fluoride related compounds are miscible in water and lipids as a result they can crosses the biological membranes (Crenzy *et al.,* 2000; Bruce spittle). The fluoride induced neurotoxicity was first reported by Mullenix *et al.,* (1995); and the related report was published by Varner *et al.,* (1998). The recent research findings suggested that the fluoride may effect on brain and other smooth tissues before its effect on skeletal system (Basha *et al.,* 2011; Hanen *et al.,* 2007; Vani and Reddy, 2000). Study also reported about fluoride's relation to oxidative stress, free radical generation, antioxidant system, brain function, IQ and behavior alterations (Seraj *et al.,* 2012; Barbiera *et al.,* 2010). The literature suggested that development of neurotoxicity is not parallel to fluorosis development, but based on the latest reports on the chemical nature of fluoride, neurotoxicity might develop at early time period of fluoride ingestion and before the onset of Fluorosis (Sieverat and Phillip, 1959; Bera *et al.,* 2007; Ailani *et al.,* 2009).

The recent findings on polyphenolic compounds, especially flavonoids, tannins, and phenylpropanoids expressing and acting as good antioxidants (Chouhan *et al.,* 2011; Heba *et al.,* 2011). It was shown in particular that some edible antioxidants are capable of moderating oxidative stress-induced neuronal cell death (Byers and Perry, 1992; Machlin and Adrianne, 1987, chiu *et al.,* 2008). Curcumin is an active polyphenolic compound present in the *Curcuma longa* Linn derived from the rhizome of this plant, and widely used as a food additive. The *Curcuma* plant has been a source of medicinal and food additives in China, India, Iran and, now nearly throughout the world (Navabi *et al.,* 2011). Recent studies on the use of natural and complementary medicines in western medicine have drawn the attention of the scientific community to this natural product (Chouhan *et al.,* 2011). It was found that Curcumin has a surprisingly wide range of beneficial effects, including anti-inflammatory, antioxidant, chemo preventive, and chemotherapeutic activities (Nabavi *et al.,* 2011, 2012). The antioxidants are capable of nullifying the stress-induced neuronal loss in some neurodegenerative disorders, such as Alzheimer's, Parkinson's diseases etc. (Mishra and Palanivelu, 2008). Till now there are no reports available on developing brain of rat with Curcumin treatment against NaF toxicity. Hence we report the efficacy of curcumin as neuroprotection with emphasis on oxidative stress against NaF in post-natal rats.

MATERIALS AND METHODS

Sodium fluoride, Curcumin, bovin albumin serum (BSA), nitroblue tetrazolium (NBT), thiobarbituric acid (TBA), tricholoro acetic acid (TCA), hydrogen peroxide and hydroxylamine hydrochloride.

The animals and pellet seed were obtained from NCLAS, National Institute of Nutrition, Hyderabad, India. The protocols of experiment were approved by the departmental ethical committee, CPCSEA. Timed pregnant rats were divided to four

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groups i) Control (Group-I), ii) Experimental 20 ppm fluoride treated (Group-II), iii) experimental 20 ppm fluoride + Curcumin 10 mg, iv) Experimental 20 ppm fluoride + Curcumin 20 mg. Each pregnant animal were kept in a separate polypropylene with 12/12hr: day/night cycle.

Control animal received the normal tap water whereas experiment animals received the DDW with 20ppm fluoride orally. Day 1 pregnant rats were allowed to 20ppm fluoride as a mode of prenatal exposure or gestational exposure to unborn until birth of pups and the post-natal rat pups received Fluoride through colostrum from mother until they are 21 days old. Curcumin (Sigma Chemicals) powder was dissolved in lukewarm corn oil to prepare stock solution; and one dose was administered orally through gavage daily to experimental group's iii & iv. The animals maintained up to 53 days including mothers gestational and pups grown up to 30 days. Day 1, 7, 14, 21 & 30th control and experimental rats were sacrificed and brains were dissected out and stored for biochemical analysis. Behavior tests were performed on 21 and 30 day old pups.

Biochemical Measurements

Estimation of total protein: The total protein content was measured by the method of Bradford using BSA as the standard.

Estimation of Lipid peroxidation: The intensity of lipid peroxidation in terms of formation of TBA reactive substances was examined by the modified technique of Ester Bauer and Cheeseman. The homogenates including 0.5 mg protein was mixed with 0.5 ml of TCA (20%) + 1 ml of TBA (0.67%) and incubated for1 h at 100°C. After cooling, samples were centrifuged, and the supernatant was collected. The absorbance of reaction mixtures were measured at 535 nm wavelength using a blank containing all the reagents except for homogenates.

Estimation of Superoxide dismutase: Superoxide dismutase (SOD) activity was determined using modified method of Marklund and Marklund (1974); Kakkar *et al.*, (1984). Reaction mixtures contained 2 ml of Sodium carbonate (50 mM), 0.8 ml of Nitroblue tetrazolium (25 µM), and 0.4 ml of freshly prepared hydroxylamine hydrochloride (0.1 mM). The reaction mixtures were mixed by inversion followed by the addition of the clear supernatant of homogenates (0.1 ml, 1:10 w/v). The absorbance in reaction samples were recorded at 560 nm wavelength.

Estimation of Catalase activity: The activities of catalase were examined spectrophotometrically by modifying the protocols of Bonaventura *et al.*, and Aebi's. 50µl of the supernatant was added to a cuvette containing 2 mL of phosphate buffer (pH 7.0) and 1 mL of 30mM H_2O_2. Catalase activity was measured spectrophotometrically at 240nm. The molar extinction coefficient of H_2O_2, 43.6M cm^{-1} was used to determine the catalase activity. One unit of activity is equal to 1 mmol of H_2O_2 degraded/ min and was expressed as units/mg of protein.

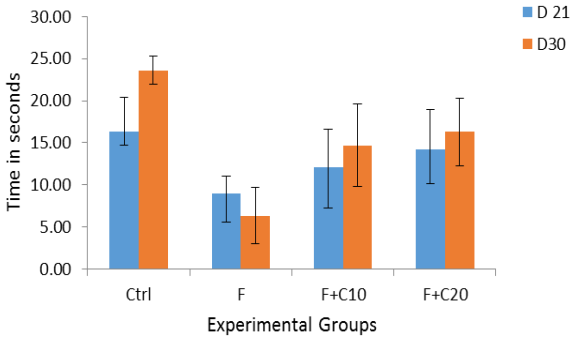
Rotarod: Total locomotor activity of the rats was studied with dolphin 4 compartment rota rod. The data was taken as total duration 30 min to each animal during the 5 min session interval.

Statistical approach

The values Mean±SD of six (n=6) individual observations; the percentage change of experimental over control calculated at concerned age of experiment day. Post Hoc test, Null Hypothesis (H_0), multiple comparison test calculated. Post hoc multiple comparisons in between experiment days values significant P<0.05.The values expressed as in rating as in points.

RESULTS

Figure 1: Effect NaF exposure on motor coordination behavior in developing rats by the RotaRod at 21 and 30th day of control (G-I), Fluoride 20 ppm (G-II), F+ Curcumin 10 mg (G-III) & F+ Curcumin 20 mg (G-IV) groups



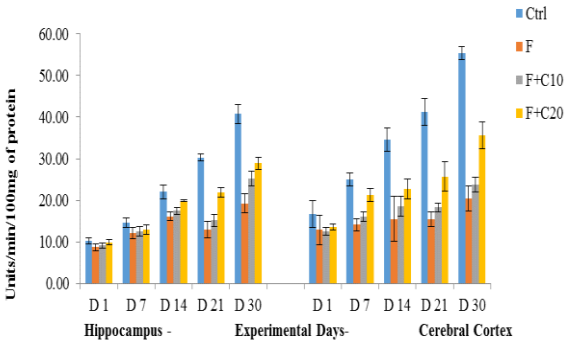
The values were statistically significant at p<0.05 level.

The fluoride treated rats showed less motor control and imbalance during the rota rod test, which was significantly less compared to control and protective receiving groups. The observations clearly indicate the efficacy of curcumin in restoring the motor control and decrease the effects of NaF (Figure 1).

The levels of SOD in fluoride treated rats depleted significantly in comparison to control and protective receiving groups. The observations indicate curcumin's efficacy as antioxidant and reduce the effects of NaF. The levels of SOD are recovering in

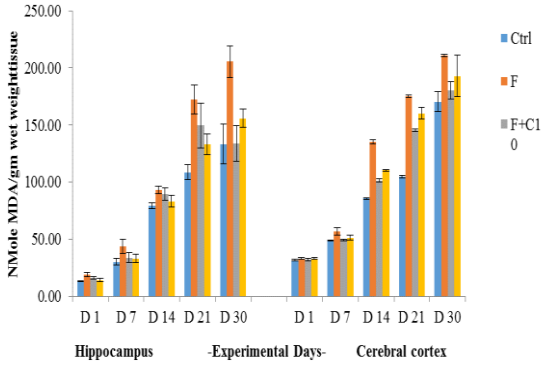
presence of curcumin dose dependently in both hippocampus and cortex regions (Figure 2).

Figure 2: Effect of prenatal and postnatal exposure of NaF, NaF + Curcumin 10mg, 20mg on Super Oxide Dismutase (SOD) activity in cerebral cortex and hippocampus of rat brain.



The values were statistically significant at p<0.05 level.

Figure 3: Effect of prenatal and postnatal exposure of NaF, NaF + Curcumin 10mg, 20mg on lipid peroxidation (Malondialdehyde – MDA) in cerebral cortex and hippocampus of rat brain.

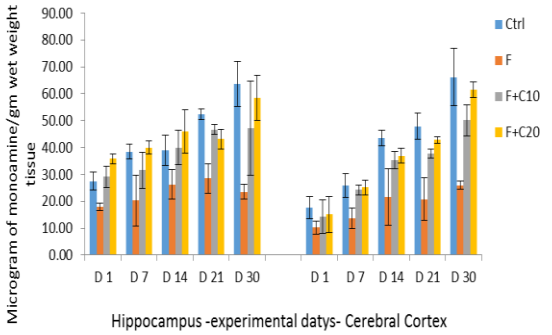


The values were statistically significant at p<0.05 level.

The lipid peroxidation increased in fluoride treated rats and depleted significantly in comparison to control and protective receiving rats indicating curcumin as excellent antioxidant against NaF induced oxidative damage. The levels of LPO decreased in presence of curcumin dose dependently in both hippocampus and cortex regions (Figure 3).

The Catalase activity diminished in fluoride treated rats in comparison to control and protective receiving rats. The levels of Catalase recovered in presence of curcumin dose dependently in both hippocampus and cortex regions. Results suggesting curcumin as excellent antioxidant against NaF induced oxidative damage (Figure 4).

Figure 4: Effect of prenatal and postnatal exposure of NaF, F + Curcumin 10mg, 20mg on Catalase activity in cerebral cortex and hippocampus of rat brain.



The values were statistically significant at p<0.05 level.

DISCUSSION

In most of the neuronal disorders and diseases the reactive oxygen species (ROS) are regarded as one of the pivotal pathologic mediators. In presence of toxin (F) the cellular antioxidant system are altered and would result in generation of free radicals (Chiu *et al.*, 2008). Enhanced oxidation of cellular macromolecules might lead to injury of the brain or result in unfavorable cellular responses in case of oxidative stress (Ailani *et al.*, 2009). Failure of brain functions in case of high oxygen consumption and neutralization of the oxidative species could be the toxic effect of fluoride. Fluoride is believed to enhance the free radical species number by inhibiting the antioxidative system (Machlin and Adrianne, 1987).

In the present study, the high levels of MDA in the cerebral cortex, and hippocampus is due to fluoride stimulated oxidative stress where in the free radicals generation is elevated in cellular system and causing extensive damage in absence of protective agents and the damage can be controlled in the presence of some defensive or neutralizing agents (Chiu *et al.*, 2008; Krishnaiah and Reddy, 2007). The previous results (Vani and Reddy, 2000) from our lab support the findings. Similarly, significant decrease (p<0.05) in levels of SOD and catalase could only indicate enhanced production of H_2O_2 and O_2. The curcumin has great ability against free radical scavenging and exert antioxidant capacity to treat medical condition including neurodegeneration, diabetes and cancer in various pathological states. In this study, the curcumin associated antioxidant activity against Fluoride mediated toxicity in brain may have efficacy in eliminating Fluoride-induced ROS radicals. The groups who received fluoride in combination with Curcumin showed significant decrease in MDA levels and increase in SOD and Catalase levels indicating the efficiency of Curcumin against fluoride oxidative stress in dose dependent manner (p<0.05). The curcumin receiving

rats have shown reversal of motor activity and protection in dose dependent manner and the motor activity was almost similar to that of the control rats.

CONCLUSION

The chronic induction of Fluoride into pregnant mothers, might have led to disruption of the pro-oxidant/antioxidant balance in the developing brain as a result of nutritional and antioxidants deficiency due to oxidative stress. As per the results and observations made during our experiments it's evident that fluoride has the ability to cause loss of energy capacity to meet an oxidative challenge and ultimately neural loss as a result of oxidative stress and Curcumin exposure has given a bolstering capacity to neutralize the reactive free radicals and fortitude the cellular antioxidant defense.

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