

Effect of maternal fluoride exposure on developing CNS of rats: Protective role of *Aloe vera*, *Curcuma longa* and *Ocimum sanctum*

N Madhusudhan, P Mahaboob Basha*, Puja Rai, Fiyaz Ahmed & G Ravi Prasad[†]
Department of Zoology and [†]Botany, Bangalore University, Bangalore 560 056, India

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Fluoride is toxic to neuronal development and its excessive intake during pregnancy cause adverse effects on neonatal development. The present study examined the presence of oxidative stress during maternal exposure of fluoride and the therapeutic strategy of *Aloe vera*, *Curcuma longa* and *Ocimum sanctum* extracts in functional prevention of fluoride led oxidative stress. The pregnant Wistar rats were exposed to 100 ppm fluoride in drinking water and pups born to them were supplemented with phytoextracts daily. On 21st postpartum day, the pups were sacrificed to analyse fluoride and oxidative stress markers. Fluoride exposure significantly increased its accumulation, lipid peroxidation and decreased the activities of catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and glutathione levels in discrete regions of the central nervous system (CNS) of pups indicating oxidative stress and inhibited antioxidant defense. The results implied the vulnerability of developing CNS to fluoride toxicity. On phytoextract supplementation, the oxidant devastation was suppressed by regaining antioxidant homeostasis near normal level proving efficacy and therapeutic strategy. Among the phytoextracts supplemented the *Ocimum sanctum* is found to be more effective.

Keywords: Fluoride toxicity, Lipid peroxidation, Oxidative stress, Phytoextracts

Fluorosis is an endemic public health problem prevailing in 23 nations around the world including India, where it is endemic in 17 out of 32 States and Union territories¹. Naturally fluoride exposure occurs through drinking water or from dietary sources, artificially by consuming fluoridated water or fluoride containing tablets and/or accidental exposure. Prolonged consumption of fluoride in excess (> 1ppm), causes dental caries, skeletal deformities, soft tissue damage viz. impairment in the functioning of liver, kidney, muscle, brain, heart, thyroid, testis, ovary, etc²⁻⁵. Fluoride passes through placenta and also appears in low concentrations in saliva, sweat, and milk^{6,7}. Moreover, fluoride exposure exhibit increased transport into the blood stream and likely across the blood-brain barrier⁸.

The brain is prone to oxidative stress due to the presence of high levels of polyunsaturated fatty acids, relatively low antioxidant capacity, the presence of redox metal ions and high oxygen utilization⁹. The neuronal growth-spurt in rats occurs in the first 3 weeks of postpartum¹⁰, which specify the

brain developmental age and time when many neuro-teratogens exert adverse effects and undergoes profound structural and functional transformations, making it vulnerable to a variety of external influences¹¹. The long term high fluoride intake enhances oxidative stress, disturbs the antioxidant defense, suggesting increased oxidative stress as one of the mediating factors in the pathogenesis caused by fluoride^{8,12}, whereas in the developmental stages of life, the mode of action of fluoride is debating and moreover its occurrence in the developing central nervous system (CNS) during maternal fluoride exposure is not elucidated.

Since, fluorosis is irreversible, its prevention by appropriate and timely intervention is necessary, therefore, a greater understanding at biochemical and molecular mechanisms of the disease and its progression are very important. Presently much attention is being paid to study the interactions of xenobiotics with one another or with dietary factors¹³. Antioxidants and chelators are widely used to minimize the toxicity/stress and the role of minerals (Ca, Zn, Se, I, etc) and vitamins (A, D, C, E) against fluoride toxicity are also documented^{13,14}. Prolonged exposure of minerals and vitamins may not be safe as their accumulation may exert other effects in addition

*Correspondent author
Telephone: +91-80-22961571
Fax: +91-80-23219295
E-mail: pmbashabub@rediffmail.com

to fluoride stress. Therefore the choice of antistress compounds should aim not only to ameliorate the stress but also be safe and economical. Medicinal plants with a long history of human use ultimately yield novel drug prototypes. Systematic and intensive search for plants for new drugs to treat fluorosis seems to be of great utility. *Ocimum sanctum*, *Curcuma longa* and *Aloe vera* are widely used to treat several diseases as they are known to exhibit antioxidant, antidepressant, antibacterial, anti-inflammatory, antirheumatic, antistress, antipyretic, antitumor, antimicrobial, antiulcerogenic, antihypertensive, analgesic, immunomodulatory and adaptogenic properties¹⁵⁻¹⁸.

Hence the present study is aimed to assess (i) fluoride intoxication to mother rats and the effect on the pups during development, (ii) the susceptibility of developing pups to oxidative stress and (iii) the therapeutic efficiency of extracts of *Aloe vera*, *Curcuma longa* and *Ocimum sanctum*.

Materials and Methods

Animals—Laboratory bred pre-mated Wistar strain female albino rats *Rattus norvegicus* were obtained from Sri Raghavendra Enterprises, Bangalore, India and acclimatized to laboratory conditions (12 h dark/light, 25° ± 2° C). Standard rodent pellet diet was given *ad libitum*. The animals were maintained in accordance with the guidelines of National Institute of Nutrition, ICMR, Hyderabad, India and approved by the Institutional Animal Ethical Committee Bangalore University, Bangalore.

Plant material—Leaves of *A. vera*, rhizomes of *C. longa* and leaves of *O. sanctum* were collected from Bio-park, Bangalore University, Bangalore, India and authenticated in the Department of Botany Bangalore University, Bangalore, India.

***Aloe vera* L.**—*A. vera* powder was prepared from *A. vera* leaf gel according to the published procedure¹⁶ with required modifications. Mature, healthy and fresh leaves of *A. vera* having a length of approximately 75 to 90 cm were washed with fresh water, cut transversely into pieces and thick epidermis was selectively removed. The solid gel in the center of the leaf was homogenized. The resulting mucilaginous, thick and straw colored homogenate was lyophilized.

***Curcuma longa* L.**—*C. longa* rhizomes were powdered and macerated in 95% (w/v) ethanol for 48 h using Soxhlet apparatus. The mixture was then

filtered using a 0.2 µm filter (SRL, India.) and concentrated to dark yellow residue in rotary evaporator.

***Ocimum sanctum* L.**—Air dried *O. sanctum* leaves were powdered at room temperature and aqueous extract was made in distilled water after adequate stirring for 24 h, the mixture was centrifuged and filtered. The supernatant was collected and lyophilized¹⁹.

All extracts were stored in dry sterilized small containers at 4°C until further use. Each extract was reconstituted daily in saline water at room temperature and used for supplementation.

100 ppm fluoride (F⁻) water—The 100 ppm fluoride water was prepared by dissolving 2.21 g of sodium fluoride in 500 ml of tap water (<1ppm F⁻) and volume was made up to 1 liter. This solution was 1000 ppm F⁻, which was diluted 10 times to achieve 100 ppm F⁻ water.

Design—The pregnant rats were assorted into 2 groups and were given following treatment throughout the study. Group I (n=6), allowed to drink tap water orally and Group II (n=24) allowed to drink 100 ppm F⁻ water *ad libitum* orally during gestation and post gestation. After parturition, fluoride exposed animals (mother along with pups) were further divided into four subgroups of 6 each. The fluoride treatment to mother rats was continued and the pups born to them were orally administered daily with reconstituted freshly prepared extracts by gavage (single administration) for 21 (postnatal) consecutive days at following doses:

Group II A: No treatment

Group II B: *A. vera* (300 mg/kg body weight /day)

Group II C: *C. longa* (100 mg/kg body weight /day)

Group II D: *O. sanctum* (250 mg/kg body weight /day)

Group I and Group II A pups were administered with saline water to simulate the physical stress of plant extract administration.

At the end of 21st postnatal day, pups from each group were randomly pooled, sacrificed and the discrete regions of CNS viz. cerebral cortex, medulla oblongata, cerebellum and spinal cord were separated and used for the determination of fluoride and oxidative stress markers. In addition, the average rate of feed and water consumption (per day) of all mother

rats and body weight of all pups on autopsy day was recorded, and the organ somatic index (OSI) was calculated using the following formula:

$$\text{OSI} = \frac{\text{weight of the organ}}{\text{weight of the body}} \times 100$$

Chemicals —Epinephrine and 5,5'-Dithiobis 2-nitrobenzoic acid (Ellman's reagent) procured from M/s Sigma Chemicals USA, and other AR grade chemicals from M/s Merck Ltd were used for the assay.

Biochemical assays —Fluoride (F⁻) was analyzed by the ion selective electrode method as described by Inkielewicz *et al.*,²⁰ and the oxidative stress markers viz., lipid peroxidation (LPO)^{21,22}, catalase (CAT)²³, superoxide dismutase (SOD)²⁴, glutathione peroxidase (GSH-Px)²⁵, glutathione-S-transferase (GST)²⁶, and glutathione (GSH)²⁷ were assayed spectrophotometrically. Protein was estimated as per Lowry *et al.*²⁸.

Statistical analysis— Results are expressed as mean \pm SE of 6 observations. Data compilation was carried out using SPSS 15.0 software by employing one way analysis of variance followed by Bonferroni's post hoc test to compare means between the different treatment groups and *P* values <0.05 were considered statistically significant.

Results

Maternal exposure of fluoride resulted in remarkable alterations on the antioxidant homeostasis, confirming the adverse effects of fluoride on developing CNS of rat pups. The rate of feed and water consumption of all the fluoride treated mother rat groups decreased significantly (*P*<0.001) in comparison to control group (Table 1). The body weight on autopsy day of all the fluoride exposed pups decreased (1.12-fold) significantly (*P*<0.001) and OSI was also decreased (1.07-fold) with less significance (*P*<0.227) (Table 2).

Exposure of fluoride to the developing pups resulted in accumulation of fluoride and as a consequence, an increased level of LPO was observed. Whereas catalase, SOD, GSH-Px, GST and GSH activity/levels were decreased in cerebral cortex, medulla oblongata, cerebellum and spinal cord (Table 3).

Region wise spinal cord was highly affected during fluoride exposure in terms of altered levels of SOD (1.54-fold), GSH-Px (1.34-fold), GST (1.59-fold) and GSH (1.65-fold). Similarly, cerebellum with decreased catalase (1.37-fold) and medulla oblongata with increased LPO (6.19-fold) were the other affected regions on fluoride exposure.

The administration of *A. vera*, *C. longa* and *O. sanctum* to fluoride exposed pups significantly reduced the toxic risk of fluoride by exhibiting restoration. In cerebral cortex *O. sanctum* was found to be effective in restoring the altered activities of all the parameters except catalase, where *A. vera* was effective. In medulla oblongata *O. sanctum* was found to be effective in all the parameters except LPO, where *A. vera* was effective, and in case of SOD *O. sanctum* and *C. longa* were equally effective. In cerebellum *O. sanctum* was found to be effective on all the parameters except fluoride, where *A. vera* was effective. In spinal cord *O. sanctum* was found to be effective on all the parameters, except LPO, where *A. vera* was effective in offering amelioration.

Table 1—Average feed / water consumption by the control and fluoride exposed pregnant (mother) rats

[Values are mean \pm SE of 6 observations. Figures in parenthesis are % over controls.]

	Feed consumption (g feed/animal/day)	Water consumption (ml water/animal/day)
Control	20.36 \pm 0.80	25.24 \pm 0.55
Fluoride (F ⁻)	16.51 \pm 0.56 (-18.91) ^b	21.38 \pm 0.53 (-15.32) ^a
F Variance	15.313	26.650

P values ^a < 0.001, ^b < 0.01 compared to control.

Table 2— Amelioration of phytoextracts on body weight and organ somatic index of developing rat during fluoride exposure.

[Values are mean \pm SE of 6 observations. Figures in parenthesis are % over controls]

	Body weight (g)	Organ somatic index
Control	18.43 \pm 0.13	5.99 \pm 0.09
Fluoride (F)	16.45 \pm 0.13 (-10.75) ^a	5.58 \pm 0.09 (-10.75)
F + <i>A. vera</i>	17.51 \pm 0.07 (-5.00) ^{ad}	5.61 \pm 0.13 (-5.00)
F + <i>C. longa</i>	17.37 \pm 0.09 (-5.73) ^{ad}	5.65 \pm 0.16 (-5.73)
F + <i>O. sanctum</i>	17.82 \pm 0.04 (-3.30) ^{ad}	5.71 \pm 0.16 (-3.30)
F Variance	54.904	1.518

P values ^a < 0.001 compared to control, ^d < 0.001 compared to fluoride group.

Table 3—Amelioration of phytoextracts on fluoride levels and oxidative stress markers in cerebral cortex of developing rat during fluoride exposure.

[Values are mean \pm SE of 6 observations each. Figures in parenthesis are of % increase(+) or decrease(-) over control]

Group	Fluoride (μg of fluoride / g tissue)	LPO (n moles of MDA / mg tissue)	CAT (μ moles / min / mg protein)	SOD (μ moles / min / mg protein)	GSH-Px (μ moles / min / mg protein)	GST (n moles GSH-CDNB formed/mg protein/min)	GSH (mg of GSH / 100g of tissue)
Cerebral cortex							
Control	0.64 \pm 0.01	1.93 \pm 0.02	125.77 \pm 4.69	4.66 \pm 0.14	3.43 \pm 0.04	85.59 \pm 2.33	49.29 \pm 1.69
Fluoride (F)	2.14 \pm 0.02 ^a (+236)	2.31 \pm 0.01 ^a (+19.78)	105.69 \pm 5.26 ^c (-15.45)	3.26 \pm 0.04 ^{ad} (-30.00)	2.73 \pm 0.06 ^{ad} (-20.39)	57.24 \pm 3.32 ^a (-33.12)	35.31 \pm 1.32 ^a (-28.36)
F + <i>A. vera</i>	1.97 \pm 0.03 ^{ad} (+208)	2.10 \pm 0.02 ^{ad} (+8.89)	123.39 \pm 4.56 (-1.90)	3.98 \pm 0.06 ^{ad} (-14.69)	3.08 \pm 0.02 ^{ad} (-10.44)	71.07 \pm 1.52 ^{bd} (-16.97)	40.54 \pm 1.55 ^{bd} (-17.74)
F + <i>C. longa</i>	2.06 \pm 0.02 ^a (+223)	2.22 \pm 0.03 ^a (+15.20)	121.34 \pm 3.27 (-3.52)	4.01 \pm 0.08 ^{ad} (-13.94)	3.13 \pm 0.02 ^{ad} (-8.79)	69.21 \pm 2.32 ^{af} (-19.14)	40.63 \pm 0.86 ^b (-17.57)
F + <i>O. sanctum</i>	1.94 \pm 0.03 ^a (+204)	2.09 \pm 0.04 ^{bd} (+8.20)	120.73 \pm 4.15 (-4.01)	4.18 \pm 0.04 ^{bd} (-10.30)	3.28 \pm 0.04 ^d (-4.51)	72.67 \pm 2.23 ^{be} (-15.09)	41.16 \pm 1.51 ^b (-16.49)
F Variance	699.503	33.192	3.177	38.389	45.613	17.510	12.572
Medulla oblongata							
Control	0.40 \pm 0.02	1.61 \pm 0.02	119.36 \pm 5.63	3.56 \pm 0.12	2.55 \pm 0.06	85.65 \pm 2.48	42.35 \pm 1.80
Fluoride (F)	1.91 \pm 0.13 ^a (+382)	2.15 \pm 0.05 ^a (+33.44)	94.68 \pm 2.37 ^a (-20.68)	3.04 \pm 0.03 ^a (-14.61)	2.15 \pm 0.03 ^a (-15.75)	58.27 \pm 1.95 ^a (-31.96)	26.38 \pm 1.22 ^a (-37.70)
F + <i>A. vera</i>	1.88 \pm 0.03 ^a (+375)	1.96 \pm 0.05 ^{af} (+21.64)	106.07 \pm 2.15 (-11.13)	3.29 \pm 0.08 (-7.63)	2.39 \pm 0.04 (-6.21)	70.59 \pm 1.32 ^{ae} (-17.58)	35.64 \pm 1.70 ^{ce} (-15.85)
F + <i>C. longa</i>	1.81 \pm 0.03 ^a (+359)	1.91 \pm 0.03 ^{ae} (+18.84)	103.99 \pm 2.04 ^c (-12.88)	3.32 \pm 0.06 (-6.79)	2.43 \pm 0.05 ^d (-4.77)	68.99 \pm 1.98 ^{ae} (-19.45)	35.91 \pm 0.83 ^{cd} (-15.22)
F + <i>O. sanctum</i>	1.74 \pm 0.03 ^a (+340)	1.98 \pm 0.04 ^a (+23.19)	110.66 \pm 3.42 ^f (-7.29)	3.33 \pm 0.03 (-6.32)	2.47 \pm 0.02 ^d (-3.20)	71.38 \pm 1.64 ^{ad} (-16.65)	36.30 \pm 1.49 ^d (-14.29)
F Variance	103.510	25.170	7.094	6.448	12.587	26.050	15.520
Cerebellum							
Control	0.27 \pm 0.01	1.94 \pm 0.05	125.21 \pm 2.14	3.31 \pm 0.04	2.46 \pm 0.04	82.93 \pm 2.28	42.48 \pm 0.88
Fluoride (F)	1.67 \pm 0.05 ^a (+523)	2.24 \pm 0.08 ^b (+15.11)	91.23 \pm 1.87 ^a (-27.14)	2.86 \pm 0.06 ^a (-13.68)	2.08 \pm 0.05 ^a (-15.40)	59.76 \pm 1.57 ^a (-27.93)	27.76 \pm 1.45 ^a (-34.67)
F + <i>A. vera</i>	1.54 \pm 0.07 ^a (+472)	2.13 \pm 0.04 (+9.79)	101.93 \pm 2.32 ^{af} (-18.50)	3.07 \pm 0.05 ^c (-7.49)	2.32 \pm 0.03 ^c (-5.70)	70.55 \pm 1.18 ^{ad} (-14.71)	36.52 \pm 1.30 ^f (-14.05)
F + <i>C. longa</i>	1.58 \pm 0.03 ^a (+488)	2.18 \pm 0.03 ^c (+12.10)	102.79 \pm 2.34 ^{af} (-17.91)	3.09 \pm 0.06 (-6.79)	2.30 \pm 0.02 ^c (-6.31)	68.70 \pm 1.17 ^{ae} (-17.15)	37.57 \pm 1.79 ^e (-11.56)
F + <i>O. sanctum</i>	1.57 \pm 0.03 ^a (+483)	2.12 \pm 0.02 (+8.93)	106.36 \pm 2.85 ^{ae} (-15.06)	3.17 \pm 0.07 ^d (-4.43)	2.36 \pm 0.04 ^d (-4.07)	72.04 \pm 1.34 ^{ad} (-13.13)	38.75 \pm 2.31 ^e (-8.79)
F Variance	216.044	4.714	28.345	8.808	13.384	28.179	10.178
Spinal cord							
Control	0.40 \pm 0.06	1.25 \pm 0.03	126.24 \pm 3.38	2.49 \pm 0.05	2.24 \pm 0.03	67.50 \pm 1.73	38.81 \pm 1.31
Fluoride (F)	1.80 \pm 0.05 ^a (+353)	1.64 \pm 0.09 ^b (+30.72)	96.25 \pm 1.35 ^a (-23.75)	1.62 \pm 0.04 ^a (-35.23)	1.67 \pm 0.04 ^a (-25.21)	42.40 \pm 1.21 ^a (-37.19)	23.46 \pm 1.23 ^a (-39.55)
F + <i>A. vera</i>	1.69 \pm 0.03 ^a (+326)	1.35 \pm 0.06 (+7.98)	106.40 \pm 3.31 ^a (-15.72)	2.13 \pm 0.04 ^{ad} (-14.71)	2.01 \pm 0.02 ^{ad} (-10.07)	57.57 \pm 2.12 ^{bd} (-14.71)	33.30 \pm 1.81 ^d (-14.21)
F + <i>C. longa</i>	1.76 \pm 0.07 ^a (+342)	1.41 \pm 0.08 (+12.10)	109.76 \pm 3.18 ^{ad} (-13.05)	2.14 \pm 0.03 ^{ad} (-14.04)	1.97 \pm 0.04 ^{ad} (-11.78)	60.83 \pm 2.00 ^d (-9.89)	34.24 \pm 1.37 ^d (-11.78)
F + <i>O. sanctum</i>	1.68 \pm 0.05 ^a (+323)	1.36 \pm 0.07 (+8.28)	115.06 \pm 2.26 ^{bc} (-8.86)	2.19 \pm 0.04 ^{ad} (-12.10)	2.07 \pm 0.03 ^{bd} (-7.46)	60.26 \pm 1.76 ^d (-10.74)	33.68 \pm 1.26 ^d (-13.23)
F Variance	107.601	4.321	15.474	63.650	43.446	26.923	15.851

P values ^a < 0.001, ^b < 0.01, ^c < 0.05 compared to control, ^d < 0.001, ^e < 0.01, ^f < 0.05 compared to fluoride group

Discussion

Feed/water consumption and body weight/organ somatic index — The animals exposed to fluoride showed significant decrease in body weight followed by considerable decrease in the rate of feed and water consumption. This could be attributed to atrophic gastritis and poor gastrointestinal absorption produced by fluoride ingestion, which may contribute to decreased food intake in experimental animals²⁹. Results also indicate suppressed appetite and disturbed nutrient digestibility that can eventually lead to excessive breakdown of cellular macromolecules causing weight loss. The decrease in OSI may be due to weight loss, degeneration of organs³⁰, and altered antioxidant system, which may be prime factor in causing fluoride toxicity. Supplementations of phytoextracts in the present study improved food intake, body weight and organ somatic index.

Fluoride —Fluoride exposure resulted in marked accumulation of fluoride in the discrete regions of CNS of pups. The accumulation of fluoride is natural due to its chronic exposure, as the exposure may commence in the maternal blood passing through the placenta to the fetus and continues during infancy through fluoride containing milk and drinking water. Moreover the immaturity of excretory or enzymatic systems in developing animals may favor the accumulation. The pups treated with phytoextracts have shown considerable reduction in fluoride levels; this may be attributed to the supplemented phytoextracts in restoring the antioxidant homeostasis to a considerable extent which, in turn may favour the elimination of fluoride from cells.

Oxidative stress markers—Fluoride exposure significantly exacerbated the levels of LPO along with the decreased activities of antioxidant enzymes like SOD, CAT, GSH-Px, GST and GSH levels in discrete regions of CNS suggestive of oxidative stress. The results observed were in agreement with the previous findings^{3,5,7,9,31}. The reactive oxygen species (ROS) formed exceeded the antioxidant capacity of a cell, which were highly toxic, react with proteins, enzymes and nucleic acids and may lead to the cell death via apoptosis or necrosis³². Further, the oxidative stress not only increases free radical injury but also enhances excitotoxicity, since ROS, reactive nitrogen species and lipid peroxidation products can trigger the excitotoxic process documented both in animal and human studies^{4,33,34}.

Region specific alterations—The developing CNS while undergoing cell differentiation and migration, synaptogenesis and subsequent pruning of synaptic tree in response to ongoing neuronal activity may be selectively affected by exposure to fluoride. The significant accumulation of fluoride ($P<0.001$) in various parts of CNS, especially in cerebellum and spinal cord caused metabolic perturbations at subcellular level, which may be due to unprotected role of blood brain barrier. Cerebellum and spinal cord appear to be more susceptible to oxidative damage than cerebral cortex and medulla oblongata as they possess low endogenous levels of vitamin E, differences in iron content, oxygen consumption rate, cell types, functions etc. Further these areas are vulnerable to sensory function deficits, motor disturbances and adverse effects on cognitive functions, such as learning and memory, indicative of brain dysfunction on experimental fluorosis. Although there are differences among regions in response to fluoride exposure that cannot be explained through a simple model, a potential role of a number of elements as biomarkers in this kind of neuronal alterations cannot be excluded³⁵.

Phyto extract treatment— Administration of natural plant extracts to fluoride intoxicated pups alleviated the altered cascade of free radical damage / oxidative stress.

Aloe vera: A remarkable recovery was observed with *A. vera* supplementation. This may be due to composition of three aloesin derivatives from aloe (isorabaichromone, feruoylaloetin, and p-coumaroylaloetin) that showed potent free radical and superoxide anion-scavenging activities¹⁸. The flavonoids in the *A. vera* extract may also protect ascorbate from degradation in the intestinal tract and then *in vivo*³⁶. *Aloe* is unique in its ability to improve the absorption of natural antioxidants like vitamin C and vitamin E³⁷ and its supplementation in present study enhanced antioxidant status to alleviate the fluoride mediated ROS.

Curcuma longa: A distinctive recovery was observed with *C. longa*. *C. longa* contains curcumin (diferuloylmethane— a polyphenolic substance), that possess the structure of two electrophilic α and β -unsaturated carbonyl groups, which by virtue of Michael reaction can react with nucleophiles, such as glutathione. In addition, curcumin and its derivatives act as chain-breaking antioxidants rather than as direct radical scavengers. In curcumin, the phenolic and the methoxy group on the phenyl ring and the

1,3-diketone system seems to be important structural features that can contribute to play a major role in the antioxidant defense. The diketone system is a potent ligand for metals such as iron¹⁷, which is vital because free iron, more than any other transition metal is implicated in undergoing redox transition *in vivo* with the consequent generation of ROS. The presence of ferrous and ferric ions causes a series of radical reactions, via the Fenton reaction leading to the formation of the hydroxyl radical (OH[•])³⁸. Further, curcumin has the potential to inhibit lipid peroxidation and effectively intercept and neutralize ROS, such as hydroxyl radical, superoxide radical, and singlet oxygen and NO-based free radicals³⁹. Thus the supplementation of *C. longa* duly eliminates fluoride generated ROS.

Ocimum sanctum: A substantial recovery was observed on supplementation with *O. sanctum*. *O. sanctum* contains six phenolic compounds including eugenol, rosmarinic acid, apigenin and three other flavonoids, which showed an excellent antioxidant activity *in vitro*¹⁵. Further the flavonoids mainly orientin and vicetin of *O. sanctum* revealed their *in vitro* antioxidant activity also working under *in vivo* conditions⁴⁰. These phytochemicals would quench the reactive intermediates and radical species rendered during fluoride exposure and constituents helping to keep normalcy of enzymic and non-enzymic antioxidants.

In conclusion, the results suggest that the fluoride intoxication to mother rats exert effects on pups through exacerbated oxidative stress and alter the cellular process. The developing animals no longer prevent the oxidative stress and are more prone to oxidative damage. The supplementation of phytoextracts concomitantly restored toxic effects to near normal levels by augmenting the antioxidant defense through scavenging activity and provides an evidence of having therapeutic role in free radical mediated nervous system disorders including neuronal fluorosis. Among the plant extracts supplemented, the *O. sanctum* was found to be more effective.

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