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Selenium and alpha-tocopherol reduces fluoride induced oxidative stress in brain and muscle of mice

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ABSTRACT

Fluoride is one of the common environmental pollutants. Its excessive exposure results in a wide array of toxicity phenotypes including oxidative stress, skeletal and soft tissue damage etc. Antioxidants such as Selenium (Se) and α-tocopherol are attractive agents for oxidative stress prevention because of their safety profile and wide availability. It is known that in combination, Se and alpha-tocopherol act synergistically against ROS formation. This study investigated the protective effects of selenium (05 μg/kg BW) and Alpha-tocopherol (2 mg/kg BW) on markers of oxidative stress in brain and muscle of mice exposed to sodium fluoride (20mg/kg BW) for 15 days. The results showed significant ($p<0.05$) alterations in markers of oxidative stress includes; an increase in xanthine oxidase activity and lipid peroxidation, a decline in SOD, CAT, GST and GPx activities in fluoride exposure group in comparison with control group indicates oxidative stress induced by fluoride. These changes were reversed modestly in Se and alpha-tocopherol alone treated groups and significantly ($p<0.05$) in the combinedly treated group indicating synergistic action in mitigation of fluoride effect.

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INTRODUCTION

Fluoride is one of the widespread environmental pollutants, especially in industrial areas (Sun *et al.*, 2017). It becomes a known reason for severe water contamination due to its highly electronegative nature and prevalence (Sharma *et al.*, 2017). Ingesting fluoride from drinking water is the most common way of fluoride toxicity. Hence, WHO suggests fluoride level should be below 1.5 mg/L in drinking water to prevent various disorders (Sun *et al.*, 2016). Its harmful or beneficial effects depend on the concentration of fluoride in the body (Sun *et al.*, 2017). Exorbitant fluoride ingestion, mostly through the drinking water, may have detrimental impacts on teeth, bone, heart, kidney, liver and brain (Ma *et al.*, 2017; Sun *et al.*, 2017). Available data suggest that excessive fluoride exposure leads to its accumulation in different parts of the brain and gastrocnemius muscle and contribute to disturbed oxygen metabolism and ROS formation, thereby induces oxidative stress, which in turn, alters the phospholipids metabolism leading to muscular and neuronal damage and death (Mesram *et al.*, 2017; Nalagoni and Karnati, 2016). In addition to stimulating oxidative stress, fluoride also reduces the antioxidant potential of various organs by decreasing the activity of antioxidant enzymes (Bhatnagar *et al.*, 2014).

Dietary supplements trap the reactive oxygen species (Jiang, 2014; Ju *et al.*, 2010) and thereby causing an increase in antioxidant vitamin levels (Batista *et al.*, 2016). Therefore, the importance

of dietary supplementation in boosting up the antioxidative defence has been attracted for decades. Selenium (Se) is a non-metallic trace element known as a nutrient essential to human health (Klein *et al.*, 2003). It regulates the synthesis of selenoproteins. Selenoproteins involve in antioxidant defences by acting as a cofactor for the antioxidant enzymes to prevent oxidative injury (Nazıroglu, 2009; Cardoso *et al.*, 2014).

Further, it has been suggested as a neuroprotectant in animal models of Alzheimer's disease (Lakshmi *et al.*, 2015). Alpha-tocopherol acts as a major lipid-soluble antioxidant in cell membranes. It specifically inhibits lipid peroxidation by scavenging various types of free radicals (Uneri *et al.*, 2006). Several studies reported a decrease in oxidative stress by individual and combined antioxidant supplementation (Block *et al.*, 2008; Devaraj *et al.*, 2008). Supplementation with selenium, vitamin C, and vitamin E effectively improved antioxidant–oxidant balance in obese children and adolescents (Murer *et al.*, 2014). It is known that in combination, Se and α-tocopherol act synergistically against the formation of reactive oxygen species (Pak *et al.*, 2002). The above information prompted us to examine the antioxidant capacity of Se and alpha-tocopherol. Thus, in this study, we intended to explore the protective effects of selenium and alpha-tocopherol individually and combinedly on oxidative stress in the brain and gastrocnemius muscle of experimental animals.

MATERIALS AND METHODS

Chemicals

Selenium and sodium fluoride from Loba Chemie and alpha-tocopherol from Merck Company were purchased.

Animal maintenance

Swiss albino mice (30$\pm$2g) were purchased from NIFLA, NIN, Hyderabad, India. The animals were housed under standard conditions (22$\pm$2^{o}C, 12 hrs light-dark cycle, pellet diet and water).

Experimental design

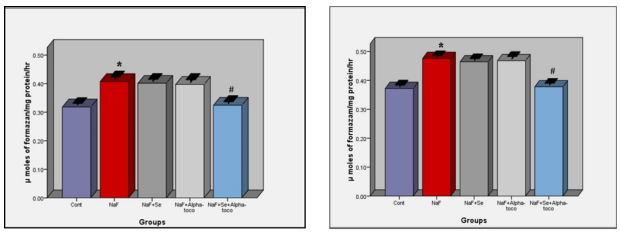
The animals were divided into five groups (6 animals each). Group-1: Saline, Group-2: NaF (20mg/ kg BW), Group-3: Selenium (5 μg/ kg BW), Group-4: Alpha-tocopherol (2 mg/kg BW) Group-5: NaF+Se+alpha-tocopherol (20 mg/kg BW+5 μg/kg BW+2mg/kg BW). The doses were given daily between 8.15 am–9.15 am for fifteen days. All the experiments were conducted as per the institutional ethical committee guidelines (No: 383/01/a/CPCSEA).

Biochemical assays

The isolated tissues were homogenised, and the supernatant was collected for biochemical studies. XOD activity was measured as described by (Govindappa and Swami, 1966). The results were expressed as μ moles of formazan formed/mg protein/hr. Lipid peroxide formation was measured by using Wills method (Wills, 1966). MDA is the end product of peroxidised polyunsaturated fatty acids. The result was expressed as nmol of MDA/mg protein/3mins. SOD activity was analysed as described by the (Kono, 1978). The results were expressed in units (U/mg protein). Catalase activity was assayed as described by (Luck, 1971). The result was expressed as μMol of H_2O_2 decomposed/min/mg/protein. The GST activity was assayed as described by (Habig *et al.*, 1974). The results were expressed as n mole of GS-CDNB/mg protein/min. Glutathione peroxidase activity was measured as described by (Lawrence and Burk, 1976). The result was expressed as μ moles of NADPH oxidised/min/mg/protein.

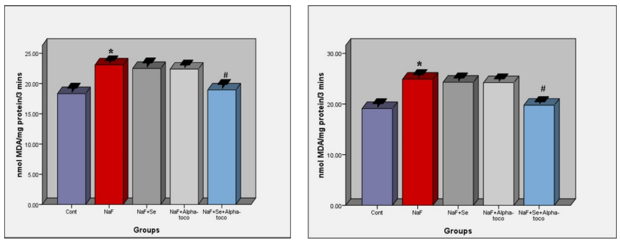
Statistical analysis

The data were analysed using one-way ANOVA, followed by Tukey's studentised range test (HSD). The significant level was considered at p=0.05.



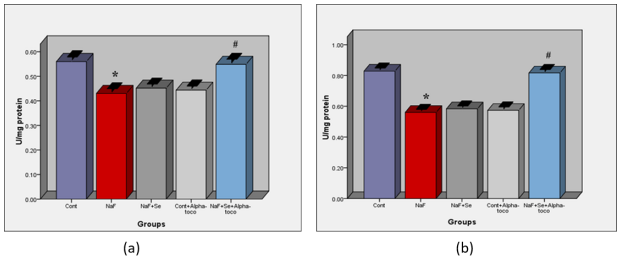
(a) (b)

Figure 1: Se and α-tocopherol effect on XOD activity of the brain (a) and muscle (b) in mice subjected to NaF. Values are means$\pm$SEM. *(p<0.05)versus control, # (p<0.05) versus NaF.



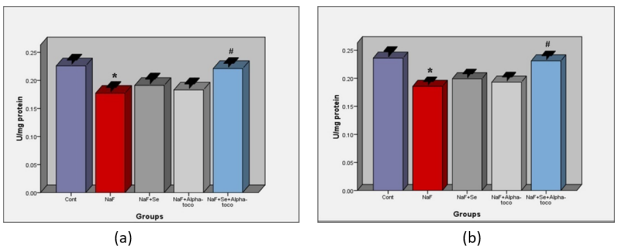
(a) (b)

Figure 2: Se and α-tocopherol effect on MDA content of the brain (a) and muscle (b) in mice subjected to NaF. Values are means$\pm$SEM. *(p<0.05) versus control, # (p<0.05) versus NaF



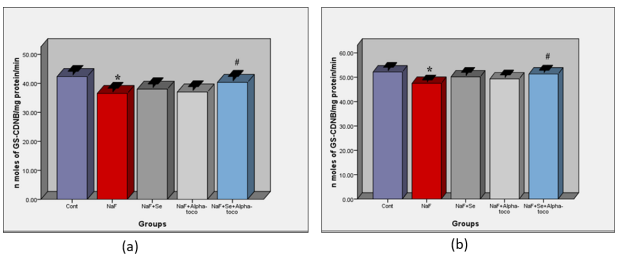
(a) (b)

Figure 3: Se and α-tocopherol effect on SOD activity of the brain (a) and muscle (b) in mice subjected to NaF. Values are means±SEM. *(p<0.05)versus control, # (p<0.05) versus NaF.



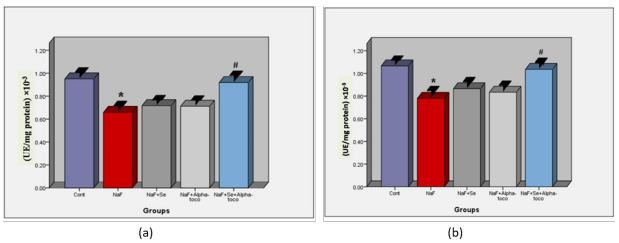
(a) (b)

Figure 4: Se and α-tocopherol effect on CAT activity of the brain (a) and muscle (b) in mice subjected to NaF. Values are means±SEM. *(p<0.05)versus control, # (p<0.05) versus NaF.



(a) (b)

Figure 5: Se and α-tocopherol effect on GST activity of the brain (a) and muscle (b) in mice subjected to NaF. Values are means±SEM. *(p<0.05)versus control, # (p<0.05) versus NaF.



(a) (b)

Figure 6: Se and α-tocopherol effect on GPx activity of the brain (a) and muscle (b) in mice subjected to NaF. Values are means±SEM. *(p<0.05)versus control, # (p<0.05) versus NaF.

RESULTS AND DISCUSSION

Xanthine oxidase activity

The XOD activity was significantly (p<0.05) enhanced in the brain (27.95%) and muscle (27.98%) in the fluoride group in comparison with the control group. In contrast, it was recovered partially in NaF+selenium, NaF+α-tocopherol and significantly (p<0.05) in NaF+Selenium+ α-tocopherol treated groups with respect of 24.73%, 25.80% and 1.61% in the brain and 26.10%, 24.84% and 1.88% in muscle indicates synergistic effects of Se and α-tocopherol (Figure 1).

Lipid peroxidation

The MDA content was significantly (p<0.05) enhanced in the brain (25.96%) and muscle (30.36%) in the fluoride group in comparison with the control group. In contrast, it was recovered partially in NaF+selenium, NaF+ α-tocopherol and significantly (p<0.05) in NaF+Selenium+α-tocopherol treated groups with respect of 22.69%, 22.09% and 3.32% in brain and 27.17%, 26.70% and 3.45% in muscle indicates synergistic effects of Se and α-tocopherol (Figure 2).

Superoxide dismutase (SOD)

The SOD activity was significantly (p<0.05) decreased in the brain (23.21%) and muscle (32.39%) in the fluoride group in comparison with the control group. In contrast, it was recovered partially in NaF+Selenium, NaF+ α-tocopherol and significantly (p<0.05) in NaF+Selenium+α-tocopherol treated groups with respect of 19.46%, 20.89% and 2.14% in brain and 29.57%, 30.78% and 1.40% in muscle indicates synergistic effects of Se and α-tocopherol (Figure 3).

Catalase (CAT)

The catalase activity was significantly (p<0.05) decreased in the brain (21.61%) and muscle (21.68%) in the fluoride group in comparison with the control group. In contrast, it was recovered partially in NaF+selenium, NaF+ α-tocopherol and significantly (p<0.05) in NaF+Selenium+α-tocopherol treated groups with respect of 15.67%, 18.22% and 2.11% in brain and 15.48%, 19.02% and 2.21% in muscle indicates synergistic effects of Se and α-tocopherol (Figure 4).

Glutathione-S-Transferase (GST)

The GST activity was significantly (p<0.05) decreased in the brain (8.93%) and muscle (13.77%) in the fluoride group in comparison with the control group. In contrast, it was recovered partially in NaF+Selenium, NaF+ α-tocopherol

and significantly (p<0.05) in NaF+Selenium+α-tocopherol treated groups with respect of 3.83%, 5.42% and 1.59% in brain and 10.22%, 12.59% and 4.72% in muscle indicates synergistic effects of Se and α-tocopherol (Figure 5).

Glutathione peroxidase (GPx)

The GPx activity was significantly (p<0.05) declined in the brain (26.82%) and muscle (30.73%) in the fluoride group in comparison with the control group. In contrast, it was recovered partially in NaF+selenium, NaF+ α-tocopherol and significantly (p<0.05) in NaF+Selenium+α-tocopherol treated groups with respect of 18.85%, 21.85% and 2.91% in brain and 24.63%, 25.15% and 3.36% in muscle indicates synergistic effects of Se and α-tocopherol (Figure 6).

Fluoride is an irresistible environmental toxic element. Its excessive exposure induces several adverse effects on human health (Meenakshi and Maheshwari, 2006). Ample of studies on animals and humans chronically exposed to fluoride reported vulnerability of all soft tissue organs (Bhatnagar *et al.*, 2006). Fluoride accumulation in the brain and muscle of mice induces stress and prevents oxidative defence mechanisms, thereby resulting in oxidative stress-induced neuronal and muscular tissues damage (Vani and Reddy, 2000). Fluoride, a known neurotoxic agent, affects brain function and development (Ge *et al.*, 2018; McPherson *et al.*, 2018). Repeated exposure to fluoride induces pathological lesions in the brain of experimental animals (Adedara *et al.*, 2017; Khan *et al.*, 2018). This study is reporting the antioxidant effects of Se and α-tocopherol on oxidative stress in the brain and muscle of experimental mice models.

It is well known that fluoride toxicity induces ROS production (Apel and Hirt, 2004). ROS includes hydrogen peroxide, superoxide and hydroxyl radicals etc. These free radicals are mainly generated by redox reactions catalysed by xanthine oxidase (XO), flavin oxidase, NADPH oxidase (NOX), cytochrome P450, and also by respiratory chain components in mitochondria (Dasuri *et al.*, 2013). In the present study, the increased xanthine oxidase activity (p<0.05) in the brain and muscle of fluoride exposure group in comparison with the control group confirms the increased ROS production by fluoride toxicity. The increased ROS production leads to lipid peroxidation, which is indicated by increased malondialdehyde content (Farmer and Mueller, 2013; Oyagbemi *et al.*, 2020). In the current study, the increased MDA content (p<0.05) in the brain and muscle of NaF group in comparison with the control group indicates the lipid peroxidation induced by increased reactive oxygen species. The increased ROS level reduces the activity of the antioxidant enzymes. Antioxidant enzymes include SOD, CAT, GST, GPx, GSH, etc., play a key role in the elimination of ROS, thereby decreasing oxidative stress. SOD and CAT are blockers of free radicals generated by toxic substances. SOD plays a crucial role in the first line of defence. It dismutases the peroxides into H_20_2 and O_2. Catalase removes H_2O_2 by converting it into O_2 and H_2O. In the present study, SOD and catalase activities were significantly (p<0.05) decreased in muscle and brain in NaF treated group in comparison with the control group. The results are following earlier studies which reported declined SOD and CAT activities in the gastrocnemius muscle, liver, heart, kidney and brain in fluoridated animals (Nalagoni and Karnati, 2016; Oyagbemi *et al.*, 2020) and people living in endemic fluoride areas (Li and Cao, 1994). GST and GPx activities were significantly (p<0.05) decreased in brain and muscle in fluoride intoxicated group in comparison with the control group. The decline in their activities could be a result of their augmented utilisation to purge hydrogen peroxide and organic hydroperoxides from both the tissues. The present study results conform with the previous studies where fluoride inhibits the activity of the antioxidant enzymes includes SOD, CAT, GPx, GST and GR (Sarkar *et al.*, 2014; Oyagbemi *et al.*, 2020). However, the Se and Alpha-tocopherol combination significantly (p<0.05) declined the elevated MDA Content, inhibited xanthine oxidase activity and restored the activities of antioxidant enzymes such as SOD, CAT, GPx, and GST than individual administration. The protective effects of Se and alpha-tocopherol could be due to the trapping free radicals and restoring the antioxidant defence (Jiang, 2014; Meydani *et al.*, 1988). Collectively, the results indicating that the muscle was more prone to the fluoride toxicity than the brain, which may be due to the protective role of the blood-brain barrier and differential sensitivity of various organs to the fluoride toxicity. However, Se and alpha-tocopherol synergistically mitigated the oxidative stress in the brain and muscle in fluoridated animals.

CONCLUSIONS

In conclusion, the fluoride exposure resulted in a significant increase in XOD activity, enhanced LPO and a decline in the antioxidant enzymes (SOD, CAT, GST and GPx) activity, thereby increasing the susceptibility of brain and muscle to imminent damage with potent free radicals. But, the oxidative damage was more in the muscle than the brain. In combination, selenium and alpha-tocopherol significantly miti-

gated the oxidative stress and restored the activities of antioxidant enzymes than individual administration. Based on these findings, this study reports that the selenium and alpha-tocopherol, in combination, effectively mitigated the fluoride-induced oxidative stress in mice models. Further studies are needed to elucidate a specific mechanism.

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Conflicts of interest

The authors declare that they have no conflict of interest for this study.

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REFERENCES

Adedara, I. A., Abolaji, A. O., Idris, U. F., Olabiyi, B. F., Onibiyo, E. M., Ojuade, T. D., Farombi, E. O. 2017. Neuroprotective influence of taurine on fluoride-induced biochemical and behavioral deficits in rats. *Chemico-Biological Interactions*, 261:1–10.

Apel, K., Hirt, H. 2004. Reactive Oxygen Species: Metabolism, Oxidative Stress, and Signal Transduction. *Annual Review of Plant Biology*, 55(1):373–399.

Batista, E. D., Andretta, A., de Miranda, R. C., Nehring, J., dos Santos Paiva, E., Schieferdecker, M. E. M. 2016. Food intake assessment and quality of life in women with fibromyalgia. *Revista Brasileira de Reumatologia (English Edition)*, 56(2):105–110.

Bhatnagar, M., Rao, P., Saxena 2006. Biochemical changes in the brain and other tissues of young adult female mice from fluoride in their drinking water. *Fluoride*, 39(4):280–284.

Bhatnagar, M., Sharma, C., Suhalka, P., Sukhwal, P., Jaiswal, N. 2014. Curcumin attenuates neurotoxicity induced by fluoride: An in vivoevidence. *Pharmacognosy Magazine*, 10(37):61–61.

Block, G., Jensen, C. D., Morrow, J. D., Holland, N., Norkus, E. P. 2008. The effect of vitamins C and E on biomarkers of oxidative stress depends on baseline level. *Free Radical Biology and Medicine*, 45(4):377–384.

Cardoso, B. R., Bandeira, V. S., Jacob-Filho, W., Cozzolino, S. M. F. 2014. Selenium status in elderly: Relation to cognitive decline. *Journal of Trace Elements in Medicine and Biology*, 28(4):422–426.

Dasuri, K., Zhang, L., Keller, J. N. 2013. Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. *Free Radical Biology and Medicine*, 62:170–185.

Devaraj, S., Leonard, S., Traber, M. G., Jialal, I. 2008. Gamma-tocopherol supplementation alone and in combination with alpha-tocopherol alters biomarkers of oxidative stress and inflammation in subjects with metabolic syndrome. *Free Radical Biology and Medicine*, 44(6):1203–1208.

Farmer, E. E., Mueller, M. J. 2013. ROS-Mediated Lipid Peroxidation and RES-Activated Signaling. *Annual Review of Plant Biology*, 64(1):429–450.

Ge, Y., Chen, L., Yin, Z., Song, X., Ruan, T., Hua, L., Liu, J., Wang, J., Ning, H. 2018. Fluoride-induced alterations of synapse-related proteins in the cerebral cortex of ICR offspring mouse brain. *Chemosphere*, 201:874–883.

Govindappa, S., Swami, K. S. 1966. Electrophoretic Characteristics and Their Relationship to the Enzyme Activities of Subcellular Components in the Amphibian Muscle Fibre (1). *Archives Internationales de Physiologie et de Biochimie*, 74(2):259–269.

Habig, W. H., Pabst, M. J., Jakoby, W. D. 1974. Glutathione-S-transferases the first enzymatic step in mercapturic acid formation. *J. Biol. Cem*, 249(22):7130–7139.

Jiang, Q. 2014. Natural forms of vitamin E: Metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic Biol Med*, 72:76–90.

Ju, J., Picinich, S. C., Yang, Z., Zhao, Y., Suh, N., Kong, A.-N., Yang, C. S. 2010. Cancer-preventive activities of tocopherols and tocotrienols. *Carcinogenesis*, 31(4):533–542.

Khan, A. M., Raina, R., Dubey, N., Verma, P. K. 2018. Effect of deltamethrin and fluoride co-exposure on the brain antioxidant status and cholinesterase activity in Wistar rats. *Drug and Chemical Toxicology*, 41(2):123–127.

Klein, E. A., Thompson, I. M., Lippman, S. M., Goodman, P. J., Albanes, D., Taylor, P. R. 2003. Select: the selenium and vitamin E cancer prevention trial Urologic Oncology. *Seminars and Original Investigations*, 21(1):59–65.

Kono, Y. 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Arch Biochem Biophys*, 186(1):189–195.

Lakshmi, B. V. S., Sudhakar, M., Prakash, K. S. 2015.

Protective Effect of Selenium Against Aluminum Chloride-Induced Alzheimer's Disease: Behavioral and Biochemical Alterations in Rats. *Biological Trace Element Research*, 165(1):67–74.

Lawrence, R. A., Burk, R. F. 1976. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochemical and Biophysical Research Communications*, 71(4):952–958.

Li, J., Cao, S. 1994. Recent studies on endemic fluorosis in China. *Fluoride*, 27(3):125–128.

Luck, H. 1971. *Methods of enzymatic analysis*. Academic Press, New York.

Ma, Y., Zhang, K., Ren, F., Wang, J. 2017. Developmental fluoride exposure influenced rat's splenic development and cell cycle via disruption of the ERK signal pathway. *Chemosphere*, 187:173–180.

McPherson, C. A., Zhang, G., Gilliam, R., Brar, S. S., Wilson, R., Brix, A., Picut, C., Harry, G. J. 2018. An Evaluation of Neurotoxicity Following Fluoride Exposure from Gestational Through Adult Ages in Long-Evans Hooded Rats. *Neurotoxicity Research*, 34(4):781–798.

Meenakshi, Maheshwari, R. C. 2006. Fluoride in drinking water and its removal. *Journal of Hazardous Materials*, 137(1):456–463.

Mesram, N., Nagapuri, K., Banala, R. R., Nalagoni, C. R., Karnati, P. R. 2017. Quercetin treatment against NaF induced oxidative stress related neuronal and learning changes in developing rats. *Journal of King Saud University - Science*, 29(2):221–229.

Meydani, M., John, B., Macauley, Jeffrey, B. 1988. Blumberg Effect of Dietary Vitamin E and Selenium on Susceptibility of Brain Regions to Lipid Peroxidation. *Lipids*, 23(5):405–409.

Murer, S. B., Aeberli, I., Braegger, C. P., Gittermann, M., Hersberger, M., Leonard, S. W. 2014. Antioxidant Supplements Reduced Oxidative Stress and Stabilized Liver Function Tests but Did Not Reduce Inflammation in a Randomized Controlled Trial in Obese Children and Adolescents. *The Journal of Nutrition*, 144(2):193–201.

Nalagoni, C. S. R., Karnati, P. R. 2016. Protective effect of resveratrol against neuronal damage through oxidative stress in cerebral hemisphere of aluminium and fluoride-treated rats. *Interdisci Toxicol*, 9(2):78–82.

Nazıroglu, M. 2009. Role of selenium on calcium signalling and oxidative stress-induced molecular pathways in epilepsy. *Neurochem Res*, 34(12):2181–2191.

Oyagbemi, A. A., Adebiyi, O. E., Adigun, K. O., Ogunpolu, B. S., Falayi, O. O., Hassan, F. O. 2020. Clofibrate, a PPAR-α agonist, abrogates sodium fluoride-induced neuroinflammation, oxidative stress, and motor incoordination via modulation of GFAP/Iba-1/anti-calbindin signaling pathways. *Environmental Toxicology*, 35(2):242–253.

Pak, R. W., Lanteri, V. J., Scheuch, J. R., Sawczuk, I. S. 2002. Review of Vitamin E and Selenium in the Prevention of Prostate Cancer: Implications of the Selenium and Vitamin E Chemoprevention Trial. *Integrative Cancer Therapies*, 1(4):338–344.

Sarkar, C., Pal, S., Das, N., Dinda, B. 2014. Ameliorative effects of oleanolic acid on fluoride induced metabolic and oxidative dysfunctions in rat brain: Experimental and biochemical studies. *Food and Chemical Toxicology*, 66:224–236.

Sharma, D., Singh, A., Verma, K., Paliwal, S., Sharma, S., Dwivedi, J. 2017. Fluoride: A review of preclinical and clinical studies. *Environmental Toxicology and Pharmacology*, 56:297–313.

Sun, Z., Nie, Q., Zhang, L., Niu, R., Wang, J., Wang, S. 2017. Fluoride reduced the immune-privileged function of mouse Sertoli cells via the regulation of Fas/FasL system. *Chemosphere*, 168:318–325.

Sun, Z., Zhang, W., Li, S., Xue, X., Niu, R., Shi, L., Li, B., Wang, X., Wang, J. 2016. Altered miRNAs expression profiling in sperm of mice induced by fluoride. *Chemosphere*, 155:109–114.

Uneri, C., Sari, M., Akboga, J., Yuksel, M. 2006. Vitamin e-coated tympanostomy tube insertion decreases the number of free radicals in the tympanic membrane. *Laryngoscope*, 116(1):140–143.

Vani, M. L., Reddy, K. P. 2000. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. *Fluoride*, 33(1):17–26.

Wills, E. D. 1966. Mechanisms of lipid peroxide formation in animal tissues. *Biochem J*, 99(3):667–676.