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Effects of Oral Administration of Sodium Fluoride on Oxidative Stress Markers and Salivary Glands (biochemical and histopathological study)

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Abstract

Aims: to investigate serum biochemical changes (malondialdehyde levels and T-AOC) and the histopathological changes of salivary glands**. Materials and Methods**: Twelve healthy mature male rabbits were included in the study. Group one (6 animals): the rabbits were maintained on a standard diet and water without any treatment for 30 days. Group two (6 animals): the rabbits were given) NaF at the dose of 20 mg/kg/day (dissolved in 400 ml of distilled water for 30 days. At the end of one month of treatment, the animals were sacrificed and (5ml) of blood samples were taken from all animals via jugular vein during the animal sacrificing. Serum was separated from all samples and stored at -20 ˚C till time of analysis by colorimetric Assay kit for both total antioxidant capacity (T-AOC) by (Elabscience, Cat. No. E-BC-K136-S, TBA method) and malondialdehyde (MDA) by (Elabscience, Cat.No.E-BC-K025-S, TBA method).Tissue samples from parotid and sub-mandibular salivary glands were isolated and examined under a light microscope for histopathological changes. **Results**: Significant differences were founded between the values of serum biochemical markers between control and treatment groups. T-AOC in control group was higher than in treatment group, while MDA level in treatment group was higher than control group. Sections of the parotid and submandibular glands taken from the control group appeared to have the normal histological structure compared to the treated group, which showed an acute inflammatory cell infiltration, Hyperemia with thickening of blood vessels wall. **Conclusions**: NaF at dose of 20 mg/kg/day for 30 days can lead to a decrease in T-AOC with considerable inflammatory changes in tissues of salivary glands of rabbits.

الخلاصة

الأهداف: فحص التغيرات الكيميائية الحيوية في المصل (مستويات malondialdehyde والقدرة الكلية المضادة للأكسدة) والتغيرات النسيجية المرضية للغدد اللعابية. **المواد وطرائق العمل**: تم تضمين اثني عشر أرنباً من الذكور الناضجين بصحة جيدة في الدراسة. المجموعة الأولى (٦ حيوانات): تم الحفاظ على الأرانب على نظام غذائي معياري والماء دون أي علاج لمدة ٣٠ يومًا. المجموعة الثانية (٦ حيوانات): أعطيت الأرانب فلوريد الصوديوم (NaF) بجرعة ٢٠ ملجم / كجم / يوم مذاب في ٤٠٠ مل من الماء المقطر لمدة ٣٠ يومًا. في نهاية شهر واحد من العلاج ، تم التضحية بالحيوانات وتم جمع عينات الدم الوريدي (٥ مل) من جميع الأرانب عبر الوريد الوداجي أثناء التضحية بالحيوان. تم فصل عينات المصل وتخزينها عند -٢٠ درجة مئوية حتى وقت التحليل بواسطة مجموعة الفحص اللوني لكل من سعة مضادات الأكسدة الكلية (T-AOC) بواسطة (Elabscience ،Cat. No. E-BC-K136-S ، طريقة TBA) و malondialdehyde (MDA) بواسطة (Elabscience ،Cat.No.E-BC-K025-S ، طريقة TBA).. تم عزل عينات الأنسجة من الغدد اللعابية النكفية وتحت الفك السفلي وفحصها تحت المجهر الضوئي للتغيرات النسيجية المرضية. **النتائج**: وجدت فروق ذات دلالة إحصائية بين قيم الواسمات البيوكيميائية في المصل بين مجموعتي التحكم ومجموعة العلاج. كانت السعة الكلية لمضادات الأكسدة في المجموعة الضابطة أعلى منها في المجموعة المعالجة ، بينما كان مستوى مالونالديهيد في مجموعة المعالجة أعلى من مجموعة السيطرة. أظهر أن أقسام الغدد النكفية وتحت الفك السفلي المأخوذة من المجموعة الضابطة لها التركيب النسيجي الطبيعي مقارنة بالمجموعة المعالجة ، والتي أظهرت ارتشاح الخلايا الالتهابي الحاد ، وفرط الدم مع سماكة جدار الأوعية الدموية. **الاستنتاجات**: فلوريد الصوديوم بجرعة ٢٠ مجم / كجم / يوم لمدة ٣٠ يومًا يمكن أن يؤدي إلى انخفاض في القدرة المضادة للأكسدة الكلية مع تغيرات التهابية كبيرة في أنسجة الغدد اللعابية للأرانب.

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INTRODUCTION

Different populations are exposed to high levels of fluorides through drinking water that, due to their chronic intake, cause several types of damage to health. So monitoring the systemic fluoride effects by evaluating the impacts of chronic/subchronic exposure through different sources on oxidative stress and different organs in necessary. Fluorine is the broadly distributed electronegative element in the periodic table and fluoride has a wide availability in the world [(1)]. Sodium fluoride compounds are used in different forms like toothpaste, mouth wash, fluoride tablets, and fluorinated water for the prevention of dental caries[(2)] consequently, the exposure of people to fluoride substances has become significant[(3)]. Fluoride deficiency has ever been recorded as a disease [(4,5)] and it has no considerable vital role in growth and development of human body also no deficiency signs have been approved [(6)]. Dental caries is not related to deficiency of Fluorine, it seems to has the advantage in the prevention of this disease, with disadvantages of teeth, and skeletal fluorosis, together with alterations in some vital organs like liver, kidney, heart and so on [(7- 9)], which depends on the whole ingested fluoride amount [(10)].

In Fluoride studies, the histological findings showed an obvious difference in which can be related in part to diversity in the fluoride compounds used, the route of their administration, and the diverse species of the animals used. Possibly Fluoride removes the normal lipase stimulus that transfer fatty acid and glycerol across the cell membrane [(11)]. Various studies propose that fluoride exposure can slow down variety of enzymes activity and lead to generation of free radicals, impeding with antioxidant defense mechanisms [(12)]. (NaF) affect antioxidant activity and may induce oxidative stress in salivary glands after few hours of intake [(12)]. So, acting against the most important metabolic pathways of the living system [(13-15)].

This study aims to investigate the effects of NaF on serum level of T-AOC and MDA levels in addition to histopathological changes of salivary glands in the rabbit model.

MATERIALS AND METHODS

This research was approved by the scientific committee/department of Dental Basic Science/College of Dentistry/University of Mosul. Twelve mature male rabbits of 10-12 months old and body weight of 1.0-1.5 Kg, bought from local market were involved in the study. Animals were housed indoors in animal house of the College. They were retained under photoperiod cycle of light: from 6:00 to 18:00 h and dark: Form 18:00 to 6:00 at room temperature of 20 ± C° . Standardized diet was used with tap water to fed animals twice daily in addition to

clinical examination by veterinarian every day until the day of slaughtering. **Experimental design:** The animals were randomly separated into 2 groups: Group one

(6 animals): The rabbits were maintained on a standard diet and water without any treatment for 30 days and were sacrificed on the 31st day. Group two (6 animals): The rabbits were given (NaF)at dose of 20 mg/kg/day dissolved in 400 ml of distilled water for 30 days and were sacrificed on the 31st day. **Biochemical Analysis:** at the end of one month of treatment, the animals were sacrificed and 5ml of blood were collected from each rabbit via jugular vein during its sacrifice and allowed to clot for 30 min at room temperature, centrifuged at 3000 rpm for 10 min, then separated serum samples were stored at -20 ˚C till time of analysis by using colorimetric Assay kit for both T-AOC (Elabscience, Cat.No.E-BC-K136-S, TBA method) and MDA(Elabscience, Cat.No.E-BC-K025-S, TBA method).

Histopathological study: for histopathological examination, tissue samples from parotid and submandibular salivary glands were isolated and fixed in 10 % formalin, cut and stained with H&E. These sections were subjected to examination under a light microscope by a pathologist in a blinded manner.

Statistical analysis: data are expressed as mean ±SD .P¯ value of less than 0.05 was accepted as being significant in all types of statistical tests .the current study was analyzed using SPSS program

RESULTS

Gross Examination of animals fluoridized animals stays alive the whole study period. They examined at autopsy showing similarities with the controls in gross appearance and the macroscopic organization of their organs. Significant differences were founded between the values of serum biochemical markers between control and treatment groups. T-AOC in control group was higher than in treatment group While MDA level treatment group was higher than control group as shown in Figure (1and2) respectively.

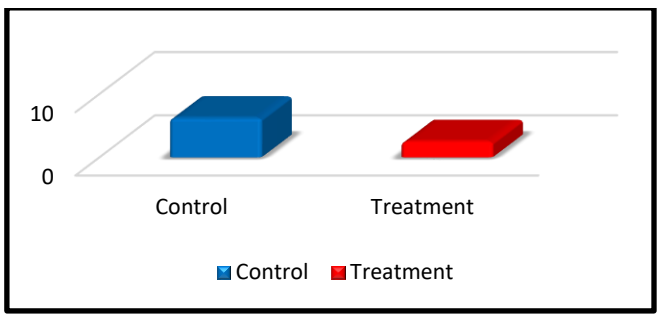

Figure (1): Comparison between T-AOC levels of both study groups

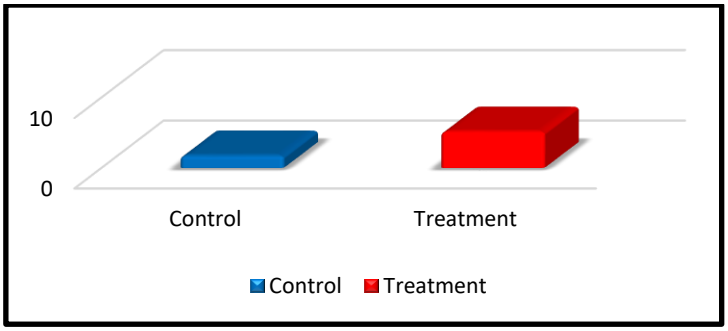

Figure (2): Comparison between MDA levels of both study groups

The histopathological results:

The histopathological examination includes submandibular and parotid salivary glands specimens for evaluation of any tissue destruction and/or ulceration, presence of inflammatory reaction with an assessment of vascularity, and blood vessels. All sections of parotid and submandibular glands taken from control group appeared to have the normal histological structure. The parotid sections which is an entirely serous secretory gland, the serous acini lined by pyramidal cells with eosinophilic cytoplasm, the nuclei are deeply basophilic and rounded in shape , situated in the basal third of the cells. The striated duct with distinct boundaries and lined by a single layer of columnar epithelium. Submandibular sections which is serous and mucous acini larger in diameter than those of the parotid gland and the boundaries of cells lining more distinct (clear). The striated ducts were larger in diameter, more in number than those of parotid gland. Figure (3 and 4).

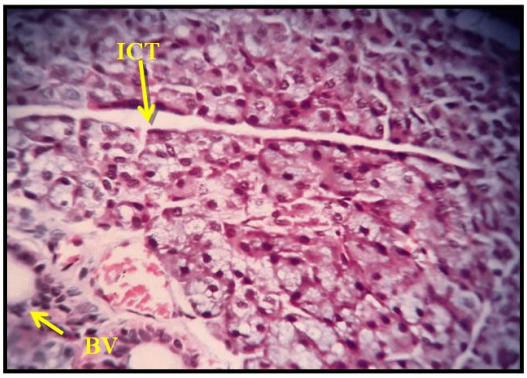

Figure (3): Photomicrograph of a parotid salivary gland section of control group. No inflammatory cell infiltration H&E 40x. ICT: inter septal connective tissue, B.V: blood vessels

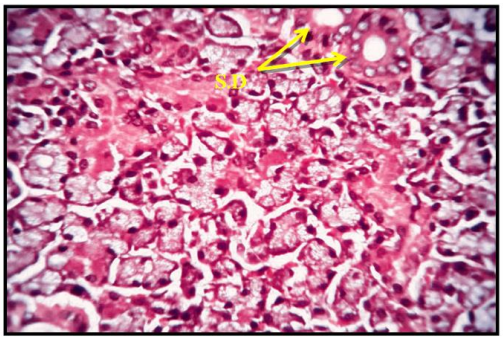

Figure (4): Photomicrograph of submandibular salivary gland section of control group. Normal acinus H&E 40x. S.D: striated duct

While all sections of the parotid and submandibular glands taken from treatment group showed similar changes, there is a marked disturbance in the architecture of acinar cells and the cells lining the striated ducts. Parotid sections showed acute inflammatory cell infiltration with the accumulation of neutrophil, hyperemia between salivary acinus. Figure (5 -8)

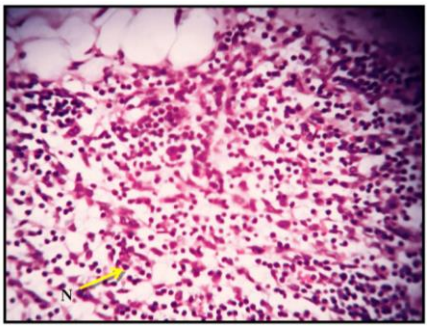

Figure (5): Photomicrograph of a parotid salivary gland section of treatment group. Acute inflammatory cell infiltration (neutrophil) H&E 40x. N: Neutrophil

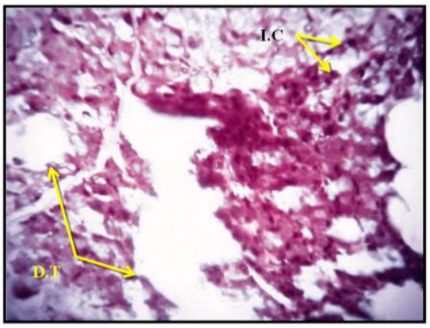

Figure (6): Photomicrograph of parotid gland section of treatment group. Less than 1mm ulceration with accumulation of neutrophil H&E 40x D.T: Destruction tissue, I.C: Inflammatory cell

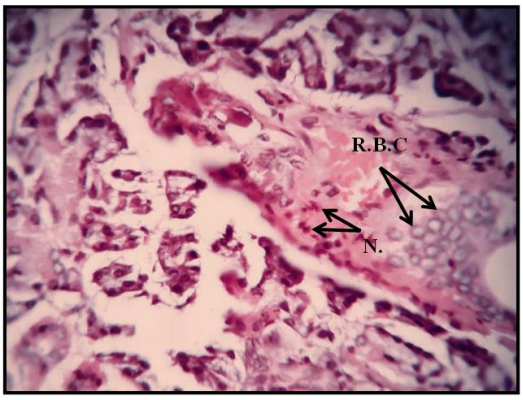

Figure (7): Photomicrograph of submandibular gland section of treatment group. Hyperemia between salivary acinus H&E 40X N: Neutrophil, R.B.C: Red blood cell.

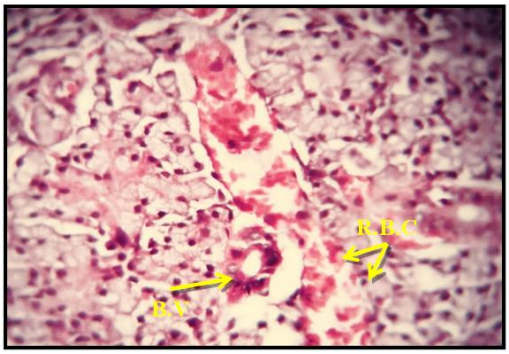

Figure (8): Photomicrograph of submandibular gland section of treatment group. Hyperemia without ulceration H&E 40X R.B.C: Red blood cell, B.V: Blood vessels

DISCUSSION

As the effectiveness of topical fluoride in the prevention of dental caries has been well established, excessive exposure to systemic fluoride showed some health problems including dental and skeletal fluorosis [16]. Unwanted side effects of fluoride can be correlated not only to the body's total fluoride intake but also to its retention in the body. Understanding fluoride metabolism and its physiological description is therefore very important to avoid or decrease side effects of systemic fluoride exposure. [17] Chronic

exposure to fluoride can produce free radicals, reactive species which leads to redox imbalance and cytotoxicity. Oxidative stress is known as a major mode of action of fluoride toxicity [18]. Absorbed fluoride causes damage of nuclear DNA, mitochondrial dysfunction, and also it alters intracellular redox status and enhances oxidation of membrane lipids and proteins [19]. In agreement with the present study, fluoride exposure can generate reactive oxygen species and result in cellular damage in animal models [18].

Fluoride inhibits the activities of numerous antioxidant enzymes, increases MDA and decrease glutathione levels. It inhibits ATPase activity leading to ATP depletion and suppression of ROS scavenging capacity of cells. Fluoride induces mitochondrial depolarization which rises ROS production and oxidative stress causing apoptotic cell death [20]. Excessive production of ROS leads to oxidative modification of macromolecules resulting in membrane injury through lipid peroxidation [21,22]. This suggests that the major mode of fluoride action depends on oxidative and nitrosative mechanisms and changes in redox homeostasis. [23.

During the presentation investigation, salivary gland sections showed necrosis and vacculation of serous acini with presentation of edema and hemorrhage. Necrosis of epithelium that lines the striated ducts with presentation of macrophages and edema of rabbits treated with sodium fluoride. In support of these results, evidences that fluoride ingestion may be a causative factor to inflammatory and degenerative changes include numerous studies supporting that fluoride can concentrate in some tissues like the eye leading to toxicity of retina [24]. Also, Fluoride was recorded to affect the epidermal tissues in mice and rabbits, but, the mechanism explain NaF toxic effects has not so far been obviously revealed [25].

Shashi et al. (2002) assesses renal damage in experimental fluorosis, they observed that the cytological structure of the kidneys revealed increased cloudy swellings, extensive vacuolization, tubular epithelium degeneration, renal tubular necrosis, glomeruli atrophy and hypertrophy, interstitial edema, and nephritis. The effects of sodium F were to create distinct changes in parotid and

submaxillary salivary glands and causes reduction in the concentration of biochemical parameters vital to the salivary physiology [26,27]. The clear pictures of derangement of cells in these glands may come from the side effects of fluoride on cellular metabolic system. Secretary function of glands is depending on the special metabolic activity of their numerous constituent cells. Fluoride can cause normal cellular functions not possible by disruption of grycolysis at a serious phase. It blocks the reformation of adenosine triphosphate by preventing maximum cellular oxidation. Possibly Fluoride removes the normal lipase stimulus that transfers fatty acid and glycerol across the cell membrane [26].

More studies are warranted to observe the impact of chronic fluoride exposure on general and oral health. Increasing of evidences would service in determining the optimal fluoride concentrations for humans.

CONCLUSION

Sodium fluoride can lead to decrease in T-AOC, increase in MDA serum levels with considerable inflammatory changes in tissues of salivary glands of rabbits. So NaF enhances the generation of free radicals and oxidative stress. The results of this study could represent a step in an attempt to delineate the molecular mode of fluoride toxicity so that suitable preventive measures can be considered.

REFERENCES

1. Lavalle-Carrasco, J.; Molina-Frechero, N.; Nevárez-Rascón, M.; Sánchez-Pérez, L.; Hamdan-Partida, A.; González-González, R.; et al. Recent Biomarkers for Monitoring the Systemic Fluoride Levels in Exposed Populations: A Systematic Review. Int. J. Environ. Res. Public Health, 2021, 18: 317. https://doi.org/10.3390/ ijerph18010317
2. Sun Z , Li S , Yu Y , Chen H, Ommati MM and Manthari RK. Alterations in epididymal proteomics and antioxidant activitof mice exposed to fluoride. Arch Toxicology, 2017. 17(2): 54 - 62.
3. Lavalle-Carrasco J, Molina-Frechero N, Nevárez-Rascón M, Sánchez-Pérez L, Hamdan-Partida A, and González-González R ., et al. Recent Biomarkers for Monitoring the Systemic Fluoride Levels in Exposed Populations: A Systematic Review. Int. J. Environ. Res. Public Health 2021, 18, 317. https://doi.org/10.3390/ijerph18010317
4. Meng X, Yao Y, Ma Y, Zhong N, Alphonse S, Pei J, Effect of fluoride in drinking water on the level of 5-methylcytosine in human and rat blood, Environmental Toxicology and Pharmacology (2020), doi: https://doi.org/10.1016/j.etap.2020.103511
5. Peckham S, and Awofeso N. (2014). Water Fluoridation: A Critical Review of the Physiological Effects of Ingested Fluoride as a Public Health Intervention Hindawi Publishing Corporation. Scientific World Journal, 14(5): 1012 – 1020
6. Waugh DT. (2019). The Contribution of Fluoride to the Pathogenesis of Eye Diseases: Molecular Mechanisms and Implications for Public

Health. International Journal of Environmental Research and Public Health, 16(8): 115 - 122.

7. Efsa J. (2013). Scientific Opinion on Dietary Reference Values for fluoride. Bioscience Engineering, 11: 3332.
8. Doull J, Boekelheide K, Farishian BG, Isaacson RL, Klotz JB and Kumar JV. (2006). Fluoride in drinking water: a scientific review of EPA's standards. National Academies Press, Washington.
9. Jagtap S, Yenkie MK, Labhsetwar N and Rayalu S. (2012). Fluoride in drinking water and defluoridation of water. Chemical Reviews, 112: 2454 – 2466.
10. Perumal E, Paul V, Govindarajan V and Panneerselvam L. (2013). A brief review on experimental fluorosis. Toxicology Letters, 223: 236 – 251
11. Barbier O, Arreola-Mendoza L and Del Razo LM. (2010). Molecular mechanisms of fluoride toxicity. Chemical and Biological Interaction, 188: 319 – 333.
12. Ogilvie AL. (1951). Histological finding in the salivary gland of rat following (NaF) administration. Biosicience, 30(5): 712 - 727.
13. Yamaguti PM, Simões A, Ganzerla E, Souza DN, Nogueira FN and Nicolau J. (2013). Effects of Single Exposure of (NaF) on Lipid Peroxidation and Antioxidant Enzymes in Salivary Glands of Rats. Hindawi Publishing Corporation, Oxidative Medicine and Cellular Longevity.
14. Shanthakumari D, Srinivasalu S and Subramanian S. (2004). Effect of fluoride intoxication on lipidperoxidation and antioxidant status in experimental rats. Toxicology, 204(2-3): 219 – 228.
15. Mittal M and Flora SJS. (2006). Effects of individual and combined exposure to sodium arsenite and (NaF) on tissue oxidative stress, arsenic and fluoride levels in male mice. Chemico-Biological Interactions, 162(2):128 – 139.
16. Ten Cate, J. M., & Buzalaf, M. A. R. (2019). *Fluoride Mode of Action: Once There Was an Observant Dentist . . . Journal of Dental Research, 98(7), 725–730.* doi:10.1177/0022034519831604
17. Mahmood, M., Azevedo, L. B., Maguire, A., Buzalaf, M., & Zohoori, F. V. (2021). *Pharmacokinetics of fluoride in human adults: The effect of exercise. Chemosphere, 262,127796.* doi:10.1016/j.chemosphere.2020.127796.
18. Barbier, O., Arreola-Mendoza, L., & Del Razo, L. M. (2010). *Molecular mechanisms of fluoride toxicity. Chemico-Biological Interactions, 188(2), 319–333.* doi:10.1016/j.cbi.2010.07.011
19. Miranda, G.H.N., Gomes, B.A.Q., Bittencourt, L.O., Aragao, ˜ W.A.B., Nogueira, L.S., Dionizio, A.S., Buzalaf, M.A.R., Monteiro, M.C., Lima, R.R., 2018. Chronic exposure to (NaF) triggers oxidative biochemistry misbalance in mice: effects on peripheral blood circulation. Oxid. Med. Cell. Longev. 2018, 8379123 https://doi.org/10.1155/2018/8379123.
20. Jothiramajayam, M., Sinha, S., Ghosh, M., Nag, A., Jana, A., & Mukherjee, A. (2014). *(NaF) Promotes Apoptosis by Generation of Reactive Oxygen Species in Human Lymphocytes. JToxicology and*

Environmental Health, Part A, 77(21),1269-1280.

21. Adedara, I.A., Ojuade, T.J.D., Olabiyi, B.F. *et al.* Taurine Ameliorates Renal Oxidative Damage and Thyroid Dysfunction in Rats Chronically Exposed to Fluoride. *Biol Trace Elem Res* **175,** 388–395 (2017).
22. Oyagbemi, A. A., Omobowale, T. O., Ola-Davies, O. E., Asenuga, E. R., Ajibade, T. O., Adejumobi, O. A., … Yakubu, M. A. (2018). *Quercetin attenuates hypertension induced by (NaF) via reduction in oxidative stress and modulation of HSP 70/ERK/PPARγ signaling pathways. Bio Factors.*
23. Maheshwari, N., Qasim, N., Anjum, R., & Mahmood, R. (2021). *Fluoride enhances generation of reactive oxygen and nitrogen species, oxidizes hemoglobin, lowers antioxidant power and inhibits transmembrane electron transport in isolated human red blood cells. Ecotoxicology and Environmental Safety, 208, 111611.*
24. Błaszczyk I, Birkner E and Kasperczyk S. (2011). Influence of methionine on toxicity of fluoride in the liver of rats. Biological Trace Element Research, 139(3): 325 – 331.
25. Sorsby A and Harding R. (1960). Experimental degeneration of the retina. Brazil Journal of Ophthalmology, 44:213 - 224.
26. Shukla N and Pandey GS. (1991). Fluoride level in cataract lenses in an urban area of India. Fluoride, 24: 40 - 43.
27. Ogilvie AL. (1951). Histological finding in the salivary gland of rat following (NaF) administration. Biosicience, 30(5): 712 - 727.