Current Trends in Biotechnology and Pharmacy
Vol. 16 (3) 365 - 372, july 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3.50

Assessment of Hematological Profile and Oxidative Stress Status in Sodium Fluoride Induced Anemia and Kidney Disorder in Rats

Anfal Djouadi[1,2], Samir Derouiche[1,2*]

[1]Department of Cellular and Molecular Biology, Faculty of natural sciences and life, University of El-oued El-oued 39000,Algeria.

[2]Laboratory of Biodiversity and Application of Biotechnology in the agricultural Field, Faculty of natural sciences and life, University of El-oued, El-oued 39000,Algeria

*Corresponding author: dersamebio@gmail.com

Abstract

The aim of this study was to evaluate the hematological profile and oxidative stress status in spleen and kidney tissues in rats exposed to fluoride. Twelve healthy adult male Wistar rats were randomly divided into 2 groups (n=6); the first group used as a control group. The second group exposed to fluoride as fluoride sodium (NaF) in drinking water (400 ppm) for 60 days. On which we measured some hematological, biochemical and oxidative stress parameters. Our findings revealed that in comparison with the control rats, fluoride exposure caused, a significant reduction ($p<0.001$) in the body weight and a significant rise ($p<0.05$) in relative spleen and kidney weight. In addition, Result showed that in fluoride intoxicated rats, a significant alteration in blood component, a significant ($p<0.01$) increase in serum Creatinin, urea, K^+, Ca^{+2} and a significant reduction ($p<0.01$) in Na^+ and Cl^- levels as compared to control. Results revealed also that sodium fluoride treatment in rats affected antioxidant defense system by decreasing GSH and CAT levels while MDA and SOD levels were significantly increased ($p<0.05$). Histopathological analysis showed that NaF induced a high structural alteration in kidney compared to control. Results demonstrated the toxic effect of sodium fluoride in drinking water by causing anemia, hemato-toxicity and oxidative stress in spleen and kidney tissues.

Keywords: NaF, Oxidative stress, anemia, hemogram, Kidney, Spleen.

Introduction

Blood represents a major target of xenobiotics and toxicants like fluoride that enter by any route and red blood cells (RBC), being the most abundant cells not only in blood but entire human body, are quickly exposed to their action (1). Fluoride is still unavoidable environmental pollutant negatively influencing human health (2). Experimental evidence showed that relentless exposure to fluoride affects the formation of blood forming cells i.e., hematopoietic cells in cavities of bone marrow and inhibits the transport of K+/Cl− ions altering the hematopoietic process (3,4). Human hematopoiesis produces the equivalent of our body weight of red blood cells (RBCs), white blood cells and platelets every 10 years of life (5). The hematopoietic system is hierarchically organized and maintained by hematopoietic stem cells (HSCs) that give rise to highly proliferative but lineage-committed hematopoietic progenitor cells (HPCs) that will differentiate into all mature blood cell types (6). Abnormalities in the normal program of blood cell formation may lead to various hematopoietic disorders (7), among them anemia in which hemoglobin (Hb) level and/or red blood cells (RBC) number are decreased. The normal Hb level depends on gender, age, gestational status, and altitude. It occurs as a consequence of

many disease processes and manifests largely as fatigue, shortness of breath, and exercise intolerance (8). Chronic kidney disease (CKD) patients are more likely to be anemic when compared to the general population (9). The pathophysiology is complex and is related to decreased Erythropoietin (EPO) production, decreased RBCs life span, and the chronic inflammatory status. EPO is an endogenous glycoprotein hormone that regulates the production, survival, and differentiation of red blood cells, this molecule is mainly produced by the fetal liver and adult kidney by hypoxia stimulus (10). As fluoride can affect hematopoietic process, it can affect kidney function because under normal physiological situations, about 60% of the daily fluoride absorbed by healthy adults is excreted through the kidney in the urine causing kidney function injury (11). Renal tubule cells have diverse regulatory and endocrine functions modulating reabsorption of sodium, water and bicarbonate (12). Therefore in this paper we will assess the hematological profile, renal-spleen oxidative stress and electrolytes levels perturbation after a sub-chronic exposure to a high dose of sodium fluoride in drinking water in rats

Materials and Methods

Animals and experimental design

In our study we used adult male Wistar rats with initial weight between 193-227 g. They were placed in two groups of 6 rats in each and kept in animal's house of faculty of natural and life sciences, University of El Oued, Algeria. The animals are carried in a laboratory place for adaptation with conditions of temperature (22.27±0.52) , humidity (72±2.01)% and photoperiod (12 hours of light/12 hours of black). Access to Standard diet and water is free for animals ad libitum during the experiments. The experimental protocols involving animals were reviewed and approved by the local Ethics Committee referenced (35 EC/DCMB/FNSL/EU2021). To reach our objective, twelve (12) healthy adult male Wistar rats were randomly divided into 2 groups (n=6);

Group I: rats were treated with normal diet and water used as a control group.

Group II: rats exposed to fluoride as fluoride sodium (NaF) in drinking water (400 ppm) for 60 days.

Evaluate the body weight and food intake were recorded weekly during the experiment.

Blood collection and tissue preparation

After of 8 weeks of NaF, rats were fasted for 16 h, then sacrificed, the blood was collected in EDTA tubes and in tubes without anticoagulants. The serum was obtained by centrifuging the blood at 3 000 rpm for 10 min and then stored at −20 °C, and used for biochemical assay. Then, the hematological parameters were estimated in EDTA tube. The kidney and spleen of rats of different groups was rapidly excised, weighed and stored at- 20 °C until use in oxidative stress evaluation.

Estimation of biochemical and hematological parameters

Kidney function markers were assessed by detecting the content of Creatinin and urea, Sodium (Na^{+}), potassium (K^{+}), chloride (Cl^{-}) and calcium (Ca^{+2}) in serum by using autoanalyzer. red blood cells (RBCs), hematocrit (HTC), hemoglobin (HBG), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were estimated using an automated hematoanalyzer.

Determination of oxidative stress markers

Malondialdehide (MDA) and reduced glutathione (GSH) levels were measured spectrophotometrically in Spleen and kidney according to methods described by Yagi (13) and Weckberker and Cory (14), respectively. Superoxide dismutase (SOD) and catalase (CAT) activities were realized according to method of Beauchamp and Fridovich (15) and Aebi (16) respectively.

Statistical analysis

Our statistical study was performed by the software (Minitab 17) using Student t test. Differences were considered statically significant at $p<0.05$.

Results

Initial body weight, body weight gain and relative kidney and spleen weight

Results showed that fluoride treatment at a dose 200 ppm caused a decrease in body weight gain and an increase in relative Spleen and kidney weights compared to control (Table 1).

Table 1: Body weight gain & relative organs weight in control and NaF rats

Parameter	Control (n=6)	NaF (n=6)
Initial Body weight	227.75 ± 1,38	198.8 ± 5,58
Weight gains (g / j / rat)	0.19 ± 0.091	-0.55 ± 0.042***
Relative Spleen Weight (%)	0,26±0,0214	0,23±0,008*
Relative Kidney Weight (%)	0,31±0,01	0,54±0,06*

Comparison with the control group: * P < 0.05, **P < 0.01, ***P < 0.001

Hematological profile

As shown in (Table 2) hematological profile present a significant ($p<0.05$) reduction in RBCs, HGB and HCT and significant elevation in Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in male fluoride exposed rats in comparison with control.

Biochemical parameters

Table 3 showed high significant ($p<0.01$) increase in serum Creatinin and urea levels as renal function biomarkers and high significant ($p<0.01$) serum potassium (K^+) and calcium (Ca^{+2}) elevation and reduction in sodium (Na^+) and chloride (Cl^-) concentrations in NaF exposed rats compared to control.

Oxidative stress parameters

Sodium fluoride enhances spleen and kidney free radicals production resulting in significant increased MDA levels and reduction in antioxidant defense lowering significantly GSH levels; and CAT activity while SOD activity was significantly increased ($p<0.05$) (table 4).

Kidney histopathological study

Histopathological analysis of kidney was shown in (Figure 1), Kidney tissue of NaF exposed group shows necrosis, tubule dilatation and degeneration, glomerular atrophy and inflammation

Table 2: Hematological profile of control and NaF groups

Parametres	Control (n=6)	NaF (n=6)
Red Blood Cells (10^12/l)	6,25±0,589	4,705±0,42*
Hemoglobin (g/dl)	14,35 ± 0,171	12,68 ± 0,508**
Hematocrite (%)	30,35± 2,02	25,93± 1,80*
Mean Corpuscular Volume (fl)	51,82±0,868	55,06±0,738*
Mean Corpuscular Hemoglobin (pg)	21,58 ± 1,86	28,7 ± 2,39*
mean corpuscular hemoglobin concentration (g/dl)	41,62±3,34	59,6±4,25**

Comparison with the control group: * P < 0.05, **P < 0.01, ***P < 0.001

Table 3: Renal function biomarkers and electrolytes levels in control and NaF group

Parametres	Control (n=6)	NaF (n=6)	
Creatinin (mg/l)	6,75 ±0,479	11,333±0,943 **	11,333±0,943 **
Urea (g/)	0,6267±0,0233	0,76±0,0309**	0,76±0,0309**
Sodium (mmol/l)	143,45±1,59	124,2±1,63**	124,2±1,63**
chloride (mmol/l)	114,45±2,25	107,1±1,15*	107,1±1,15*
Potassium (mmol/l)	5,98±0,190	35,82±1,15**	35,82±1,15**
Calcium (mg/l)	109±1,1	118±2,19**	118±2,19**

Comparison with the control group: * P < 0.05, **P < 0.01, ***P < 0.001

Table 4. Oxidative stress markers in control and NaF groups

Parametres		Control (n=6)	NaF (n=6)
MDA (nmol/g of tissue)	Spleen	57,94± 4,08	72,79 ± 2,00 **
	Kidney	35,88 ± 4,49	58,09 ± 7,01 *
GSH (nmol/g of tissue)	Spleen	16,76 ± 2,27	9,43 ± 0,39***
	Kidney	7,916±0,917	4,52±0,72*
SOD (UI/g of tissue)	Spleen	0,67±0,01	0,916±0,01*
	Kidney	0,95±0,02	1,619±0,131*
CAT (UI/g of tissue)	Spleen	0,021±0,002	0,014±0,001**
	Kidney	0,037±0,004	0,020±0,002**

Comparison with the control group: * P < 0.05, **P < 0.01, ***P < 0.001

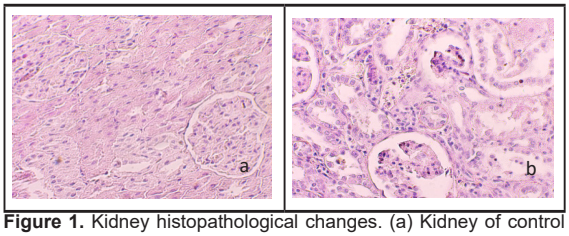

Figure 1. Kidney histopathological changes. (a) Kidney of control group (b) Kidney of sodium fluoride exposed group

Discussion

In current study hematological profile present a reduction in RBCs, HGB and HCT in male fluoride exposed rats in comparison with control. Several studies performed on hematological alteration induced by fluoride in animals(17, 18) also in human where study performed by Ruhi Anjum *et al.* (19) on human isolated red blood cells (RBCs) indicate that sodium fluoride induce lysis and damage in human RBCs via oxidative stress which in hand may be the cause of RBC number reduction in our study and in another hand, may be caused because of high affinity of fluoride to bone which lead to intoxication of bone marrow resulting into alterations in normal hematological profile (20), which confirmed by the increase in serum calcium level in NaF exposed rat as a result of its release from bone after fluoride fixation. Fluoride can bound to heme forming heme-fluoride complexes (21, 22) causing HGB degradation and then its level reduction in NaF exposed rats compared to control. Spleen functions include storing blood cells in response to hemorrhage and hypoxia or other stress, filtering or clearing old or damaged blood cells and pathogens, and maintaining iron metabolism (23). We observed

in our results an increase in MDA and SOD levels and a decrease in GSH and CAT levels of spleen in rats exposure of NaF compared to control indicating an oxidative state; these results are similar to the study of Chen et al. (24), which present also accumulation of fluoride ion in spleen cells resulting in cellular death and spleen damage explained the decrease in relative spleen weight in NaF exposed group compared to control. In another study where MDA level, superoxide dismutase SOD and CAT activities evaluated in children with iron deficiency anemia (IDA) (25); In our experimentation NaF induced anemia maybe via hemolysis, hematopoiesis inhibition or via reducing iron availability. In hand iron deficiency in human cells especially in the red blood cells can lead to membrane damage associated with free radicals generation, in another hand erythropoiesis needs to be controlled, so there is a balance between RBC production and destruction. It is well documented that balanced hematopoietic growth factors and hematopoietic inhibitory factors are crucial to an effective hematopoietic system as they regulate the growth, differentiation, and proliferation of hematopoietic stem cells (HSCs) to produce blood cells (26). We hypothesize that NaF-induced anemia is the result of a wide variety of causes that often coexist together to induce an experimental anemia model by altering many key sites. In addition the secretion of hormones that participate in the regulation of systemic and renal hemodynamics, The kidneys also secrete hormones that participate in red blood cell production (EPO) (27) which is hormone that regulates the production, survival, and differentiation of red blood cells. Inflammation in chronic kidney diseases increases ferritin and hepcidin independent of iron statuses, which reduce iron availability (28). In current study an increase in serum renal function biomarkers levels in NaF exposed rats compared to control with increase in relative kidney weight in that group indicate the renal dysfunction simultaneously with oxidative stress statue by increasing of MDA and SOD and decreasing of GSH and CAT levels in kidney of rats treated with fluoride compared to control. Accumulation of hydrogen peroxide (H_2O_2) may be attributed to decrease in catalase activity (29). Nephromegaly is a result of inflammation; thus the loss of renal function after NaF exposure can lead to reduction of iron availability and absence of EPO which resulting in hematopoiesis inhibition. Thus the reduction of HCT may be referring to reduction to RBCs number in those rats. Elevation in Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in NaF exposed fluoride rats compared to control. High significant serum sodium (Na^+), potassium (K^+), chloride (Cl^-) and calcium (Ca^{+2}) concentrations changes in NaF exposed rats compared to control. The nephrons, kidneys structural and functional units, affect changes to blood plasma via filtration, reabsorption, secretion and excretion (30). Through these mechanisms the kidneys maintain homeostasis of electrolyte concentrations, fluid volume, osmolality and acid-base balance (31). Hyperkalemia is the most common life-threatening electrolyte derangement as a cause of acute kidney injury (32). It is plausible that critically ill patients presenting with kidney injury may have an inadequate sodium and water control, presenting higher prevalence of dysnatremia (33).

Conclusion

The present study exhibits the toxic effects of NaF on kidney and spleen by induced oxidative stress which could also prove that fluoride in high doses in drinking water can cause hematotoxicity and anemia in rats.

Funding

No funding from external agencies.

Conflicts of interest

The authors declare that there is no conflict of interest in this study.

Acknowledgement

This research was made by the

D01N01UN390120190001 research project, which was funded by the Algerian Ministry of Higher Education and the Directorate General for Scientific Research and Technological Development.

References

1. 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. *Ecotoxicol. Environ. Saf.* 208 :111611.

2. Djouadi, A., & Derouiche, S. (2017). Study of fluoride-induced haematological alterations and liver oxidative stress in rats. W*orld j. pharm. pharm. sci.;* 6(5): 211-221.

3. Ameeramja, J., Raghunath, A. & Perumal, E. (2018). Tamarind seed coat extract restores fluoride-induced hematological and biochemical alterations in rats. *Environ. Sci. Pollut. Res.* 25 (26) :26157–66.

4. Abbas, M., Siddiqi, M. H., Khan, K., Zahra, K. & Naqvi, A. un N. (2017). Haematological evaluation of sodium fluoride toxicity in oryctolagus cunniculus. *Toxicol. Reports* 4 (March) :450–4.

5. Dale, D. C. & Mackey, M. C. (2015). Understanding, Treating and Avoiding Hematological Disease: Better Medicine Through Mathematics? *Bull. Math. Biol.* 77 (5) :739–57.

6. Gerosa, R. C., Boettcher, S., Kovtonyuk, L. V., Hausmann, A., Hardt, W. D., Hidalgo, J., Nombela-Arrieta, C. & Manz, M. G. (2021). CXCL12-abundant reticular cells are the major source of IL-6 upon LPS stimulation and thereby regulate hematopoiesis. *Blood Adv.* 5 (23) :5002–15.

7. Chatterjee, R., Chattopadhyay, S. & Law, S. (2016). Alteration of classical and hematopoiesis specific p53 pathway in the bone marrow hematopoietic stem/progenitor compartment facilitates leukemia progression in experimental mice. *Leuk. Res.* 47 :70–7.

8. Alshamsi, I. (2022). Extended Literature Review of the role of erythropoietin stimulating agents (ESA) use in the management of post renal transplant anaemia. *Transplant. Reports* 7 (2) :100097.

9. Derouiche, S., Cheradid, T., & Guessoum, M. (2020). Electrolyte Analysis of drinking water of Djamaa (Algeria) region and study its effect on the kidneys in Wistar rats. *Asian J. Research Chem*., 13(3):219-224.

10. Silva, I., Alípio, C., Pinto, R. & Mateus, V. (2021). Potential anti-inflammatory effect of erythropoietin in non-clinical studies in vivo: A systematic review. *Biomed. Pharmacother.* 139.

11. Wu, L., Fan, C., Zhang, Z., Zhang, X., Lou, Q., Guo, N., Huang, W., Zhang, M., Yin, F., Guan, Z., Yang, Y. & Gao, Y. (2021). Association between fluoride exposure and kidney function in adults: A cross-sectional study based on endemic fluorosis area in China. *Ecotoxicol. Environ. Saf.* 225 :112735.

12. Derouiche, S., Serouti, A., & Rezzag-mohcen, O. S. (2019). risk of metribuzin (triazinone herbicide) on hematological and renal structure and function of pregnancy rabbits. *Asian J.Biol. Sci*., 12:192-198.

13. Yagi, K., (1976). A simple fluorometric assay for lipoperoxide in blood plasma. Biochem. Med., 15: 212-216.

14. Weckberker, G. & Cory, J.G. (1988). Ribonucleotide reductase activity and

growth of glutathione-depleted mouse leukemia L1210 cells in vitro . Cancer Lett., 40: 257-264.

15. Beauchamp, C. & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem., 44: 276-287.

16. NAebi, H. (1984). Catalase in vitro . Meth. Enzymol., 105: 121-126.

17. Fatima, B., Saeed, U., Hussain, D., Jawad, S. e. Z., Rafiq, H. S., Majeed, S., Manzoor, S., Qadir, S. Y., Ashiq, M. N. & Najam-ul-Haq, M. (2022). Facile hydrothermal synthesis of NiTe nanorods for non-enzymatic electrochemical sensing of whole blood hemoglobin in pregnant anemic women. *Anal. Chim. Acta* 1189.

18. Pan, C., Yan, M., Jin, H., Guo, H. & Han, X. (2021). Chronic exposure to MC-LR increases the risks of microcytic anemia: Evidence from human and mice. *Environ. Pollut.* 288 (August) :117966.

19. Karadeniz, A. & Altintas, L. (2008). Effects of panax ginseng on fluoride-induced haematological pattern changes in mice. 41 (March) :67–71.

20. Eren, E., Özturk, M. & Mumcu, E. F. (2005). Fluorosis and its hematological effects. *Toxicol. Ind. Health* 21 (9) :255–8.

21. Anjum, R., Maheshwari, N. & Mahmood, R. (2022). 3,4-Dihydroxybenzaldehyde mitigates fluoride-induced cytotoxicity and oxidative damage in human RBC. *J. Trace Elem. Med. Biol.* 69 (July 2021) :126888.

22. Kant, V., Verma, P. K., Pankaj, N. K., Kumar, J., Kusum, Raina, R. & Srivastava, A. K.(2009). Haematological profile of subacute oral toxicity of fluoride and ameliorative efficacy of aluminium sulphate in goats. *Toxicol. Int.* 16 (1) :31–5.

23. Hallez, F., Combès, A., Desoubries, C., Bossée, A. & Pichon, V. (2022). Analysis of long-lived sulfur mustard-human hemoglobin adducts in blood samples by red blood cells lysis and on-line coupling of digestion on an immobilized-trypsin reactor with liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* 1665 :462830.

24. Chen, W.-L., Nishita, Y., Nakamura, A., Kato, T., Nakagawa, T., Zhang, S., Shimokata, H., Otsuka, R., Su, K.-P. & Arai, H. (2022). Hemoglobin Concentration is Associated with the Hippocampal Volume in Community-Dwelling Adults. *Arch. Gerontol. Geriatr.* 101 (February) :104668.

25. Wei, Y., Wang, T., Liao, L., Fan, X., Chang, L. & Hashimoto, K. (2022). Brain-spleen axis in health and diseases: A review and future perspective. *Brain Res. Bull.* 182 :130–40.

26. Shashi, A. & Thakur, S. (2022). Gene Expression and Alterations of Antioxidant Enzymes in Spleen of Rats Exposed To Fluoride. *J. Trace Elem. Med. Biol.* 72 (August 2021) :126966.

27. Sharif Usman, S., Dahiru, M., Abdullahi, B., Abdullahi, S. B., Maigari, U. M. & Ibrahim Uba, A. (2019). Status of malondialdehyde, catalase and superoxide dismutase levels/activities in schoolchildren with iron deficiency and iron-deficiency anemia of Kashere and its environs in Gombe State, Nigeria. *Heliyon* 5 (8) :e02214.

28. Iqubal, A., Syed, M. A., Haque, M. M., Najmi, A. K., Ali, J. & Haque, S. E. (2020). Effect of nerolidol on cyclophosphamide-induced bone marrow and hematologic toxicity in Swiss albino mice. *Exp. Hematol.* 82 :24–32.

29. Derouiche, S., Ahmouda, I. & Moussaoui, R. (2021). Effectiveness of a Novel

SeNPs synthetized by Aquilaria malaccensis extract compared to selenium acetate on lead induced metabolic disorder and oxidative stress in pregnant rats. I*nternational Journal of Chemical and Biochemical Sciences.* 19: 50-57.

30. DEROUICHE, S., CHERADID, T., & Guessoum, M. (2020). Heavy metals, Oxidative stress and Inflammation in Pathophysiology of Chronic Kidney disease - A Review. Asian Journal of Pharmacy and Technology. 2020; 10(3): 202-206. DOI: 10.5958/2231-5713.2020.00033.1

31. UEDA, N. & TAKASAWA, K. (2018). Impact of inflammation on ferritin, hepcidin and the management of iron deficiency anemia in chronic kidney disease. *Nutrients* 10 (9).

32. GOODRICH, E. L. & BEHLING-KELLY, E. (2019). Clinical Pathology of Donkeys and Mules. *Vet. Clin. North Am. - Equine Pract.* 35 (3) :433–55.

33. MCGREGOR, T. & JONES, S. (2021). Fluid and electrolyte problems in renal dysfunction. *Anaesth. Intensive Care Med.* 22 (7) :406–9.