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Mitigating Effects of Triphala on Fluoride Blood Toxicity in Rat

KEYWORDS: Triphala, Sodium fluoride, Fluoride Toxicity, Blood

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ABSTRACT *Healthy adult female rats (Rattus norvegicus) were administered sodium fluoride (NaF) at the dose of 10 mg/kg body weight and triphala at 30 mg/kg body weight orally. Both doses were given individually and also in combination for 30 days to investigate the fluoride toxicity in blood and its reversal by triphala. NaF feeding also brought about a significant decline in hemoglobin level, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and red blood cell (RBC), which suggested occurrence of anemia and probable effect on haemopoetic system. Significant increase in erythrocyte sedimentation rate (ESR) indicates toxic effects in the body. Increase in white blood cells (WBC) count significantly suggests adverse effect of NaF treatment on the immune system of the rats. Co-administration of triphala to fluoride treated rats resulted in a partial effects in blood and body weight due to its probable protective role. Thus, triphala mitigated NaF induced toxicity marginally in a rat model.*

INTRODUCTION

Fluorine is one of the most active elements, which belong to halogen group with a atomic number 9. The mass number of its isotopes are 18 and 19 but only the natural isotope 19F is stable.[1] Fluoride (F^-) in water sources is known to induce both useful and harmful influences on human body. Excessive amount of F can cause fluorosis.[2] It is one of the most electronegative ions commonly used in the pharmaceutical and agrochemical industries.[3] Fluoride intoxication causes different disorders in the body including hepatotoxicity, nephrotoxicity, neurotoxicity, and cardiotoxicity,[3,4] associated with oxidative stress and altered anti-oxidant defense mechanisms. Previous studies from our laboratories and others have indicated that, consumption of antioxidant, such as *Triphala*, may mitigate the fluoride-induced oxidative stress in different tissues of rats.[5] It also appears to be beneficial in its free-radical scavenging actions beyond its stimulatory effects on antioxidant enzyme system.[6,7] The current study was undertaken to scrutinize mitigative role of *Triphala* on fluoride induced blood toxicity.

METHODS AND MATERIALS

Animals: Mature female Wistar rats (*Rattus norvegicus*) weighing between 250 and 350gm were procured from Zydus-Cadila Health Care, Ahmedabad under the Animal Maintenance and Registration No. 167/PO/C/99/CPCSEA from the Ministry of Social Justice and Empowerment, Government of India. The animals were housed under standard temperature (24±1°C) at a 12-hr dark/light cycle. They were fed standard rodent food (Pranav Agro Industries, Vadodara, India) and water *ad libitum*.

Experimental design: After a 15-day adaptation period, the animals were divided into five different groups (Table 1) of 15 each and caged separately. Based on our earlier studies,[1] the following doses were given for 30 days. Group I (control) rats were maintained on standard diet. Group II was treated with *Triphala* alone (30mg/kg bw) orally. Group III was administered a dose of sodium fluoride (10mg/kg bw) orally. Group IV was given 10 mg/kg bw dose of NaF along with *Triphala* 30mg/kg bw orally.

After 30 days, the rats were fasted overnight and sacrificed under mild ether anesthesia. The weight of the body were recorded, and blood was colored by cardiac puncture and it was allowed to clot for 1 hour at room temperature and thereafter serum was separated by centrifugation and used for various serum parameters.

Table 1. Experimental Protocol

Group	Treatment and daily dose (15 rats in each group)	Duration (days)	Day of autopsy
I	Untreated control	-	Sacrificed with treated
II	*Triphala* alone (30 mg/kg bw, orally)	30	31st
III	Sodium fluoride (10 mg/kg bw, orally)	30	31st
IV	NaF treated (10 mg/kg bw, orally) + *Triphala* (30 mg/kg bw, orally)	30	31st

Biochemical Analysis:- Erythrocyte Sedimentation Rate (ESR), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC) were done by using Automated Haematological Analyzer Sysmex-Kx 21 (Japan).

Statistical Analysis: Data are presented as mean ± SEM. One-way analysis of variance (ANOVA) with Turkey's significant difference post hoc test was used to compare differences among groups. Data were analyzed statistically by Graph Pad Prism 5.0 statistical software. P values <0.05 were considered significant.

RESULTS

BODY WEIGHTS

The body weight of experimental rats showed a significant ($P<0.05$) reduction after the treatment with NaF for 30 days. Treatment of triphala along with sodium fluoride treated rats did not show change as compare to control. Triphala alone had no effect. (Table.2)

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BLOOD PARAMETERS

Hematological parameters had manifested alterations after fluoride treatment in all the groups of rats. In NaF treated group, the hematological parameters including Hb, RBC, MCHC and PLT showed a significant ($p<0.001$) decrease. Ht, MCV and MCH also showed a significant ($p<0.001$) reduction, whereas WBC and ESR were elevated significantly ($p<0.001$). These variations were remarkably ameliorated in all the parameters following co-supplementation of triphala except ESR, MCH and MCHC, which showed a partial mitigation. Treatment of thriphala alone revealed no changes as compared to control. (Tables 3).

Table2. Gravimetric parameters of control and experimental groups

Parameter	Control	Triphala	NaF	NaF + Triphala
Body weight (gm)	276 ±4.28	280 ±5.78^{NS}	236 ±5.55*	271 ±7.19^{NS}

Values are Mean± S.E., NS= Non significant;
* P<0.05;** P<0.01; + P<0.001

Table3. Haematological parameters of control and experimental groups

Parameters	Control	Triphala	NaF	NaF + Triphala
HB (gm %)	13.76 ±0.21	14.00 ±0.39^{NS}	10.33 ±0.19^{+}	12.70 ±0.12*
RBC (Millions /cu.mm)	4.90 ±0.15	4.98 ±0.09^{NS}	4.03 ±0.06^{+}	4.47 ±0.09*
WBC (Thousands /cu.mm)	7740 ±87.66	7780 ±111.19^{NS}	9160 ±437.55^{+}	8660 ±279.73*
Erythrocyte Sedimentation Rate (ESR) (mm/hr)	5.39 ±0.17	5.91 ±0.05^{NS}	18.02 ±0.27^{+}	6.14 ±0.17*
Mean Corpuscular Volume (MCV) (fl)	51.15 ±0.20	51.55 ±0.12^{NS}	44.60 ±0.37^{+}	49.94 ±0.26*
Mean Corpuscular Haemoglobin (MCH) (pg)	16.26 ±0.14	16.49 ±0.17^{NS}	12.95 ±0.21^{+}	15.53 ±0.18*
Mean Corpuscular Haemoglobin Concentration (MCHC) (g/dl)	30.77 ±0.35	31.36 ±0.33^{NS}	25.84 ±0.25^{+}	29.32 ±0.21*

Values are Mean± S.E., NS= Non significant;
* P<0.05; ** P<0.01; + P<0.001

DISCUSSION

In the present study, a significant reduction in body and organ weight of toxicant treated animals was recorded in comparison to controls. Similar results were reported by our laboratory and others[8,9,10] in rats and mice fed with different concentrations of fluoride. Zhang et al.[11] also observed that the surface of rat incisors fed with fluoride had chalky color change and cross striations could be seen on the enamel surface. Same trends of decrease in gravimetric of NaF treated rats have been accounted from our laboratory,[1,9, 12-13] which are related to less food intake and also hormone imbalance.

The treatment of sodium fluoride brought about a reduction in body weight of female rat which could be attributed to low food consumption, altered protein and energy metabolisms[9,14] and electrolyte imbalance due to adrenal dysfunction.[15] Similar results were also reported by other authors on mice and rats by fluoride treatment.

Haematological parameters such as total RBC, Hb, MCV, MCH, MCHC were significant decline, whereas enhancement were observed in total WBC count and ESR as a effect of fluoride intoxication.[16] This reduction in Hb, RBC, MCV, MCH, MCHC with increase in the WBC and ESR indicated alterations in haemopoitic tissue. Bone marrow cell number could be affected and are in agreement with the data of others studied on mice and rats.[17-19] Sharma et al.[20-21] documented that rats exposed to fluoride water (3, 4.5, 5.8 and 6 ppm) for 15, 30, 60 and 120 days and observed weight loss, reduced total erythrocytes count, Hb percentage and haematocrit value and increased total leucocyte count in support our findings. The changes in erythrocyte count and haemoglobin concentrations have been correlated with cytotoxic effect of sodium fluoride on erythropoiesis. Due to decline in the total RBC count and Hb concentration, concomitant decrease in MCH an MCHC was observed in the present study.

Maheswaram et al.[22] reported increased WBC count in a dose dependent manner in collaboration of our observation. This enhancement of WBC could be attributed to a stimulation of immune system in response to tissue damage caused by sodium fluoride. However, the intoxication of sodium fluoride on total WBC count and ESR has been observed to be significantly increased after treatment with sodium fluoride due to leucocytosis. Leucocytosis is an outcome of proliferation of haemopoitic cells leading to progressive infiltration in peripheral blood.[23] Sodium fluoride increases the ESR due to the decreased total RBC count as total erythrocyte count depends on the rouleux formation of erythrocytes. Furthere the rouleux formation is an indication of increase in the density of its mass which along with reduced erythrocyte count, is responsible for increased erythrocyte sedimentation rate.[24] However, reduction in hemoglobin concentration may be due to production of reactive oxygen species under the influence of sodium fluoride, which results in destruction of the cell membrane and its function. O' Connor and Fromm[25] reported a decrease in RBC count due to hemolysis as a consequence of sodium fluoride toxicity. In support our data Rao et al.[26] also documented that fluoride is known to induce haemolysis in vitro in human.

Supplementation of triphala to NaF treated rats, the gravimetric and blood cell counts were compensated to certain extent comparable to control level indicating mitigate role of triphala It is well documented that *Emblica officinalis* and its active components like ascorbate, emblicanins, polyphenols etc. are powerful antioxidants in prevention of free radical production and quenching these radicals by enhancing the defense system during stress to combat tissue structure and function.[27,28] Since the Triphala is reported to be strong antioxidant and this antioxidant property may be related to the presence of gallic acid, vitamin C and flavonoid contents in it.[29]

Hence, Triphala treatment along with NaF reduced the toxic effects of fluoride in blood, since all the above-mentioned heamopoetic parameters were not appreciably altered as compared to the controls, confirming the anti-oxidative properties of triphala.

In conclusion, our results demonstrated that F induced heamatopoetic dysfunction is mediated by increase of oxidative stress affecting its internal milieu in rats. However, the administration of triphala extract to treated rats attenuates F- generated heamatopoetic dysfunction due to its ameliorative effect. Thus, the herbal product triphala is beneficial to overcome F induced lymphatic toxicity in animals.

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REFERENCE

1. Underwood EJ. 1977. Aluminium. In: trace elements in human and animal nutrition. 4th edition. New York, Academic press, PP-430-433. | 2. Rao MV, Bhatt RN. Protective effect of melatonin on fluoride-induced oxidative stress and testicular dysfunction in rats. Fluoride 2012;45(2):116-124. | 3. Ayoob S, Gupta AK. Fluoride in drinking water: a review on the status and stress effects. Crit Rev Environ Sci Technol 2006;36:433–487. | 4. Nabavi SF, Habtemariam S, Jafari M, Sureda A, Nabavi SM. Protective role of gallic acid on sodium fluoride induced oxidative stress in rat brain. Bull Environ Contam Toxicol 2012;89:73–7. | 5. Reddy VD, Padmavathi P, Gopi S, Paramahamsa M, Varadacharyulu NC. Protective Effect of Emblica officinalis Against Alcohol-Induced Hepatic Injury by Ameliorating Oxidative Stress in Rats. Indian J Clin biochem 2010;25(4):419-424. | 6. Hiraganahalli BD, Chinampudur VC, Dethe S, Mundkinajeddu D, Pandre MK, Balachandran J, Agrawal A. Hepatoprotective and antioxidant activity of standardized herbal extracts. Pharmcogn Maq 2012;49(11):1128-36. | 7. Golechha M, Bhatia J, Ojha S, Arya DS. Hydroalcoholic extract of Emblica officinalis protects against kainic acid-induced status epilepticus in rats: evidence for an antioxidant, anti-inflammatory, and neuroprotective intervention. Pharm Bio 2011;49(11):1128-36. | 8. Vani ML, Reddy, KP. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice. Fluoride 2000;33:17-26. | 9. Chawla SL, Yadav R, Shah D, Rao MV. Protective action of Emblica officinalis against fluoride-induced hepatotoxicity in adult female mice. Fluoride 2008;41:44-51. | 10. Basha PM, Rai P, Begum S. Evaluation of fluoride-induced oxidative stress in rat brain: a multigeneration study. Biol Trace Elem Res 2011;142(3):623-637. | 11. Zhang XL, Sh X, Guo XY, Cheng GY, Zhang Y. Effect of fluoride on the expression of MMP-20/TIMP-2 in ameloblast of rat incisor and the antagonistic effect of melatonin against fluorosis. Shanghai. Kou. Qiang. Yi. Xue. 2011;20(1):10-5. | 12. Rao MV, Chawla SL, Patel N. Emblica officinalis reduction of fluoride-induced nephrotoxicity in mice. Fluoride 2009;42(2):110-6. | 13. Chawla SL, Rao MV. Protective effect of melatonin against fluoride induced oxidative stress in the mouse ovary. Fluoride 2012;45(2):125-132. | 14. Jhala DD, Nair SB, Chinoy NJ. Reversible toxicity of fluoride and arsenic in ovary of mice. Fluoride 2004;37(2): 71-79. | 15. Susheela AK, Kumar AA. study of the effect of high concentrations of fluoride on the reproductive organs of male rabbits, using light and scanning electron microscopy J Reproduction and Fertility 1991;92: 353-360. | 16. Chinoy NJ, Shah SD. Synergistic action of vitamins and calcium in mitigation of fluoride and arsenic induced hematological toxicity in mice. Ind J Environ Toxicol 2004;14(1): 1-7. | 17. Ravichandran, B. Ecotoxicological study of fluorides and its impact on human health. 2001. Ph.D. Thesis, Jadavpur University, Reginal Occupational Health Centre Indian Council of Medical Research, Calcutta, India. | 18. Chinoy NJ. Studies on fluoride, aluminium and arsenic toxicity in mammals and amelioration by some antidotes. In: Tripathi G. (Ed.), Modem Trends in Environmental Biology. CBS Publishers, New Delhi, 2002;pp: 164-193. | 19. Eren E, Oztuk M, Mumcu EF, Canatan D. fluorosis and its haematological effects. Toxicol Ind Health 2005;21: 255-258. | 20. Sharma JD, Jain R, Solanki D, Solanki M. Effects of ethanolic seed extract of Allium cepa, vitamin C and withdrawal treatment on vat deferens and sex accessory glands of albino rats. National Sem and Applic Biotech, 2004;23-24. | 21. Sharma JD, Sharma MK, Agrawal P, Sohu D, Jain P. Physiological and biochemical alterations in vital organs of albino rats following fluoride water treatment. Ind J Environ Sci 2006;10(2): 125-130. | 22. Maheswaran R, Devapaul A, Murlidharan S, Velmurugan B, Ignacimuthu S. Haematological studies of fresh water fish, Clarias batrachus (L.) exposed to mercuric chloride. Int J Integrative Biol 2008;2:49-54. | 23. Sharma S, Sharma A, Singh PK, Soni P, Sharma S, Sharma P, Sharma K.P. Impact of distillery soil leachate on haematology of Swiss albino mice. Bull Environ Contam Toxicol 2007;79:273-277. | 24. Agarwal R, Chaurosia JP. Chronic effects of Mercuric chloride ingestion on rat adreno cortical function. Bull Environ Contain Toxicol 1989;43: 481-484. | 25. O' Connor DV, Fromm P.O. The effect of methyl mercury on gill metabolism and blood parameters of Rainbow trout. Bull Environ Contam Toxocol 1975;13: 406-411. | 26. Rao MV, Vyas DD, Meda RB, Chawla SL. In vitro protective role of melatonin against hemolysis induced by sodium fluoride in human blood cells. Fluoride 2011;44: 70-75. | 27. Yokozawa T, Kim HY, Kim HJ, Okubo T, Chu DC, Juneja LR. Amla (Emblica officinalis Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. Br J Nutr 2007;97(6):1187-1195. | 28. Bharti VK, Srivastava RS. Effect of pineal proteins at different dose level on fluoride-induced changes in plasma biochemicals and blood antioxidants enzymes in rats. Biol Trace Elem Res 2011;141(1):275-282. | 29. Dhanalakshmi S, Devi RS, Srikumar R, Manikanda, S, Thangaraj R. Protective effect of Triphala on cold stress-induced behavioral and biochemical abnormalities in rats. Yakugaku Zasshi 2007;127(11): 1863-1867. |