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Haemato- Biochemical Alterations in Subacute Oral Toxicity of Sodium Fluoride in Wistar Rats

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ABSTRACT

The present study was carried out to determine the extent of haemato-biochemical alterations in 28 days repeated dose oral toxicity of sodium fluoride (NaF) at three dose levels in Wistar rats. The administration of NaF caused dose dependent reduction ($p \leq 0.01$) in the levels of haemoglobin, packed cell volume, total erythrocyte count and lymphocyte percent while an increase in mean corpuscular volume, mean corpuscular haemoglobin and neutrophils of all the rats belonging to group II (5 mg/kg body weight, *per os*), group III (25 mg/kg body weight, *per os*) and group IV (50 mg/kg body weight, *per os*) as compared to rats of control group (group I). Macrocytic- normochromic anaemia was recorded in NaF intoxicated rats. Biochemical alterations were dose dependent elevation ($p \leq 0.01$) in plasma enzyme activities of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in group II, III and IV in comparison to rats of control group. Hyperglycaemia ($p \leq 0.01$) was recorded in rats of group III and IV. Hypoproteinaemia ($p \leq 0.01$) and hypoglobulinaemia ($p \leq 0.01$) were seen in Wistar rats of group II, III and IV as compared to control group. Moreover, increased ($p \leq 0.05$) albumin to globulin ratio, blood urea nitogen, creatinine and uric acid levels were observed in the intoxicated rats of group II, III and IV as compared to control group. Thus, it is concluded that 28 days subacute toxicity of NaF in wistar rats has adverse effects on haemato-biochemical profile.

Keywords: Haemato- Biochemical, Rats, Sodium fluoride, Toxicity

Fluoride is ubiquitous in the environment and therefore, sources of drinking waters are likely to contain at least some amount of fluoride. Fluoride being a cumulative poison, prolonged ingestion of drinking water containing 1.5 - 3 ppm of fluoride ion causes deleterious effects on skeletal, dental and soft tissues. The mean fluoride concentration in ground and surface water varies with geographical location *viz.* 0.48 ± 0.05 ppm to 1.30 ± 0.16 ppm at Nayagarh, Orissa (Maiti *et al.*, 2003) whereas 0.2 ± 0.02 ppm to 13.2 ± 1.2 ppm in Durg district of Chhattisgarh (Giri *et al.*, 2013). Concentration of fluoride above the permissible limits causes several harmful effects on body tissues leading to impairments in normal physiology. Blood is the most important connective tissue of the body which helps in transportation of several molecules. The haematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal (Mukinda and Syce, 2007). Thus any changes in tissues may cause some alterations in the blood cells and blood plasma constituents. The present communication reports the extent of haemato-biochemical alterations in repeated dose oral subacute toxicity of sodium fluoride in Wistar rats.

MATERIALS AND METHODS

The experimental investigation was planned to adjudge the toxicopathological effects of sodium fluoride on haemato-biochemical profile in Wistar rats after obtaining approval from Institutional Animal Ethics Committee (IAEC). The experimental rats were maintained under regular supervision in controlled environment with 12 hours light dark cycle and provided with standard feed and

water *ad libitum* throughout the experimental period. The test chemical Sodium fluoride (NaF) of 99% purity was obtained from Merck Limited, Mumbai- 400018. Forty eight healthy Wistar rats, weighing 195 - 215 g (females) and 220 – 235 g (males) were randomly divided into four different groups having 12 (6 males + 6 Females) rats each. Rats of group I were kept as control and were given only distilled water, orally. Rats of group II, III and IV were administered NaF, dissolved in distilled water, at the dose rate of 5.0, 25.0 and 50.0 mg/kg body weight, orally for 28 days respectively.

At the end of experiment on day 28 under diethyl ether anaesthesia blood samples were collected in heparinised and non-heparinised vials from retroorbital venous plexus of rats as described by Moore (2000). The samples were analysed for various haematological parameters viz. haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), differential leucocyte count (DLC) and the erythrocytic parameters (MCH, MCHC and MCV) by standard methods. Enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and levels of glucose, total protein, albumin, blood urea nitrogen (BUN), uric acid and creatinine were determined using the standard diagnostic kits (Autopak, Siemens Healthcare Diagnostic Ltd. Baroda) and semi autoanalyzer (RA - 50, Chemistry System, Bayer) as per the manufacturer's instructions. The data obtained was subjected to statistical analysis by complete randomized design (CRD) and presented as Mean ± S.E.

RESULTS AND DISCUSSION

The findings of haematological profile, enzyme and biochemical profile is summarised in tables 1, 2 and 3**.** The administration of NaF orally for 28 days to Wistar rats caused dose dependent reduction ($p \leq 0.01$) in the levels of Hb, PCV, TEC and percent lymphocytes whereas an increase in MCV, MCH and neutrophils % (Table 1) of all the rats belonging to group II, III and IV as compared to rats of control group (group I). The mean values of TLC were found to be increased in rats of group III ($p \leq 0.05$) and group IV ($p \leq 0.01$) as compared to rats of group I and II. Moreover, macrocytic– normochromic anaemia was observed in NaF intoxicated rats. Biochemical alterations comprised of dose dependent elevation ($p \leq 0.01$) in plasma enzyme activities of ALT, AST and ALP (Table 2),

Table 1. Effect of subacute sodium fluoride toxicity on haematological parameters in Wistar rats (n = 6)

Parameters	Group I	Group II	Group III	Group IV
Hb (g/dl)	13.90 ± 0.13 ^{a}	12.15 ± 0.17 ^{c}**	12.20 ± 0.08 ^{b}**	10.55 ± 0.13 ^{d}**
PCV (%)	39.98 ± 0.34 ^{a}	34.20 ± .44 ^{b}**	32.25 ± 0..26 ^{c}**	30.50 ± 0.12 ^{d}**
TEC (Millions/ cu.mm)	8.65 ± 0.05 ^{a}	6.84 ± 0.06 ^{b}**	5.76 ± 0.09 ^{c}**	4.23 ± 0.02 ^{d}**
MCH (pg)	16.08 ± 0.10 ^{d}	17.77 ± 0.12 ^{c}**	19.47 ± 0.34 ^{b}**	24.92 ± 0.19 ^{a}**
MCV (fl)	46.24 ± 0.20 ^{d}	50.03 ± 0.21 ^{c}**	56.06 ± 1.03 ^{b}**	72.06 ± 0.10 ^{a}**
MCHC (g/dl)	34.77 ± 0.08 ^{a}	34.53 ± 0.12 ^{a}	34.73 ± 0.18 ^{a}	34.59 ± 0.30 ^{a}
TLC (Thousands/ cu.mm)	7.01 ± 0.01 ^{c}	7.02 ± 0.05 ^{c}	7.20 ± 0.04 ^{b}*	7.42 ± 0.09 ^{a}
Lymphocyte (%)	66.75 ± 0.25 ^{a}	58.00 ± 0.82 ^{b}**	48.00 ± 0.41 ^{c}**	41.25 ± 0.25 ^{d}**
Monocyte (%)	4.75 ± 0.25 ^{a}	4.25 ± 0.28 ^{a}	4.25 ± 0.24 ^{a}	4.25 ± 0.25 ^{a}
Neutrophil (%)	24.75 ± 0.48 ^{d}	33.75 ± 0.75 ^{c}**	44.25 ± 0.48 ^{b}**	51.25 ± 0.48 ^{a}**
Eosinophil (%)	3.75 ± 0.25 ^{a}	4.00 ± 0.41 ^{a}	3.50 ± 0.29 ^{a}	3.25 ± 0.25 ^{a}
Basophil (%)	00.00	00.00	00.00	00.00

Values indicate Mean ± S.E. Superscripts may read row wise for comparison of means. Mean values with similar superscripts do not differ significantly from each other. *significant at $p \leq 0.05$ and **significant at $p \leq 0.01$ from control group (group I).

Table 2. Effect of daily oral administration of sodium fluoride on plasma enzymes in Wistar rats (n = 6)

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
I	44.75 ± 0.68 ^{d}	96.0 ± 0.82 ^{d}	115.75 ± 1.11 ^{d}
II	49.75 ± 1.28 ^{c**}	136.25 ± 1.75 ^{c**}	137.25 ± 1.11 ^{c**}
III	64.75 ± 0.84 ^{b**}	161.0 ± 1.08 ^{b**}	193.25 ± 1.38 ^{b**}
IV	126.75 ± 2.35 ^{a**}	200.75 ± 1.38 ^{a**}	328.75 ± 1.11 ^{a**}

Values indicate Mean ± S.E. Superscripts may read column wise for comparison of means. Mean values with similar superscripts do not differ significantly from each other.

*significant at $p \leq 0.05$ and **significant at $p \leq 0.01$ from control group (group I).

In the present study the findings of haematological alterations might be attributed to NaF induced stress, inhibition of Na+/K+ ATPase activity in cells, decreased erythropoiesis due to high level of fluoride in serum and bone for a prolonged period or nutritional imbalance or reduction in the size of medullary canal due to osteosclerosis (Santhakumari and Subramanian, 2007). The other reason might also be due to the toxic damage to the vital organs particularly spleen, liver, kidney and bone which are directly or indirectly related with haematopoiesis (Sharma *et al.*, 2010, Rao and Vidyunmala, 2010). Indeed, the transaminases (AST and ALT) are well- known enzymes used as good indicators of liver function and as biomarkers predicting possible toxicity. Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood. ALP is a marker enzyme of fluoride toxicosis and bone pathology. The increase in the activity of ALP might be

Table 3. Effect of daily oral administration of NaF to Wistar rats for 28 days on plasma biochemical parameters (n= 6)

Parameters	Group I	Group II	Group III	Group IV
Glucose (mg/dl)	69.50 ± 0.87^{c}	70.50 ± 1.05^{c}	87.75 ± 0.83^{b**}	96.75 ± 0.95^{a**}
Total Protein (g/dl)	7.25 ± 1.10^{a}	6.88 ± 1.26 ^{b**}	6.33± 1.05^{c**}	5.73 ± 1.38^{d**}
Albumin (g/dl)	3.95 ± 0.93^{b}	4.13 ± 0.85^{a**}	3.90 ± 0.81 ^{bc}	3.80 ± 0.94^{c*}
Globulin (g/dl)	3.30 ± 0.12^{a}	2.75 ± 0.53 ^{b**}	2.43 ± 0.66 ^{c**}	1.93 ± 0.79^{d**}
Albulin: Globulin	1.20 ± 0.65^{c}	1.50 ± 0.32 ^{b**}	1.61 ± 0.25 ^{b**}	1.99 ± 0.31^{a} **
Creatinine (mg/dl)	0.73 ± 0.35^{b}	0.78 ± 0.22^{b}	0.93 ± 0.51 ^{b} *	2.88 ± 0.96^{a**}
BUN (mg/dl)	35.14 ± 0.66^{c}	35.93 ± 0.81^{c}	58.65 ± 0.95^{b**}	100.07 ± 0.98^{a**}
Uric Acid (mg/dl)	0.80 ± 0.24^{d}	1.25 ± 0.36^{c*}	2.38 ± 0.31^{b**}	1.99 ± 0.11^{a**}

Values indicate Mean ± S.E. Superscripts may read row wise for comparison of means. Mean values with similar superscripts do not differ significantly from each other. *significant at $p \leq 0.05$ and **significant at $p \leq 0.01$ from control group (group I).

hypoproteinaemia ($p \leq 0.01$) and hypoglobulinaemia ($p \leq 0.01$) in rats of group II, III and IV in comparison to rats of control group. Dose dependent increased ($p \leq 0.01$) glucose was recorded in rats of group III and IV. Apart from these observation there were also an increased ($p \leq 0.05$) albumin to globulin (A: G) ratio, BUN, creatinine and uric acid concentration (Table 3) in the intoxicated rats of group II, III and IV as compared to those of control group.

due to the fluoride induced cell injury to osteoblasts and osteocytes initiate a repair response resulting in increased proliferation of osteoblasts, matrix formation and ALP production. Hyperglycaemia noticed in the present study corroborates well with Menoyo *et al.* (2008) who reported that after intake of NaF, the plasma fluoride increases and plasma insulin decreases, accompanied by an increase in serum glucose levels. The decreased levels of total plasma protein and globulin in rats of group II, III and IV in our

study might be attributed to liver damage, nephropathy and malabsorption due to gastroenteropathy. BUN, creatinine and uric acid concentration in plasma are important indices of kidney function and are related to the glomerular filtration rate (GFR). The plasma levels get increased as the GFR decreases due to renal malfunctions. In the present study, BUN, creatinine and uric acid were increased in all NaF intoxicated groups. These observations might be attributed to fluoride induced renal failure. Our findings on haematology and various biochemical parameters is in accordance with the earlier reports of natural cases of fluorosis in cattle (Maiti and Das, 2004; Upadhyay *et al.*, 2005), goat (Singh *et al.*, 2002), buffaloes (Sharma *et al.*, 2010) as well as in experimental studies in rats (Sharma *et al.*, 2007; Bharti and Srivastava, 2011) and mice (Rao and Vidyunmala, 2010). On the basis of this study it is concluded that 28 days subacute toxicity of NaF in wistar rats has adverse effects on haemato-biochemical profile in a dose dependent manner.

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