

## TOXICITY OF FLUORIDE TO DIABETIC RATS

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**SUMMARY:** Wistar rats were given 20 ppm fluoride in drinking water, or single administration of 115 mg/kg alloxan i.m. to induce diabetes, or single administration of 115 mg/kg alloxan i.m. followed by 20 ppm fluoride for 31 days. Blood sugar level increased in rats given alloxan and alloxan + fluoride. Body weight gain in rats given alloxan + fluoride decreased significantly compared to other groups. Decrease in hæmoglobin and glutamic oxaloacetate transaminase (GOT) was seen only in rats given alloxan + fluoride. In this group alkaline phosphatase, the target enzyme in fluoride toxicosis, increased considerably. The toxicity of fluoride in diabetic rats was further reflected in organ weight data. This investigation shows that fluoride toxicity is greater in diabetic rats.

Key words: Alkaline phosphatase; Body weight; Diabetes mellitus; Fluoride toxicity; Hæmoglobin.

### INTRODUCTION

Endemic fluorosis remains one of the most important water-borne diseases in our country.<sup>1</sup> Over 50% of the groundwater sources in India have been contaminated by fluoride. The problem of fluorosis has been reported in various states in India, affecting more than 150,000 villages seriously.<sup>2</sup>

A survey conducted in 1977 revealed that over two million Indians were affected by diabetes.<sup>3</sup> The International Federation of Diabetes and the World Health Organization pointed out the steadily growing incidence of diabetes mellitus and introduced it as a broader based non-communicable disease control programme.<sup>4</sup> Diabetes is not a single disease but a heterogeneous group of disorders in glucose intolerance.<sup>5</sup> Diabetes mellitus affects anabolic, catabolic and mineral metabolism.<sup>6</sup>

Considering the large population suffering from diabetes mellitus and the high level of fluoride in 45% of drinking water sources in India,<sup>7</sup> it may be presumed that people who suffer from diabetes mellitus may intake abnormal levels of fluoride. To the best of our knowledge, not many studies have been carried out to understand the manifestation of fluoride toxicity in people suffering from diabetes mellitus. In the present investigation, diabetes mellitus (NIDD - non-insulin-dependent diabetes) was induced in rats and thereafter they were exposed to fluoride in drinking water. Body weight changes, organ weights, hæmatology and blood chemistry were considered in this study.

### MATERIALS AND METHODS

Female albino rats of Wistar strain (113-133 g) procured from the Institute's animal house were acclimated to laboratory conditions for 7 days, and divided into 4 groups, each of 4 animals. Group 2 was given: 20 ppm fluoride in drinking water, Group 3 115 mg/kg alloxan i.m. and Group 4 115 mg/kg alloxan i.m. followed by 20 ppm fluoride in drinking water. The control (Group 1) was given only ordinary drinking water.

The fluoride solution was prepared by dissolving an appropriate quantity of NaF (Ranbaxy Laboratories Ltd, Punjab, India) in distilled water. The dose of alloxan

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(S.d fine-Chem Ltd, Bombay, India) was prepared in sterilized water for injection. Daily body weight gain, feed and water consumption of the animals were recorded.

At the end of the experiment, *i.e.* on day 32, blood samples were collected from anaesthetised rats from orbital sinus for haematological measurements: white blood corpuscles (WBC), red blood corpuscles (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and biochemical investigations (glucose, protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, glutamic pyruvate transaminase (GPT), glutamic oxaloacetate transaminase (GOT) and alkaline phosphatase (ALP).

The animals were sacrificed under anaesthetic ether, and brain, heart, liver, spleen, kidney, gonad and adrenal were isolated for weight determination. Haematological parameters were determined on an Erma Particle Counter (Erma Inc, Tokyo, Japan) and the biochemical parameters were estimated using reagent kits (Span Diagnostics, Surat, India) on Erba-Chem-5 plus semiauto analyser (Transasia Biochemicals Ltd, Bombay, India). The data were subjected to a Barlett test for homogeneity, followed by Anova and 't' test.<sup>8</sup>

### RESULTS AND DISCUSSION

Two spontaneous mortalities occurred (one on day 3, the other on day 30) in rats given 115 mg/kg alloxan *i.m.* (Group 3), and one occurred (on day 31) in rats given 115 mg/kg alloxan *i.m.* + 20 ppm fluoride in drinking water (Group 4).

The present study does not show any effect on feed consumption of rats treated with fluoride (Group 2) or alloxan (Group 3) or alloxan + fluoride (Group 4) (Table 1). Similar results have been reported when rats were given 10 ppm fluoride in drinking water.<sup>9</sup>

TABLE 1. Feed and water consumption in control and treated rats

Group	Feed consumption (g)	Water consumption (mL)
1 Control	11.28 ± 1.47	17.88 ± 2.78
2 Fluoride	10.93 ± 2.09	15.73 ± 2.18
3 Alloxan	14.15 ± 2.02	36.73* ± 6.49
4 Alloxan + fluoride	16.73 ± 1.44	62.97 <sup>□</sup> ± 8.18

Values are mean ± S.D. \* Significantly different from Control ( $P < 0.05$ )

<sup>□</sup> Significantly different from Control as well as Alloxan group ( $P < 0.05$ )

An increase in water consumption was recorded in rats given alloxan + fluoride, compared to control animals. Water consumption in rats given alloxan + fluoride was significantly higher than that of the alloxan group. This finding has significance because in an area endemic for hydrofluorosis intake of large quantities of drinking water directly increases fluoride intake. Reports have shown that the intake of excessive amounts of fluoride causes polydipsia.<sup>10</sup> Body weight tended to gain in both control and treated groups, though the gain was less in the latter groups. In rats treated with alloxan (Group 3) the body weight gain decreased until day 8, then increased but the rate of increase was less than that of the control and fluoride

treated groups. In rats treated with fluoride + alloxan the body weight gain decreased from day 2 of the experiment and these animals did not recover until day 31, when the experiment terminated (Figure 1). It has been shown that fluoride decreases body weight gain in laboratory animals.<sup>11,12</sup> The present study shows that a decrease in body weight gain induced by fluoride was greater in diabetic rats, which is further evident from the negative slope of the regression equation (percent body weight gain vs day) (Table 2).

FIGURE 1. Daily percent body weight gain in treated rats

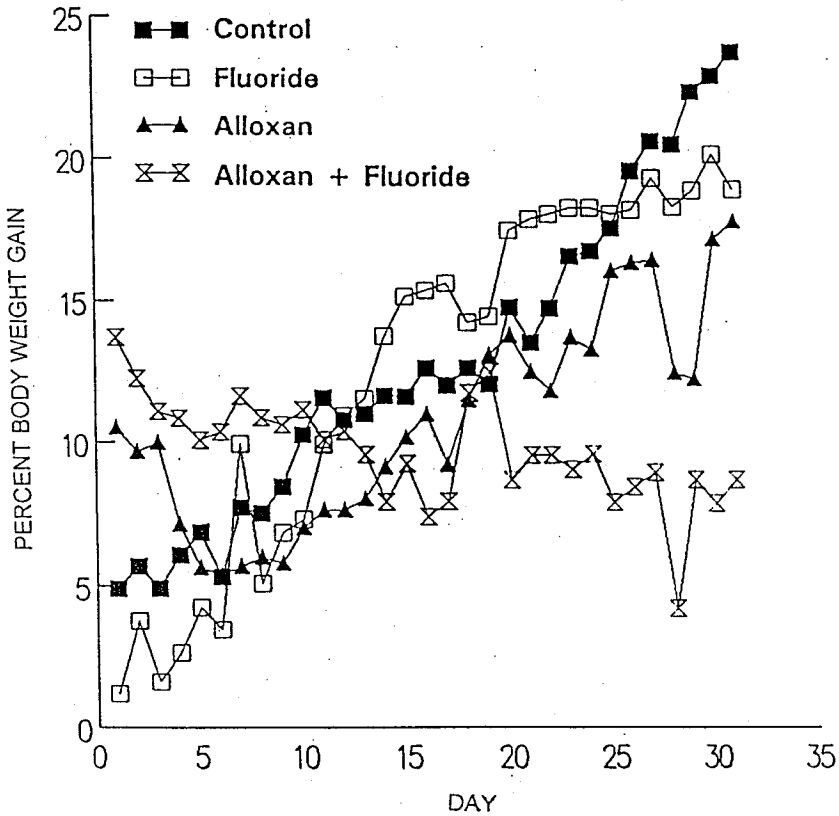


TABLE 2. Regression equation between body weight (g%/day) and treatment period (day)

Animals	Regression equation*	R <sup>2</sup>	S.E. of slope
Control	Y = 3.1663 + 0.5992X	0.95	0.0244
Fluoride	Y = 2.0860 + 0.6501X	0.91	0.0390
Alloxan	Y = 5.3308 + 0.3150X	0.66	0.0418
Diabetes + fluoride	Y = 11.8876 - 0.1381X	0.46	0.0277

\* Y = a + bX: [Y = Body weight gain (g%/day); a = Intercept; b = Slope; X = days]

Hæmatological parameters evaluated in the present study - viz. WBC, MCV, MCH and MCHC were not affected by the treatments, but a decrease in RBC and HCT was evident in rats treated with fluoride, HCT in rats treated with alloxan, and RBC, HGB and HCT in rats treated with alloxan + fluoride. HGB, which was not significantly different from other groups in rats treated with fluoride or alloxan, significantly decreased in those given alloxan + fluoride, which indicates the toxicity to HGB by fluoride in diabetic rats (Table 3). Reports have shown that fluoride decreases RBC and HGB.<sup>13,14</sup> However, in the present investigation rats given 20 ppm fluoride (Group 2) did not show any change in these parameters, but in diabetic rats given 20 ppm fluoride they significantly decreased. This result indicates that diabetic rats are prone to exhibit alterations in RBC and HGB in fluoride toxicosis.

TABLE 3. Hæmatology of control and treated rats

Group	WBC ( $\times 10^3 \mu\text{L}$ )	RBC ( $\times 10^6 \mu\text{L}$ )	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control (n=4)	8.25 $\pm 2.1$	7.15 $\pm 1.37$	14.89 $\pm 2.99$	41.69 $\pm 9.51$	55.83 $\pm 1.59$	20.75 $\pm 1.35$	37.28 $\pm 2.41$
Fluoride (n=4)	8.53 $\pm 3.59$	5.49* $\pm 1.01$	12.42 $\pm 3.25$	28.81* $\pm 4.98$	53.24 $\pm 4.42$	19.98 $\pm 1.61$	38.40 $\pm 2.87$
Alloxan (n=2)	8.50 $\pm 4.67$	5.81 $\pm 1.46$	11.85 $\pm 3.54$	29.93* $\pm 8.80$	51.34 $\pm 2.29$	20.20 $\pm 1.13$	37.75 $\pm 2.76$
Alloxan + fluoride (n=3)	6.97 $\pm 2.51$	4.43* $\pm 0.93$	9.12* $\pm 2.09$	25.00* $\pm 7.70$	53.90 $\pm 2.46$	22.77 $\pm 3.42$	39.17 $\pm 4.78$

Values are mean  $\pm$  S.D.

\* Significantly different from control ( $P < 0.05$ )

Administration of 115 mg/kg alloxan i.m. significantly increased blood glucose level. Induction of diabetes mellitus which reflects an increase in blood sugar level by the administration of alloxan has been studied by several workers.<sup>15</sup> In rats treated with alloxan + fluoride the blood sugar increased, compared with that of control, but it was on a par with that of the rats treated with alloxan (Table 4). A significant increase in protein and globulin observed in rats given fluoride and those given alloxan + fluoride may indicate hepatic disorders.<sup>16</sup> An increase in protein was reported in ventral prostate of rats given 10 mg/kg b.w. fluoride orally for 30 days.<sup>17</sup> BUN significantly increased in rats given alloxan and those given alloxan + fluoride, whereas creatinine was unaffected in any of the treated groups compared to that of control. An increase in BUN which may be due to renal dysfunction is another characteristic change in diabetes.<sup>18</sup> An increase in GPT was evident in rats given alloxan but in those given alloxan + fluoride the GPT level was similar to that of control animals. GOT significantly decreased in the alloxan + fluoride group. It has been shown that 10 ppm of fluoride given in drinking water for 12 weeks decreased GOT in rabbits.<sup>19</sup> However, in the present study rats given 20 ppm of fluoride (Group 2) did not show any change in GOT level. The decline of GOT in rats given alloxan + fluoride (Group 4) may suggest the magnification of fluoride toxicity in diabetic rats as also indicated by alkaline

TABLE 4

BIOCHEMICAL ESTIMATIONS OF RATS GIVEN FLUORIDE (20 ppm)ALLOXAN (115 mg/kg/b.w.)  
AND ALLOXAN (115 mg/kg/b.w.) + FLUORIDE (20 ppm)

Groups	glucose (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	BUN (mg/dl)	Creatinine (mg/dl)	GPT IU/l	ALP IU/l	GOT IU/L
CONTROL (n=4)	105.82 ± 9.31	6.95 ± 0.71	3.92 ± 0.77	2.78 ± 0.78	19.57 ± 4.85	2.77 ± 0.24	64.61 ± 17.57	276.43 ± 46.47	149.08 ± 33.15
FLUORIDE (n=4)	116.08 ± 3.46	8.05 * ± 1.13	3.73 ± 1.35	4.46 * ± 2.47	16.62 ± 1.36	2.37 ± 0.19	81.24 ± 6.75	460.27 * ± 50.86	133.05 ± 18.71
ALLOXAN (n=2)	363.84 * ± 15.49	6.68 ± 0.91	3.33 ± 0.29	3.88 ± 0.14	39.29 * ± 5.31	2.59 ± 0.16	91.58 * ± 14.99	251.90 ± 30.26	145.75 ± 37.55
ALLOXAN + FLUORIDE (n=3)	397.85 * ± 57.12	8.86 * ± 1.30	3.95 ± 0.26	4.83 * ± 0.53	39.69 * ± 1.69	3.14 ± 0.91	76.37 ± 3.19	620.07 * ± 189.47	105.59 * ± 17.93

Values are expressed as mean ± S.D.; Values given in parentheses are number of animals.

\* Significantly different from control (P < 0.05)

TABLE 5  
 PERCENT ORGAN WEIGHTS OF RATS GIVEN FLUORIDE (20 ppm) ALLOXAN (115 mg/kg b.w.)  
 AND ALLOXAN (115 mg/kg b.w.) + FLUORIDE (20 ppm)

Groups	Brain	Heart	Liver	Spleen	Adrenal @	Kidney @	Ovary @
CONTROL	1.68 ± 0.82 (n=4)	0.63 ± 0.29 (n=4)	6.67 ± 3.43 (n=4)	0.87 ± 0.41 (n=4)	0.04 ± 0.01 (n=8)	0.69 ± 0.34 (n=8)	0.09 ± 0.01 (n=8)
FLUORIDE (n=4)	2.38* ± 0.21 (n=4)	0.76 ± 0.13 (n=4)	8.53 ± 1.47 (n=4)	1.40 ± 0.22 (n=4)	0.04 ± 0.01 (n=8)	0.87 ± 0.12 (n=8)	0.04 ± 0.01 (n=8)
ALLOXAN (n=2)	2.99* ± 0.40 (n=2)	0.63 ± 0.01 (n=2)	8.42 ± 0.35 (n=2)	0.76 ± 0.06 (n=2)	0.04 ± 0.01 (n=4)	0.98 ± 0.17 (n=4)	0.05 ± 0.02 (n=4)
ALLOXAN + FLUORIDE (n=3)	1.84 ± 0.04 (n=3)	0.73 ± 0.18 (n=3)	9.72 ± 1.83 (n=3)	0.70 ± 0.6 (n=3)	0.11* ± 0.01 (n=6)	1.06* ± 0.12 (n=6)	0.05 ± 0.01 (n=6)

Values are expressed as mean g%/weight ± S.D.; Values given in parentheses are number of samples.

\* Significantly different from control (P < 0.05).

@ Both left and right

phosphatase, which has been reported as the target enzyme in fluoride toxicosis.<sup>20</sup> Higher activity of serum alkaline phosphatase reflects abnormal formation of bone due to stimulated osteoblastic activity as suggested by Blood and Radostits.<sup>21</sup> In the present study, alkaline phosphatase level in fluoride-treated rats (Group 2) significantly rose and the level shot up in diabetic rats given fluoride (Group 4).

Pathological findings of the present study also indicate toxicological magnification by fluoride in diabetic rats. Weight ratios of adrenals and kidneys significantly increased compared to those of control (Table 5). Both diabetes<sup>22</sup> and fluorosis<sup>23</sup> are major disorders which affect several organ systems. It may be concluded from the present study that toxicity by fluoride is greater in diabetes.

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