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Fluoride-induced Haematological Changes in Rabbits

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Although the effect of fluoride ingestion on the blood cell profile in experimental animals was studied earlier by HIRAO (1972) who pointed out changes in hemoglobin content, population of erythrocytes, reticulocytes, platelets, leucocyte, and hematocrit, the information is inadequate to provide a scientific rationale for the wide spectrum of deviations observed both in experimental and human fluorosis. According to HIRAO (1972), anaemia was a result of fluoride ingestion, and the finding was corroborated by the reduction in body weight of the animal model. However, the conclusions drawn were based on studies conducted on 10, 30, and 50 mg doses of NaF administered to rabbits for a shorter duration, i.e. 5 mo only. The information available on short-term exposure to NaF is inadequate to elucidate the manifestations of fluoride toxicity.

The present investigation is designed to collect the baseline data on blood cell counts and hemoglobin content of rabbits born to fluorosed rabbits as well as adult rabbits administered with 10 mg NaF/kg body weight for 6 and 12 mo.

MATERIALS AND METHOD

Adult, healthy, female rabbits maintained on standard diet (Hindustan Lever, Bombay) and water ad libitum were given through intragastric route 10 mg NaF/kg body weight daily for 12 mo. Control rabbits were of the same sex and having body weight within the range of the experimental animals. The control animals were maintained under similar laboratory conditions as experimental animals but were deprived of NaF. Blood was drawn from the ear vein of the rabbits after 6 and 12 mo. Blood samples from 5 mo old rabbits born to fluorosed rabbits (mothers treated with 10 mg NaF/kg body weight for 7 mo and not the young ones) were also collected in a similar manner. Sodium salt of heparin was used as the anticoagulant. Blood cells were counted by the method of CARTWEIGHT (1968) as described below.

Erythrocytes : Blood was diluted in red blood cell pipette using erythrocyte diluting fluid. The hemocytometer was charged carefully, and red blood cells were counted under high power in the four corners and central square of the central large square.

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Total Leukocytes : Blood was diluted in white blood cell pipette using leukocyte diluting fluid (Tuirk's solution : Glacial acetic acid 4 mL; Gentian violet (1% aqueous sol) 1 mL; Distilled water 100 mL). Hemocytometer was charged and leukocytes were counted in each of four large corner squares.

Eosinophils : Blood was diluted in white blood cell pipette with eosinophil diluting fluid (pilot's solution). The eosinophils in all 9 large squares in each of two chambers were counted.

Platelets : Blood was diluted with 1% ammonium oxalate solution in a red blood cell pipette. Hemocytometer was charged and kept in a moist chamber for 15 min. Platelets were counted in the four small squares and central square of the central large square in each of the chambers.

Lymphocytes, Monocytes, Basophils and Neutrophils : Blood smear was differentially stained using Weight's stain. Approximately 400 to 500 differentially stained cells were counted from a large field. The total number of individual cell poputation was calculated.

Hemoglobin content of the blood was estimated using Drabkin's Reagent (Sigma) Colorimetrically.

The statistical significance of the data was evaluated by student "t" test.

RESULTS AND DISCUSSION

It is evident from Table 1 that in all the 4 groups of animals investigated an increase in body weight has been recorded. The blood cell counts also revealed changes due to excessive ingestion of fluoride.

It is evident from Table 2 that the erythrocyte and leukocyte population revealed a significant reduction after 6 and 12 mo after fluoride ingestion. However, the young ones born to those animals which were on NaF for 7 mo did not reveal any deviation either in erythrocyte or leukocyte population.

The lymphocytes and basophils significantly altered in 6 and 12 mo treated rabbits as well as in rabbits born to fluorosed animals. However, the lymphocyte population at 6 mo after NaF ingestion revealed an increase while in the other groups the lymphocyte population was reduced.

The thrombocytes (platelets), monocytes and neutrophils were reduced in 6 and 12 mo treated rabbits but not in the young ones born to rabbits intoxicated with NaF.

The hemoglobin content was significantly reduced in all the three experimental groups of animals investigated.

Table 3 reveals the fluoride content in plasma in the various groups of animals investigated. It is noteworthy that the young rabbits had fluoride in the normal limits while the 6 mo treated animals have higher fluoride content

Table 1 : Showing the Body Weight of the Rabbits Before and After NaF Ingestion.

	Initial Body Weight (When NaF Administration was Started)	Final Body Weight (When Blood was drawn)
Controls rabbits	-	1200 - 1306 g
Rabbits born to fluorosed mothers (mothers treated for 7 mo)	50 - 60 g (Body weight of the young ones at the time of birth. Young ones were not exposed to NaF after birth)	1240 - 1360 g (5 mo old)
Control rabbits	600 - 700 g	1650 - 1800 g
6 mo treated	650 - 750 g (Body weight when NaF treatment was started)	2000 - 2160 g
12 mo treated	620 - 680 g (Body weight when NaF treatment was started)	2400 - 2450 g

In each group 5 animals were investigated.

Table 2 : Effect of NaF Administration (10 mg/kg body wt. at 24 h interval) on Blood Cell Count and Hemoglobin Content.

Blood Cell Population		Control Mean ± S.D.	Rabbits born to Fluorosed Rabbits Mean ± S.D.	Duration	
				6 months Mean ± S.D.	12 months Mean ± S.D.
Erythrocytes	(M/cmm)	5.9 ± 0.33	5.66 ± 0.41	3.68 ± 1.2*	4.5 ± 0.74*
Leukocytes	(Th/cmm)	10.78 ± 0.965	10.466 ± 0.611	4.76 ± 0.522*	6.44 ± 0.434*
Lymphocytes	"	3.12 ± 0.239	2.68 ± 0.39*	4.52 ± 1.11*	2.54 ± 0.55*
Thrombocytes	"	428.9 ± 15.09	408.7 ± 9.93	340.0 ± 43.0*	375.2 ± 25.75*
Monocytes	"	0.691 ± 0.029	0.707 ± 0.116	0.367 ± 0.045*	0.512 ± 0.024*
Neutrophils	"	3.16 ± 0.416	3.02 ± 0.40	1.12 ± 0.303*	1.76 ± 0.40*
Eosinophils	"	0.248 ± 0.37	0.072 ± 0.044	0.098 ± 0.057	0.056 ± 0.052
Basophils	"	0.304 ± 0.059	0.12 ± 0.01*	0.12 ± 0.083*	0.1 ± 0.07*
Blood Hemoglobin	(g/dL)	14.68 ± 0.729	11.16 ± 1.107*	10.18 ± 0.867*	13.2 ± 1.44*

In each group 5 sets of animals were carried out; S.D. = Standard deviation

* $P < 0.01$

which was found to be significant at P value $<$ 0.01. Although in the present investigation, there are no data for fluoride content for 12 mo duration, there is evidence from earlier reports to suggest that the fluoride content in serum/plasma increase as the duration of NaF administration increase (SUSHEELA et al. 1982).

Table 3 : Showing the Fluoride Content (in ppm) in Plasma in Young Rabbits Born to Fluorosed Mother and Adult Rabbits Treated with NaF (10 mg/kg body wt/day) for Six Months.

	Control	Young Rabbits Born to Fluorosed Mother	Adult Rabbits Treated for 6 mo
	0.06	0.07	0.38
	0.06	0.06	0.32
	0.09	0.08	0.4
		0.06	0.42
		0.05	0.36
Mean $\pm$ S.D.	0.07 $\pm$ 0.02	0.064 $\pm$ 0.011	*0.37 $\pm$ 0.038

* P $<$ .01; S.D. = Standard deviation.

The significant reduction in the population of lymphocytes, basophils and hemoglobin content of the young rabbits could possibly be due to toxic manifestations as a result of placental transfer of fluoride. Placental transfer of fluoride has been confirmed by other investigators(MAPLESDER et al. 1960; ERICSSON & MALMNAS 1962). However, the plasma fluoride level being within normal limits, it is possible that fluoride deposition may have occurred in the developing osseous tissues.

The reduction in the blood cell counts could possibly due to the II-oxygenated adrenal cortical steroids other than aldosterone as it is known to influence the number of blood lymphocytes, erythrocytes and eosinophils as well as the structure and function of lymphoid tissue (WHITE et al. 1978). The reduction in the number of lymphocytes and erythrocytes during 12 mo of NaF exposure may possibly be due to the reduction of adrenal cortical steroids other than aldosterone. RAO & SUSHEELA (1979) have demonstrated hypertrophy of adrenal gland and reduction in the activity of delta 5, 3 beta steroid dehydrogenase activity due to excessive fluoride ingestion.

The reduction in hemoglobin is most unlikely due to inadequate nutrition as reported by SUTTIE (1968) as there is an increase in body weight of the animals investigated. It is likely that hypofunction of adrenal gland may be responsible for anaemia.

The thrombocytopenia observed during 6 and 12 mo of fluoride ingestion may possible lead to decreased formation of active thromboplastin with deficient production of thrombin. Enhanced fibrinogen levels in plasma during fluoride intoxication due to calcium and thrombin deficiency has been reported by SUSHEELA & SHARMA (1981).

In conclusion it should be stated that excessive ingestion of fluoride exerts its toxic influence on haematological profile of adult rabbits and has equally adverse influence on the young rabbits born to fluorosed mothers. Placental transfer of fluoride to the growing foetus is known to occur although the plasma fluoride level is latent. The hematological studies is suggestive of the involvement of adrenal cortex in fluoride intoxication.

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