FLUORIDE TOXICITY ON RAT TESTIS AND CAUDA EPIDIDYMAL TISSUE COMPONENTS AND ITS REVERSAL

Niloufer J Chinoy, Shruti Shukla, Amita S Walimbe and Shrobona Bhattacharya
Ahmedabad, India

SUMMARY: The toxic effects were evaluated of sodium fluoride (NaF) ingestion on the physiology of tissue components of testis and epididymis of adult, male albino rats, and the possible reversal of the effects by use of some antidotes. The results revealed that the testis and cauda epididymal proteins were altered, with disappearance of some proteins and induction of some new ones. This is the first report of such changes. Phosphatidylcholine and sphingomyelin remained unaltered during NaF ingestion, but a significant decrease occurred in phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. Significantly low levels of glutathione after 30 days of treatment were also obtained. On comparing the alterations in protein profile, phospholipids and glutathione in both tissues, it was evident that the protein profile was disturbed more in testis than in cauda epididymis, whereas phospholipids and glutathione levels were affected more in cauda than in testis. The investigations into reversibility showed that ascorbic acid (vitamin C) and calcium could ameliorate fluoride toxicity.

Key words: Ascorbic acid; Calcium; Epididymis; Fluoride toxicity; Phospholipids; Rat; Reversibility; Testis.

INTRODUCTION

The susceptibility of the male reproductive system to toxic insults leading to reduced fertility and abnormal fetal development has become a major concern recently. Among toxic agents known to affect reproduction, the chemical or physical factors that influence germ cells are the most prevalent.[1] Studies from our laboratory[1-12] have revealed that fluoride, one of the naturally occurring toxicants, affects the structural and functional integrity of reproductive organs in male and female rodents and guinea pigs, and leads to the loss of fertility. It has been reported[1] that sodium fluoride (NaF) affects the histoarchitecture of testis causing disintegration of the germinal epithelium, with denudation and vacuolation of seminiferous epithelial cells. This disturbs spermatogenesis, and thus reduces spermatozoa density. The structure of cauda epididymis was also altered and its histology revealed damage to the secretory epithelium with intensely pycnotic nuclei, loss of stereocilia and absence of sperm in the lumen. In rodents the cauda epididymal sperm mobility and fertility rate decreased.

The present work was undertaken to elucidate, in the light of earlier work from our laboratory,[4,5,12] the effects of fluoride toxicity on some androgen dependant parameters of rat testis and cauda epididymis and its possible reversibility by feeding ascorbic acid (AA) and or calcium (Ca^{2+}).

MATERIALS AND METHODS

Adult, male, albino rats (*Rattus norvegicus*) weighing between 150-200 gm were maintained on standard chow and water which was given *ad libitum*. The animals were grouped into five groups and treatment was administered as shown in the experimental protocol.

Dr N J Chinoy, Professor and Head, Zoology Department, School of Sciences, Gujarat University, Ahmedabad 380 009, India. Telephone (91) 79 442362, Fax (91) 79 441654.

Route: oral.
Source and purity: NaF -Loba Chemie, Bombay 99% purity.
AA - Loba Chemie, Bombay, 99% purity
Ca^{2+} - Calcium phosphate, Glaxo India Limited, Bombay, 99% purity.
All the other chemicals used were from Sigma, Fluka or E Merck.

EXPERIMENTAL PROTOCOL

Group	Treatment and dosages	No of animals	Duration (days)	Day of autopsy
I	Control	15		Sacrificed along with NaF-treated
II	NaF treated (10 mg/kg body wt.	15	30	31
III	NaF treatment as in group II, withdrawal after 30 days and from day 31 administration of AA (50 mg/animal /day) for another 30 days.	15	30	61st
IV	NaF treatment as in group II, withdrawal after 30 days and from day 31 administration of Ca^{2+} (62.5 mg/animal/day) for another 30 days.	15	30	61st
V	NaF treatment as in group II, withdrawal after 30 days and from day 31 administration of AA + Ca^{2+} dosages same as in group III and IV.	15	30	61st

Dose: To experimental animals, NaF was administered at a dose of 10 mg/kg body weight for 30 days. The dose was mainly based on LD_{50} value (250 mg/kg body weight in rats) and earlier studies.[5,6] This is $^1/_{25}$th of the LD_{50} value. In a different set of experiments, the treatment was withdrawn after 30 days of NaF ingestion and administration of therapeutic agents, *viz*, ascorbic acid and/or calcium was introduced.

Duration: All the animals were treated for a period of 30 days since earlier studies have revealed that NaF treatment at a dose of 10 mg/kg body weight is found to be effective 30 days after treatment.

At the end of each treatment, the animals were autopsied after cervical dislocation, and the required tissues were carefully excised, blotted free of blood, weighed on a Roller Smith (USA) torsion balance, homogenized in suitable solvents and utilised for respective parameters as follows:

Protein profile: Polyacrylamide gel electrophoresis (PAGE) of cauda epididymis and testis proteins was carried out following the method of Laemmli.[15] Densitometric scanning of SDS-PAGE gels was carried out using a Pharmacia LKB Ultra XL densitometer. Silver staining of the gel was carried out by the method of Heukeshover and Dernick[16] using methanol-formaldehyde fixative.

Phospholipids: The phospholipids were extracted with lipid solubilizing and protein denaturing solvents (methanol followed by chloroform) then separated by using two-dimensional thin layer chromatography.[17] followed by quantitative estimation (percent phospholipids) by the method of Bartlett[18] with Amino Naphthol Sulphonic Acid (ANSA) colouring reagent. The blue colour was read at 820 nm on a Systronics

UV VIS Spectrophotometer 118.

Statistics: In all the experiments a minimum of 8-10 replicates were done and the data were analysed statistically using ANOVA and Scheffe's test.

RESULTS

Protein profile

In testis, 11 protein bands of molecular weights ranging from 15.8-83.7 kD (kilo daltons) were observed after densitometric scanning. In NaF treated rat testis (group II), proteins of molecular weights 21.4 kD and 64.7 kD were absent. However, six extra protein bands of molecular weights 17.1 kD, 29.9 kD, 31.3 kD, 34.4 kD 51.1 kD and 92.9 kD were observed in testis after NaF treatment while eight protein were not affected corresponding to 15.8 kD, 25.8 kD, 39.5 kD, 40.4 kD, 45.2 kD 55.1 kD, 57.6 kD and 83.7 kD.

Withdrawal of treatment and administration of AA (Group III) showed that extra bands of 13.7 kD and 62.0 kD were observed which were not seen in testis of either control or NaF-treated animals. Protein bands of molecular weights 34.4 kD, 51.1 kD, 75.2 kD and 92.9 kD which were observed in the NaF treated group were found in this group (III). However, all the proteins were not found at a normal level.

In group IV, withdrawal of NaF treatment and administration of calcium also showed recovery in the protein band corresponding to 21.4 kD. A band at 13.7 kD which was not observed in control or NaF treated animals but found in the ascorbic acid treatment was also present. Extra protein bands of molecular weights 29.3 kD, 30.7 kD, 32.2 kD and 34.1 kD were present in this group and protein of molecular weight 64.7 kD was not recovered as compared to control.

Withdrawal of NaF treatment and administration of Ca^{2+} and AA simultaneously (group V) again showed the protein band at 13.6 kD (not found in control and treated group). Extra protein bands of molecular weight 29.3 kD, 30.7 kD, 34.1 kD, 43.2 kD and 61.5 kD still persisted (Table 1).

In cauda epididymis, 13 protein bands of molecular weights ranging from 10.9 kD to 58.6 kD were observed. In NaF treated (group II) proteins in molecular range of 12.2 kD, 15.6 kD, 20.5 kD, 36.2 kD, 55.1 kD and 58.6 kD (total 6) were not found. On the other hand, some new proteins were induced, *viz*, 44.9 kD, 50.7 kD and 53.1 kD, respectively.

The withdrawal of NaF treatment and administration of AA (group III) showed 14 bands of which 11 bands corresponded to normal proteins found in control, along with some extra bands corresponding to 44.9 kD, 50.5 kD and 52.9 kD. However, protein in molecular range 15.6 kD was still absent.

In group IV, withdrawal of NaF treatment and administration of Ca^{2+} for 30 days, 13 bands were observed of which 10 bands corresponded to proteins of control animals and extra bands at 48.0 kD, 50.5 kD and 52.9 kD (Table 2) as in group III. Proteins in the molecular range 12.2 kD, 15.6 kD and 55.1 kD present in control were not found.

In group V, nine protein bands corresponding to control were observed and the new induced proteins were absent. However, proteins corresponding to 15.6 kD, 25.1 kD and 58.6 kD found in the control were not present.

On the whole, the number of induced proteins was greater in the testis than in cauda epididymis. The protein bands corresponding to molecular weight of 64.7 kD in testis and one protein band corresponding to 15.6 kD in cauda epididymis were highly sensitive to NaF treatment as they were not present. On the other hand, proteins corresponding to 15.8 kD, 25.8 kD, 45.2 kD, 55.1 kD, 57.5 kD and 83.7 kD (total 6) in testis were not altered throughout treatment. Similarly, in epididymis proteins at 10.9 kD, 13.9 kD, 18.0 kD, 25.1 kD, 31.8 kD, 41.6 kD and 47.0 kD (Total 7) were not affected. The testis protein profile was more adversely altered by NaF treatment than the protein profile of cauda epididymis.

Phospholipids

Phosphatidylcholine and sphingomyelin levels remained unaltered in testis after 30 days of NaF treatment, whereas, a significant decrease was observed in phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol. The administration of AA (group III) for 30 days induced recovery at 86.3% in phosphatidylethanolamine, 92.7% in phosphatidylserine and 60.7% in phosphatidylinositol in testis. The administration of Ca^{2+} after NaF treatment (group IV) also revealed a similar picture. However, recovery was better in group III than in group IV. The phospholipid profile was almost to a normal state after the administration of Ca^{2+} and AA simultaneously in group V (Table 3).

The cauda epididymal plasma membrane phospholipids also revealed a significant decrease in phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol in NaF-treated animals. The administration of AA and Ca^{2+} alone (groups III and IV) caused no recovery in phosphatidylinositol and phosphatidylserine while recovery occurred in phosphatidylethanolamine (79.9, 86.6%), phosphatidylserine (71.7, 70%) and phosphatidylinositol (44.7, 43.7%) as compared to control. Administration of AA and Ca^{2+} together (group V) caused significant recovery almost to normal levels for phosphatidylethanolamine, (95.2%), phosphatidylserine (101.2%) but comparatively less for phosphatidylinositol (56.1%) levels (Table 4). On the whole, the recovery was better in testis than in cauda epididymis and especially with respect to phosphatidylinositol (Tables 3 and 4).

Glutathione

The testis glutathione levels decreased significantly in NaF treatments after 30 days. The level recovered significantly after withdrawal of treatment and administration of AA and/or Ca^{2+} (group III and IV). Recovery was more in group III (83.66%) than when Ca^{2+} alone was administered (67.06%) (group IV). The recovery was maximum when Ca^{2+} as well as AA were administered together (88.46%) (group V) (Table 5). The cauda epididymal glutathione levels were also significantly decreased by NaF treatment after 30 days. The levels recovered after treatment with AA and/or Ca^{2+} alone (groups III and IV) (57.7% and 53.0%) respectively. However, recovery was very significant in group V where Ca^{2+} and AA were administered simultaneously (Table 5) (72.7%).

On the whole, recovery was better in testis than in cauda epididymis. Comparing the effects of NaF on testis and cauda epididymis phospholipids and glutathione, it was evident that cauda epididymis was by and large, affected more than testis, wherein recovery was also better in the testis than the cauda epididymis.

TABLE 1. Proteins of following molecular weights (kilo daltons) have been observed on SDS-PAGE in testis of rats of control and treated groups (I-V).

GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
-	-	13.7	13.7	13.6
15.8	15.5	15.4	15.3	15.2
-	17.1	16.8	16.8	16.6
21.4	-	-	20.2	-
25.8	26.4	26.1	25.8	-
-	29.9	29.5	29.3	29.3
-	31.1	30.8	30.7	30.7
-	-	-	32.2	-
-	34.4	-	34.1	34.1
39.5	37.6	-	-	-
40.4	41.4	-	41.0	-
-	-	43.4	-	43.2
45.2	45.0	45.9	45.5	45.5
-	51.1	-	-	-
55.1	54.4	53.9	53.6	53.7
57.6	55.9	57.9	57.2	57.2
64.7	-	-	-	-
-	-	62.0	61.5	61.5
-	75.2	-	74.3	74.0
77.0	-	-	-	-
83.7	82.8	82.2	82.1	82.1
-	92.9	-	-	-

Group I Untreated control
Group II NaF treatment for 30 days
Group III NaF treatment as in group II withdrawal from day 31st and fed Ascorbic acid for another 30 days.
Group IV NaF treated as in group II withdrawal from day 31st and fed Calcium for another 30 days.
Group V Combined treatment as in groups III and IV.

TABLE 2. Proteins of following molecular weights (kilo daltons) have been observed on SDS-PAGE in cauda epididymis of rats of control and treated groups (I-V)

GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
10.9	11.5	10.1	10.1	10.0
12.2	-	12.9	-	-
13.9	13.2	12.9	13.9	12.8
15.6	-	-	-	-
18.0	17.2	16.9	17.2	17.0
20.5	-	19.6	19.5	19.3
25.1	24.0	23.0	24.6	-
31.8	30.6	30.2	30.9	30.0
36.2	-	34.7	34.8	34.7
41.6	40.1	39.7	39.7	39.6
-	-	44.9	-	-
47.0	45.6	45.3	45.3	45.1
-	-	48.0	48.0	-
-	50.7	50.5	50.5	-
-	53.1	52.9	52.9	-
55.1	-	-	-	-
58.6	-	56.8	56.8	56.5

TABLE 3. Testicular plasma membrane phospholipids (%) in control group (I) and treated groups (II-V) groups of rats

Groups	Phosphatidyl--choline	Phosphatidyl--ethanolamine	Sphingo-myelin	Phosphatidyl--serine	Phosphatidyl--inositol
Group I	41.79±1.06	28.72±1.36 (100%)	7.98±0.98	15.24±1.24 (100%)	8.27±0.87 (100%)
Group II	40.52±1.02	22.75±1.45 (79.2)	7.87±0.88	11.79±1.13 (77.4)	4.14±0.64 (50.1)
Group III	40.78±1.21	24.78±1.13 (86.3)	7.97±0.99	14.12±1.04 (92.7)	5.02±0.68 (60.7)
Group IV	40.18±1.24	23.12±1.18 (80.5)	8.10±1.10	13.13±1.13 (86.2)	4.98±0.34 (60.2)
Group V	41.89±1.30	27.75±1.63 (95.6)	7.70±1.04	15.13±1.24 (99.3)	8.16±0.79 (98.6)

Values are membrane phospholipids (%) ± S.E. Values in parenthesis are % of control values.

TABLE 4 Cauda epididymal plasma membrane phospholipids (%) in control group (I) and treated groups (II-V) of rats

Groups	Phosphatidyl--choline	Phosphatidyl--ethanolamine	Sphingo-myelin	Phosphatidyl--serine	Phosphatidyl--inositol
Group I	44.24±1.04	22.83±1.24 (100%)	15.19±1.18	11.75±1.01 (100%)	4.99±0.39 (100%)
Group II	44.14±0.98	14.79±1.21 (64.8)	15.10±0.70	8.64±0.62 (73.5)	2.13±0.19 (42.7)
Group III	43.93±0.88	18.24±1.08 (79.9)	15.19±0.86	8.42±0.79 (71.7)	2.23±0.24 (44.7)
Group IV	44.10±0.92	19.78±1.19 (86.6)	15.72±0.92	8.23±0.74 (70.0)	2.18±0.22 (43.7)
Group V	44.21±0.92	21.74±1.44 (95.2)	15.74±1.28	11.89±0.97 (101.2)	2.80±0.20 (56.1)

Values are membrane phospholipids (%) ± S.E. Values in parenthesis are % of control values.

TABLE 5. Glutathione levels in testis and cauda epididymis of control group (I) and treated groups (II-V) of rats

	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V
Testis	50.62±3.96	12.34±1.56	42.35±2.28	33.95±2.18	44.78±3.06
	(100%)	(24.4%)	(83.7%)	(67.1%)	(88.5%)
Cauda	36.33±2.98)	11.35±1.58	20.96±2.14	19.25±2.94	26.42±1.42
	(100%)	(31.2%)	(57.7%)	(53.0%)	(72.7%)

Values are µg/100 mg fresh tissue weight ± S.E. Values in parenthesis are % of control values.

TABLE 3B. Testis phospholipids ANOVA

Source of variation	SS	dF	MS	f(cal)	F(tab)
Phosphatidylcholine					
Groups	9.0	4	2.25	8.43	4.89
Residual	4.0	15	0.26		
Phosphatidylethanolamine					
Groups	119.8	4	29.95	112.31	4.89
Residual	4.0	15	0.26		
Sphingomyelin					
Groups	1.2	4	0.30	0.46	4.89
Residual	9.7	15	0.65		
Phosphatidylserine					
Groups	36.8	4	9.20	23.00	4.89
Residual	6.0	15	0.40		
Phosphatidylinositol					
Groups	64.7	4	16.17	69.32	4.89
Residual	3.5	15	0.23		

SS: sum of squares, dF: degree of freedom, MS: mean of squares, Significance: (1% level) $P < 0.001$.

TABLE 4B. Cauda epididymal ANOVA

Source of Variation	SS	dF	MS	f(cal)	F(tab)
Phosphatidylcholine					
Groups	0.20	4	0.05	0.16	4.89
Residual	4.75	15	0.32		
Phosphatidylethanolamine					
Groups	159.50	4	39.87	56.96	4.89
Residual	10.50	15	0.70		
Sphingomyelin					
Groups	2.80	4	0.70	1.83	4.89
Residual	5.75	15	0.38		
Phosphatidylserine					
Groups	63.80	4	15.95	50.37	4.89
Residual	4.75	15	0.31		
Phosphatidylinositol					
Groups	24.30	4	6.07	21.44	4.89
Residual	4.25	15	0.28		

SS: sum of squares, dF: degree of freedom, MS: mean of squares.
Significance at 1% level, $P < 0.001$.

TABLE 5B. Glutathione ANOVA

Source of Variation	SS	dF	MS	f(cal)	F(tab)
Cauda Epididymis					
Groups	1402.50	4	350.62	66.36	4.89
Residual	79.25	15	5.28		
Testis					
Groups	3531.80	4	882.95	124.06	4.89
Residual	106.75	15	7.12		

SS: sum of squares, dF: degree of freedom, MS: mean of squares.
Significance at 1% level, $P < 0.001$.

DISCUSSION

The protein profile revealed that two protein bands were missing in testis and six in cauda epididymis after NaF treatment. This could be due to an inactivation/inhibition of some enzymes or proteins or alteration of protein synthesis.[20] The treatment also resulted in the induction of some new proteins which were not present in control animals: 6 extra bands were observed in NaF-treated rat testis and 2 in cauda epididymis. This induction of new proteins might also be related to decrease/increase in DNA and RNA levels after NaF treatment.[21]

The induction of new proteins was more in testis than in cauda epididymis. In cauda epididymis the protein corresponding to the molecular weight of 15.6 kD, and in testis 64.7 kD, were the most sensitive to NaF treatment. They did not recover at all, whereas others were resistant to treatment or they were not affected. To the best of our knowledge this is the first report of its kind.

As the proteins of testis and cauda epididymis are known to be involved as androgen carrier proteins, in testicular functions and in sperm motility,[22] it follows that NaF treatment might affect the levels of these proteins as well as alter sperm motility and viability.[23,24] The detailed effect on testicular protein and nucleic acid levels need to be studied in the future as the proteins of the testis were affected more that those of cauda epididymis.

A significant decrease in the phospholipids especially phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol levels obtained in testis and cauda epididymis after NaF treatment would affect the activity of membrane bound enzymes by disturbing membrane fluidity which is more in cauda epididymis than in testis. Phosphatidylinositol is known to be involved in hormone receptor (HR) interaction[25] and signal transduction. The changes in phosphatidylinositol in testis and cauda epididymis suggest that the HR interaction might be affected which would lead to other metabolic alterations in these organs.

The significantly lower levels of glutathione after 30 days treatment of NaF could possibly be due to the stress imposed by NaF. Glutathione is known to have a role in the formation of reduced AA from dehydroascorbic acid. The reduced AA together with its free radical, monodehydroascorbic acid (MDHA), are powerful reducing agents which help in overcoming stress in several tissues.[13,14] Hence, alterations in the glutathione levels after NaF treatment would affect epididymal and testicular oxido-reductase metabolism.

Fluoride-testis interrelationships have been investigated by several workers. Chinoy and Sequeira[1] reported that 30 days of treatment with NaF (10 mg/kg body weight) to mice resulted in sloughing off of the spermatogenic cells in the luminal region of seminiferous tubules of the testis leading to disorganisation of their epithelium. This caused a complete absence of spermatogenesis in the testis. Shashi[26] has observed similar changes in rabbit testis after NaF treatment, while Narayana and Chinoy[12] have reported that steroidogenesis and spermatogenesis were hampered by NaF treatment in rats.

The epididymis is the site for maturation of spermatozoa, where they acquire motility and fertilizability,[22,27-29] and therefore normal internal milieu of the epididymis is necessary for maintenance of sperm structural and functional integrity. NaF treatment disturbs the homeostasis in the epididymis of rats, mice and rabbits,[9,10,12] leading to alteration in sperm motility, viability and subsequently their fertility.[3,7,12] In the present study too, the internal milieu of epididymis was affected as evident by changes in proteins, phospholipids and glutathione, which in turn would affect the spermatozoa.

Earlier reports[4,5,10,12,13] have indicated that AA and Ca^{2+} can mitigate the fluoride toxicity effects and bring about reversal. Ascorbic acid is a biological antioxidant which is known to activate numerous hydroxylating enzymes, participate in metabolic processes as a supplementary source of energy in several tissues including sperms.[30] It also inhibits phosphodiesterase and hence enhances C-AMP levels which would bring about activation of several kinases and have an indirect metabolic effect.[4,5]

Fluoride toxicity was also reduced by administering Ca^{2+} to fluoride-treated rats for 30 days (group IV). It has been demonstrated that calcium ingestion by fluoride-intoxicated rabbits and rats caused a significant recovery in the NaF-inhibited activities of some specific enzymes (ATPase, SDH and ACPase) in cauda epididymal sperm.[10,12] The action of Ca^{2+} and C-AMP are interdependent with each other and could control the rate of Ca^{2+} transfer across the plasma membrane.[31] The Ca^{2+} probably made the fluoride less soluble through the

formation of CaF_2. This has been shown frequently. Another mechanism by which Ca^{2+} could have aided in recovery might be through inhibition of phosphodiesterase (PDE) activity like ascorbate, leading to enhanced C-AMP levels, since it is a known inhibitor of this enzyme.[32]

The present data show that AA administration (50 mg AA/animal/day) during the withdrawal period (group III) was beneficial and revealed much better recovery, in almost all the parameters, than by Ca^{2+} treatment alone (group IV). The combined treatment of Ca^{2+} and AA (group V) resulted in more recovery than by individual treatments (group III and IV) of testicular and cauda epididymal metabolism, especially in the levels of phospholipids and glutathione. The recovery might have been due to the additive action between AA and Ca^{2+}, consistent with our earlier data.[7,12,13] The increased levels of C-AMP might be one of the mechanisms at cellular level responsible for better recovery in group V than in groups III and IV. Hence C-AMP levels need to be determined in future studies.

The present study indicates that Ca^{2+} and AA have a significant beneficial role in mitigating fluoride toxicity in rat testis and cauda epididymis, and manifest an additive action in recovery of NaF-induced effects.

Acknowledgment: The help rendered by Ms Dhruva Patel for preparation of the manuscript is gratefully acknowledged.

REFERENCES

1 Chinoy NJ, Sequeira E. Effects of fluoride on the histoarchitecture of reproductive organs of the male mouse. *Reproductive Toxicology 3* (4) 261-267 1989.
2 Chinoy NJ and Sequeira E. Fluoride induced biochemical changes in reproductive organs of male mice. *Fluoride 22* (2) 78-85 1989.
3 Chinoy NJ, Sequeira E. Reversible fluoride induced fertility impairment in male mice. *Fluoride 25* (2) 71-76 1992.
4 Chinoy NJ. Effects of fluoride on physiology of some animals and human beings. *Indian Journal of Environmental Toxicology 1* (1) 17-32 1991.
5 Chinoy NJ. Effects of fluoride on some organs of rats and their reversal. *Proceedings of Zoological Society, Calcutta 44* (1) 11-15 1991.
6 Chinoy NJ. Fluoride toxicity in female mice and its reversal. In: Saxena AK, Ramamurthy R, Srirama Reddy G, Saxena VL. (Eds) *Recent Advances in Life Sciences.* Manu Publications, Kanpur India 1992 pp 39-50.
7 Chinoy NJ, Sequeira E, Narayana MV. Effects of vitamin C and calcium on the reversibility of fluoride induced alterations in spermatozoa of rabbit. *Fluoride 24* (1) 29-39 1991.
8 Chinoy NJ, Rao MV, Narayana MV, Neelakanta E. Microdose vasal injection of sodium fluoride in rat. *Reproductive Toxicology 5* (6) 505-512 1991.
9 Chinoy NJ, Pradeep PK, Sequeira E. Effects of fluoride ingestion on the physiology of reproductive organs of male rats. *Journal of Environmental Biology 13* (1) 55-61 1991.
10 Chinoy NJ, Reddy VVPC, Michael M. Beneficial effects of ascorbic acid and calcium on reproductive functions of prepubertal male rats. *Fluoride 27* (2) 71-79 1994.
11 Narayana MV, Chinoy NJ. Effects of fluoride on rat testicular steroidogenesis. *Fluoride 27* (1) 7-12 1994.
12 Narayana MV, Chinoy NJ. Reversible effect of sodium fluoride ingestion on spermatozoa of the rat. *International Journal of Fertility 39* (6) 337-346 1994.

13 Chinoy NJ. Ascorbic acid turnover in animal and human tissues. *Journal of Animal Morphology and Physiology (Silver Jubilee Commemorative Volume)* 68-85 1978.
14 Chinoy NJ, Sharma M, Michael M. Beneficial effects of ascorbic acid and calcium on reversal of fluoride toxicity in male rats. *Fluoride 26* (1) 45-56 1993.
15 Laemmli UK. Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature 227* 680-685 1970.
16 Heukeshover J, Dernick R. *Electrophoresis 6* 103 1985.
17 Marinetti GV. Chromatographic separation, identification and analysis of phospholipids. *Journal of Lipid Research 3* 1-11 1962.
18 Bartlett GR. Phosphorus assay in the column chromatography. *Journal of Biological Chemistry 243* 466-468 1959.
19 Grunert RR, Phillips PH. A modification of the nitroprusside method of analysis for glutathione. *Biochemistry 30* 217-225 1951.
20 Vesco C, Colombo B. Effects of sodium fluoride on protein synthesis. *Journal of Molecular Biology 47* 335-337 1970.
21 Shashi A. Preliminary observations on alterations in rabbit ovary DNA and RNA content in experimental fluorosis. *Fluoride 27* (2) 76-80 1994.
22 Chinoy NJ. Structure and function of epididymis in relation to vulnerable points of intervention for male fertility regulation. *Indian Review of Life Sciences 4* 37-68 1984.
23 Chinoy NJ, Narayana MV. *In vitro* fluoride toxicity in human spermatozoa. *Reproductive Toxicology 8* (2) 155-159 1994.
24 Chinoy NJ, Narayana MV, Dalal V *et al.* Amelioration of fluoride toxicity in some accessory reproductive glands and spermatozoa of rat. *Fluoride 28* (2) 75-86 1995.
25 Fain JN. Involvement of phosphatidylinositol breakdown in elevation of cytosol Ca^{2+} by hormones and interrelationship to prostaglandin formation. In: Kohn LD (Ed). *Hormone Receptors.* John Wiley and Sons, New York 1982 pp 237-276.
26 Shashi A. Histopathological changes in rabbit testis during experimental fluorosis. *Folia Morphologica 38* (1) 63-65 1990.
27 Prasad MRN, Rajalakshmi M. Target sites for suppressing fertility in male. In: Singhal RL, Thomas JA (Eds). *Cellular Mechanism Modulating Gonadal Hormonal Action* (Vol 2). University Park Press, Baltimore, 1976 p236.
28 Prasad MRN, Rajalakshmi M. Recent advances in the control of male reproductive function. In: Greep RO (Ed). *International Review of Physiology II* (Vol 13). University Park Press, Baltimore 1977 pp 153-199.
29 Cooper TG. *The Epididymal Sperm Maturation and Fertilisation.* Springer-Verlag, New York 1986 p281.
30 Chinoy NJ, Buch RP. Metabolic significance of ascorbic acid in human semen. *Indian Journal of Experimental Biology 15* 921-923 1977.
31 Pasternak CA. *An Introduction to Human Biochemistry.* Oxford University Press, New York 1979 p212.