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Toxicity of Fluoridated Water on Biochemical Markers and Male Reproductive Function in Rats

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Abstract

Healthy adult male albino rats of the Wistar strain (*Rattus norvegicus*) were exposed to fluoride (5.8 ppm F) contaminated drinking water for 270 days. The body and organ weights and tissue biochemistry of the testis, epididymis, vas deferens, seminal vesicle, and ventral prostate were carried out. The result revealed that fluoride water ingestion for a longer duration caused a reduction in body and reproductive organs (testis, cauda epididymis, seminal vesicle, and coagulating gland) weight compared to the control group. The concentration of protein, sialic acid, fructose, ascorbic acid, glycogen, and enzyme activity of acid phosphatase and alkaline phosphatase declined in the reproductive organs studied. However, the cholesterol concentration of the testis was found to be increased significantly following fluoride water treatment. These findings suggest that long-term fluoride exposure negatively affects male reproductive health by altering organ weight and key biochemical markers.

Keywords: Fluoride contaminated water, testis, biochemical changes, reproductive health.

Introduction

Fluoride is an essential trace element that supports dental and skeletal health. The maximum acceptable fluoride concentration may range between 1.0 and 1.5 m g/l (WHO, 1984) [40]; it strengthens bones and prevents dental caries by enhancing enamel resistance. But its excess is equally detrimental and causes severe damage to various body systems. Drinking water is the main fluoride source for intake (Murutu *et al*., 2012) [21]. At low concentrations, fluoride stimulates bone formation (Richards *et al.,* 1994) [26] and has beneficial effects on the teeth by hardening the enamel and reducing the incidence of caries (Fung *et al*., 1999) [11]. At low levels (<2 ppm), soluble fluoride in the drinking water may cause mottled enamel during the formation of the teeth, but at higher levels, other toxic effects may be observed. However, excessive fluoride intake has a toxic effect on multiple organ systems, including the liver, kidneys, brain, and reproductive system.

High fluoride affects teeth, bones, and reproduction (Susheela and Kumar 1991) [37]. The NaF water caused a reduction in the weight of male reproductive organs of the testis, prostate, and seminal vesicle (Ghosh *et al*., 2002) [14] and affects the reproductive biochemical parameters in male rats (Chinoy and Mehta, 1999). The adverse effects of fluoride on male reproduction are attracting increasing attention due to the overall decline in fertility worldwide (Barbier *et al.,* 2010) [2]. Fluoride effects on Leydig cells and decreased serum testosterone were observed in rats' exposure to NaF at 10 mg/kg for 50 days (Narayana and Chinoy, 1993) [22]. Susheela and Jethanandani (1996) [36] reported decreased serum testosterone levels in 30 men having skeletal fluorosis. Despite these findings, most research has focused on artificial fluoride compounds rather than naturally occurring fluoride in drinking water.

All these studies are based on artificially created fluoride salts and/or combinations of other electronegative elements. In this study, fluoride samples for animal experimentation are naturally present and are used for daily consumption by the animals and people living there. Our experiment aims to investigate the effects of high fluoride exposure on reproduction when administered at different intervals. But, in this research paper, we only discuss about 270 days of fluoride water treatment. However, there is a paucity of experimental studies on the effects of long-term exposure to fluoride-contaminated drinking water on the reproductive capacity of male rats to understand the adverse impacts of fluoride on humans. Therefore, the present investigation was undertaken to highlight the harmful effects of fluoride-contaminated drinking water on the reproductive organs of male albino rats.

Materials and Methods

The test samples were collected from the village of Sanganer Tehsil, which is endemic for fluoride contamination. Drinking water samples were collected from various sources, such as tube wells, open wells, hand pumps, and PHEDs. All water samples were collected in bottles (PE and PP grade; used after rinsing with 8 M HNO3 solution and deionized water thrice)

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to prevent contamination from other sources.

Water Analysis: The drinking water samples were collected from each subject and controlled in clean polyethylene bottles in all seasons between 2011 and 2013. Temperature was determined in the field. The pH (pH meter), turbidity (Nephelometer), and total dissolved solids (by evaporation method) were determined. Total alkalinity, dissolved oxygen, bicarbonates, hardness, free CO2, magnesium, calcium, nitrates, chloride, and fluoride were analyzed using volumetric titration. A digital flame photometer measured sodium and potassium content in water (APHA. AWWA, and WPCF, 1976).

Fluoride Estimation: Fluoride concentration was determined in the water sample using the standard SPANDS colorimetric method. This method is based on the reaction between fluorine and a zirconium-dye lake. Drinking water served as the control sample and was collected from nearby local areas (tap water from the university campus (1.0 ppm) and water samples from Watika village of Sanganer Tehsil, Jaipur City, Rajasthan, India, were utilized for analyzing fluoride (5.8 ppm) toxicity in male albino rats.

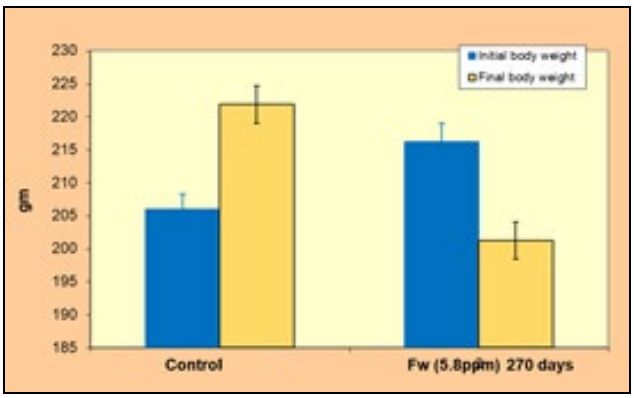
Healthy adult male albino rats (*Rattus norvegicus*; body weight: 200-250 g; age: 02 months) were divided into two groups of 20 rats each. The rats in Group I were used as control samples and treated with tap water (fluoride content of 1.0 ppm). Group II was exposed to fluoride-contaminated drinking water (5.8 ppm) for 270 days. All rats were maintained under standard husbandry conditions and fed *ad libitum* on a standard diet (Ashirwad Ltd., Chandigarh, India) and water (as described earlier) with 14 hours of daylight. The animals in each group were subjected to biochemical tests after the fluoride exposure period.

Biochemical Estimations: On day 271st the animals were weighed and autopsied. The blood was extracted through cardiac puncture. The reproductive organs testis, cauda epididymis, vas deferens, seminal vesicle, ventral prostate, and coagulating gland were excised, weighed (blotted free of blood), and stored for biochemical analysis. Protein quantification (Lowry *et al.*, 1951) [17], Fructose (Foreman *et al.*, 1973) [10], sialic acid (Warren., 1959) [39], glycogen (Montgomery., 1957) [20], cholesterol (Zlatkis *et al.*, 1953) [42], ascorbic acid (Roe and Kuether., 1943) [27], and enzyme activity of acid and alkaline phosphatases (Oser., 1965).

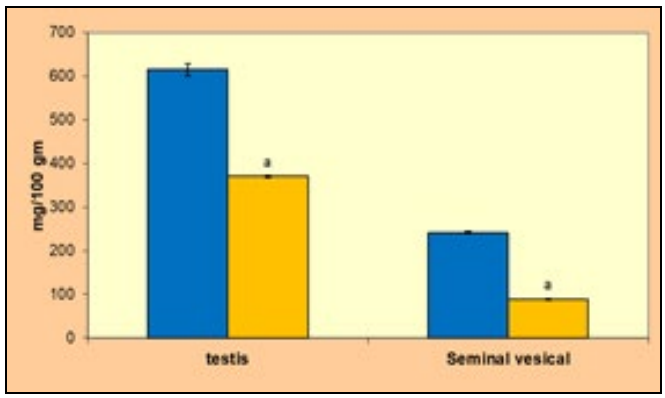
Statistical Analysis: The data were summarized as Mean ± SD. The acceptance level of significance was $p< 0.05$. A minimum of six replicates were done for each tissue and parameter. The results were analyzed statistically using the students 't-tests.

Results and Discussion

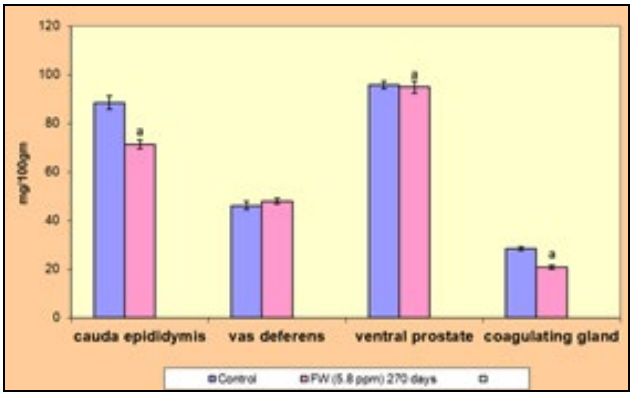
The data revealed that the fluoride (5.8 ppm F) water treatment in rats for 270 days reduced body (Table 1, graph-1) and reproductive organ weights of the testis, cauda epididymis, seminal vesicle, and coagulating gland significantly ($P< 0.001$) as compared to the control group (table 1, graph-2, 3). Similarly, our previous study showed reduced body and reproductive organ weight with 5.8 ppm fluoridated water after 60, 120, 180, treatment to male and 15, 30, and 60 days to female albino rats (Solanki M and Solanki D., 2025; Solanki M., 2024, Sharma *et al.,* 2006, Solanki, M., 2021) [34, 32, 35]. Gare. M. B., *et al*. (2021) [12] found that mice exposed to 100 ppm fluoride in drinking water significantly decreased body weight gain compared to the controls. The exposure to NaF in albino rats with different doses for 15 days showed decreased body weight in rats (Patil and Dhurvey 2015) [25]. The study by Tsunoda M., *et al*. (2005) [38] shows that the administration of NaF significantly reduces body weight gain.



Graph 1: Shows the initial body weight of control and fluoride water-treated rats and final weight after 270 days of fluoride water consumption by the male rats.



Graph 2: The testis and seminal vesical weight is significantly reduced ($P<0.001$) in fluoide-treated group compared to the control group.



Graph 3: Showing weight of cauda epididymis, vas deference, ventral prostate, and coagulating gland significantly reduced ($P<0.001$) compared to the control group.

Table 1: Body and organ weights of control and fluoride water (5.8 ppm) treated rats.

Parameter		Control	Fluoride water (5.8 ppm for 270 days)
Body wt. (gm)	Initial	206.00 ± 2.30	216.25± 2.26
	Final	221.87^{b}±2.81	201.87^{a}± 2.81
Organ wt. (mg/100 gm b.wt.)	Testis	615.05± 13.88	370.50^{a}± 13.0
	Cauda epididymis	88.49± 2.73	71.32^{a}± 1.90
	Vas deferens	46.19± 1.60	48.02± 1.18
	Seminal vesicle	241.67± 2.76	88.84^{a}± 2.04
	Ventral prostate	95.89± 1.56	94.92± 2.59
	Coagulating gland	28.48± 0.85	20.83^{a}± 0.86

Values are mean ± SE a=p<0.001, b=p<0.01

Several studies suggest that fluoride exposure can lead to a reduction in body weight. This effect is likely due to Metabolic Changes-Fluoride has been shown to affect energy metabolism by interfering with thyroid function, enzyme activity, and mitochondrial function. Reduced Food and Water Intake: Some animal studies report that high fluoride intake can decrease appetite and food consumption. Oxidative Stress: Fluoride-induced oxidative stress may contribute to weight loss by affecting tissue metabolism and energy utilization.

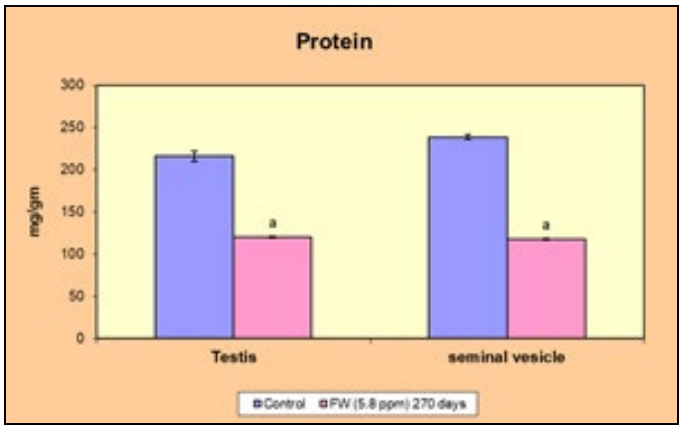
Fluoride has been reported to negatively impact the male reproductive system, with effects including Reduced Testicular Weight. Studies in rats and mice show that fluoride exposure can lead to a decrease in testicular weight, possibly due to germ cell apoptosis, reduced testosterone levels, and disrupted spermatogenesis. Decreased Epididymal and Prostate Weight. The epididymis and prostate are also affected by fluoride toxicity, which can lead to lower sperm motility and function.

Mechanisms Behind Fluoride-Induced Changes Endocrine Disruption:-Fluoride can interfere with testosterone production, affecting reproductive organ growth and function. Oxidative Stress and DNA Damage: Fluoride exposure generates reactive oxygen species (ROS), which can damage cellular structures in reproductive organs.

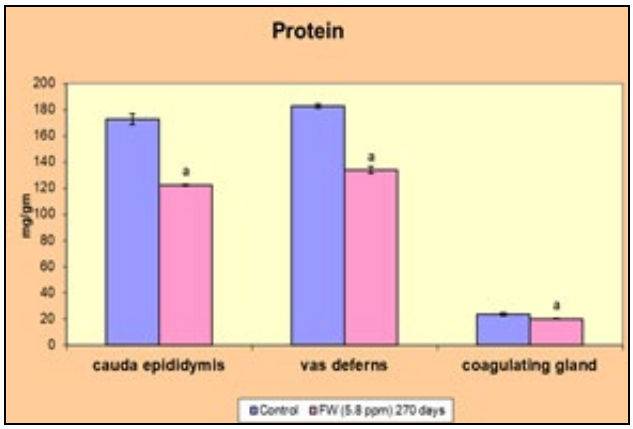
Effects on Biochemical Parameters

The androgen-dependent parameters (protein, ascorbic acid, sialic acid, fructose, acid, and alkaline phosphatases) declined significantly (P<0.001) (Graph 4-11). The protein content in the testis, seminal vesicle, cauda epididymis, vas difference, and coagulating gland decreased significantly (P<0.001) compared to the control group. Similarly, our previous study showed reduced protein concentration in study reproductive organs with 5.8 ppm fluoridated water after 120 and 180 days of treatment in male albino rats (Solanki M and Solanki D., 2025, Solanki M., 2024) [33, 32] and female rates after 60 days of treatment (Solanki M., 2024) [32]. Reduction in protein concentration may be due to protein oxidation or caused lesions in reproductive organs by fluoride. According to Mahdi *et al.,* (2011) [18], reduced protein concentration may be responsible for infertility.

Fluoride exposure has been associated with metabolic, functional, and structural impairments, particularly affecting protein and glucose metabolism. Research data strongly suggests that fluoride inhibits protein secretion and/or synthesis, leading to weight reduction (Sharma JD. 2009) [29].



Graph 4: Showing protein concentration in testis and seminal vesical significantly (P<0.001) decline after fluoride water treatment.

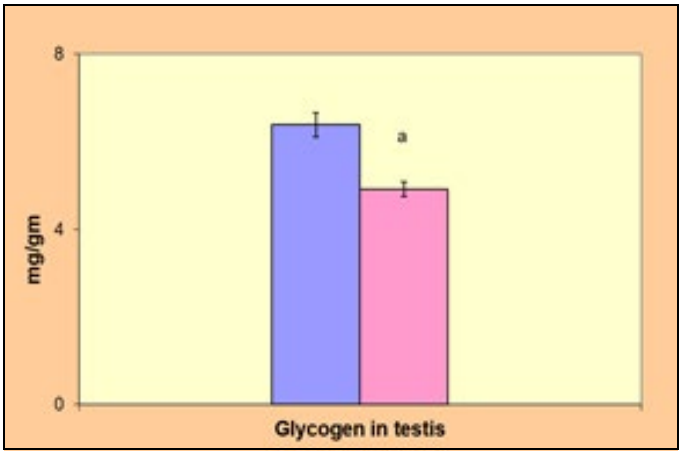


Graph 5: Shows that protein concentration in cauda epididymis, vas deference, and coagulating gland significantly (P<0.001) declined after fluoride water treatment.

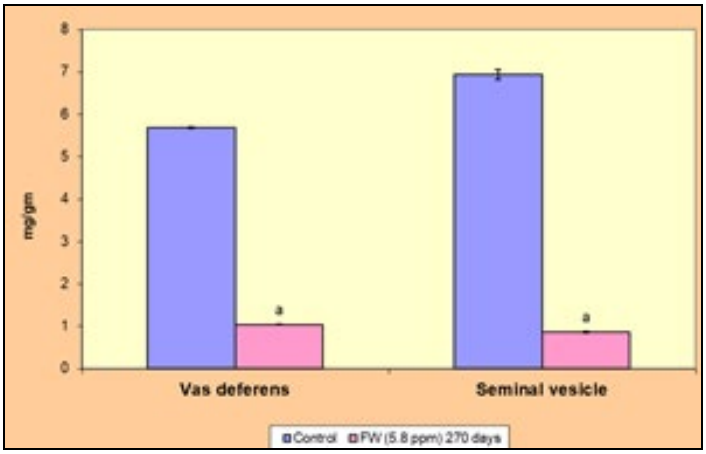
Research evidence strongly suggests fluoride inhibits protein secretion and synthesis while influencing critical signaling pathways involved in cell proliferation and apoptosis (Kuang P., *et al*., 2016) [16].

Glycogen concentration in the testis decreased significantly (P<0.001) (graph 6) following fluoride treatment. Chinoy and Mehta (1999) reported that feeding a protein-deficient diet to male mice treated for 30 days with NaF (10, 29 mg/kg/b.wt.) caused a significant decrease in glycogen concentration in the vas deferens. Fluoride exposure has been shown to disrupt glycogen metabolism in the male reproductive system, leading to significant reductions in glycogen storage within the testes (Kessabi *et al*., 1985) [15]. This depletion may be attributed to fluoride-induced inhibition of key metabolic pathways, including glycolysis and the Krebs cycle, as evidenced by decreased succinate dehydrogenase (SDH) activity in fluoride-treated animals (Momin, 2005) [19]. Since SDH is a crucial mitochondrial enzyme, its impairment can compromise oxidative metabolism, exacerbating energy deficits. The decline in SDH activity further suggests a disruption in energy production, exacerbating metabolic stress and impairing both glucose homeostasis and protein metabolism.

Fructose in vas deference and seminal vesical decreased significantly (P<0.001) (graph 7). The same finding was observed by Chinoy *et al*. (1994b) decreased fructose levels in vas deferens and seminal vesicle with sodium fluoride (NaF, 10 mg/kg b.wt.) treated rats.



Graph 6: Glycogen concentration in testis significantly (P<0.001) reduced after fluoride water treatment.

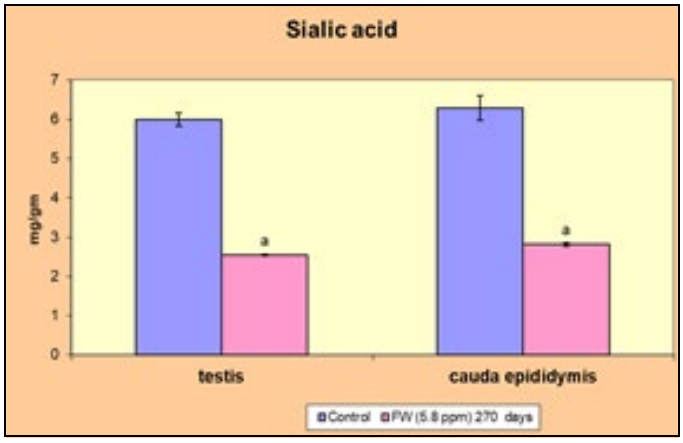


Graph 7: Fructose concentration in vas deference and seminal vesical (p<0.001) declined after fluoride water exposure to rats for 270 days.

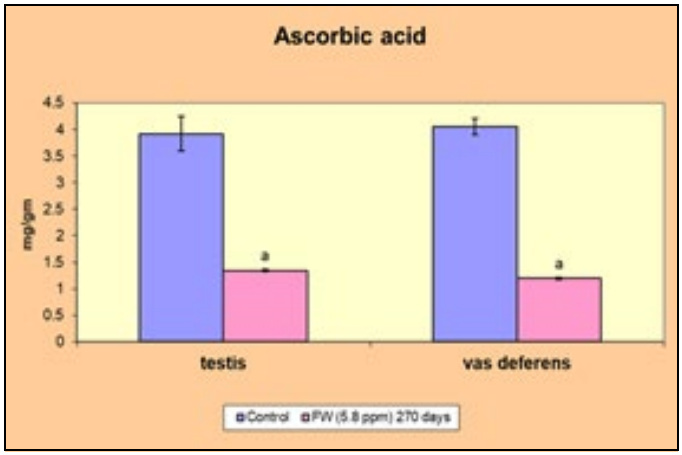
Fructose has a vital role in providing energy to sperm (Curry and Arherton, 1990) [7], and reduced levels of fructose after the treatment may be due to the direct suppression in testicular androgen secretion (Sarkar *et al.*, 2000) [28]. Furthermore, prolonged fluoride exposure has been associated with lower fructose concentrations in the vas deferens and seminal vesicles, which may adversely affect sperm motility and function (Momin, 2005) [19]. Additionally, the importance of sugar composition in seminal plasma correlates with fertility, as emphasized by Garner *et al*. (2001) [13].

Fructose plays a key role in providing energy to sperm cells, and reduced fructose availability can lead to impaired sperm motility and viability. The most important free sugar secreted by the seminal vesicle is fructose; this sugar can serve as a good index to evaluate the secretory activity of the seminal vesicle. In this study, the decreased fructose levels in the vas deferens and seminal vesicles could indicate a disruption in sperm energy metabolism, which is a crucial factor in sperm motility and overall fertility.

The sialic acid content in fluoride water-treated rats decreased in the testis and cauda epididymis; this decline is significant (P<0.001). Similarly, there was reduced sialic acid in testes, cauda epididymis, and vas deference with 5.8 ppm fluoridated water after 120 and 180 days of treatment in male albino rats (Solanki M and Solanki D., 2025, Solanki M., 2024) [33, 32]. Chinoy and Sequeira (1992) [4] observed inhibition of sialic acid concentration in the testes of mice. Chinoy *et al*. (1994) [6] reported decreases in sialic acid concentration of caput and cauda epididymis with sodium fluoride water (NaF, 10 mg/kg b.wt.) exposure for 30 days. Sialic acid is essential for sperm membrane stability, immune protection, motility, capacitation, and fertilization. Its deficiency or altered metabolism can contribute to male infertility. Low sialic acid levels in sperm are linked to poor motility, abnormal morphology, and decreased fertility.



Graph 8: Similarly, sialic acid content in fluoride water-treated rats decreased in the testis and cauda epididymis; this decline is significant (P<0.001).

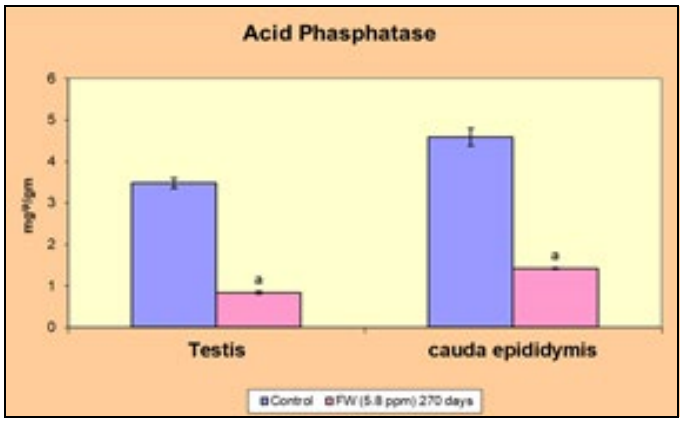


Graph 9: Ascorbic acid in the testis and vas deference significantly declined (P<0.001) in fluoride-contaminated drinking water-treated rats compared to control rats.

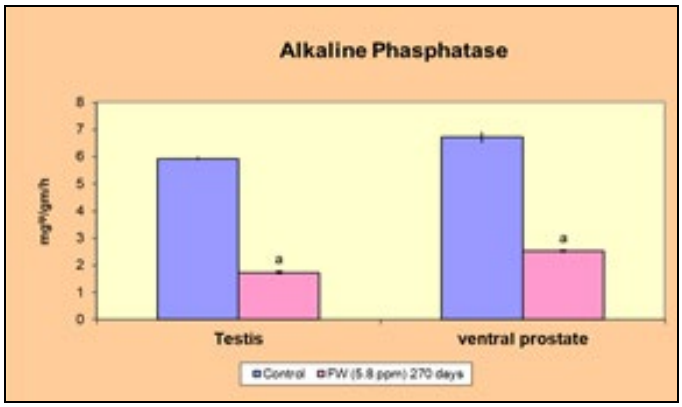
Sialic acid is a component of sialoglycoproteins on the sperm plasma membrane. It helps maintain membrane stability, fluidity, and structural integrity which are critical for sperm motility and function. It is involved in sperm maturation, particularly in the epididymis, where sperm acquire functional competence.

Ascorbic acid is a labile component of most tissues, and previous studies have indicated that decreases in its tissue concentration might act as a sensitive indicator of physiological and metabolic stress. The significantly reduced ascorbic acid in testis and vas deference after 120 days of fluoride water (5.8 ppm) exposure to male albino rats (Solanki M and Solanki D., 2025) [34]. Solanki M., (2024) [32] found a reduction in testis ascorbic acid with fluoride water for 180 days of treatment. Ascorbic acid is crucial for numerous metabolic functions in animals. While most species can synthesize it, some require dietary supplementation. It acts as an antioxidant, aids collagen and neurotransmitter synthesis, enhances immune function, and plays a key role in iron metabolism.

The enzyme activity of acid phosphatase significantly decreased ($p<0.001$) in the testis, cauda epididymis (graph 10), and alkaline phosphatase enzyme activity declined significantly ($p<0.001$) in the testis and ventral prostate after fluoride water treatments (graph 11).

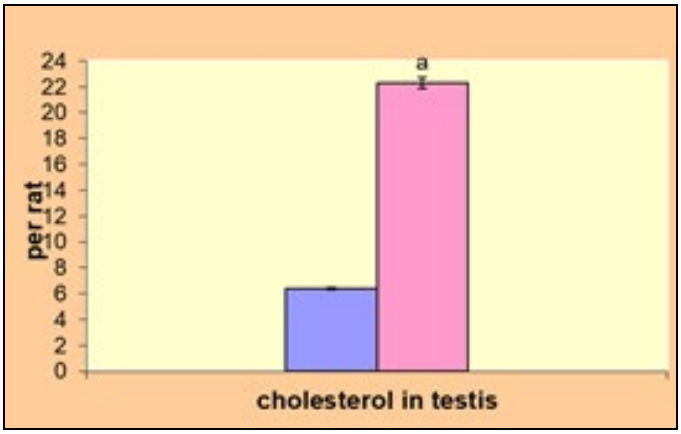


Graph 10: Acid phasphtase content in fluoride water-treated rats decreased significantly ($P<0.001$) in the testis and cauda epididymis



Graph 11: Similarly, alkaline phosphatase content in fluoride water-treated rats decreased in the testis and cauda epididymis; this decline is significant ($P<0.001$).

The decrease in acid and alkaline phosphatase enzyme activity may be due to increased lysosomal activity and the antiandrogenic manifestation of fluoride water. Acid phosphatase has been traditionally used as a lysosomal marker enzyme. Similarly, Chinoy *et al*. (1994) [6] reported inhibition in acid phosphatase activity of the ventral prostate with sodium fluoride (10 mg/kg b.wt.) treatment in rats for 30 days. Chinoy and Shah (2004) [5] reported decreased acid and alkaline phosphatases in the kidneys with sodium fluoride (5 mg/kg/body weight) treated mice. This observation concurs with the phosphatases findings of Narula and Jocob (1992) [23]; they reported a general decline in the alkaline phosphatase content of the various reproductive and accessory organs of the male mice after androgen estrogen therapy.



Graph 12: Cholesterol content in fluoride water-treated rats increased significantly ($P<0.001$) in the testes.

However, the cholesterol concentration of the test is enhanced significantly ($P<0.001$) (graph 12) following fluoride water exposure in rats. Similarly, our previous study showed an increase in cholesterol concentration in testes with 5.8 ppm fluoridated water after 120 and 180 days of treatment in male albino rats (Solanki M and Solanki D., 2025, Solanki M., 2025) [33, 34]. Solanki M, (2021, 2024) [32] and Sharma *et al.,* (2007, 2008) [30, 31] investigated enhanced cholesterol concentration in the studied tissue in male and female albino rats with 5.8 ppm of fluoride water exposure to rats for 15, 30, 60, and 120 days. The increase in testicular cholesterol levels observed following fluoride exposure in this study could point to significant changes in lipid metabolism, particularly in sperm function and overall semen quality. Cholesterol is a vital component of spermatozoa membranes, influencing structural integrity and functional characteristics, such as motility and capacitation. As referenced, cholesterol and phospholipids undergo dynamic modifications during spermatogenesis, sperm maturation, and capacitation (Flesch and Gadella, 2000; Flesch *et al*., 2001) [9, 8]. The membrane composition of spermatozoa, which is heavily reliant on cholesterol, plays a role in the fluidity, permeability, and fusion properties of the sperm membrane. This is essential for sperm motility and its ability to undergo capacitation, which allows sperm to fertilize an egg.

Increased cholesterol levels in the testis could suggest an imbalance in lipid metabolism. The excessive accumulation of cholesterol might result in altered sperm membrane properties, impairing motility and reducing sperm viability. Additionally, cholesterol excess may contribute to delayed seminal plasma liquefaction time, which has been linked to diminished semen quality. Fluoride exposure has been associated with various negative impacts on male reproductive health, as demonstrated in findings. The significant deterioration in semen quality, including the negative effects on sperm motility, concentration, and viability, aligns with previous studies. For example, Beer-Ljubic (2009) [3] highlighted that elevated cholesterol levels can serve as a predictive marker for poor semen quality, which could indicate that fluoride's effect on cholesterol metabolism may directly impair reproductive outcomes. Fluoride's impact on lipid metabolism could disrupt sperm membrane integrity, impair motility, and reduce overall metabolism.

Conclusion

The longer duration of exposure to fluoride-contaminated drinking water in rats induced diminished body growth and reproductive organ weights and caused reduced androgen-dependent biochemical parameters. Overall, excessive fluoride intake has been associated with decreased body weight and adverse effects on the male reproductive system, including reduced organ weights and impaired fertility. However, the effects depend on dosage, duration, and species. More human studies are needed to confirm these findings.

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