



FLOURIDE AFFECTING SPERM MOTILITY AND COUNT OF MALE *Mus musculus*

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ABSTRACT

Fluoride being common non-biodegradable and long lasting contaminant that causes major health hazards are difficult to treat at low levels of pollution. The World Health Organisation (WHO) warn the use of flouride upto 1.5 ppm as upper limit in drinking water. Fluoride greatly affect male reproductive system by inhibiting sperm function and motility. It interrupts acrosomal reaction and capacitation, affecting quantity and quality of sperm. The present study has aimed to investigate the effect of inorganic pollutant, fluoride in sperm count and motility. Male mice were divided into two groups consisting of 10 animals in each group. The first group was served as control providing with normal water the second group were treated with 2ppm sodium fluoride in water with the help of gavage, for 30 days at the interval of 24 hours. The present study demonstrates the fluoride showing a significant decrease and harmful effects in sperm count and motility.

KEYWORDS: fluoride, motility, sperm count, toxicity, infertility.

INTRODUCTION:

Numerous issues with reproductive health in both people and animals have increases as a result of increased exposure to environmental contaminants (Toft G et.al., 2006 and Hansel et.al., 2010). The link between fluoride consumption and reproductive structure in animal models have been the subject of numerous studies (Xu R et.al., 2010). Fluoride is a poison that is slightly more potent than lead. It can be said as one of the bone hunting elements ever discovered by humans (Joshi 2003). Fluoride enters the body with food, through respiration and products that contains fluoride (Cagetti et.al., 2003). Based on research papers and some overdose cases, the probable toxic dose was defined as 5mg per kg of body mass. PTD is said to be the minimal dose that leads to serious and life haunting signs and symptoms, and requires immediate treatment and hospitalisation (Waitford 2011). The World Health Organisation warned the use of fluoride up to 1.5 ppm as upper limit in drinking water. Intestine can easily absorb water soluble fluoride and quickly assimilated in the human body (Chinoy et.al., 1991). Mostly, out of the inorganic fluorides sodium fluoride (NaF) is widely used as fluoridation agent in drinking water, glass frosting agent, steel aluminium and magnesium manufacturing factories and industries, component of glues and adhesives, in dental laboratories, in veterinary medicines and anthelmintic, components used in wood preservatives (Windholz 1976).

Studies shows that sperm function and morphology is inhibited by fluoride, and also it inhibits motility, also induces sperm apoptosis and interrupts sperm capacitation, hyperactivation and acrosomal reaction, all of which place crucial steps in fertilisation process both in vivo and in vitro (Dvoraokovao, -Hortavao K et.al., 2008). Fluoride also plays negative role for endocrine activities by disrupting normal endocrine functions, it perhaps affects sperm function by attaching to its receptors or spermatozoa , according to the National Research council annual reports (Sharader Frechette K ,2007). Over exposure of fluoride leads to male reproductive toxicity through multiple pathways (Long et.al.,2009). But few studies shows that there is no negative role of fluoride on male reproduction and shows contradiction (Taos and Suttie JW,1976). Because of this controversial reports, it seems important to be acknowledge and assess with the effect of fluoride on male *Mus musculus* reproductive system.

METHOD AND MATERIALS:

Twenty adult *Mus musculus* ,6 weeks old weighing 30±5kg ,were Obtain from House of zoology department of TMBU Bhagalpur, Bihar. All mice were housed in temperature controlled rooms under 30 ±5 degrees Celsius all animals were treated in accordance with the principles of laboratory animal care. All mice were fed a standard diet and water. Thereafter the male mice (n= 20) where randomly divided into two groups a control groups (n=10) and the sodium fluoride group (n=10). The sodium fluoride groups receive 2ppm\mice\day (Kumar 2009) by gavage method for 30 days. The control groups was administered with normal food and distilled water.

Preparation of Sodium Fluoride Solutions:

Sodium fluoride aqueous solution was prepared by dissolving 100mg sodium fluoride in 100ml distilled water as stock solution. After that 2ml from stock solution was taken out and dissolved in 100ml of distilled water to make 2ppm sodium fluoride.

Surgical Procedure:

For the collection of samples on day 30, treated animals where sacrifice according to the guideline of Ethical committee.

Epididymal sperm count:

The cauda portion of each split epididymis was taken out and put in watch glass with 1ml of physiological saline solution. With a pair of sharp scissor, each portion was immediately macerated, and it's spermatozoa was released into the saline solution , after a brief waiting period . The enhanced Neubauer chamber was used to count sperm under the microscope (Sauza et.al., 2004).

Sperm Motility:

Two drops of heated sodium citrate where added after a drop of semen was placed on the slide. A coverslip was placed over the slide, and the sperm motility was seen under the microscope with a × 40 objectives, for the identification of sperm motility (Chinoy NF et.al.,1995).

RESULT:

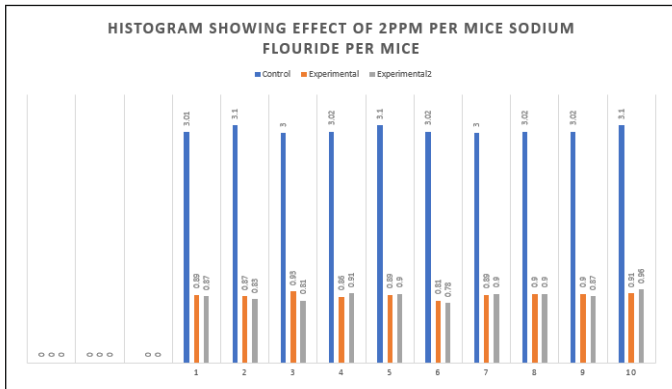
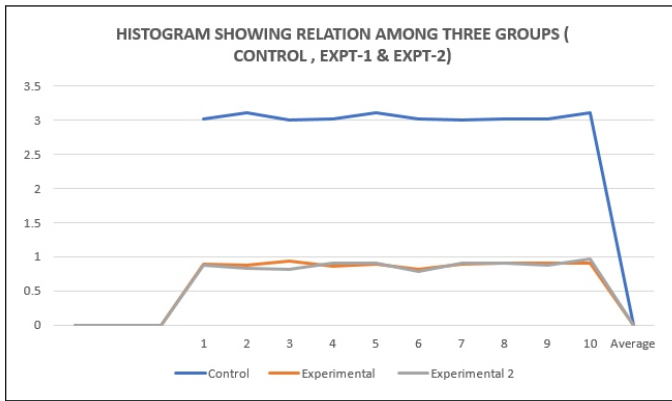
According to the result presented in table on 1, there is significant difference seen in sperm count between the groups. Administration of 2ppm\mice, sodium fluoride for 30 days, significantly decreased sperm count, in the experimental group compared to control group. Significant decrease in epididymal sperm count shows that, exposure to high fluoride affect the sperm count.

Table 1: The effect of 2PPM per mice, Sodium Fluoride on sperm count. The Experimental group in comparison to control group. N= 10

Sl. No.	Control Sperm Count (million/mm ³)	Experimental Sperm Count: 1 (million/mm ³)	Experimental Sperm Count: 2 (million/mm ³)
01	3.01	0.89	0.87
02	3.10	0.87	0.83
03	3.00	0.93	0.81
04	3.02	0.86	0.91
05	3.10	0.89	0.9
06	3.02	0.81	0.78
07	3.00	0.89	0.9
08	3.02	0.90	0.9
09	3.02	0.90	0.87
10	3.10	0.91	0.96
Average	3.03 ± 0.01	0.88 ± 0.01	0.86± 0.01

The t-value is 126.39042. The p-value is < .00001. The result is significant at p < .05.

The t-value is 100.14717. The p-value is < .00001. The result is significant at p < .05.



Effect of Sperm motility:

In the current study, it shows that there was significantly decreased in a sperm motility in the group fed with sodium fluoride with 2ppm for 30 days as compared with those of controls. Numerous more research using rats and mice revealed similar findings (Chinoy NJ and Sharma A, 1998 and Narayana MV and Chinoy NJ,1994 and Bataineh HN and Busier M,2006). Various studies reveals that fluoride could harm the motile apparatus without showing damage on other metabolic system (Zakrzewska H et.al., 2002). Fluoride may also affect sperm motility by decreasing the level of androgen carrier proteins (Chinoy NJ et.al.,1997). The seminal vesicle and vas deferens may lower levels of fructose, which fuels motility, as result of change in carbohydrate, which could be one cause (Chinoy NF et.al., 1995 and Zakrzewska H et.al., 2002). Fluoride may also exert its effects by inhibiting a variety of enzymes like fluoride bind with Co-factors such as mg, Ca, Zn and Se, which prevents glycolysis, respirations and sperm motility (Zakrzewska H et.al., 2002).

Effect on sperm count:

Fluoride toxicity may affect spermatogenesis because it lowers testosterone levels, which in turn lower testicular zinc levels. This affect the angiotensin converting enzymes activity, which in turn inhibits spermatogenesis (Sprando RL et.al., 1998).Fluoride also suppresses sertoli cell and androgen receptors ,mRNA expressions, which result in reduction in androgen receptor, testosterone function(Huang C et.al., 2008).

DISCUSSION:

The present study was carried out to explore the effects of fluoride on male sperm count an motility. One of the toxicology fields' most rapidly expanding areas of interest is reproductive defects caused by toxic exposure in males. According to reports, exposure to fluoride cause oxidative stress since, there is a higher concentration of lipid peroxide product in the testes, epididymis and Epididymal sperm compared to controls(Susheela AK and Kumar A,1997 and Ghosh D et.al., 2002).

As from this study we come to observe that there was significant decrease in a sperm count and also lower the sperm motility in the sodium fluoride treated mice between the groups for 30 days. Reactive oxygen species are produced when fluoride is present which has been shown to enhance lipid peroxidation (Chauhan DS et.al., 2013). Because Polyunsaturated fatty acids make up the sperm plasma membrane, which is specially sensitive to lipid peroxidation and protein oxidation(Chauhan DS et.al., 2013). The sperm membrane contained Polyunsaturated fatty acids that ROS can directly attack, causing lipid peroxidation, sperm membrane integrity damage, axoneme structure, destruction and ultimately reduction in sperm activity and fertility(Saleh RA and Agarwal,2002 and Kai SH et.al., 2008). Sperm with any aberrant morphology could perform poorly, and have a negative effect on fertilisation success.

CONCLUSION:

Male mice exposed to fluoride suffer from negative impact on their ability to reproduce, by showing significant decrease in a sperm count and motility. And

may this effects become more prominent, the longer the exposure period.

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