

## THE PROTECTIVE ROLE OF VIT-C (ASCORBIC ACID) AGAINST SODIUM FLUORIDE (NaF) TOXICITY ON REPRODUCTIVE SYSTEM OF ADULT MALE ALBINO RAT.

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### ABSTRACT

Fluorosis is an endemic public health problem in nearly 22 nations around the world. The present study has been undertaken to show the effect of Sodium fluoride (NaF) on male reproductive system and also to establish the protective role of ascorbic acid (Vitamin C) on this system. Vitamin C partially attenuated some male reproductive system dysfunctions in hyperglycemic rats. It has been shown that oxidative stress plays a possible role in the apoptosis of Sertoli cells induced by NaF, and that these effects can be suppressed with vitamin C. In our present study, NaF at a dose of 20mg/kg body weight significantly reduced the body weight, testicular weight and epididymal weight as compared to NaF+Vit-C treated group. Vit-C significantly increased

the body weight, testicular weight and epididymal weight at a dose of 20mg/kg body weight as compared to NaF+Vit-C treated group. NaF at a dose of 20mg/kg body weight significantly reduced the sperm count as well as sperm motility as compared to NaF+Vit-C treated group. Vit-C significantly increased the sperm count as well as sperm motility at a dose of 20mg/kg body weight as compared to NaF+Vit-C treated group and NaF treated group respectively.

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## INTRODUCTION

Fluoride is an environmental toxin, naturally found in many countries of world like Kenya, Tanzania, China, Australia, New Zealand & India for so many years.<sup>[1]</sup> It has been reported that 45% of drinking water in India was contaminated by fluorides. Humans are exposed to sodium fluoride (NaF)<sup>[2]</sup> from a number of sources, including water, medicines, pesticides, insecticides, fertilizer residues, dental restorative materials, dental products (tooth pastes and mouth rinses), pediatric supplements, beverages prepared with fluoridated water and food. Fluorosis is an endemic public health problem in nearly 22 nations around the world.<sup>[3]</sup> The World Health Organization (WHO) guideline is that 1.5 ppm of fluoride is the desirable upper limit in drinking water. The magnitude of problem of fluorosis in India is roughly estimated to be of 66.62 million people at risk. The recommended levels of fluoride in drinking water are 0.5–0.8 mg/L. Fluoride levels above 1.5 mg/L may lead to dental fluorosis while levels above 3–6 mg/L during the life time may lead to skeletal fluorosis.<sup>[4]</sup>

Reproductive dysfunction or malfunction is generally induced by the supplementation or intake of several elements or compounds. Such things or chemicals are actually ingested by various routes. Some of them are negligence of people and others are compulsive ignorance. This present study has been undertaken to show the effect of Sodium fluoride (NaF) on male reproductive system and also to establish the protective role of ascorbic acid (Vitamin C) on this system. Previous works have been done to show the potentiality of NaF and other such chemicals to prove the effect on various systems of the body.<sup>[5]</sup> Study has been done on fluoride toxicity in various systems with different animals. Ingestion of fluoride can produce gastrointestinal discomfort at doses at least 15 to 20 times lower (0.2– 0.3 mg/kg) than lethal doses.<sup>[4]</sup> Although helpful for dental health in low dosage, chronic exposure to fluoride in large amounts interferes with bone formation. In this way, the most widespread examples of fluoride poisoning arise from consumption of ground water that is abnormally fluoride-rich. Currently, in advanced countries, most cases of fluoride exposure are due to the ingestion of dental fluoride products. Other sources include glass-etching or chrome-cleaning agents like ammonium bi-fluoride or hydrofluoric acid. Involvement of the reproductive organs due to fluorosis in animals had also been studied extensively.<sup>[6]</sup>

The low levels of fluoride in food rendered mice infertile, while a high-fluoride diet improved their fertility. It has been reported that sodium fluoride treatment in mice caused an alteration in the histology of reproductive organs and morphology of sperm and induced biochemical changes. These reports were contradicted by Tao and Suttie, whose experiments showed that fluoride did not play any essential role in reproduction.<sup>[7]</sup> Few studies have also been reported with supplementation of few vitamins proving their protective role on male reproductive system after being hampered by ingestion of few chemicals. It has been reported that vitamin C partially attenuated some male reproductive system dysfunctions in hyperglycemic rats. Concurrent administration of cimetidine and vitamin C could be encouraged to reduce the adverse reproductive effects of cimetidine. It has been reported that the antioxidant vitamins, C and E are protective in terms of sperm counts, motility and morphology. Vitamin appeared to ameliorate the adverse effects of sodium arsenite on epididymal sperm number and some morphometrical parameters of the adult rat testis.<sup>[8]</sup> Another study showed that oxidative stress plays a possible role in the apoptosis of Sertoli cells induced by NaF, and that these effects can be suppressed with vitamin C. In earlier studies, it has been shown that, vitamin C is a strong antioxidant, with an ability to neutralize free radicals, and is widely distributed throughout the body. It also plays a protective role against oxidative stress, stimulates cell division and reproduction, protects sperm from harmful oxidative processes, and improves fertility. Present study has also its aim to evaluate the efficacy of Vit-C to ameliorate all reproductive parameters.

## MATERIALS AND METHODS

**Procurement:** Healthy, adult male albino rats (*Musculus*) Weighting between 140 to 180 gm of Wistar strain rats were taken for this experiment.

**Maintenance of Animals:** Animals were maintained as per National guidelines and protocols. The animals were kept in polypropylene cages; saw dust was put on the bottom of cages. The cages were cleaned daily and were maintained in a controlled environmental temperature ( $22\pm 2^{\circ}\text{C}$ ) in an animal house under a photoperiod of 12 hours of light and 12 hours of darkness with free access to water. Animals were fed on standardized normal diet (20% protein) which consists of wheat, sattu, milk powder, and water ad libitum.

**Source of Drug:** Sodium fluoride was obtained from laboratory.

**Source of Ascorbic Acid:** 500mg of Ascorbic acid was collected from market and it was dissolved in 25ml of distilled water.

**Preparation of NaF solution:** Sodium fluoride (NaF) was used to prepare the required aqueous NaF solution. 2mg Sodium Fluoride (NaF) was dissolved in 100ml of distilled water and 1ml of Sodium Fluoride (NaF) solution is prepared.

**Experimental design:** The rats were equally divided into following groups.

**Group I (Control):** This group comprised the control group. These will be provided with standardized normal diet and they received distilled water ad-libitum.

**Group II (NaF treated animals):** The animals of this group received sodium fluoride (NaF). Animals were given sodium fluoride (NaF) using a feeding tube attached to a hypodermic needle in the dose of 20mg/kg body weight/day for 28days.

**Group III (NaF + Vit-C treated animals):** The animals of this groups were treated with sodium fluoride (NaF) and in addition to NaF, animals were given ascorbic acid (Vitamin-C) in the dose of 20mg/kg body weight/day for 28 days.

**Group IV (Vit-C treated animals):** In this group animals were given Vit-C (ascorbic acid) at a dose of 20mg/kg body weight for 28 consecutive days.

**Measurement of parameters:** After completion of 28 days of treatment, the rats were anaesthetized one after another with anaesthetic with ether & cut them.

#### **Reproductive parameters estimation**

Testis & epididymis of each rat was dissected out & treated off adipose tissues & weights were taken from sacrifices rat.

**Sperm count:** From each separated epididymis, the cauda part is removed & placed in a petridish containing 5 ml of pH buffer solution. Then the cauda treated with pH buffer solution stitched by the niddle till it became turbid. Then the solution is taken with the help of WBC-pipette into the neubauer chamber. At last the sperms are counted under the microscope through high magnification.

**Sperm Motility:** A few drops of solution are taken to the group-slide from the above mentioned turbid solution .The slide is covered with a cover slip & examined under microscope with high power.

**Histopathology of testis:** From the dissected rat a cleaned testis was taken and was kept in 4% formaldehyde solution for preservation. At the end the slide is prepared and examined under the microscope.

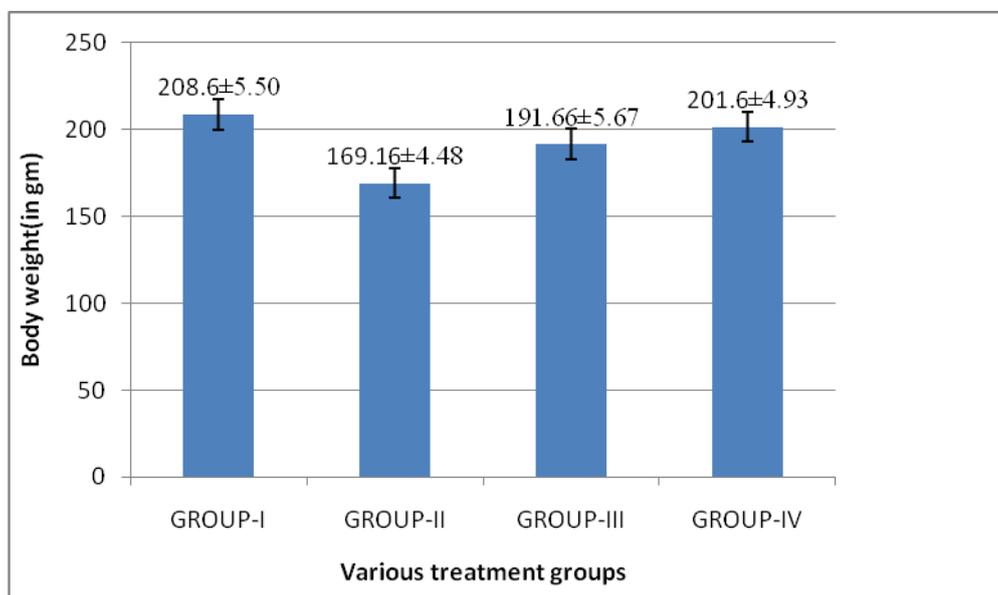
### Statistical analysis

Data were represented as mean  $\pm$  SEM and finally results were compared to the respective controls with the help of student 't' test.

## RESULTS

### Body weight

Animals of group-I have gained the body weight after 28 days treatment while group-II animals of NaF treatment have lost their body weight. Animals of group-III of NaF+Vit-C treatment have recovered their body weight significantly increase ( $p < 0.005$ ) compare to group I animals.



**Figure1:** Body weight values are mean  $\pm$  SEM (in gm), n=6 rats in each group.

### Testicular weight

Testicular weight of NaF treated animals (group-II) was reduced significantly ( $p < 0.05$ ) comparing to their control counterpart and on the other hand testicular weight has been recovered significantly increase ( $p < 0.05$ ) in group-III animal compare to group-I animals.

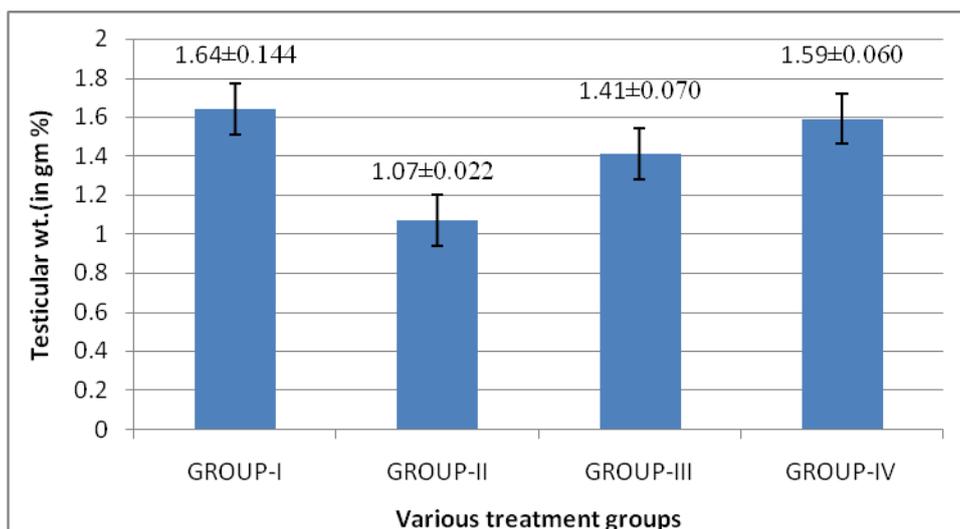


Figure 2: Testicular weight values are mean  $\pm$  SEM (gm %), n=6 rats in each group.

### Epididymal weight

NaF treated animals (group-II) have suffered from testicular weight loss in respect to control group animals significantly ( $p < 0.005$ ). Weight loss has been regained significantly ( $p < 0.005$ ) in those animals supplemented with Vit-C (group-III).

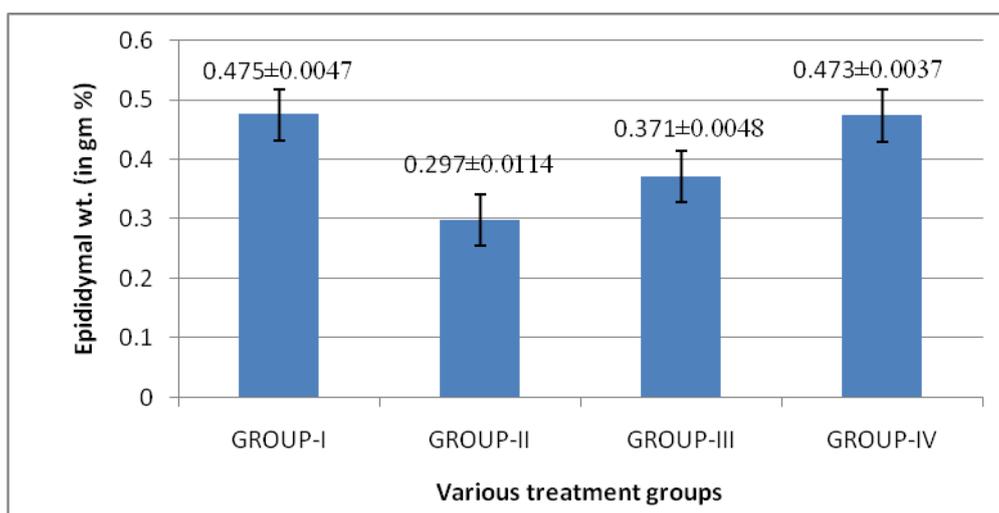
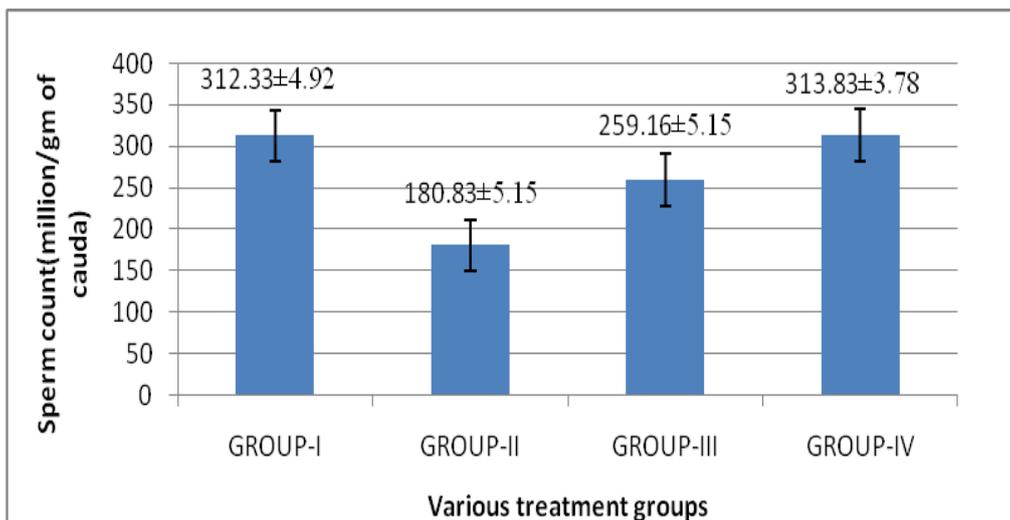


Figure 3: Epididymal weight values are mean  $\pm$  SEM (gm %), n=6 rats in each group.

### Sperm count

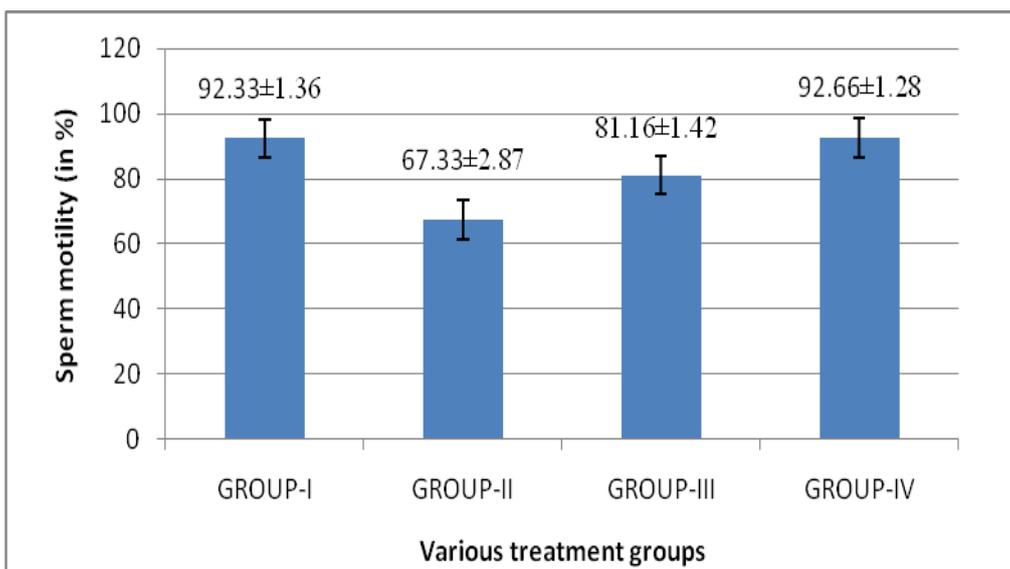
Comparing the control group animals, sperm count has been reduced significantly ( $p < 0.005$ ) in group-II animals and significant ( $p < 0.005$ ) increase is also found in group-III.



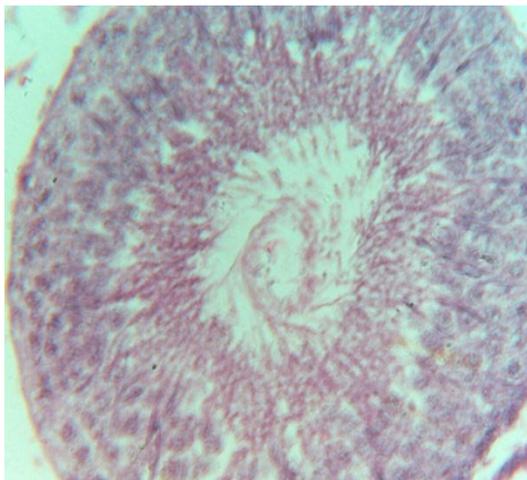
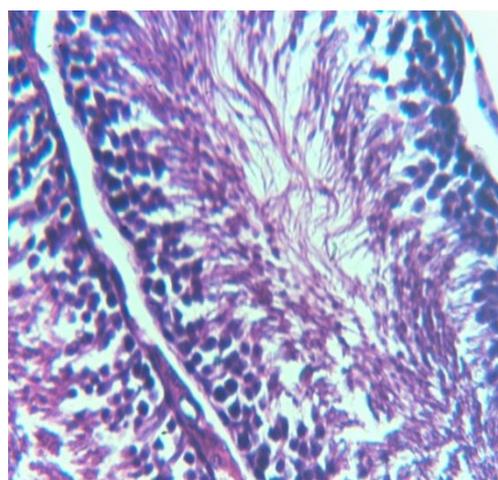
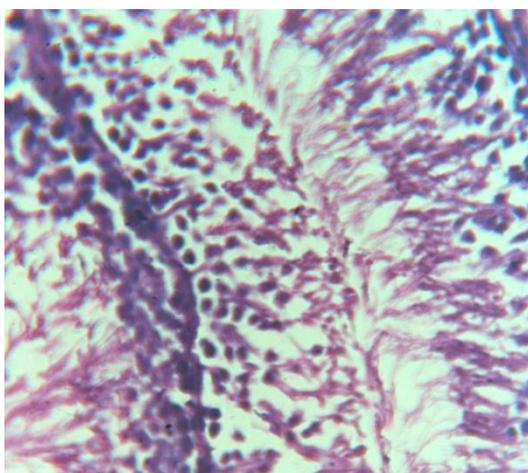
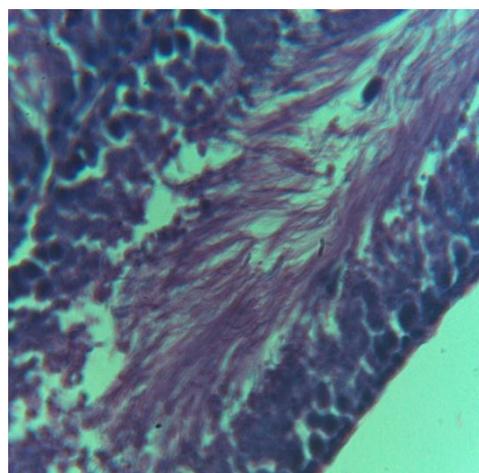
**Figure 4:** Sperm count values are mean  $\pm$  SEM (million/gm of cauda), n=6 rats in each group.

### Sperm motility

Sperm motility has been reduced significantly ( $p < 0.005$ ) in NaF treated animals in comparison with normal animals. On the contrary percentage of sperm motility has been improved significantly ( $p < 0.01$ ) in Vit-C supplemented group in respect to the Group-I animals.



**Figure 5:** Sperm motility values are mean  $\pm$  SEM (in %), n=6 rats in each group.

**Histopathology of the testis****Fig 6: Control testis.****Fig 7: NaF treated testis.****Fig 8: NaF+Vit-C treated testis.****Fig 9: Vit-C treated testis.**

From those above pictures histology of those testis show normal spermatogenesis and different stages of differentiation & maturation of spermatogenesis.

**DISCUSSION**

The present study shows contradictory reports regarding the effect of reproductive system.<sup>[14]</sup> Therefore, the study is performed to establish the link between fluoride & it's effect on body wt., weight of testis, weight of epididymis, sperm count, sperm motility& histopathology of testis.

In our study there are a significant decrease in the body weight compare to thegroup I when NaF treated on animals. The present study clearly shows the effect of NaF on general growth

pattern of the body. It is also significant that the co-administration of the Vit-C has recovered the general growth of the body in experimental animals.<sup>[22]</sup>

Decrease in testicular weight takes place comparing to the normal group of animals when NaF has been introduced but administration of the Vit-C along with NaF have improved the testicular weight on that particular group of animals.

Epididymal weight has been significantly reduced in NaF treated animals compared to the normal group. Similarly, in another study, rabbits fed on fluoride were having a significant decrease in epididymal weight.<sup>[23]</sup> Also, the weight of the cauda epididymis in fluoride-treated mice declined significantly compared with the normal groups. In group III animals, supplementation of Vit-C increased the epididymal weight compared to that of the group II animals.

In present observation sperm count has been declined in greater extent in NaF treated animals compared to normal group animals. Similar results has been observed in rats, mice and rabbits in studies performed earlier.<sup>[24]</sup> The effect of fluoride toxicity on spermatogenesis may be due to fluoride reduces the testosterone levels & by reducing the testicular zinc levels, it impairs angiotensin-converting enzyme (ACE) activity and hence causes inhibition of spermatogenesis.<sup>[25]</sup> Vit-C supplementation has improved the sperm count compare to the NaF treated group significantly as the protective role of Vit-C has also been shown in previous study.<sup>[25]</sup>

In present study the motility of the sperm has been reduced significantly in group II animals (NaF treated) compare to the normal group. Similar results were observed in rats and mice in many other studies. Mechanism behind the reduction in sperm motility may be the decline in the fructose level, which provides energy for motility in the seminal vesicle and vas deferens due to alteration in carbohydrate. Another reasons may be due to decrease in androgen carrier protein.<sup>[26]</sup> Administration of Vit-C as a supplement in group III animals has proved its efficacy after improving the motile capacity of sperm significantly in comparison with NaF treated animals which is well supported by the experiment of early researcher.

## CONCLUSION

From this experimental study it is concluded that fluoride toxicity produce definite alteration in many Reproductive parameters, which were dose and duration dependent. Withdrawal of

NaF treatment and administration of Ascorbic Acid revealed significant recovery in all the parameter suggested that effects induced by NaF treatment were transient and reversible and hence no permanent damage occurred.

## REFERENCES

1. Hu Long, Ying Jin, Mu Lin, Yu Sun, Liang Zhang and Carole Clinch. Fluoride Toxicity in the male reproductive system. *Fluoride*, 2009; 42(4): 260-276.
2. Seyed M. Nabavi, Seyed F. Nabavi, Akbar H. Moghaddam, William N. Setzer and Morteza Mirzaei. Effect of silymarin on sodium fluoride-induced toxicity and oxidative stress in rat cardiac tissues. *Anais da Academia Brasileira de Ciências*, 2012; 84(4): 1121-1126.
3. Muthu K and Krishnamoorthy P. Effect of Vitamin C and Vitamin E on Mercuric Chloride -Induced Reproductive Toxicity in Male Rats. *Biochemistry & Pharmacology*, 2012; 1(7): 1-5.
4. Elias Adikwu and Oputiri Deo. Hepatoprotective Effect of Vitamin C (Ascorbic Acid). *Pharmacology & Pharmacy*, 2013; 4(1): 84-92.
5. Shweta Parihar, Anil Choudhary and Shalini Gaur. Toxicity of fluoride in liver of Albino rat and Mitigation after adopting artificial (Vitamin C and D) and natural (Aloe vera) food supplementations. *International Journal of Advancements in Research & Technology*, 2013; 2(2): 1-11.
6. Ismael Abdel Aziz and Atef Masad. Fluoride-induced Hematological and Biochemical Changes in Albino Rat. The Therapeutic Action of Vitamin C and Olive Oil on Fluoride Effects. *Journal of Al Azhar University-Gaza (Natural Sciences)*, 2013; 15: 31-50.
7. D. K. Giri, R. C. Ghosh, D. K. Kashyap and Govina Dewangan. Evaluation of subacute sodium fluoride toxicity on spermatozoa & testicular tissue of male wistar rats. *The Bioscan*, 2013; 8(3): 983-987.
8. Kavitha N, Sandeep V, Praveena M, Ravi Sekhar P and Jayantha Rao K. Role of vitamin 'C' against sodium fluoride toxicity on haematological profile in albino mice. *International Journal of Review In Life Sciences*, 2013; 3(2): 27-31.
9. Bashir Ahmad Shah MS, Gh. Mohd. Bhat MS, Mohd. Saleem Itoo MD, Nasseer Ahmad Shah MD, Shaheen Shadad MD and Javed Ahmad Khan MD. Effect of Fluoride Ions on the Microanatomy of Lungs in Albino Rats. *IOSR Journal Of Environmental Science, Toxicology And Food Technology*, 2013; 6(3): 75-78.

10. Raghu Jetti, Raghuveer CV, Mallikarjuna Rao C, Somayaji SN, Prakash Babu B. Neuroprotective effect of Ascorbic acid and Ginkgo biloba against Fluoride caused Neurotoxicity. *IOSR Journal Of Environmental Science, Toxicology And Food Technology*, 2014; 8(1): 30-36.
11. Imran Khan & Anita Ranga. Biochemical changes in Sodium Fluoride Induced kidney of Swiss Albino mice and it's Amelioration by Ascorbic Acid. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 2014; 3(4): 948-956.
12. Vijay K. Bharti, R. S. Srivastava, H. Kumar, S. Bag, A. C. Majumdar, G. Singh, S. R. Pandi-Perumal, and Gregory M. Brown. Effects of Melatonin and Epiphyseal Proteins on Fluoride-Induced Adverse Changes in Antioxidant Status of Heart, Liver, and Kidney of Rats. *Advances in Pharmacological Sciences*, 2014; 2014: 1-6.
13. Banu Orta Yılmaz and Melike Erkana. Effects of Vitamin-C on Sodium Fluoride Induced Oxidative Damage In Sertorli Cells. *Fluoride*, 2015; 48(3): 241-251.
14. Arpita Samanta, Bidyut Bandyopadhyay, Nirmalendu Das. Fluoride Intoxication and Possible Changes in Mitochondrial Membrane Microviscosity and Organ Histology in Rats. *International Journal of Scientific Research*, 2016; 5(6): 42-45.
15. Shyamal Kanti Das, Soumendra Nath Karmakar and Debarati Singha Roy. Efficacy of ascorbic acid as protective substance on sodium fluoride induced reproductive impairment. *European Journal of Biomedical and Pharmaceutical Sciences*, 2016; 3(11): 265-271.
16. Adamma A Emejulu, Chinwe S Alisi, Emeka S Asiwe, Chidi U Igwe, Linus A Nwogu and Viola A Onwuliri .Renal and Hepato-Protective Effects of Irvingia gabonensis Juice on Sodium Fluoride-Induced Toxicity in Wistar Rats. *Journal of Clinical Toxicology*, 2016; 6(2): 1-6. 2016.
17. Mohammad Ali Takhshid, Ali Reza Tavasuli, Yazdan Heidary, Mojtaba Keshavarz and Hussain Kargar. Protective Effect of Vitamins E and C on Endosulfan-Induced Reproductive Toxicity in Male Rats. *Iranian Journal of Medical Sciences*, 2016; 37(3): 173-180.
18. Shyamal Kanti Das, Soumendra Nath Karmakar and Debarati Singha Roy. Protective role of tri calcium phosphate [Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>] against sodium fluoride (NaF) toxicity on reproductive system of male albino rat. *International Journal of Novel Trends in Pharmaceutical Sciences*, 2016; 6(6): 120-125.

19. Naseer Ahmad Baba, R Raina, Pawan K Verma, M Sultana, S Prawez, Nisar Ahmad Nisar and R. S. Pura. Toxic Effects Of Fluoride & Chlorpyrifos On Antioxidant Parameters In Rats: Protective Effects Of Vitamins C & E. *Fluoride*, 2013; 46(2): 73–79.
20. P Ekambaram, T Namitha, S Bhuvanewari, S Aruljothi, D Vasanth and M Saravanakumar. Therapeutic Efficacy of Tamarindus indica (L) to protect against fluoride-induced oxidative stress in the liver of female rats. *Fluoride*, 2010; 43(2): 134–140.
21. Mona Helal and Mai El Dakdoky. Fetotoxicity of fluoride in rats and the protective action of some antioxidants. *Fluoride*, 2006; 39(3): 202–210.
22. Kaur K, Koul ML and Koul RI. Histological changes in liver following sodium fluoride ingestion. *Fluoride*, 1981; 14: 119-123.
23. Flora SJS, Dhawan M and Tandon SK. Effects of combined exposure to aluminium and ethanol on aluminium body burden and some neuronal, hepatic and haematopoietic biochemical variables in the rat. *Human Exp. Toxicol*, 1991; 10: 45-48.
24. Huang C, Yang H, Niu R, Sun Z, Wang J. Effect of sodium fluoride on androgen receptor expression in male mice. *Fluoride*, 2008; 41: 10–7.
25. Fatma GU, Suna K, Dilek D, Filiz D and Yusuf K. Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E. *Food and Chemical Toxicology*, 2009; 47: 1903–1908.
26. Sonmez M, Turk G and Yuce A. The effects of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male wistar rats. *Theriogenology*, 2005; 63(7): 2063–2072.