



EFFICACY OF ASCORBIC ACID AS PROTECTIVE SUBSTANCE ON SODIUM FLUORIDE INDUCED REPRODUCTIVE IMPAIRMENT

Shyamal Kanti Das, Soumendra Nath Karmakar* and Debarati SinghaRoy

Post Graduate Department of Physiology, Krishnath College (Under K.U), Berhampore, Murshidabad, West Bengal, India.

***Corresponding Author: Soumendra Nath Karmakar**

Post Graduate Department of Physiology, Krishnath College (Under K.U), Berhampore, Murshidabad, West Bengal, India.

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ABSTRACT

This study deals with the evaluation of ascorbic acid (Vit-C) as protective agent against Sodium fluoride (NaF) toxicity on male reproductive system. Present Observation clearly reveals the toxic effect of NaF on male reproductive system as body weight, testicular weight, epididymal weight, sperm count, sperm motility, SGPT, SGOT and serum testosterone have been hampered in significant manner. Besides this, improvement or recovery has taken place significantly on these parameters after co-administration or supplement of Vit-c along with NaF. This proves the protective efficacy of Vit-c.

KEY WORDS: Sodium fluoride, ascorbic acid, sperm count, sperm motility, testosterone.

INTRODUCTION

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. Humans are exposed to NaF from a number of sources including water, medicines, insecticides, pesticides, fertilizers, few dental restorative agents, dental products like tooth paste and often from few beverages.^[1] 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.^[2] 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.^[3] Currently, in advanced countries, most cases of fluoride exposure are due to the ingestion of dental fluoride products.^[4] Other sources include glass-etching or chrome-cleaning agents like ammonium bi-fluoride or hydrofluoric acid.^[5,6] Involvement of the reproductive organs due to fluorosis in animals had also been studied extensively. Messar et

al., reported that the low levels of fluoride in food rendered mice infertile, while a high-fluoride diet improved their fertility.^[7] 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.^[8] These reports were contradicted by Tao and Suttie, whose experiments showed that fluoride did not play any essential role in reproduction.^[9] 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.^[10] Concurrent administration of cimetidine and vitamin C could be encouraged to reduce the adverse reproductive effects of cimetidine.^[11] It has been reported that the antioxidant vitamins, C and E are protective in terms of sperm counts, motility and morphology.^[12] Vitamin E appeared to ameliorate the adverse effects of sodium arsenite on epididymal sperm number and some morphometrical parameters of the adult rat testis.^[13] 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.^[14] 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.^[15-17] Present study has

also its aim to evaluate the efficacy of Vit-C to ameliorate few reproductive parameters.

MATERIALS AND METHODS

Experimental protocol

Adult (90±10 days) male albino rats (120±10 gm) of Wistar strain were taken for this experiment. Animals were maintained as per National guidelines and protocols. Animals were housed in clean polypropylene cages and were maintained in a controlled environmental temperature (22±2°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 70% wheat, 20% gram, 5% fish meal powder, 4% dry yeast powder and 1% oil and water ad libitum.

Animal treatment

Rats were equally divided into three groups (n=12). Initial body weights of all the rats were recorded.

Group-I: Rats were treated as control group and were maintained on standard diet and water ad libitum for 30 days.

Group-II: 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 30 days.

Group-III: In addition to NaF, animals were given Vitamin-C in the dose of 15mg/kg body weight/day for 30 days.

Measurement of parameters

After completion of 30 days of treatment, final body weights of all the rats were taken and the rats were anaesthetized one after another with anaesthetic ether and blood was collected directly from hepatic portal vein and allowed to coagulate. Clear serum was collected and stored in 20°C. Testis and epididymis of each rat were dissected out and trepanned off adipose tissues and weights were taken. After scattering it, sperms were dispersed into the buffer solution and it was taken for the

count of sperm and its motility through the process of Majumder and Biswas.^[18]

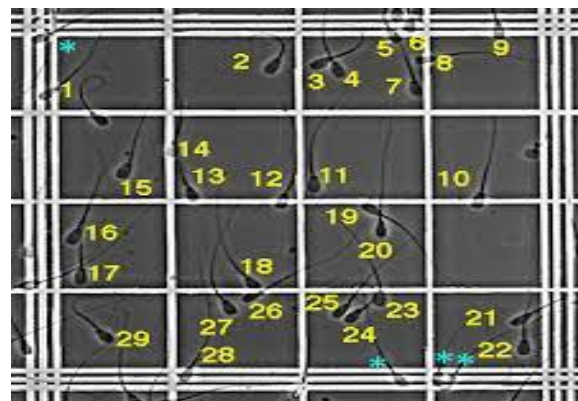


Fig: 1: Method of sperm count

Hormonal level like testosterone in serum of all animals was estimated with the help of ELISA method. Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) were measured of all the control and experimental animals through the process of Kind and King.^[19]

Statistical analysis

Data have been represented as mean±SEM and finally results were compared with the respective controls with the help of student's "t" test (Das 2005).^[20]

RESULTS

Body weight

Animals of group-I have gained the body weight after 30 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 ($p<0.05$) compare to group-II animals (fig:2).

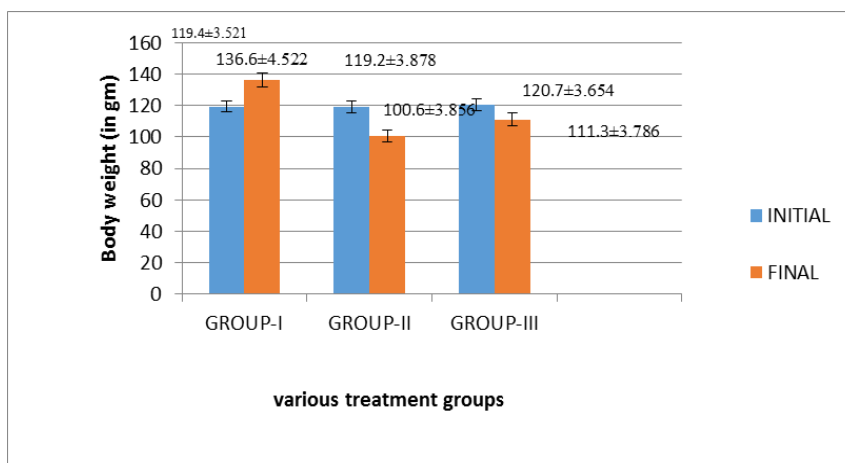


Fig: 2: Initial & final body wt. values are mean ± SEM (in gm), n=12 rats in each group.

Testicular weight

Testicular weight of NaF treated animals (group-II) was reduced significantly ($p<0.001$) comparing to their

control counterpart and on the other hand testicular weight has been recovered significantly ($p<0.01$) in group-III animal compare to group-II animals (fig:3).

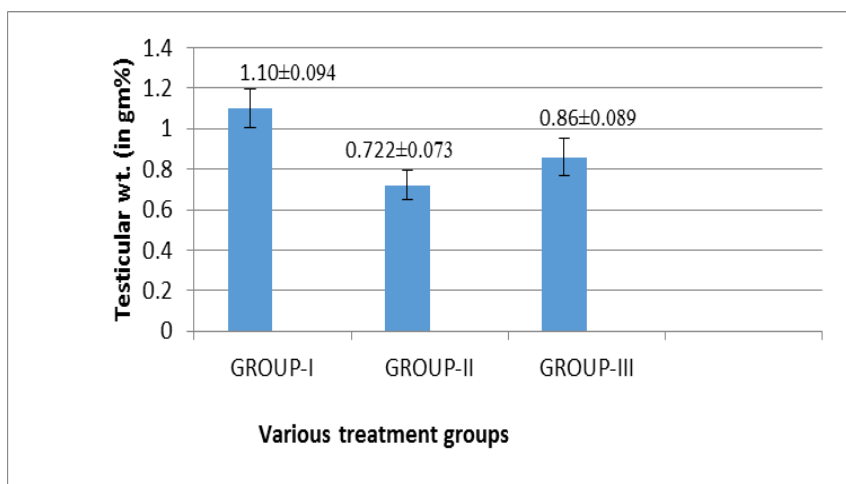


Fig: 3: Presentation of testicular wt. Values are mean±SEM (gm%), n=12 animals in each group.

Epididymal weight

NaF treated animals (group-II) have suffered from testicular weight loss in respect to control animals

significantly ($p < 0.001$). Weight loss has been regained significantly ($p < 0.005$) in those animals supplemented with Vit-C (group-III) (fig:4).

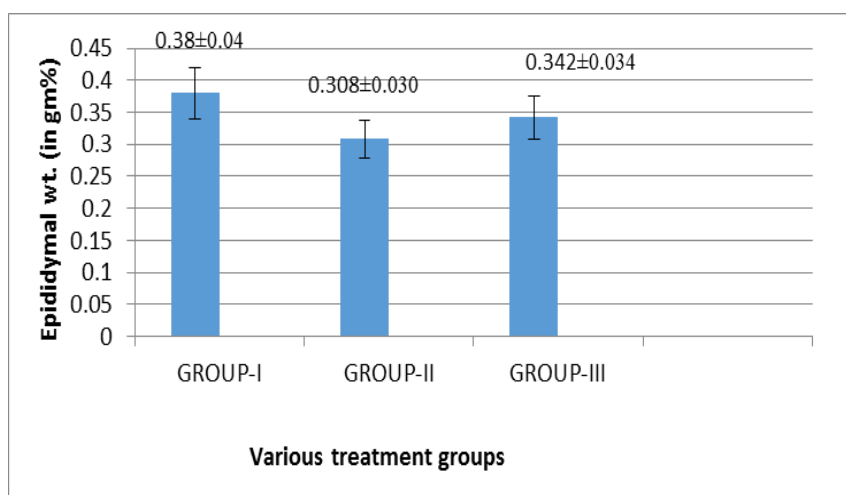


Fig: 4: Presentation of epididymal weight. Values are mean ± SEM (gm%), n=12 animals in each group.

Sperm count

Comparing the control group animals, sperm count has been reduced significantly ($p < 0.001$) in group-II animals

and significant ($p < 0.001$) increase is also found in group-III animals comparing to the group-II animals (fig:5).

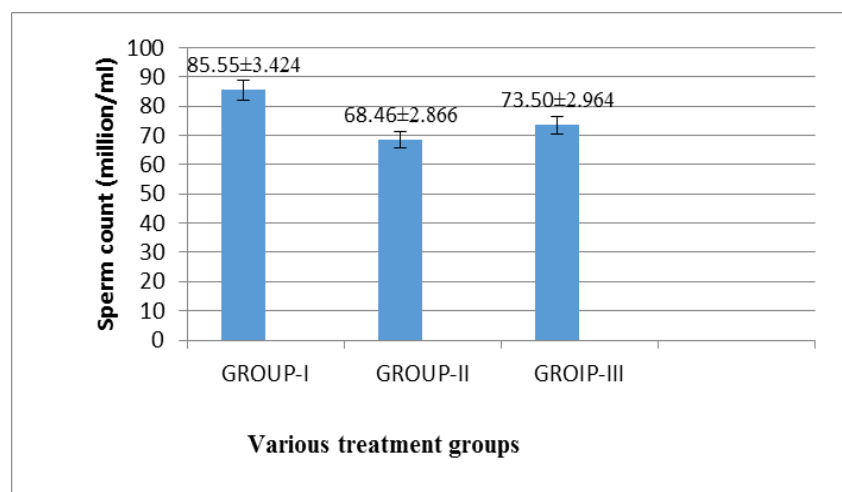


Fig: 5: presentation of sperm count. Values are mean ± SEM (million/ml), n=12 rats in each group.

Sperm motility

Sperm motility has been reduced significantly ($p < 0.001$) in NaF treated animals in comparison with control animals. On the contrary percentage of sperm motility

has been improved significantly ($p < 0.001$) in Vit-C supplemented group in respect to the NaF treated animals (fig:6).

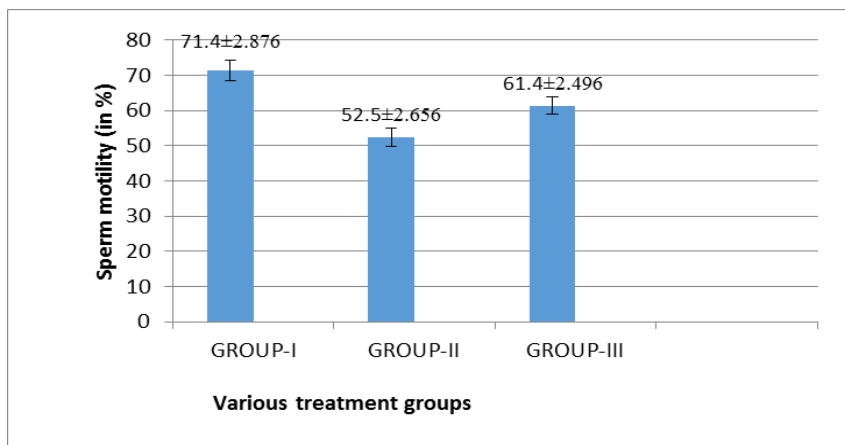


Fig:6: Presentation of sperm motility. Values are mean $\pm$ SEM (in %), n=12 rats in each group.

SGPT

Serum level of SGPT has been increased in group-II animals significantly ($p < 0.001$) in respect to group-I.

Significant difference ($p < 0.001$) in recovery is also found in group-III animals compare to that of their respective NaF treated animals (group-II) (fig:7).

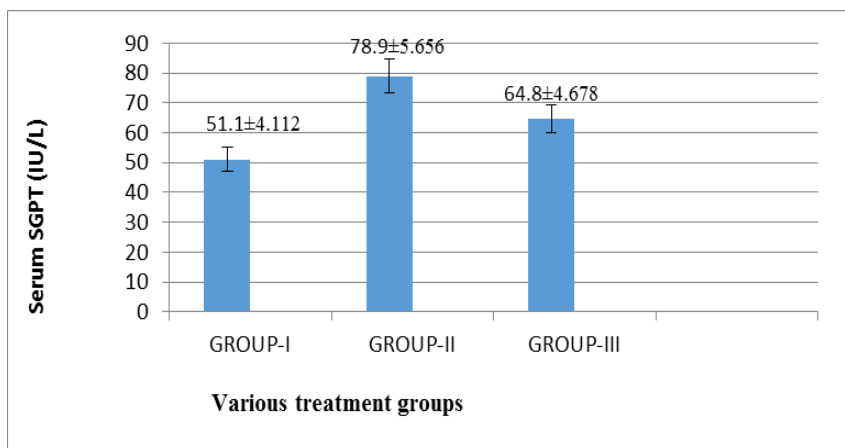


Fig:7: Presentation of SGPT level. Values are mean $\pm$ SEM (IU/L), n=12 rats in each group.

SGOT

Significant increase ($p < 0.001$) in serum SGOT level is observed between group-I and group-II animals. There

significant ($p < 0.01$) recovery is also found between group-II and group-III animals (fig:8).

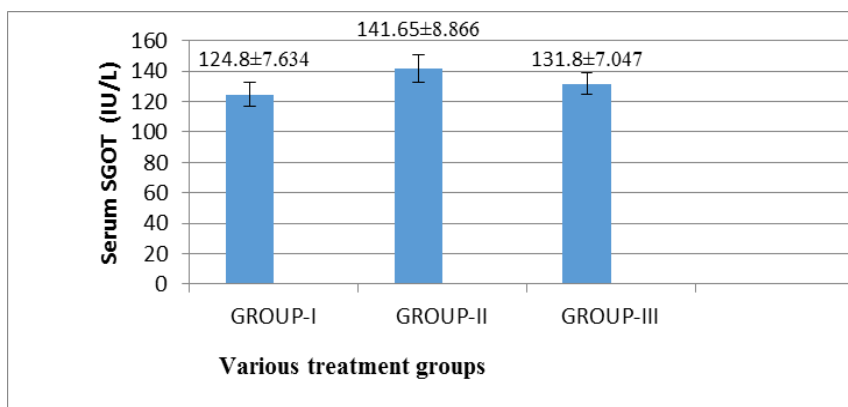


Fig:8: Presentation of SGOT level. Values are mean $\pm$ SEM(IU/L), n=12 rats in each group.

Serum testosterone

Significant decrease ($p<0.01$) in serum level concentration is found between control and NaF treated

animals. Significant ($p<0.05$) increment is found in serum testosterone level between NaF treated and Vit-C supplemented group (fig:9).

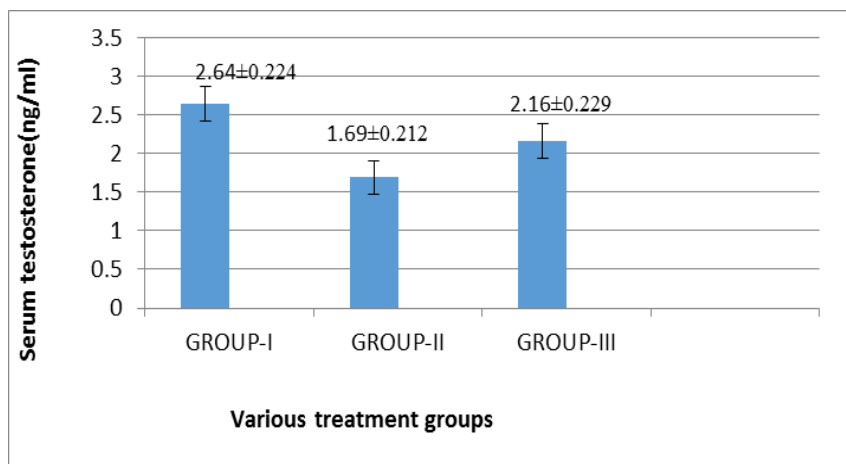


Fig: 9: Presentation of testosterone conc. Values are mean±SEM (ng/ml), n=12 rats in each group.

DISCUSSION

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.

Decrease in testicular weight takes place comparing to the control 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 control group. Similarly, in another study, rabbits fed on fluoride were having a significant decrease in epididymal weight.^[21] Also, the weight of the cauda epididymis in fluoride-treated (10 mg/kg for 30 days) mice declined significantly compared with the control groups.^[22] In group III animals supplementation of Vit-C has 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 control animals. Similar results has been observed in rats, mice and rabbits in studies performed earlier^[22-24]. The effect of fluoride toxicity on spermatogenesis may be due to fluoride reduces the testosterone levels and by reducing the testicular zinc levels, it impairs angiotensin-converting enzyme (ACE) activity and hence causes inhibition of spermatogenesis.^[1] 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.^[25]

In present study the motility of the sperm has been reduced significantly in group II animals (NaF treated) compare to the control group. Similar results were observed in rats and mice in many other studies.^[22,26-28]

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.^[23,29] Another reasons may be due to decrease in androgen carrier protein.^[30] 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.^[25]

Present observation has showed significant increment in serum SGPT and SGOT level in NaF treated animals compare to that of their respective control animals. Similar alteration has taken place in other experiments establishing the hepatic damage by fluoride activity.^[31] Fluoride and aluminium alone cause a similar increase in these serum transaminases.^[32,33] This observation has also established the significant recovery of these transaminases level after supplementation of Vit-C.

Serum testosterone level is decreased after administration of NaF comparing to the control group. It has been postulated that apart from the direct effect on testosterone level^[1], fluoride inhibits androgen receptor (AR) mRNA expression in sertoli cells and causes a decrease in AR through which testosterone acts.^[34] Vit-C introduction has recovered the destructive effect of NaF significantly in present study as Vit-C plays key role in testosterone synthesis.^[35,36]

CONCLUSION

Present study clearly establishes the destruction of male reproductive system of rat after treatment of NaF. But it has also been proved that supplementation of Vit-C has its potentiality to recover the damaging effect of NaF.

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