Protective role of tri calcium phosphate \([\text{Ca}_3(\text{PO}_4)_2]\) against sodium fluoride (NaF) toxicity on reproductive system of male albino rat

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Abstract
Current study is about the evaluation of Tri Calcium phosphate \([\text{Ca}_3(\text{PO}_4)_2]\) as protective agent against Sodium fluoride (NaF) toxicity on male reproductive system. Observation of this experiment clearly shows toxic effect 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 after NaF administration. Besides this, improvement or recovery has taken place significantly on these parameters after co-administration or supplement of \(\text{Ca}_3(\text{PO}_4)_2\) with NaF. This proves the protective efficacy of \(\text{Ca}_3(\text{PO}_4)_2\).

Keywords: Sodium fluoride, tri calcium phosphate, sperm count, sperm motility, testosterone.

INTRODUCTION
In recent years, population is facing some problems regarding their health from some chemicals. These chemicals are often taking their routes into human system through life schedule of the persons. These chemicals commonly take entry sometimes due to negligence of people or unconsciousness of handling of few materials. The present experiment has been proposed to elucidate the destructive role of sodium fluoride (NaF). Besides this, the project has also its aim to search the protective efficacy of tri calcium phosphate \([\text{Ca}_3(\text{PO}_4)_2]\) against NaF induced reproductive failure. 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 works have been done earlier to show their protective efficiency on NaF induced toxicity. Protective role of quercitin was found on NaF induced oxidative stress in rat’s liver [10]. It has been investigated the anti-oxidative properties of aqueous extract of the bark of Terminalia arjuna (TA) on sodium fluoride (NaF) induced oxidative damages in the livers and kidneys of Swiss albino mice [11]. The effect of dietary Ca in response to fluoride (F) treatment was investigated in rats [12]. The nephron-protective effect of gallic acid isolated from Pelliphyllum peltatum was examined in

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sodium fluoride (NaF) treated rats [13]. Intensive-swimming-induced oxidative stress causes dysfunctions in the male reproductive system, which can be protected by the co-administration of sodium selenite and zinc sulfate [14]. Withania somnifera may be having antioxidant property; protecting male reproductive organs from ROS (Reactive Oxygen Species) and can avoid infertility [15]. The present study has been designed to resolve some conflicts arising from previous works and for searching new chemicals having protective role on reproductive toxicity.

MATERIALS & 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 polypylene 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 Ca₃(PO₄)₂ in the dose of 25mg/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 treamed 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 [16]. 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 [17].

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 [18].

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+[Ca₃(PO₄)₂] treatment have recovered their body weight significantly (p<0.05) compare to group-II animals (fig:1).

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:2).

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 [Ca₃(PO₄)₂] (group-III) (fig:3).

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 animal comparing to the group-II animals (fig:4).

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
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(p<0.001) in [Ca$_3$(PO$_4$)$_2$] supplemented group in respect to the NaF treated animals (fig:5).

**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:6).

**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:7).

**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 [Ca$_3$(PO$_4$)$_2$] supplemented group (fig:8).

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**Fig 1. Initial& final body wt. values are mean±SEM (in gm), n=12 rats in each group**

**Fig 2. Presentation of testicular wt. Values are mean±SEM (gm%), n=12 animals in each group**

**Fig 3. Presentation of epididymal weight. Values are mean±SEM (gm%), n=12 animals in each group**

**Fig 4. Presentation of sperm count. Values are mean±SEM (million/ml), n=12 rats in each group**
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**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 \([\text{Ca}_3(\text{PO}_4)_2]\) 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 \([\text{Ca}_3(\text{PO}_4)_2]\) 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 [19]. Also, the weight of the cauda epididymis in fluoride-treated (10 mg/kg for 30 days) mice declined significantly compared with the control groups [20]. In group III animals supplementation of \([\text{Ca}_3(\text{PO}_4)_2]\) 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 [21-22]. The effect of fluoride toxicity on spermatogenesis may be due to fluoride.
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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]. \(\text{Ca}_3(\text{PO}_4)_2\) supplementation has improved the sperm count compared to the NaF treated group significantly.

In present study the motility of the sperm has been reduced significantly in group II animals (NaF treated) compared to the control group. Similar results were observed in rats and mice in many other studies [20, 23-25](Chinoy and Sharma 1998, Narayana and Chinoy 1994, Narayana and Chinoy 1994, Bataineh and Nusier 2006). 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 [21, 26]. Another reason may be due to decrease in androgen carrier protein [27]. Administration of \(\text{Ca}_3(\text{PO}_4)_2\) 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.

Present observation has showed significant increment in serum SGPT and SGOT level in NaF treated animals compared to that of their respective control animals. Similar alteration has taken place in other experiments establishing the hepatic damage by fluoride activity [28]. Fluoride and aluminium alone cause a similar increase in these serum transaminases [29-30]. This observation has also established the significant recovery of these transaminases level after supplementation of \(\text{Ca}_3(\text{PO}_4)_2\). This finding is well supported by earlier experiment [31].

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 [32]. \(\text{Ca}_3(\text{PO}_4)_2\) introduction has recovered the destructive effect of NaF significantly in present study.

CONCLUSION

Present study obviously establishes the destruction of male reproductive system of rat after treatment of NaF. It has also been proved that supplementation of \(\text{Ca}_3(\text{PO}_4)_2\) has its potentiality to recover the damaging effect of NaF.

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CONFLICT OF INTEREST

No conflict of interest.

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