

**TAMARIND PULP EXTRACT MEDIATED RECOVERY OF FLUORIDE INDUCED
REPRODUCTIVE TOXICITY ON MALE ALBINO RAT.**

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ABSTRACT

The present study was under taken to evaluate the possible protective action of the tamarind pulp extract on sodium fluoride (NaF) mediated reproductive toxicity on albino Wistar rat (male). In present study, different experimental animal groups were treated with NaF (2mg/ml/100gm), both NaF (2mg/ml/100gm) and tamarind pulp extract (1mg/100gm body weight) along with control group animals treated with water respectively. NaF treatment caused drastic reduction in physiological, biochemical and reproductive parameters in comparison with control group of animals. Body weight along with testicular and epididymal weight have been increased after co administration of tamarind pulp extract in comparison with their respective NaF treated group. In the same manner, recovery has taken place in serum protein, serum glucose, sperm count, sperm motility and serum testosterone concentration after tamarind pulp extract co supplementation with NaF. Histological structure has also been improved after tamarind pulp extract administration. It can be concluded that tamarind pulp extract has potency as protective agent against NaF induced reproductive toxicity on albino rats.

KEYWORDS: Sodium fluoride, tamarind pulp, sperm count, sperm motility, histology.

1. INTRODUCTION

The presence of fluoride in ground water is governed by several factors like igneous rock formation, hydrothermal fluids, metamorphic rocks and weathering processes.^[1] Although fluorosis has been regarded in the past as disease affecting only bone and teeth, recent studies have given evidence that fluoride toxicity adversely affects most of the soft organ.^[2] Various evidences accumulated in the recent years indicate that fluoride is detrimental to male reproductive system. For instance Ghosh et al.,2002^[3] reported a significant diminishment in the relative weight of testis and prostate in sodium fluoride treated animals. Besides this, sperm count was also decreased significantly in rats. A study on rabbits by Sussheela et al.,1991^[4] showed that when these are exposed to 10mg NaF/kg of body weight for 29 months, the spermatogenic cells in the seminiferous tubule were disrupted, degenerated and devoid of spermatozoa.

The pharmacological investigations revealed that the Tamarindus indica pulp extract is antifungal, hypoglycemic, cholesterolemic, cytotoxic, anti-inflammatory, gastrointestinal and hypolipomic in its activities.^[5-7] However it has been postulated that fluoride could decrease the fructose level, which provides energy to sperm for motility^[8] and fluoride

toxicity led to the decline in sperm count via hormonal imbalance, alterations in epididymis and accessory reproductive glands.^[9] Tamarindus indica pulp extract has its possible reversibility on the testis of albino rats. So, this present study has been undertaken to evaluate the therapeutic potency of tamarind pulp extract on fluoride induced male reproductive toxicity.

2. MATERIALS AND METHODS

2.1 Experimental protocol

Adult (90±10 days) male albino rats (110±10 gm) of Wistar strain were taken for this experiment and approved by IACUC. Animals were housed in clean polypropylene cages and were maintained in a controlled environmental temperature (25±2°C) in an animal house under a balanced photoperiod 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.

2.2 Preparation of drugs

1gm of NaF was added with 500ml of distilled water to have the final solution before administration. 100gm tamarind pulp was added with 100ml distilled water to

get the desired extract before supplementation to the experimental animals.

2.3 Animal treatment

Rats were equally divided into three groups (n=10). 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 for 21 days.

Group-II: Animals were given sodium fluoride (NaF) using a feeding tube attached to a hypodermic needle in the dose of 2mg/ml/100gm of body weight/day for 21 days.

Group-III: In addition to NaF applied in above dose, animals were given tamarind pulp extract in the dose of 1mg/ml/100gm of body weight/day for 21 days.

2.4 Animal sacrifice and measurement of parameters

After completion of 21 days of treatment, final body weights of all the rats were taken by the electronic balance. The rats were then anaesthetized one after another with anaesthetic ether followed by cervical dislocation and blood was collected directly from hepatic portal vein and allowed to coagulate. Clear serum was collected and stored in 20°C. Testis of each rat was dissected out and weights were taken with the help of

electronic balance. Testis from each experimental animal was processed for histology and 5µ thick sections were taken and stained with haematoxyline and eosin^[10] for further observation. Serum glucose was measured using the standard kits (COGENT). The total serum protein was estimated by Lowry method with a standard curve of BSA.^[11] Sperm count and sperm motility were measured by the process of Biswas and Majumder.^[12] The serum testosterone concentration was measured by the standard ELISA (abcam) kit.

2.5 Statistical analysis

The statistical analysis was carried out by student's 't' test^[13] to generalize the results of various biochemical parameters of experimental groups in comparison to their respective control group and $p < 0.05$ was considered as significant result.

3. RESULTS

3.1 Body weight

Treatment of rat for 21 days with NaF caused significant reduction in body weight relative to control. There is significant ($*p < 0.05$) improvement found in the tamarind pulp extract treated groups in comparison with NaF treated animals.

Table 1: Results of body weight of different experimental groups including control group. Values are mean±SEM (gm, n=10) followed by two tail 't' test.

	Group-I (Control)	Group-II (NaF)	Group-III (NaF+Tamarind pulp)
Initial	115.40±3.314	111.90±3.104	112.10±3.561
Final	133.00±4.012	*100.20±2.911	*107.30±2.921

3.2 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 ($*p < 0.05$) in group-III animal compare to group-II animals.

Table 2: Results of testicular weight of different experimental groups including control group. Values are mean±SEM (gm%, n=10) followed by two tail 't' test.

Group-I (Control)	Group-II (NaF)	Group-III (NaF+Tamarind pulp)
1.03±0.019	*0.78±0.012	*0.96±0.016

3.3 Epididymal weight

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

significantly ($*p < 0.05$). Weight loss has been regained significantly ($*p < 0.05$) in those animals supplemented with tamarind pulp (group-III).

Table 3: Results of epididymal weight of different experimental groups including control group. Values are mean±SEM (gm%, n=10) followed by two tail 't' test.

Group-I (Control)	Group-II (NaF)	Group-III (NaF+Tamarind pulp)
0.32±0.041	*0.13±0.026	*0.30±0.039

3.4 Sperm count

The sperm count decreased significantly (*p<0.05) after 21 days of fluoride treatment. But increased significantly

(*p<0.05) after the tamarind pulp extract supplementation.

Table 4: Results of sperm count of different experimental groups including control group. Values are mean±SEM (million/ml, n=10) followed by two tail 't' test.

Group-I (Control)	Group-II (NaF)	Group-III (NaF+Tamarind pulp)
82.30±1.932	*70.10±1.622	*78.50±1.577

3.5 Sperm motility

The sperm motility decreased significantly (**p<0.001) after fluoride treatment of 21 days. But after tamarind

pulp extract treatment sperm motility increased significantly (**p<0.001) in comparison with NaF treated group of animals.

Table 5: Results of sperm motility of different experimental groups including control group. Values are mean±SEM [no. of motile sperm/100 sperm (%), n=10] followed by two tail 't' test.

Group-I (Control)	Group-II (NaF)	Group-III (NaF+Tamarind pulp)
74.10±1.710	**63.30±1.581	**69.80±1.685

3.6 Serum glucose level

Treatment of rat for 21 days with NaF caused significant decrease in glucose level relative to control. Significantly

(*p<0.05) increased result was found in the tamarind pulp extract supplemented group after 21 days treatment.

Table 6: Results of serum glucose level of different experimental groups including control group. Values are mean±SEM (gm/100ml, n=10) followed by two tail 't' test.

Group-I (Control)	Group-II (NaF)	Group-III (NaF+Tamarind pulp)
88.32±1.325	*76.28±1.126	*86.30±1.239

3.7 Serum total protein

In rats treated with NaF, total protein decreased significantly as compared to the control group. On the

other hand significant increase (*p<0.05) in total serum protein was observed following administration of tamarind pulp extract with NaF.

Table 7: Results of serum total protein of different experimental groups including control group. Values are mean±SEM (gm/100ml, n=10) followed by two tail 't' test.

Group-I (Control)	Group-II (NaF)	Group-III (NaF+Tamarind pulp)
7.25±0.430	*5.16±0.380	*6.61±0.490

3.8 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 after supplementation of tamarind pulp extract along with NaF.

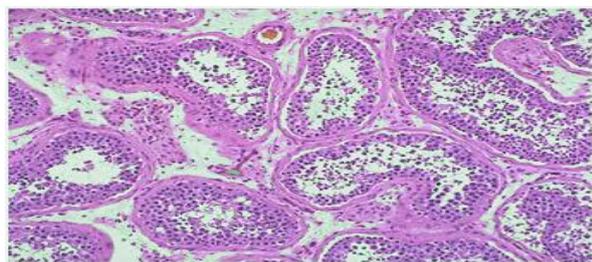
Table 8: Results of serum testosterone of different experimental groups including control group. Values are mean±SEM (ng/ml, n=10) followed by two tail 't' test.

Group-I (Control)	Group-II (NaF)	Group-III (NaF+Tamarind pulp)
2.31±0.274	**1.42±0.201	*1.98±0.214

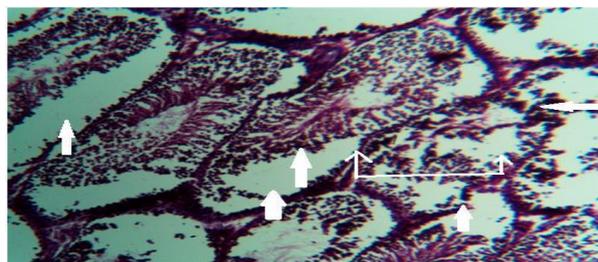
3.9 Histological study

Histological structure has been changed in NaF treated group in comparison with the animals of control group. Changes have taken place basically with disintegration of

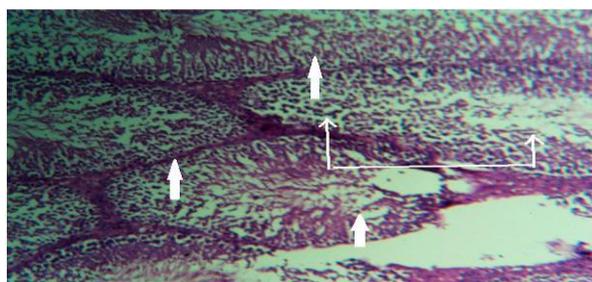
seminiferous tubules. It has also been found that reduction in accumulation of spermatozoa has taken place in the animals of both the treated group.



A



B



C

A. Histological section of testis of control rats.

B. Histological section of fluoride induced testis of rats showing (disruption of seminiferous tubular diameter; loss of sertoli cells, spermatocytes, total disruption of the structure of seminiferous tubule).

C. Histological section of fluoride and tamarind treated testis of rats showing (some improvement of histology of the testis, but not fully recovered the structure of seminiferous tubule, presence of sertoli cells).

Figure:1 (A,B,C): Histological section of testis including control and experimental rats.

4. DISCUSSION

The present investigation was carried out to explore the effects of potential toxicity of fluoride on the testis of albino rat and its possible reversibility upon treatment of *Tamarindus indica* pulp extract. In present study, after application of NaF for 21 days, the body weight has been decreased significantly. The weight of testis and epididymis has also been decreased significantly after NaF administration in comparison to control animals. This alteration is similar type of change in previous study.^[14-15] *Tamarindus indica* pulp extract supplementation caused significant improvement in body weight, testicular weight and epididymal weight.

In present study the results revealed that the fluoride hampered the sperm count whereas, tamarind pulp extract treatment on the other hand increased the sperm count significantly. The fluoride toxicity leads to the decline in sperm count via hormonal imbalance precisely due to reduction in testosterone level as well as testicular Zn level and accordingly impairs the spermatogenesis.^[16] Similar findings in relation to the effect of fluoride on sperm count have been observed in rats, mice and rabbits in various studies performed earlier.^[4,8-9] It also causes structural and functional defects in spermatozoa^[9,17] and alterations in epididymis and accessory reproductive glands.^[9] Fluoride toxicity causes oxidative damage, disturbed signal transduction and suppresses testosterone level which has been well supported by our previous work.^[18] Present study also shows decreased sperm motility in treated animals which has been well supported by other studies^[8,19-21] in rats and mice. Fluoride directly interferes with spermatogenesis by

depressing EGF and EGFR,^[22] modifying G-protein signaling,^[23] diminishing levels of androgen receptor (AR) disturbing levels of estradiol and increases the levels of FSH and LH. This has reflection on the result of the present study as the level of testosterone has been decreased in NaF treated group in comparison with control group animals. After tamarind pulp extract supplementation, the concentration of testosterone in serum has been significantly increased in comparison with NaF treated group. However, the ascorbic acid which is also a constituent of *Tamarindus indica* is a biological oxidative agent which is known to active numerous hydroxylating enzymes, which participate in metabolic processes as supplementary source of energy in several tissues including spermatozoa. This metabolic advantage has been reflected on significant increment in serum glucose and serum total protein after tamarind pulp supplementation in this study.

Sperm motility showed significant decrease due to potential toxicity of fluoride, while the *Tamarindus indica* treatment hampered fluoride induced toxic effects thereby increased the sperm motility in significant way. The earlier findings revealed that the fluoride acts directly on the motile apparatus without substantially affecting other metabolic pathways as it inhibits ATPase in spermatozoa.^[24] However, it has been postulated that fluoride could decrease the fructose level, which provides energy to sperm for motility,^[8] by inhibiting many enzymes like enolases, acid and alkaline phosphatases by first binding with cofactors like Mg, Ca, Zn and Se^[25] and thus inhibits glycolysis, respiration and motility of sperm. In histology of testis there were

disruption of the total structure of seminiferous tubule & seminiferous tubular diameter, lack of sertoli cells, spermatocytes in the fluoride induced groups which is similar as previous study.^[14] But in the tamarind treated groups there were some improvement in the histology of testis. So, the activities of the medicinal plant *Tamarindus indica* pulp extract showed the significant improvement in recovering from damage caused by fluoride on male reproductive system in rat.

5. CONCLUSION

From this experiment it may be concluded that decreased body weight, testicular weight, epididymal weight, sperm count and sperm motility of treated rat after NaF application has been well improved after therapeutic treatment of tamarind pulp extract. It also shows improved testicular histology and general biochemical parameters.

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CONFLICTS OF INTEREST

No conflicts of interest declared by the authors.

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