



BENEFICIAL EFFECT OF *TAMARINDUS INDICA* ON THE TESTES OF ALBINO RAT AFTER FLUORIDE INTOXICATION

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ABSTRACT

The Present investigation covers the scientific comprehension of the potential effects of fluoride toxicity on body and testes weight and the spermatozoal activities in male albino rat and the beneficial effect of novel medicinal plant *Tamarindus indica* therapeutic treatment (10gm/kg body weight) after fluoride water intoxication. Three groups of male albino rats five of each were intoxicated with ground water fluoride (14.29 mg/L fluoride ion concentration) collected from fluoride zone of Agra. The other three groups were given the subsequent treatment of *Tamarindus indica* after fluoride water intoxication. The fluoride water exposure led to the significant reduction in the body and organ weight, whereas the sperm count, motility and sperm sluggishness were the decline of sperm count and its activities. However, the therapeutic treatment of *Tamarindus indica* pulp extract manifested the improvement in the recovery and mitigation of devastating toxic effects of fluoride on the concerned parameters in male albino rat.

KEYWORDS : Beneficial effect, Fluoride toxicity, Sperm count, Sperm motility and Sperm sluggishness, *Tamarindus indica*.



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INTRODUCTION

The major sources of fluoride in ground water are fluoride bearing rocks such as fluor spar, Cryolite, fluorapatite and hydroxyl apatite¹. The presence of fluoride in ground water is governed by several factors like igneous rock formation, hydrothermal fluids, metamorphic rocks and weathering processes².

Ground water is one of the most important sources of drinking water and contamination of ground water with fluoride is increasingly becoming a matter of grave concern, as 17 states in India have been declared endemic for fluorosis, and of these 5 states have indicated hyperendemicity for fluorosis³. In thousands of villages across Uttar Pradesh it is causing death and severe illness. Although fluorosis has been regarded in the past as disease affecting only bone and teeth, recent studies have given ample evidence that fluoride toxicity adversely affects most of soft organ^{4,5}. This disturbs spermatogenesis and thus reduces spermatozoa density.⁶

Fluoridation of water supplies is practiced in many parts of world, where fluoride is recognized as the most effective caries preventive agent⁷. Among different effects fluoride can produce in different organ systems of body, the reproductive tract is susceptible to disruption by fluoride at a concentration sufficient to produce other manifestations of toxicity⁸.

Medicinal plants form the backbone of traditional system of medicine in India. Medicinal plants are rich source of novel drugs that form the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs. *Tamarindus indica* is a slow growing, long-lived massive tropical tree of the family Fabaceae⁹. The pharmacological investigations revealed that the *Tamarindus indica* pulp is antibacterial, antifungal, hypoglycemic, cholesterolemic, cytotoxic, anti-inflammatory, gastrointestinal, laxative, hypolipomic and antioxidative in its activities^{10,11,12}.

The current therapeutic approach to fluoride poisoning is to increase the excretion of fluoride by chelation. Co-administration of antioxidants such as vitamin C and E or N-acetyl cysteine during chelation therapy had been found to be beneficial in increasing fluoride mobilization and assisting the recovery of altered biochemical variables¹³. A number of recent investigations revealed that protein molecules from various plant sources possess antioxidant activities^{14,15}. *Tamarindus indica* showed beneficial effects in recovering from fluoride toxicity in dogs, and over defluoridated water supply to adolescent boys in fluorotic area^{16,17}. The present investigation was undertaken to elucidate, in the light of earlier research findings^{12,16,17}, the fluoride toxicity on spermatozoal parameters of albino rat testes and its reversibility by giving the treatment of *Tamarindus indica* pulp extract.

MATERIALS AND METHODS

The male albino rats (*Rattus norvegicus*) of wistar strain, weighing between 120 ± 25gm were obtained from the colony of albino rats bred in the animal house of Zoology Department, School of Life Sciences, Khandari Campus, Agra, were used for experimental purpose.

The albino rats were housed in polypropylene cages measuring 45 × 25 × 15 cm and maintained at controlled temperature of 25 ± 2°C, humidity of 65 ± 10% and proper circadian rhythm.

The acclimatized animals were divided into seven different groups and caged separately. They were maintained on standard diet - Goldmohar Brand Feed and water *ad libitum*.

The fluoride water was collected from fluoride zone in Agra region from as usual water sources like hand pumps in polypropylene bottles. The fluoride concentration in water samples were estimated by the method of ion selective method. However, the fruits of *Tamarindus indica* were procured from local market and were subjected for the taxonomical

identification to the Department of Botany, School of Life Sciences, Khandari Campus, Dr. B.R. Ambedkar University, Agra. The selected therapeutic dose of *Tamarindus indica* for entire research was 10gm/kg body weight given to albino rats^{16,18}.

The selected thirty five albino rats of almost equal weight and size were divided into seven groups of five albino rats each. The one group of albino rats were treated as control for 3, 7, 14 and 21 days; the next three groups were intoxicated with ground water fluoride for 7, 14 and 21 days; while other three groups were first intoxicated with fluoride water in the same way and then treated with *Tamarindus indica* dose for 3, 7 and 14 days respectively.

At the end of respective treatment albino rats were fasted over night separately on 7, 14 and 21 days after fluoride water intoxication while 3, 7 and 14 days *Tamarindus indica* treatment along with fluoride water intoxication respectively. On the next day of each control and experimental groups, the albino rats were first weighed then, autopsied by decapitation and the

required organs were carefully excised, blotted free of blood, rinsed in normal phosphate saline buffer (pH 7.4) and weighed on a Roller Smith (USA) torsion balance, homogenized in suitable solvents and utilized for respective parameters.

The sperm count was performed with the Neubauer hemocytometer described in WHO protocol MB-50, (1983), whereas the sperm motility was assessed by using Trans Membrane Migration Ratio (TMMR) method¹⁹, and sperm sluggishness was determined by using Eosin Stain²⁰. All the data were statistically analyzed, by using student's 't' test and ANOVA. All the results were expressed as mean \pm SEM.

RESULTS

In whole experiment results of fluoride water intoxicated groups alone were compared with that of control group, whereas the results of *Tamarindus indica* treated along with fluoride water ingestion groups with that of fluoride water intoxicated groups respectively.

Table 1
Beneficial effect of *T. indica* on the testes of albino rat after fluoride intoxication

Group	Body weight	Organ weight	Organ – body weight ratio
Control	135.8 \pm 1.428	1.752 \pm 0.0326	0.0129 \pm 0.0002
7 days F.W.I.	130 \pm 1.4142*	1.464 \pm 0.02315***	0.0115 \pm 0.0002***
3 days T.I. treatment after 7 days F.W.I.	134 \pm 1.4142*	1.62 \pm 0.0141***	0.0121 \pm 0.0007 ^{NS}
14 days F.W.I.	120 \pm 1.4142***	1.374 \pm 0.0172***	0.0114 \pm 0.00005***
7 days T.I. treatment after 14 days F.W.I.	130.8 \pm 1.019***	1.536 \pm 0.00927***	0.0117 \pm 0.0001*
21 days F.W.I.	114.6 \pm 1.326***	1.33 \pm 0.0182***	0.0094 \pm 0.002 ^{NS}
14 days T.I. treatment after 21 days F.W.I.	128.4 \pm 1.166***	1.48 \pm 0.0130***	0.0115 \pm 0.0001 ^{NS}

(n = 5) (a) \Rightarrow P value \geq 0.05, (b) \Rightarrow P value \leq 0.05, (c) \Rightarrow P value \leq 0.01, (d) \Rightarrow P value \leq 0.001

Body Weight: The body weight of albino rats decreased significantly ($p \leq 0.05$) after 7 days while very highly significantly ($p \leq 0.001$) after 14 and 21 days of fluoride water ingestion. However, body weight increased significantly ($p \leq 0.05$) after 3 days, while very highly

significantly ($p \leq 0.001$) after 7 and 14 days of *Tamarindus indica* treatment (Table 1).

Organ Weight: The organ weight decreased very highly significantly ($p < 0.001$) after 7, 14 and 21 days of fluoride water ingestion. However, organ weight

increased very highly significantly ($p \leq 0.001$) after, 3, 7 and 14 days of *Tamarindus indica* treatment (Table 1).

Organ-body Weight Ratio: The organ-body weight ratio decreased very highly significantly ($p \leq 0.001$) after 7 and 14 days,

while non-significantly ($p \geq 0.05$) after 21 days of fluoride water ingestion. However, organ-body weight ratio increased non-significantly ($p \geq 0.05$) after 3 and 14 days while increased significantly ($p \leq 0.05$) after 7 days of *Tamarindus indica* treatment (Table 1).

Table 2
Beneficial effect of *T. indica* on the testes of albino rat after fluoride intoxication

Group	Sperm count	Sperm motility	Sperm sluggishness
Control	47.4 ± 1.777	36.8 ± 1.356	17.2 ± 1.067
7 days F.W.I.	38.8 ± 0.08602**	30 ± 0.7071**	11.4 ± 0.5099***
3 days T.I. treatment after 7 days F.W.I.	43.2 ± 0.8602**	34.6 ± 0.927**	15 ± 1.000**
14 days F.W.I.	29.8 ± 1.2806***	22.6 ± 1.077***	8 ± 0.707***
7 days T.I. treatment after 14 days F.W.I.	38.2 ± 1.2806***	31.8 ± 1.428***	14 ± 0.707***
21 days F.W.I.	21 ± 1.000***	17.4 ± 1.0295***	5.6 ± 0.5099***
14 days T.I. treatment after 21 days F.W.I.	35 ± 1.000***	30.4 ± 1.0295***	14.2 ± 1.2806***

(n = 5)(a) ⇒ P value ≥ 0.05, (b) ⇒ P value ≤ 0.05, (c) ⇒ P value ≤ 0.01, (d) ⇒ P value ≤ 0.001

Sperm Count: The sperm count decreased highly significantly ($p \leq 0.01$) after 7 days, while very highly significantly ($p \leq 0.001$) after 14 and 21 days of fluoride water ingestion. However, the sperm count increased highly significantly ($p \leq 0.01$) after 3 days while very highly significantly after 7 and 14 days of *Tamarindus indica* treatment (Table 2).

Sperm Motility: Sperm motility decreased highly significantly ($p \leq 0.01$) after 7 days, while very highly significantly ($p \leq 0.001$) after 14 and 21 days fluoride water ingestion. However, sperm motility increased highly significantly ($p \leq 0.01$) after 3 days, while very highly significantly ($p \leq 0.001$) after 7 and 14 days of *Tamarindus indica* treatment (Table 2).

Sperm Sluggishness: The sperm sluggishness decreased highly significantly ($p \leq 0.01$) after 7 days, while very highly significantly ($p \leq 0.001$) after 14 and 21 days of fluoride water ingestion. However, Sperm sluggishness increased highly significantly ($p \leq 0.01$) after 3 days, while very highly significantly ($p \leq 0.001$) after 7 and 14 days of *Tamarindus indica* treatment (Table 2).

DISCUSSION

The present investigation was carried out to explore the effects of potential toxicity of ground water fluoride on the testes of albino rats and its possible reversibility upon treatment of *Tamarindus indica* pulp extract.

The ground water fluoride intoxication led to the decline in the body weight. Similar findings were reported by other researchers in mice, and rats^{21, 22}. However, the increased due to beneficial effect of *Tamarindus indica* treatment which also had been reported in dogs, mice, and rats^{16, 18, 21}.

The organ weight and their ratio manifested the decrease upon fluoride water intoxication while the beneficial effect of therapeutic *Tamarindus indica* pulp extract played the significant role in the reversal of the same. This decrease might be due to impairment of protein metabolism/synthesis²³. Similar demonstrations were made in the

rabbit, mice, and rats^{24, 25, 26}.

In the present study the results revealed that fluoride water intoxication hampered the sperm count whereas, *Tamarindus indica* treatment on the other hand increased the total sperm count. The fluoride toxicity led to the decline in sperm count via hormonal imbalance²⁷, structural and functional defects in spermatozoa^{28, 29}, alterations in epididymis and accessory reproductive glands²⁹. The fluoride toxicity resulted in sloughing off the spermatogenic cells in seminiferous tubules of testes leading to disorganization of their epithelium³⁰, hampered steroidogenesis and spermatogenesis³¹. Fluoride intoxication causes oxidative damage³¹, zinc deficiency, disturbed signal transduction²⁷ and suppresses testosterone level³². F directly interferes with the spermatogenesis by depressing EGF and EGFR³³, modifying G-protein signaling³⁴, diminishing levels of androgen receptor (AR), disturbing levels of estradiol and increases the levels of FSH and LH²⁷. However, the ascorbic acid which is also a constituent of *Tamarindus indica* is a biological oxidative agent which is known to activate numerous hydroxylating enzymes, which participate in metabolic processes as supplementary source of energy in several tissues including spermatozoa³⁵.

Sperm motility showed the significant decrease due to potential effects of fluoride water toxicity, while the *Tamarindus indica* treatment hampered fluoride induced toxic effects thereby increased the sperm motility 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 spermatozoa³⁶.

The mechanism by which fluoride affects sperm motility has not been clearly elucidated. However, It has been postulated that fluoride could decrease the fructose level, which provides energy to sperm for motility²³, by inhibiting many enzymes like enolases,

acid and alkaline phosphatases by first binding with cofactors like, Mg, Ca, Zn, and Se³⁷ and thus inhibits glycolysis, respiration and motility of sperm²⁷. The testicular oxidative stress caused by fluoride resulted in the damage of sperm cell membrane which might be accountable for inhibition of testicular spermatogenesis with reduced sperm activity³⁸.

The another parameter sperm sluggishness manifested the significant reduction upon fluoride water intoxication whereas a thereaaptic supplement of the *Tamarindus indica* pulp extract depicted the mitigation of the toxic impacts on the physiological activities of spermatozoa caused by the fluoride ingestion. Similar evaluations were reported in mice, rats, and humans^{18,21,23} that the reduction sperm motility, viability and changes in sperm metabolism. Spermatozoa are particularly vulnerable to lipid peroxidation because plasma membrane is lodged with large quantities of polyunsaturated fatty acids²⁷. Fluoride induced lipid peroxidation could unable the antioxidant enzymes to protect plasma membrane that surround the mitochondria, tail, and acrosome from defects^{42, 43}.

The epididymis is the site necessary for the maintainance of structural and functional integrity of sperm and homeostasis as well were reported in mice, rats, and rabbits^{31, 44, 45}. However, the chelating and antioxidant activities of *Tamarindus indica* pulp extract showed the significant improvement in recovering from damage disruption caused by fluoride water toxicity.

CONCLUSION

From this investigation it could be concluded that the *Tamarindus indica* is beneficial in the medicinal perspective against the potential toxic effects by disrupting the normal architecture and functions of spermatozoa of the concerned ground water toxicity.

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