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PROTECTIVE ROLE OF SPIRULINA AND TAMARIND FRUIT PULP ON SPERMS OF FLOURIDE EXPOSED SWISS ALBINO MICE

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Abstract

The protective role of antioxidants rich supplements viz. Spirulina and tamarind fruit pulp (@230mg/kg bodyweight) were screened on sperm morphology, their counts and motility in Swiss albino mice exposed to sub-acute (@ 190 mg / kg body weight) and sub-chronic (94 mg / kg body weight) doses of fluoride for 7 days and 90days respectively. The recovery of sub-chronic group was also examined after 90days of fluoride with drawl. Fluoride exposure induced morphological abnormalities in sperms and reduced their motility and counts; and alterations were relatively higher in the mice reared only on standard diet in comparison to those standard diet + diet supplement. Fluoride with drawl led to faster recovery of diet supplement groups. Among supplements, combination of Spirulina + tamarind fruit pulp was relatively more ameliorative than either of them.

Key word: Fluoride toxicity, sperm morphology, counts, motility

INTRODUCTION

Fluoride impairs spermatogenesis (Pushpalatha et al. 2005, Sarkar et al. 2006, Sharma et al. 2015) which induces structural defects in spermatids and epididymal spermatozoa (Chinoy and Narayana 1994, Kumar and Susheela 1995) affecting their motility (Huang et al. 2007, Wang et al. 2009) and fertility (Spittle 2008, Izquirdo-Vega et al. 2008). These defects were ascribed to fluoride induced oxidative damage to spermatozoa (Huang et al. 2007, Spittle 2008) since their plasma membrane contains large quantities of polyunsaturated fatty acids (Alvarez and Storey 1995, Sikka 1996) while cytoplasm is low in reactive oxygen species (ROS) scavenging enzymes (De Lamirande and Gagnon 1995).

Antioxidants and chelators have been widely used to minimize fluoride toxicity (Susheela and Bhatnagar 2002, Chinoy and Shah 2004). â-carotene present in algae and leafy green vegetables has greater anti-oxidant effects than synthetic â-carotene (Amotz 1987). Spirulina, a blue - green algae, is rich in phytonutrients such as protein, minerals, essential fatty and amino acids, vitamins, and carotenoids (Wu et al. 2016). It's application as a diet supplement ameliorates toxicity of heavy metals (El-Tantawy 2016), fluoride (Sharma et al. 2014, 2015, Ghoneim et al. 2015, Yadav et al. 2016) and azo dye methyl red (Sharma et al. 2005). Since it is safe for human consumption, it has been considered as a supplement in human food (Hirashi et al. 2002).

Tamarind (*Tamarindus indica* L) fruit pulp is rich in antioxidants like polyphenols and flavanoids (Martinello et al. 2006). Its intake mobilizes fluoride deposit from bone finally lost via urinary excretion (Khandare et al. 2002, Ranjan et al. 2009). Thus, fortifying diet of fluoride exposed organism with antioxidant rich supplements and improving kidney efficiency for fluoride removal will minimize toxic effects of fluoride. Since Spirulina and tamarind are bestowed with these properties, we therefore, screened their protective role separately and also in combination on fluoride induced toxicity on epidydimal spermatozoa of Swiss albino mice including toxicity reversal, and important findings are reported in this communication.

MATERIALS AND METHODS

Animals- Swiss albino mice *(Mus musculus* L.) were reared in a well-ventilated animal house as per regulations of the Institutional Animal Ethical Committee of the University (1678/GO/a/12/CPCSEA). The investigation was carried out in the following four phases.

Phase -1- Following an acclimation period of one week, 60 healthy young male mice (age = 75 - 80 days, weight = 30 ± 0.5g) were allotted randomly to six groups of 10 mice each housing five mice in a polypropylene cage [50 cm (length) x 25 cm (width) x 15 cm (height)].

Treatments- Animals of group I (sub-acute group) received orally through gavage (0.5 mL/mice) sub-acute dose of sodium fluoride {@ 190 mg / kg body weight as fluoride for 7 days} dissolved in distilled water. Group II and III (sub-chronic groups) were similarly administered sub-chronic dose of fluoride {@ 94 mg/kg body weight as F^- for 90days}. After 90

days of exposure, F^- treatment of group III was withdrawn and mice were allowed to recover in standard conditions for another 90 days and referred to as post-treated group hereafter in the text. The respective controls of sub-acute (group IV), sub-chronic (group V) and post-treatment (group VI) studies received an equivalent amount of vehicle (distilled water) for the exposure period. All groups had free access to potable water (pH = 7.1; ER = 0.55M&!/cm; Total hardness = 198 mg/L; Chlorides = 30 mg/L; Fluoride = 0.9 mg/L; Aluminum = nil) and standard laboratory diet (Ashirwad Ltd., Chandigarh, India) *ad libitum*.

Phase 2- 4: Young male mice (30-35 days old) received diet supplements for 45 days prior to entry into experimental protocol: Spirulina in Phase-2, tamarind fruit pulp in Phase-3, and Spirulina + tamarind in combination in Phase- 4 (@ 230 mg/kg body weight). All groups had free access to potable water and standard laboratory diet. Thereafter, general lay out of the experiments was similar to Phase-1 i.e. 60 mice (age = 75 - 80 days, weight = 30 ± 0.5g) of each phase (2-4) were divided in to 6 groups as stated earlier.

Fine suspensions of *Spirulina platensis* (Source: Sunova capsule, Dabur Ltd.) and Tamarind fruit pulp were prepared and administered through gavage (0.5mL/day) to mice as described elsewhere (Sharma et al. 2014**).**

Both control and fluoride treated mice of phase I – IV were sacrificed by cervical dislocation on 8th day in sub-acute, 91st day in sub-chronic and 181st day in post-treatment studies.

Sperm motility and counts

Surgically removed cauda epididymis was crushed in 0.9% saline (5mL). Few drops were observed to count motile and non motile sperms in 5 microscopic fields (10 ×10X) per mice using hemocytometer.

Sperm abnormalities

Sperm smears stained with Ehrlich's hematoxylin were observed for morphological abnormalities according to the criteria of Akira (1961) and Pagulayan et al. (1994), and percentages of sperms with head, tail and combined abnormalities were calculated.

RESULTS

Morphologically normal and abnormal sperms were counted in the controls, fluoride treatments and post-treatments (Table 1). Among control mice, percentage of normal sperms were higher in adult mice of sub-chronic (47.7 – 60.5%) and post-treatments (30.5-52.5%) in comparison to young mice of sub-acute treatments (22.0-35.3%), with the exception of Spirulina + tamarind group having maximum percentage (64.3%) of normal sperms even in the young mice among all controls (Table 1). One possible explanation for higher percentage of abnormal sperms in the young mice may be that these were released prior to maturation. The percentage of normal sperms was almost similar to standard feed controls (27.3- 57.8%) in F^- treatments of tamarind groups (22.0-55.0%) but higher in the Spirulina (35.3- 60.5%) and Spirulina + tamarind (47.7 – 64.3%) groups. Thus Spirulina in combination with tamarind had more favorable effect on spermatogenesis.

Fluoride exposure decreased percentage of normal sperms ("!8-21%) in both standard feed and diet supplement groups, except tamarind groups having percentage either almost similar to control or even higher (19%; Table 1). Thus tamarind affecting sperm morphology adversely in the young mice (sub-acute control) had protective role in F^- treatments. It is interesting to note that percentage of normal sperms in the treated mice of diet supplement groups (27-55%) was almost similar to standard feed controls (27-58%) possibly on account of protective role of diet supplements on spermatogenesis.

Unlike post-treated mice of diet supplement groups, post-treated mice of standard feed group failed to recover since percentage of normal sperms (19%) was lower in comparison to sub-chronic exposure (37%) suggesting extension of F^- toxicity even after it's with drawl (Table 1).

Fluoride exposure caused morphological abnormalities, being higher in the head (25-59%) region of the sperms than the tail (10-24%) while combined head and tail abnormalities (<1-7%) were meagerly present (Table 1). Further percentage of head abnormalities was higher than the respective controls, except tamarind groups though tail and combined abnormalities differed little with respective controls, except tamarind group (Table 1). The recovery of post treated mice of diet supplement groups was relatively better (Table 1). It is evident that diet supplement tamarind causing adverse effects on sperm morphology in the control mice was however, protective in F^- treatments and post-treatment.

Sperm Counts

The adult control mice of sub-chronic (1079-1319) and post treatments (2194-2323) had higher sperm counts than the young mice (499-863) of sub-acute groups. Only combination of diet supplements had favorable effect on sperm counts in the young mice (Table 2).

Sub-acute F^- exposure reduced sperm counts (21-43%), except tamarind group (Table 2). Sub-chronic exposure did not affect sperm counts in the diet supplement groups though it decreased in the standard feed group.

Sperm Motility

Unlike morphology, mice age did not affect sperm motility in the control groups (sub-acute controls = 52-64%; sub-chronic controls = 49-65%) though it was little lower in the diet supplement groups (Table 2). F^- exposure decreased sperm motility, particularly in the sub-acute treatments (22-35%).

Table 1. Percentage of morphologically normal and abnormal sperms in controls, fluoride treatments and post-treatments

Feeding groups	Treatments	Normal (%)	Abnormalities (%)		
			Head	Tail	Combined
Sub-acute exposure					
Standard feed	Control	27.3 ±4.4	55.7±5.4	13.6±1.8	3.4 ±0.8
	Treatment	14.8 ±3.2 (-13%)	57.9 ±5.6 (+2%)	24.4 ±4.4 (+11%)	3.0 ± 0.6 (-0.4%)
Spirulina	Control	35.3 ±5.0	42.5 ±6.1	21.3 ±2.9	0.9 ±0.1
	Treatment	27.0±4.3 (-8%)	58.4 ±6.7 (+16%)	15.2 ±4.7 (-6%)	3.2 ±1.4 (+2%)
Tamarind	Control	22.0±10.4	59.4 ±9.7	18.4 ±6.9	7.2±1.7
	Treatment	41.4 ±6.5 (+19%)	46.7 ±5.5 (-13%)	11.4 ±1.4 (-7%)	1.1 ±0.5** (-6%)
Spirulina + Tamarind	Control	64.3 ±2.9	25.4 ±2.4	9.9 ±0.8	0.5 ±0.1
	Treatment	42.9 ±7.2* (-21%)	39.4 ±6.1 (+14%)	16.6±4.3 (-7%)	1.1 ±0.5 (-0.6%)
Sub-chronic exposure					
Standard feed	Control	57.8±1.1	32.0±1.6	10.1±1.3	0.1±0.1
	Treatment	37.0±3.7*** (-21%)	47.9±2.7 ** (+16%)	14.5±2.0 (+4%)	0.6±0.2 (+0.5%)
Spirulina	Control	60.5±2.3	22.8±2.0	16.5±2.3	0.3±0.2
	Treatment	50.9±4.1 (-10%)	32.6±4.5 (+10%)	15.8±0.9 (-0.7%)	0.6±0.3 (+0.3%)
Tamarind	Control	55.0±3.3	30.3±2.9	14.1±4.7	0.5±0.2
	Treatment	55.0±1.3 (nil)	33.4±2.9 (+3%)	10.8±1.9 (-3%)	1.0±0.3 (+0.5%)
Spirulina + Tamarind	Control	47.7±10.9	30.9±2.5	10.3 ±1.1	0.4±0.3
	Treatment	40.2±5.6 (-8%)	43.0±5.3 (+12%)	13.5±2.3 (+3%)	3.3±0.8** (+3%)
Post-treatments					
Standard feed	Control	42.9±1.6	41.1±2.1	15.3 ±2.4	0.2±0.1
	Treatment	19.0±6.0** (-24%)	64.9±6.8** (+24%)	13.3±2.5 (-2%)	2.8±1.3* (+3%)
Spirulina	Control	52.5±2.0	22.3±6.1	20.5±1.0	0.1±0.1
	Treatment	52.5±4.0 (nil)	32.6±4.6 (-10%)	13.5±1.4** (-7%)	1.4±0.3** (+1%)
Tamarind	Control	30.5±6.8	43.6±2.6	21.6±4.4	4.2±0.7
	Treatment	34.4±5.2 (+4%)	35.3±3.3 (-8%)	28.0±2.9 (+6 %)	2.4±0.9 (-2%)
Spirulina + Tamarind	Control	43.7±4.1	42.2±2.4	11.9±1.8	1.6±0.9
	Treatment	60.1±2.3** (+16%)	21.6±2.5*** (-21%)	17.6±1.8* (+6%)	0.7±1.5 (-1%)

Data in parenthesis indicate percentage change in values in comparison to control, *p<0.05, **p<0.01 and ***p<0.001

Sub-chronic exposure did not affect sperm motility in tamarind and Spirulina + tamarind groups but it decreased in others in comparison to their respective controls. Similar to counts, sperm motility recovered only in tamarind and Spirulina + tamarind post-treatments. Fluoride exposure also decreased duration of motility and toxic effects were minimum in the tamarind and Spirulina + tamarind groups.

Table 2. Sperm counts, their motility and mean time of motility in controls, fluoride treatments and post-treatments

Feeding groups	Treatments	Counts (x 10^4/mL)	Motility (%)	Mean time (min.)
Sub-acute exposure				
Standard feed	Control	499±133	60.6± 2.0	N.A.
	Treatment	231±90 (-43%)	26.0±3.8*** (-35%)	20.0±2.8
Spirulina	Control	495±155	63.9±4.0	45.0±7.1
	Treatment	177±45 (-42%)	35.3±5.0** (-29%)	13.8±3.3** (-69%)
Tamarind	Control	366±85	51.6±5.9	32.0±5.7
	Treatment	431± 59 (+18%)	30.1±1.4** (-22%)	16.2±2.0* (-49%)
Spirulina + Tamarind	Control	863±111	59.7±3.1	45.4±9.0
	Treatment	680±216 (-21%)	33.1±3.6*** (-27%)	40.0±4.0 (-12%)
Sub-chronic exposure				
Standard feed	Control	1319±182	65.3±3.8	N.A.
	Treatment	993±110 (-25%)	48.6±1.9** (-17%)	N.A.
Spirulina	Control	1271±191	63.4±0.8	N.A.
	Treatment	1323±129 (+4%)	54.8±0.7*** (-9%)	N.A.
Tamarind	Control	1132±160	61.1±1.4	34.2±3.3
	Treatment	1777±270 (+57%)	61.6±2.0 (+0.5%)	35.0±3.0 (+2%)
Spirulina + Tamarind	Control	1079±149	49.3±2.85	63.4±4.8
	Treatment	1266±144 (+17%)	66.6±3.94** (+17%)	37.0±1.6*** (-42%)
Post- treatments				
Standard feed group	Control	2333±103	69.5±5.6	N.A.
	Treatment	1512±135** (-35%)	47.3±3.7** (-22%)	59.3±5.2
Spirulina	Control	2194±89	70.3±4.2	N.A.
	Treatment	1524±15** (-31%)	63.1±1.4 (-7%)	N.A.
Tamarind	Control	NA	43.4±12.2	50.3±7.5
	Treatment	603±156	61.5±3.7* (+18%)	40.2±6.1 (-20%)
Spirulina + Tamarind	Control	NA	38.4±10.4	48.0±9.0
	Treatment	689±197	65.5±2.0* (+27%)	50.3±7.5 (+5%)

Data in parenthesis indicate percentage change in values in comparison to control, *p<0.05, **p<0.01 and ***p<0.001

DISCUSSION

Fluoride induced oxidative stress causes lipid peroxidation in the plasma membrane that adversely affected its fluidity and integrity (Huang et al. 2007, Spittle 2008). The presence of large quantities of polyunsaturated fatty acids in the plasma membrane of spermatozoa (Alvarez and Storey 1995, Sikka 1996) and lower concentrations of reactive oxygen species (ROS) scavenging enzymes in their cytoplasm make them vulnerable to oxidative stress (De Lamirande and Gagnon 1995). The excessive reactive oxygen species (ROS) damage sperms (Agarwal and Sushil 2005) which reduce their motility

(Pushpalatha et al. 2005, Huang et al. 2007, Kumar et al. 2010) as observed in the present study. Antioxidants rich Spirulina and tamarind reduced structural abnormalities in the sperms of fluoride treatments (Table 1).

Fluoride exposure decreased sperm counts (Table 2, Huang et al. 2007, Kumar et al. 2010, Singh et al. 2012) due to histopathological alterations in testes (Sharma et al. 2015).

Ahmad et al. (2012) reported recovery in sperm morphology of post treatment with Jambul fruit extract while Singh et al. (2012) reported improvement in their count and motility after treatment with *Tamarindus indica* fruit pulp extract. Kumar et al. (2012) observed protective role of vitamin D and E on sperm count and their motility in fluoride exposed rabbit. The maximum protection was accorded when both vitamin D and E were administered together. In the present study also, diet supplements had protective role on testes, sperm morphology including their counts and motility, particularly in combination as also reported in our earlier studies on hematology (Sharma et al.2014) and kidney (Yadav et al. 2016).

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