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AMELIORATIVE ROLE OF SPIRULINA AND TAMARIND FRUIT PULP ON GENERAL HEALTH AND LIVER OF FLUORIDE EXPOSED SWISS ALBINO MICE

Anil Pandey, Shweta Sharma[a*] and Nirmala Yadav
Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India
[a] Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India
***Corresponding Author: Email-shwetdr@gmail.com**

Abstract

Hepatoprotective role of 2 diet supplements {Spirulina and tamarind fruit pulp (@ 230 mg/kg body weight)} is reported in the male Swiss albino mice exposed to fluoride (sub-acute @ 190 mg/kg bwt. for 7 days and sub-chronic @ 94 mg/kg bwt. for 90 days) including recovery of sub-chronic group after fluoride with drawl. The diets served were; standard chow alone and in combination with Spirulina/ tamarind fruit pulp/ Spirulina + tamarind fruit pulp. Standard protocols were followed for assessing histopathology and tissue biochemistry of liver and selective parameters of serum biochemistry. Compared with controls, there were reductions in feed intake (6-16%), body (18%) and liver (25%) weights of F^- treated and post-treated mice of standard feed groups. Histopathological disorders in the liver were; hydropic degeneration and vacuolization of hepatocytes, dilation of portal and central vein (12-123%), mononuclear and periportal inflammation and increase in Kupffer cell counts (21-25%) particularly in F^- treatments of standard feed groups. F^- exposure increased protein in the liver. AST and ALT levels declined in the serum of sub-acute treatments of standard feed groups were raised in sub-chronic treatments. In comparison to sub-chronic exposure, sub-acute exposure was found to be more toxic to the mice. Fluoride toxicity was comparatively less in the diet supplement groups and Spirulina in combination with tamarind was found to be the most protective.

Key words: Fluoride toxicity, Swiss albino mice, Spirulina, Tamarind, liver histology

INTRODUCTION

Fluoride is an essential trace element preventing dental and enamel caries in the human beings, but when consumed in excess via drinking water, it enters into blood after passing passively through the intestinal mucosa and transported to liver causing oxidative stress and cell necrosis (Ekambaram et al. 2001, Guo et al. 2003, Kanbur et al. 2009). Because of wide spread pollution in the environment, liver diseases are increasing at an alarming rate and therefore, worldwide search for hepatoprotective molecules is going on from various sources. Several plant species bestowed with antioxidant properties have also been found hepatoprotective against toxin-induced oxidative stress (Manna 2007, Niu et al. 2018, Al-Daihan et al. 2019). Spirulina ameliorates $CdCl_2$ and $HgCl_2$ induced hepatotoxicity (Jeyprakash and Chinnaswamy 2005, Bashandy et al. 2011) whereas fruits of *Tamrindus indica* Linn. (Caesalpiniaceae) are used in jaundice and other liver complaints in the folk medicine (Ross 2004). With this backdrop, hepatoprotective role of Spirulina and tamarind fruit pulp has been studied in fluoride exposed Swiss albino mice.

MATERIALS AND METHODS

The investigation was carried out in the following four phases.

Phase -1- Sixty healthy male mice (age = 75 - 80 days, weight = 30 ± 0.5g) acclimated for one week prior to entry into the experimental protocol were divided into six groups supplied with different treatments. Group I mice (sub-acute group) orally received through gavage (0.5 mL/ mice) sub-acute dose of fluoride (@ 190 mg/kg body weight for 7 days); and of group II and III sub-chronic dose (@94 mg / kg body weight for 90 days). Their respective controls (group IV = sub-acute, group V and group VI = sub-chronic) received an equivalent amount of vehicle (distilled water) for the study period as described earlier (Yadav et al. 2016).

Animals kept in a well ventilated, noiseless environment (Temperature 24 ± 3^0C; humidity = 40 – 60 %; 12 h light: dark cycle as per INSA 2010 guidelines) had free access to standard chow (Ashirwad Ltd., Chandigarh, India) and potable water (pH = 7.1; ER = 0.55MΩ/cm; total hardness = 198 mg/L; chlorides = 30 mg/L and fluoride = 0.9 mg/L) *ad libitum*.

Mice of group I and IV were sacrificed on day-8; and of group II and V on day-91. F^{-1} exposed animals of group III were allowed to recover for 90 days under control conditions and sacrificed on day-181 along with respective control animals (group VI), and are referred to as post- treatment groups hereafter in the text. **All regulations of the Institutional Animal Ethical Committee of the University of Rajasthan, Jaipur (1678/GO/a/12/CPCSEA) were followed during experiments.**

Phase 2 - 4 - Young male mice (30-35 days old) received diet supplements (@ 230 mg/kg body weight) along with standard chow for 45 days prior to entry into experimental protocol (phase-2: Spirulina, phase-3: tamarind fruit pulp, phase - 4: Spirulina + tamarind fruit pulp). Thereafter, general lay out of the experiments was similar to phase-1 i.e. 60 mice of each phase (2-4) were divided in to 6 groups as described earlier.

The fine suspensions of spray-dried powder of *Spirulina platensis* (Sunova capsule, Dabur Ltd.) and semidried pulp of Tamarind fruit pulp (ripe) were prepared as described elsewhere (Yadav et al. 2016) and administered orally through gavage (0.5mL/day/mice). The mice reared on standard chow are referred to as standard feed group hereafter in the text while those fed on diet supplement/s (+ Standard chow) as Spirulina, tamarind and Spirulina + tamarind groups respectively.

Animals were observed at least twice in a day for clinical signs and symptoms of toxicity. Their feed and water consumption were recorded after every 24 hour.

Autopsy- All animals of Phase 1-4 were weighed prior to their sacrifice (by cervical dislocation). Cardiac blood samples were collected into vials for serum biochemistry, and blotted free liver weighed.

Serum biochemical estimation- Diagnostic kits (Span Diagnostics Ltd., India) were used for estimating aspartate amino transferase (AST), alanine amino transferase (ALT), acid (ACP) and alkaline phosphatase (ALP), serum total protein, albumin and globulin.

Tissue biochemistry- Tissue homogenates of liver were analyzed for protein, glycogen and acid and alkaline phosphatase (Lowry et al. 1951, Montgomery 1957, Sadasivam and Manickan 1996).

Histological and Morphometric studies- Liver lobes fixed immediately in Bouin's fluid were processed to cut 6 µm thick sections which were stained with hematoxylin-eosin and examined under light microscope for histopathology (Humason 1972). Central veins were measured using oculometer standardized with stage micrometer.

Data analysis- The data expressed as mean ± the standard error of the mean were analyzed by one way ANOVA using a Systat 5.0 software program.

RESULTS AND DISCUSSION

Alterations in feed intake, body and liver weight

Fluoride exposure reduced food intake (5-43%) and body weights (10-18%) of mice except for diet supplement groups of sub-chronic exposure. Vitamins also had similar protective role in the fluoride treated Wistar rats because of increase in protein synthesis (Chinoy and Sharma 1998).

Liver weights were affected little in F^- treated (sub-acute and sub-chronic) as well as post-treated mice of diet supplement groups were however, reduced (21-25%) significantly in the standard feed groups in comparison to respective controls, and findings corroborate with earlier study (Chawla et al. 2008).

Histopathology of liver

The hepatic lobule is structural and functional unit of liver. Portal triads located at the periphery of lobule had dilated portal veins with detached epithelial lining (Plates 1, 2). Perera et al. (2018) also reported dose dependent portal dilation in the fluoride treated rats. Bile ducts were also dilated in F^- treatments (except Spirulina + tamarind groups; Plate 1, 2) to carry bile juice for digestion and detoxified (by hepatocytes) pollutant (F^-) for excretion (Cullen 2005).

F^- exposure dilated (20-120%) central veins notably in the standard feed groups (Plate 3,4; Table 1). Hepatocytes with severe hydropic degeneration and vacuolization were mostly observed in the F^- treatments of standard feed groups (Plate 3, 4) similar to earlier studies (Parashar et al. 2016, Niu et al. 2018). Nuclei in few hepatocytes were either triangular or having ruptured nuclear membrane thus showing sign of degeneration and findings are in agreement with earlier reports (Parashar et al. 2016, El Din et al. 2018).

Sinusoids were dilated in the F^- treatments (Plate 3, 4) similar to other studies (Parashar et al. 2016, El Din et al. 2018). Kupffer cells were distributed uniformly in the controls and F^- treatments of Spirulina and Spirulina + tamarind groups but aggregated with neutrophil around portal (mononuclear cell infiltration) and central veins (periportal inflammation) in other F^- treatments (Plate 1- 4). Their counts were also higher than controls in F^- treatments (standard feed groups = 21-

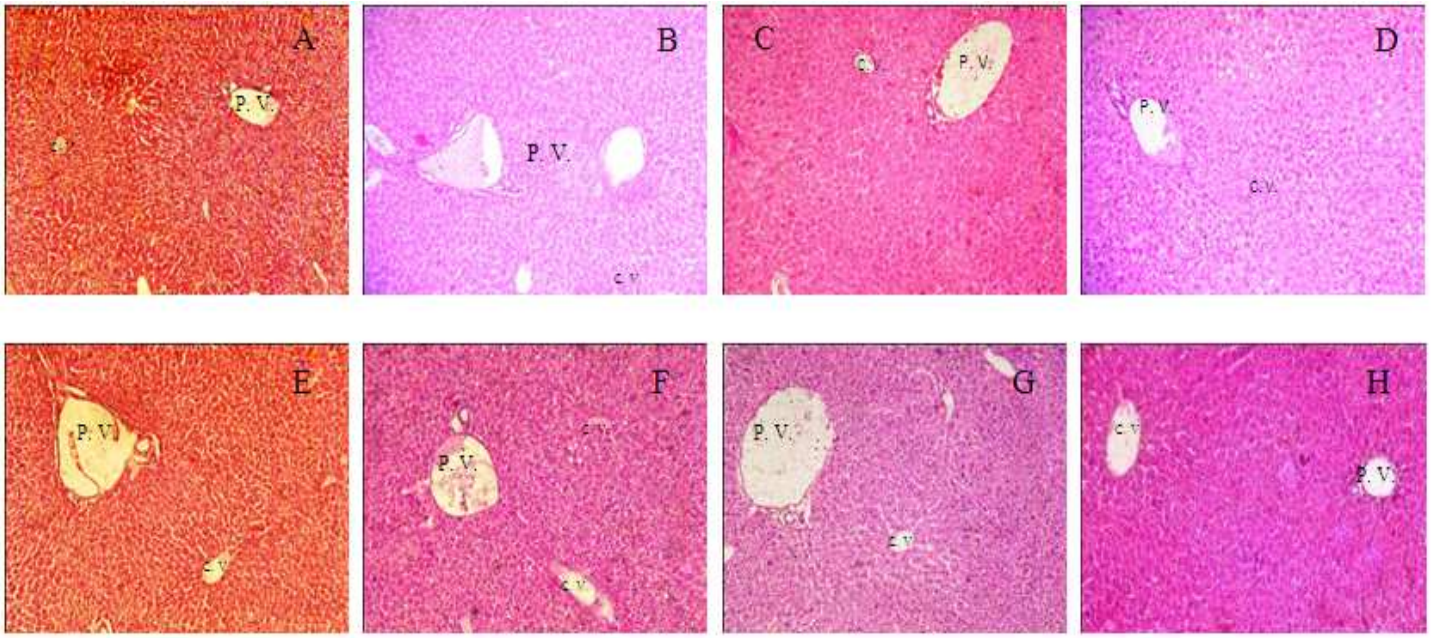

Plate1. Liver T.S. (10 × 10X) showing histological damages in fluoride treated (sub-acute) mice

A-D Controls: **A:** Standard feed group**, B:** Spirulina group**, C:** Tamarind group and **D:** Spirulina + Tamarind group **s**howing normal structure of portal triad and central vein (C.V.), **E-H Fluoride treatments: E:** standard feed group showing dilated portal triad with ruptured portal vein, **F:** Spirulina group showing intact portal veins (P.V) with periportal inflammation, **G:** tamarind group showing dilated portal vein and **H:** Spirulina + tamarind group showing intact portal veins.

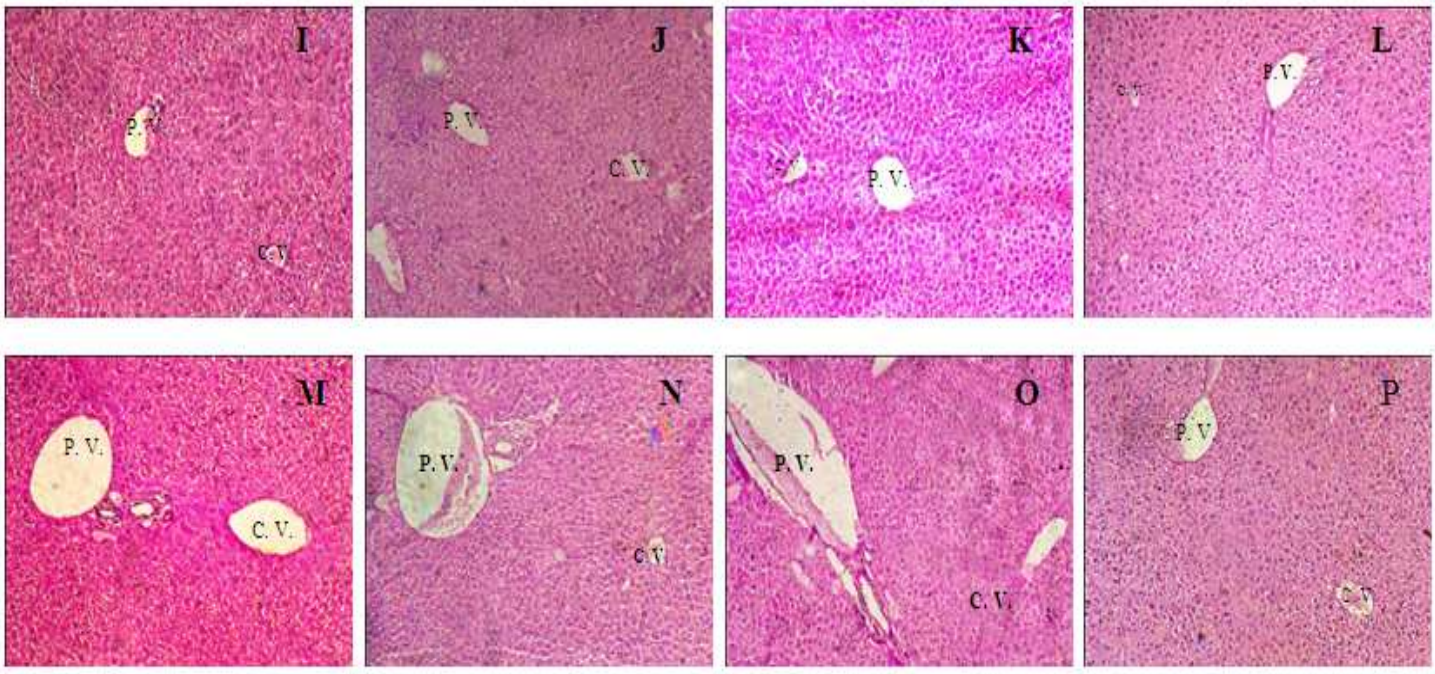

Plate 2. Liver T.S. (10 × 10X) showing histological damages in fluoride treated (sub-chronic) mice

I-L Controls: **I:** Standard feed group**, J:** Spirulina group**, K:** Tamarind group and **L:** Spirulina + Tamarind group **s**howing normal structure of portal triad and central vein

M-P Fluoride treatments: M: standard feed group showing dilated portal vein with periportal inflammation, N**:** Spirulina group showing dilated portal veins with periportal inflammation, O**:** tamarind group showing dilated portal triad and P: Spirulina + tamarind group showing almost normal portal veins with intact wall

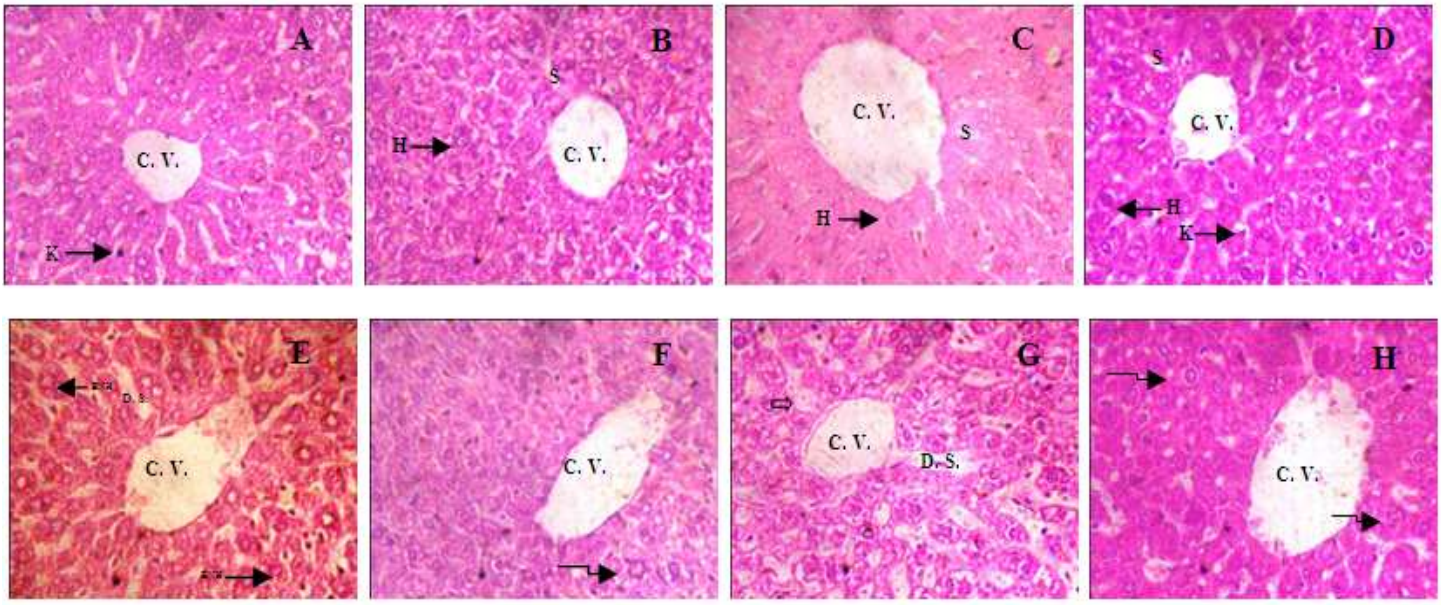

Plate 3. Liver T.S. (10 × 40X) showing histological damages in fluoride treated (sub-acute) mice

A-D Controls: **A:** Standard feed group**, B:** Spirulina group**, C:** Tamarind group **and D:** Spirulina + Tamarind group **s**howing normal structure of central vein

E-H Fluoride treatments: E: standard feed group showing dilated central vein, some binucleate hepatocytes (BNH) and dilated sinusoids (D.S.), **F:** Spirulina group showing dilated central vein with some karyomegaly (➥) cells **G:** tamarind group showing shrunken central veins with dilated sinusoids (D.S.) and **H:** Spirulina + tamarind group showing dilated central veins with some karyomegaly cells ( ), Kuffer cell (K), Hepatocyte (H).

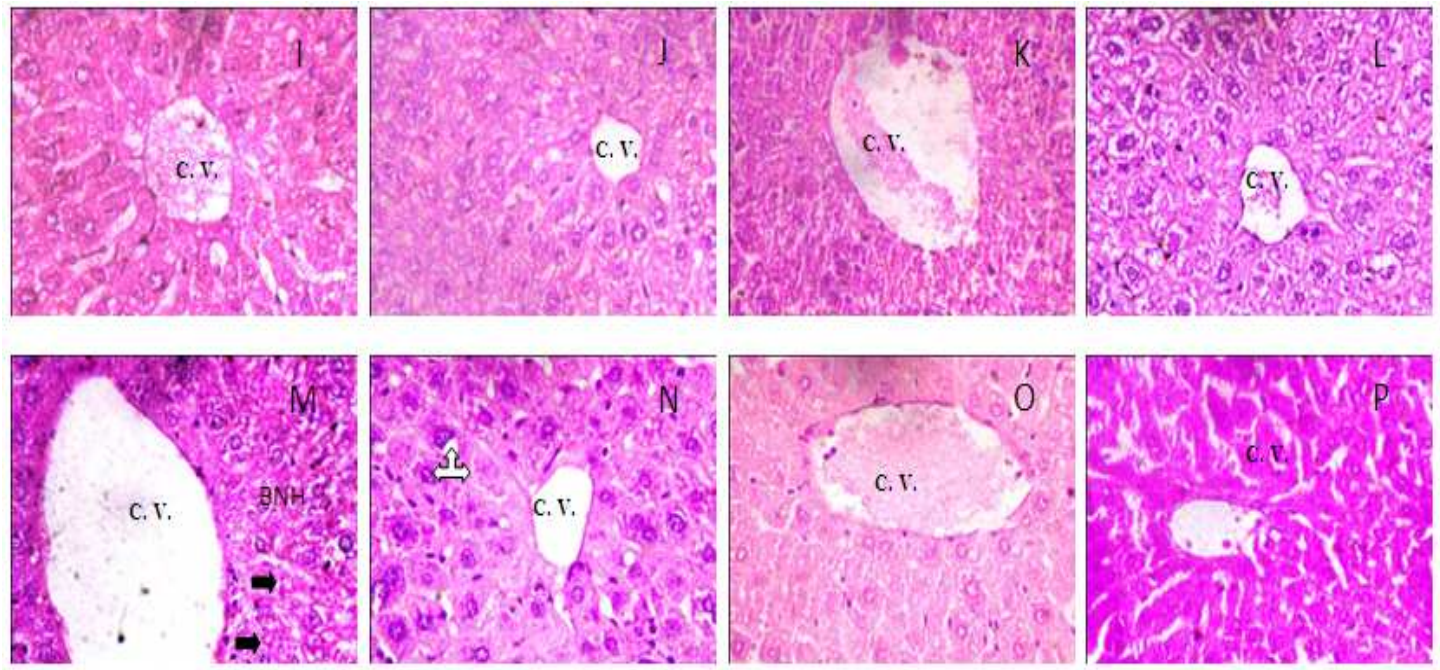

Plate 4. Liver T.S. (10 × 40X) showing histological damages in fluoride treated (sub-chronic) mice

I-L Controls: **I:** Standard feed group**, J:** Spirulina group**, K:** Tamarind group **and L:** Spirulina + Tamarind group **s**howing normal structure of central vein

M-P Fluoride treatments: M: standard feed group showing dilated central vein and degenerating hepatocyte nuclei ( ), N**:** Spirulina group showing normal central veins and bean shaped nuclei ( ), O**:** tamarind group showing dilated central vein and P: Spirulina + tamarind group showing normal central vein with dilated sinusoids

25%; diet supplement groups = 6-8%) to engulf and digest cell debris (Ding et al. 2003, Table 1). Our findings on hepatoprotective role of diet supplements are agreements with others (Jeyprakash and Chinnaswamy 2005, Bashandy et al. 2011, Ross 2004). Diet supplements in combination were however, found more ameliorative in the present study.

Post-treatments

The post-treated mice of standard feed group had dilated central and portal veins (with mononuclear cell infiltration) but these were similar to respective post-controls in the post-treated mice of diet supplement groups (Table 1). Histopathological abnormalities common in all post-treated mice were; poor integrity of epithelial lining in portal veins, hepatocytes with degenerating nuclei and dilated sinusoids and higher Kupffer cell counts higher (6-15%) (Table 1).

Tissue and Serum Biochemistry

Protein

i. Liver

Tissue protein content found almost similar to respective controls in the sub-acute treatments were raised (57-104) in the sub-chronic treatments, particularly in the standard feed group. Spirulina + tamarind group was however exception having protein levels almost similar to control (Table 2). Fluoride with drawl led to reduction in tissue protein levels in the post-treated mice of diet supplement groups possibly because of their recovery but it continued to be higher to be higher in the post-treated mice of standard feed group when compared with respective post-controls (Table 2). Bhatnagar et al. (2006) also reported dose dependent increase in protein content in the liver tissue of fluoride treated Swiss albino mice. Being an important detoxifying organ, liver may direct synthesis of new compound/s associated with detoxification of pollutant. One such compound Hsp-70, a stress protein, has already been reported in the liver of fluoride intoxicated animals (Chattopadhyay et al. 2010). Although no such protein has been explored in the present study, but significant increase in protein content of the fluoride treated mice particularly in the standard feed group suggests possibility of stress protein synthesis. The increase in protein levels in the diet supplement groups was comparatively poor possibly because antioxidant rich diet supplements detoxified

Table 1. Kupffer cell counts and area of central vein in the liver of controls, fluoride treated and post-treated mice in standard feed and diet supplement groups

	Standard feed		Spirulina group		Tamarind group		Spirulina + tamarind group	
	Control	Fluoride	Control	Fluoride	Control	Fluoride	Control	Fluoride
Kupffer cell counts								
Sub-acute	331±20	401±18** (21%)	283±14	418±16*** (48%)	372±14	396±15 (6%)	393±13	376±13 (-4%)
Sub-chronic	306±12	383±14*** (25%)	348±11	377±13 (8%)	323±12	326±11 (1%)	276±12	295±13 (7%)
Post-treated	341±11	362±13 (6%)	322±12	340±11 (5%)	283±11	325±13* (15%)	294±10	321±11 (9%)
C.V. area (μm^2)								
Sub-acute	2700.1 ±380.1	6015.2 ±691.1*** (+123%)	5117.5 ± 1045.7	6623.7± 991.4 (+29%)	6398.7 ±932.8	6686.5 ±1029.8 (+5%)	7884.3 ±1248.3	9185.5 ±967.0 (+17%)
Sub-chronic	5575.7 ±635.3	6261.4 ±865.0 (+12%)	4563.5 ±549.2	10180.0 ±839.2*** (+123%)	5861.1 ±940.3	8186.9 ±1164.8 (+34%)	7701.6 ±947.6	9059.8 ±1074.2 (+18%)
Post-treated	4051.5 ±734.6	5695.9± 1072.6 (+41%)	7913.9 ±1064.5	5702.5± 1119.8 (-28%)	5743.3 ±933.8	6400.4 ±997.7 (+11%)	5156.5 ±573.4	3931.5 ±459.5 (-24%)

Data in parenthesis are percent change in comparison to control; Significant at * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$)

Table 2. Tissue biochemistry of liver in controls and fluoride treated and post-treated mice of standard feed and diet supplement groups

	Standard feed		Spirulina group		Tamarind group		Spirulina + tamarind group	
	Control	Fluoride	Control	Fluoride	Control	Fluoride	Control	Fluoride
ACP (µmole/mg)								
Sub-acute	79±6.0	91±5* (15)	176±14	103±4** (-41)	115 ±4	108±3*** (-10)	109±9	123±7*** (13)
Sub-chronic	68± 3	77± 2 (13)	61±4	54± 4 (-11)	77 ± 5	91±4 (18)	99±2	132±4*** (33)
Post-treated	68±2	93±2*** (37)	47±3	45±3 (-4)	79±5	37±4*** (-53)	82±3	134±9*** (63)
ALP (µmole/mg)								
Sub-acute	44± 4	38± 1 (-14)	62± 1	39 ± 3*** (-37)	41 ± 1	39 ± 3 (-5)	37± 4	52± 6 (41)
Sub-chronic	26±1	37±1** (42)	17± 1	32±2*** (88)	28± 4	33 ± 5 (18)	33± 1	68 ± 3*** (106)
Post-treated	29±1	30± 2 (3)	25±4	30 ± 5 (20)	35± 2	26 ± 3 (-26)	31± 2	37±2 (19)
Protein (mg/g)								
Sub-acute	5.3±0.5	6.2±0.8 (17)	4.8±0.2	5.1±0.2 (6)	4.8 ±0.3	5.0±0.2 (4)	5.1±0.1	4.8±0.3 (-6)
Sub-chronic	2.5±0.2	5.1 ±0.1*** (104)	2.8±0.2	4.4±0.3*** (57)	3.6± 0.1	6.0±0.2*** (67)	5.9 ±0.3	5.6±0.2 (-5)
Post-treated	2.5±0.1	7.2 ±0.6 *** (188)	2.4 ±0.2	2.9 ± 0.1 (21)	7.0±0.3	5.3±0.3** (-24)	6.1±0.5	4.8±0.1 (-21)
Glycogen (mg/g)								
Sub-acute	7.3±2.7	6.8±0.9** (-7)	4.4±0.8	7.5±0.8 (+70)	3.4±0.6	8.8±0.7** (+159)	12.4±1.5	3.7±0.3** (-70)
Sub-chronic	6.4±0.7	5.1±1.3 (-20)	9.8±2.8	6.9±1.0* (-30)	28.9±4.6	9.6±2.3* (-67)	3.7±0.5	Not available
Post-treated	8.1±3.3	8.1±1.0 (Nil)	5.8±0.4	10.1±2.5* (+74)	4.8±2.1	8.1±1.8 (+69)	5.5± 1.3	5.3±0.9 (-4)

Data in parenthesis are percent change in comparison to control; Significant at * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$)

toxic effect of fluoride. Further, tamarind also reduces body burden of F^- (Khandare et al. 2002, 2004).

Fluoride exposure has little response in sub-acute treatment possibly due to short term exposure of mice whereas response in sub-chronic treatments was comparatively lesser in the diet supplement groups in comparison to standard feed group possibly due to ameliorative role of diet supplements. Further, diet supplements in combination were more protective.

Serum

Albumin: Serum

Fluoride exposure altered albumin levels in the sub-chronic treatment of standard feed group while in sub-acute and post-treatments of diet supplement groups (Table 3). Among treatments, alterations were minimum in the Spirulina + tamarind group. Other workers also made similar findings (Banupriya et al. 1997, Bouaziz et al. 2006). Since liver regulates albumin concentration effectively within narrow limits in the serum (Miller et al. 1951), alterations in its levels may therefore possibly be related to hepatic disorders.

Globulin: Serum

Globulin levels were altered in both F^- treated and post-treated mice of standard feed and diet supplement groups with the exception of Spirulina + tamarind groups (Table 3) and findings are in agreement with earlier studies (Banupriya et al. 1997, Bouaziz et al. 2006). Since Kupffer cells in association with hepatocytes regulate globulin concentration in the serum, alterations in its levels therefore suggest hepatic disorders.

It is evident from the ongoing accounts that albumin and globulin levels were affected little in the serum of fluoride treated as well as post-treated mice of Spirulina + tamarind groups suggesting supplements in combination were more ameliorative.

Table 3. Serum biochemistry of controls and fluoride treated and post-treated mice of standard feed and diet supplement groups

	Standard feed		Spirulina group		Tamarind group		Spirulina + tamarind group	
	Control	Fluoride	Control	Fluoride	Control	Fluoride	Control	Fluoride
AST (U/L)								
Sub-acute	126±7	79±4** (-37)	148±6	140±5 (-5)	159±6	123±9* (-23)	75±5	96±7** (28)
Sub-chronic	160±10	264±15*** (65)	92±6	140±11*** (52)	98±14	105±18 (7)	381±45	156±17*** (-59)
Post-treated	136±10	115±6* (-15)	190±17	167±12* (-12)	72±7	108±16** (50)	156±19	192±21* (23)
ALT (U/L)								
Sub-acute	36±2	36±1 (NIL)	64±4	56±3* (-13)	48±4	36±3** (-25)	33±4	43±5 (30)
Sub-chronic	48±5	60±7* (25)	72±5	86±6* (19)	36±2	45±6* (25)	84±11	48±6** (-43)
Post-treated	58±5	40±7** (-31)	49±4	59±4* (20)	16±2	45±8*** (181)	75±12	93±14* (24)
ALP (U/L)								
Sub-acute	234±10	270±15* (15)	344±44	440±34** (28)	294±35	288±27 (-2)	267±19	289±15 (8)
Sub-chronic	96±7	116±10* (21)	231±15	282±18** (22)	152±15	150±21 (-1)	136±15	224±19** (65)
Post-treated	202±17	140±14** (-31)	187±21	308±31** (65)	195±14	441±29 (126)	204±19	177±12 (-13)
ACP (U/L)								
Sub-acute	4.3±0.3	4.3±0.4 (NIL)	7.4±0.9	7.7±0.8 (4)	6.2±0.9	4.4±0.5* (-29)	4.2±0.8	4.5±0.8 (7)
Sub-chronic	3.7±0.7	4.5±0.6* (22)	8.0±1.0	5.5±1.0** (-31)	7.1±1.4	7.4±1.0 (4)	7.0±1.0	7.4±1.0 (6)
Post-treated	7.1±0.8	6.0±0.6* (-15)	7.4±1.1	8.8±1.0* (19)	2.9±0.3	17.1±3.7 (490)	7.3±0.5	7.4±0.5 (1)
Albumin (g/dL)								
Sub-acute	2.9±0.1	2.9±0.1 (Nil)	5.6±0.6	3.7±0.5** (-34)	4.8±0.5	3.3±0.3* (-31)	2.3±0.3	2.7±0.6 (17)
Sub-chronic	3.4±0.1	4.2±0.4* (24)	4.2±0.5	4.3±0.7 (2)	2.7±0.2	2.8±0.2 (4)	3.9±0.3	4.4±0.4 (13)
Post-treated	3.8±0.2	3.5±0.5 (-8)	4.6±0.6	3.8±0.3** (-17)	2.7±0.2	3.7±0.5 (37)	2.5±0.1	2.3±0.1 (-8)
Globulin (g/dL)								
Sub-acute	1.4±0.1	1.2±0.1 (-14)	2.7±0.3	3.5±0.3** (30)	2.9±0.4	2.1±0.3* (-28)	2.0±0.8	2.4±0.3 (20)
Sub-chronic	2.2±0.2	2.8±0.31** (27)	2.6±0.2	2.2±0.1* (-15)	1.8±0.2	1.5±0.2* (-17)	4.4±2.2	4.4±0.5 (Nil)
Post-treated	2.8±0.2	3.8±0.23** (36)	3.5±0.4	2.7±0.2** (-23)	1.0±0.1	5.2±1.8 (420)	2.7±0.6	2.5±0.4 (-7)

Data in parenthesis are percent change in comparison to control; Significant at * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$)

Acid phosphatase (Liver and Serum)

Acid phosphatase (ACP), a lysomal enzymes associated with autolysis of cells, had higher activities in the diet supplement controls that possibly resulted in relatively healthier mice than standard feed control because of replacing of ageing cells with the new healthy ones in the tissue through autophagy (Rabinowitz and White 2010).

In contrast to controls, ACP activities followed opposite trend in the fluoride exposed mice, being often higher in the liver and serum of treated mice of standard feed groups due to cell lysis in necrotic tissue (Table 3) whereas altered little in the diet supplement groups possibly due to lesser necrosis due to ameliorating role of diet supplements. It is evident that ACP induced autophagy reduces degenerative changes in the liver.

Alkaline phosphatase (Liver and Serum)

The primary importance of measuring alkaline phosphatase (ALP) is to check possibility of liver and bone disease because it's a target enzyme in fluoride toxicosis (Ranjan et al.2009). ALP levels found lower in the sub-acute treatments of liver were often higher in the liver and serum of fluoride treated as well as post-treated mice particularly of diet

supplement groups (Table 2, 3). Such alterations have been ascribed to hepatic disorders (Singh and Kanwar 1981, Chawla et al. 2008).

AST/ALT (serum)

AST and ALT associated with liver parenchymal cells are the marker enzymes for liver function and integrity (Adaramoye et al. 2008). Short-term fluoride exposure reduced their levels (AST = 5-37%, ALT = 13-25%) while opposite trends were observed in sub-chronic (AST = 52-58%, ALT = 19-25%) and post-treated mice (AST = 23-50%, ALT = 20-183%, Table 3), with exception of Spirulina + tamarind groups having their higher levels (28-30%) in the sub-acute treatment but lower in sub-chronic (43-53%). The reduction may be possibly related to their poor synthesis in necrosed liver of sub-acute treatments (Plate 3) while increase to leakage in the serum from damaged hepatocytes in sub-chronic treatments (He et al. 2014, Niu et al. 2018, Perera et al. 2018, El Din et al. 2018).

The alterations in AST and ALT levels suggest hepatotoxicity in the fluoride treated mice. It is interesting to note that perturbations in enzyme levels were relatively less in the treated mice of diet supplement groups possibly on account of their hepatoprotective role as also reported in other study (Niu et al. 2018, El Din et al. 2018).

Glycogen: Liver

Fluoride exposure altered glycogen contents in the liver particularly in the diet supplement groups having higher levels in comparison to respective controls in both sub-acute and post-treated mice while reduction in the sub-chronic treatments (Table 2). Our findings are in agreement with other workers in Al^{+3} and fluoride treated albino mice (Chinoy and Patel (1999, Chinoy and Memom 2001). Sinha et al. (2011) reported significant reduction in the activity of glucose-6-phosphatase enzyme in the Al^{+3} exposed Swiss albino mice which may have affected conversion of glycogen into glucose. Such alterations in glycogen levels of treatments may be related to hepatic disorders.

It is evident that both short-term and long-term fluoride exposure adversely affected general health of Swiss albino mice, their feed and water intake, liver histology and its biochemistry including important makers of liver functions. Such changes were more pertinent in the standard feed groups compared with diet supplement groups. Among diet supplement groups, minimum fluoride toxicity was found in Spirulina + tamarind treatments. The protective role of Spirulina may be ascribed to its richness in protein, minerals, essential fatty and amino acids, vitamins and carotenoids (Wu et al. 2016) and of tamarind fruit pulp in zinc, minerals (calcium, phosphorus, iron, magnesium, and sodium), thiamine, riboflavin, niacin, vitamin C (Glew et al. 2005) and antioxidants like polyphenols and flavonoids (Martinello et al. 2005). Tamarind fruit intake also delays progression of fluorosis since it mobilizes fluoride lost finally via urinary excretion (Khandare et al. 2002, 2004). This explains greater effectiveness of combination of Spirulina and tamarind fruit pulp in ameliorating fluoride toxicity.

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