FISHERY RESEARCH ARTICLE

Arthrospira platensis (Spirulina) and *Morinda citrifolia* (Noni) dietary supplementation can restore the aluminium and fluoride toxicity induced alteration of histopathology in the freshwater fish, *Cyprinus carpio* L.

Ram Krishna Das*

Department of Industrial Fish and Fisheries, Asutosh College, Kolkata 700 026, West Bengal, India

*Corresponding Author's E mail ID: ramkrishna.das@asutoshcollege.in

Abstract: The effect of dietary supplementation by Spirulina and Noni on histopathology of intestine, liver, kidney and ovary were investigated in common carp exposed to sub-lethal concentration of aluminium and fluoride. The sub-lethal concentration of aluminium and fluoride caused severe degenerative changes in intestine, liver, kidney, and ovary in fish. Dietary supplementation by Spirulina and Noni improved the aluminium and fluoride toxicity induced alteration of histopathology by reducing the occurrences of all the degenerative changes in the intestine, liver, kidney and ovary of common carp. The Spirulina at 1% and Noni at 0.25% showed the best response in Spirulina and Noni dietary supplementation groups respectively.

Keywords: Intestine; Kidney; Liver; Ovary; Pollution

1. Introduction

The aquaculture production is adversely affected by overcrowding, poor water quality and nutritional status due to inadequate high quality fish feed [1,2]. Population explosion coupled with rapid industrialization has led to the contamination of aquatic environment by various pollutants, which adversely affect the physico-chemical equilibrium of the aquatic body as well as bring about morphological, physiological and cytological changes of aquatic inhabitants [3]. Industrial pollution causes accumulation of potentially toxic trace elements in fishes negatively affecting the latter [4].

Aluminium, a non-essential and the most abundant metal, is the 3rd most abundant element, representing 8% of the earth's crust [5-7]. Aluminium adversely affects the haematological [8-9], biochemical [10-13] and histopathological parameters [14] of fish.

Fluorine is the lightest member of the halogen group and the most electronegative (4.0 in pauling scale) element, forms fluoride ion (F^-) in solution. It has both beneficial as well as detrimental effect in both humans and other animals.

The environmental stressor which adversely affects the growth and quality of fish fillet may be ameliorated by incorporating different plant based dietary supplement into the fish feed [15-16]. Dietary supplement is a concentrated nutrient containing vitamins, minerals, proteins and

carbohydrate that is intended to support the nutritional needs and/or supply elementary deficiencies in the ordinary diets [17]. The dietary supplements that gained popularity in recent times are microalgae like spirulina, coffee beans, garlic, Noni (*Morinda citrifolia*), Mangosteen (*Garcinia mangostana*) etc [15, 17-20].

Arthrospira platensis (commonly called Spirulina), an ubiquitous organism (blue- green algae), is rich with protein (60-70%) containing a considerable number of essential amino acids, essential fatty acids (γ-linolenic acid), vitamins (Vitamin B complex, Vitamin C, Vitamin E), Minerals (Fe, Mg, Zn, Se) and photosynthetic pigments (β-carotene, phycocyanin) [21-24].

Noni (*Morinda citrifolia*; Rubiaceae) has been used by Polynesian for over 2000 years as a traditional medicine, and food and dye [25-26]. Different parts of the Noni (*M. citrifolia*) contain 200 phytochemicals including anthraquinones, flavonoids, iridoids glycosides, organic acids, alkaloids, lignans etc [27-28].

The effect of spirulina and noni dietary supplementations against aluminium and fluoride toxicity induced alteration in histopathology in fish is scanty in literature. Thus, the ameliorative effects of these two dietary supplements against aluminium and fluoride induced toxicity in the histopathology of common carp was investigated in the present study.

2. Materials and methods

2.1. Experimental diets

The source of Spirulina (*A. platensis*) was the 'Spirulina capsules', manufactured by the Surya Herbal Ltd, India. The proximate composition of the Spirulina powder was analyzed before incorporation in the diet [29]. The maximum tolerable limit of Spirulina (*A. platensis*) to common carp (*C. carpio*) fingerlings was determined for a period of 14 days following Organization for Economic Cooperation and Development (OECD) guideline, and the value was 50 g/kg (5%). From this range of tolerance, Spirulina was incorporated at 0, 2.5 g/kg, 5.0 g/kg, 7.5 g/kg and 10 g/kg level to prepare control or basal diet, 0.25%, 0.50%, 0.75%, and 1% Spirulina diet respectively (Table 1) which were analyzed by standard methods [29].

Table 1: Proximate composition of Spirulina diets (g/kg); values are in mean±SE, (n=3 per sample)

Ingredients (g/Kg)	Control or basal diet (g/kg)	0.25%Spirulina diet (2.5 g/kg)	0.50% Spirulina diet (5 g/kg)	0.75%Spirulina diet (7.5 g /kg)	1% Spirulina diet (10 g /kg)
GNO Cake[1]	600	600	600	600	600
Fish Meal[2]	200	200	200	200	200
Rice Bran[3]	100	100	100	100	100
Vit. & Min. Premix	10	10	10	10	10
Wheat Flour	90	87.5	85	82.5	80
Spirulina (*A. platensis*)[4]	-	2.5	5	7.5	10
Proximate composition					
Crude Protein	40.673±0.014	40.300±0.028	40.190±0.037	40.546±0.050	40.266±0.012
Crude fat	8.656±0.017	8.446±0.014	8.456±0.017	8.203±0.014	8.100±0.017
Fiber	6.140±0.036	6.420±0.017	6.206±0.023	6.306±0.034	5.793±0.021
Ash	8.093±0.023	8.244±0.031	8.130±0.011	8.243±0.034	8.430±0.011
Moisture	11.546±0.024	11.250±0.028	11.803±0.014	11.506±0.040	10.430±0.005
NFE[5]	24.856±0.014	25.123±0.039	25.220±0.011	25.626±0.040	26.970±0.011
GE (kcal/100 g feed)[6]	372.592	369.977	369.959	370.891	374.144

Composition of vitamin & mineral mixture(premix): Each 1 kg contains Vitamin A 8,00,000 IU, Vitamin D_3 80,000 IU, Vitamin E 0.6g, Nicotinamide 1.2 g, Cobalt 2.2g, Copper 4.7g, Iodine 0.6g, Iron 2.2g, Magnesium 6.5g, Manganese 3.3g, Potassium 0.2g, Sodium 0.04g, and Zinc 10g; [1] Ground nut oil (GNO) cake contains 55.43% proteins and 14.45% fat; [2] Contains 51.65% proteins and 7.6% fat.; [3] Contains 9.25% proteins and 8.3% fat; [4] Contains 60% proteins; [5]NFE (Nitrogen Free Extract)=100-(Protein+Fat+Ash+Crude fiber); [6]GE (Gross Energy): Estimated according to NRC (1993)[32] as 4.64, 9.44 and 4.11 Kcal/g for protein, fat and carbohydrate respectively.

The source of Noni (*M. citrifolia*) fruit extract was the 'Noni capsules', manufactured by the Cosmic Nutracos Solutions Pvt. Ltd. The proximate composition of the Noni extract was analyzed before incorporation in the diet [29]. The common carp (*C. carpio*) fingerlings can tolerate the Noni (*M. citrifolia*) fruit extract up to 30 g/kg (3%) which was determined before start of the experiment as per the guidelines of Organization for Economic Cooperation and Development (OECD) for 14 days. From this range of tolerance, Noni (*M. citrifolia*) fruit extract was incorporated at 0, 2.5 g/kg, 5.0 g/kg, 7.5 g/kg and 10 g/kg level to prepare control or basal diet, 0.25%, 0.50%, 0.75%, and 1% Noni (*M. citrifolia*) diet respectively (table 2). The proximate composition of the experimental diets and the ingredients were estimated as per the standard methods [29].

Table 2: Proximate composition of Noni diets; Values are means ± SEM, (n = 3 per treatment group)

Ingredients(g/kg)	Control or basal diet (g/kg)	0.25% Noni diet (2.5 g/kg)	0.50% Noni diet (5.0 g/kg)	0.75% Noni diet (7.5 g/kg)	1% Noni diet (10g/kg)
GNO cake[1]	600	600	600	600	600
Fish meal[2]	200	200	200	200	200
Rice bran[3]	100	100	100	100	100
Wheat flour	90	87.5	85	82.5	80
Noni (*M. citrifolia*)[4]	-	2.5	5.0	7.5	10
Vit. & Min. mix	10	10	10	10	10
Proximate composition					
protein	40.7 ± 0.117^{a}	40.2 ± 0.0416^{b}	40.5 ± 0.136^{ab}	40.4 ± 0.04^{ab}	40.5 ± 0.0829^{ab}
fat	8.68 ± 0.0737^{b}	8.66 ± 0.0273^{b}	8.39 ± 0.151^{b}	8.47 ± 0.0498^{b}	9.99 ± 0.0379^{a}
fiber	6.16 ± 0.026^{b}	6.2 ± 0.0603^{b}	6.62 ± 0.0612^{a}	6.16 ± 0.0379^{b}	6.58 ± 0.0702^{a}
ash	8.13 ± 0.0624^{b}	8.21 ± 0.0586^{b}	8.93 ± 0.107^{a}	8.2 ± 0.0603^{b}	8.89 ± 0.0384^{a}
moisture	11.6 ± 0.0265^{a}	10.9 ± 0.0929^{b}	9.47 ± 0.122^{cd}	10.1 ± 0.276^{c}	9.26 ± 0.0491^{d}
NFE[5]	24.7 ± 0.164^{c}	24.3 ± 0.103^{c}	26.1 ± 0.041^{a}	25.6 ± 0.249^{ab}	24.9 ± 0.0874^{bc}
GE (kcal/g)[6]	372.304	368.151	374.392	372.628	384.564

Values in the same row with different superscript letters are significantly different ($p < .05$).

Composition of vitamin & mineral mixture(premix): Each 1 kg contains Vitamin A 8,00,000 IU, Vitamin D_3 80,000 IU, Vitamin E 0.6g, Nicotinamide 1.2 g, Cobalt 2.2g, Copper 4.7g, Iodine 0.6g, Iron 2.2g, Magnesium 6.5g, Manganese 3.3g, Potassium 0.2g, Sodium 0.04g, and Zinc 10g.

[1] Ground nut oil (GNO) cake contains 55.43% proteins and 14.45% fat.

[2] Contains 51.65% proteins and 7.6% fat.

[3] Contains 9.25% proteins and 8.3% fat.

[4] Contains 2.84% proteins, 2.5% fat, 4.5% ash and 7.1% moisture.

[5]NFE (Nitrogen Free Extract) =100- (Protein+Fat+Ash+Crude fiber)

[6]GE (Gross Energy): Estimated according to NRC (1993) as 4.64, 9.44 and 4.11 Kcal/g for protein, fat and carbohydrate respectively.

2.2. Experimental Design

The experimental study was conducted as per the internationally accepted laboratory animal use and care, and guidelines (guiding principles in the use of animals in toxicology, adopted by the Society of Toxicology in 1989), and as per the guidelines of the Institutional Animal Ethics Committee, University of Calcutta, West Bengal, India. In this experiment 10% of the 96 h LC_{50} of fluoride (i.e. 67.5 mg/L of NaF) and aluminium [i.e. 22.4 mg/L of $Al_2(SO_4)_3$) were used as the sub-lethal dose. Common carp fingerlings were purchased from a local fish market and then brought to the laboratory in plastic bag with sufficient oxygen. The collected fingerling of common carp were stocked in a large glass aquariums and acclimated for a total period of one month. During this period of acclimation, fingerlings of common carp were fed commercial diet at 3% of body weight daily and the water was continuously aerated. There was a total of two experiments. Each experiment

comprised of seven experimental groups like T1, T2, T3, T4, T5, T6, and T7. The T1 was the control group fed on control diet reared in normal water, T2 was the toxic control or negative control group fed on control diet and exposed to 10% of LC_{50} of aluminium or fluoride, T3 was served as plant control or positive control fed on Spirulina(1%) or Noni(0.25%) diet and reared in normal tap water, T4 to T7 were the treatment groups fed on Spirulina or Noni dietary supplementation at 0.25%, 0.50%, 0.75% and 1% respectively and exposed to 10% of the LC_{50} of aluminium or fluoride. In each experiment, a total of 210 acclimated common carp fingerlings of three-month old were randomly allocated into 7 experimental groups each with 30 fish divided into three replicate (10 fish/ replicate). The experiment or each trial was continued for a total period of 30 days. Water was aerated continuously with the replacement of water in every alternate day. Fishes were provided feed @ 3% of their body weight at 9.00 hrs daily. Standard method [30] was followed to estimate the physico-chemical parameters of water namely dissolve oxygen, free CO_2, total alkalinity, total hardness, water temperature, pH, fluoride and aluminium (Table 3).

Table 3: Physico-chemical parameters of the water; values are means ± SEM, (n = 3 per treatment group)

Sl. No.	Water quality parameters	Values
1.	Dissolve Oxygen	4.146±0.260 mg/L
2.	Free Carbon dioxide	4.633±0.120 mg/L
3.	Total Alkalinity	192.333±4.333 mg/L
4.	Total Hardness	125±1.154 mg/L
5.	Water Temperature	30.333±0.440°C
6.	pH	7.466±0.145
7.	Fluoride	0.813±0.008 mg/L
8.	Aluminium	<0.01 mg/L

2.3. Histopathology

After the end of the experiment, tissues from intestine, liver, kidney and ovary were collected from the fish by dissection and fixed in bouins fluid for 24 hours. After fixation, tissues were dehydrated in a ascending series of ethyl alcohol like 30% (2 hrs), 50% (2 hrs), 70% (6 hrs), 90% (3 hrs) and 100% (3hrs). The dehydrated tissues were then dealcoholyzed using in xylene (30 min), ½ xylene + ½ paraffin (10 min) and then infiltrated using molten paraffin (2 hrs). The block of tissues was made using paraffin. The sectioning of the tissue was done using microtome apparatus. The tissue section in paraffin ribbon was then stretched over a glass slide using mayer's albumin and a hot plate. The stretched slide was double stained using haematoxylene and eosine as per the method of Slaoui and Fiette [31] with minor modification. The stained slide was then mounted using D.P.X. [31].

3. Results

3.1. Intestine histopathology

The Transverse Section (T.S.) of intestine of common carp (T1) showed the normal architecture of intestine with muscles (longitudinal, circular), serosa and villi. Exposure of sub-lethal concentration of aluminium caused severe degenerative changes in the intestine which includes necrosis in the absorptive epithelium of the villi, and blunting of villi. Dietary supplementation with Spirulina and Noni improved the intestine histopathology by reducing the occurrence of these changes in dietary supplementation groups (Figure 1 & 2) compared to T2 (Table 4 & 5).

Table 4: Intestine histopathology of common carp fed on Spirulina diets and exposed to sublethal concentration of aluminium

Histopathology	**Stages**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Fusion and blunting (FB) of villi	I	0^+	+++	0+	+	0	0	0+
Necrosis and sloughing of villi (NS)	II	0	++	0	0	0	0+	0
degeneration of villous epithelium (DVE	II	0	+++	0	0	0+	0	0+
Degeneration of villi	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

Table 5: Intestine histopathology of common carp fed on Noni diets and exposed to sub-lethal concentration of aluminium

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Fusion and blunting (FB) of villi	I	0	+++	0+	0+	0+	0	0+
Necrosis and sloughing of villi (NS)	II	0+	++	0	0	0	0+	0
degeneration of villous epithelium (DVE	II	0	+++	0+	0	0+	0	0
Degeneration of villi	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

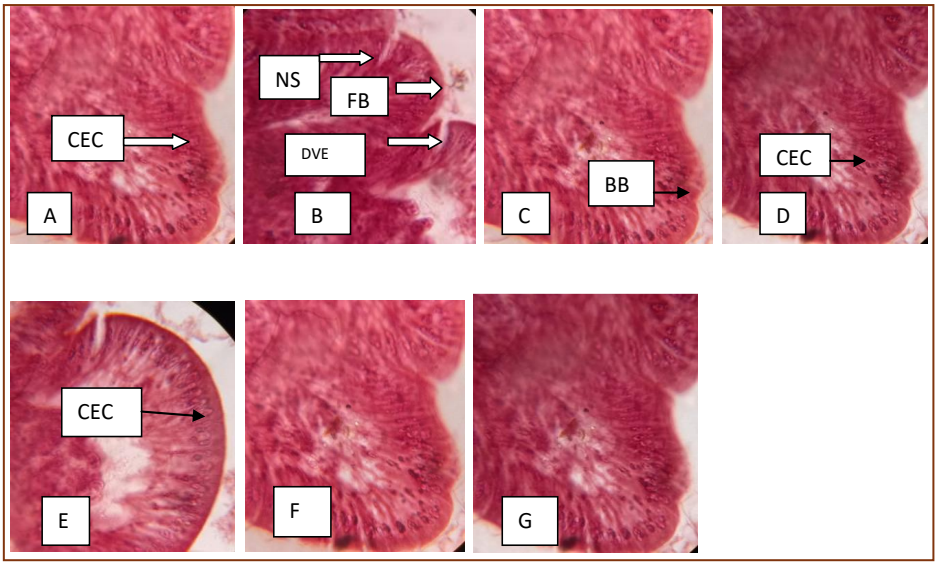

Figure 1 (A-G): T.S of Intestine of common carp fed on Spirulina diet and exposed to sub-lethal concentration of aluminium (A: Control, B: Al, C: SP, D: 0.25% SP+Al, E: 0.50%SP + AL, F: 0.75%SP+ Al, G: 1%SP + Al; NS: Necrosis and Sloughing, FB: Fusion and Blunting, DVE: Degeneration of Vilous Epithelium, CEC: Columner Epithelium Cells, BB: Brush Border; H&E x100)

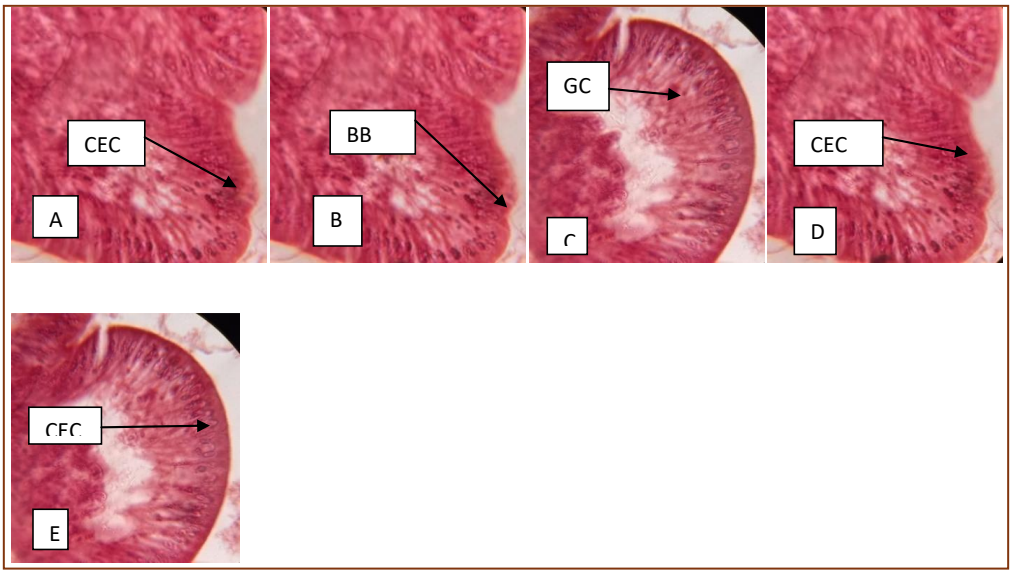

Figure 2 (A-E): T.S of Intestine of common carp fed on Noni diet and exposed to sub-lethal concentration of aluminium (A: 0.25%N, B: 0.25% N+Al C: 0.50% N + Al, D: 0.75% N+Al, E: 1% N + Al; CEC: Columner Epithelium Cells, BB: Brush Border, GC: Goblet Cells; H&E x100)

Exposure of sub-lethal concentration of fluoride caused severe degenerative changes in the intestine which includes necrosis in the absorptive epithelium of the villi, massive fusion and blunting of villi (6 A). Dietary supplementation with Spirulina and Noni improved the intestine histopathology by reducing the occurrence of these changes in T4, T5, T6, and T7 compared to T2 (Fig 3,4) (Table 6,7).

Table 6: Intestine histopathology of common carp fed on Spirulina diets and exposed to sublethal concentration of fluoride

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Fusion and blunting (FB) of villi	I	0+	++	0	0+	0	0	0+
Necrosis and sloughing of villi (NS)	II	0	++	0+	0	0	0+	0+
degeneration of villous epithelium (DVE	II	0	+++	0	0	0+	0+	0
Degeneration of villi	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

Table 7: Intestine histopathology of common carp fed on Noni diets and exposed to sub-lethal concentration of fluoride

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Fusion and blunting (FB) of villi	I	0+	++	0+	0+	0+	0+	0+
Necrosis and sloughing of villi (NS)	II	0	++	0	0+	0	0+	0
degeneration of villous epithelium (DVE	II	0	+++	0	0	0	0	0+
Degeneration of villi	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

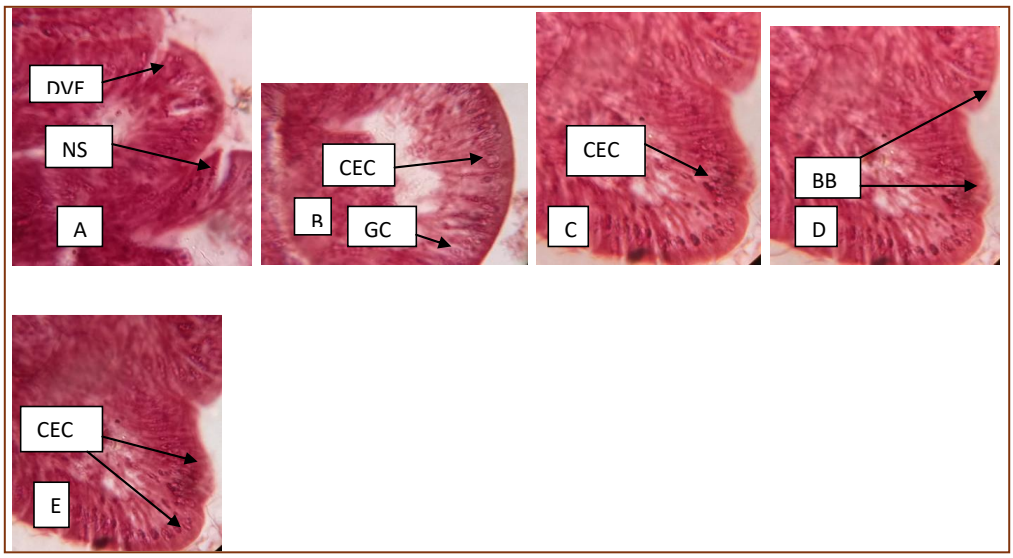

Figure 3(A-E): T.S of Intestine of common carp fed on Spirulina diet and exposed to sub-lethal concentration of fluoride. (A: Fluoride, B: 0.25% SP+F, C: 0.50% SP+F, D: 0.75% SP+F, E: 1%SP + F) (NS: Necrosis and Sloughing, FB: Fusion and Blunting, DVE: Degeneration of Vilous Epithelium, CEC: Columner Epithelial Cells, BB: Brush Border, H&E x100)

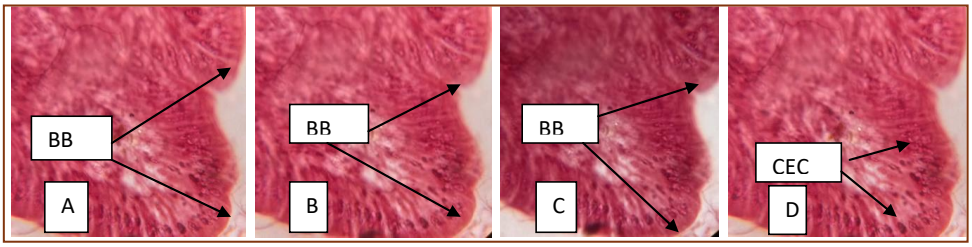

Figure 4 (A-D): T.S of Intestine of common carp fed on Noni diet and exposed to sub-lethal concentration of fluoride. (A: 0.25%N+ F, B: 0.50% N+F, C: 0.75% N + F, D: 1% N+F; BB: Brush Border, CEC: Columner Epithelial Cells, H&E x100)

3.2. Liver histopathology

The Transverse Section (T.S.) of liver of common carp (T1) showed the polygonal hepatocytes with distinct nucleus which were arranged in chord. The stained T.S. of liver also showed the central vein and sinusoids. There was no occurrence of hypertrophy of hepatocytes. The exposure of sub-lethal concentration aluminium caused hypertrophy and degeneration of hepatocytes. It also causes the occurrence of vacuoles in the hepatocytes. However, dietary supplementation of Spirulina and Noni improved the liver histopathology by reducing the occurrence of hypertrophy and vacuolization in the hepatocytes (Table 8 & 9), (Figure 5 & 6).

Table 8: Liver histopathology of common carp fed Spirulina diets exposed to sublethal concentration of aluminium

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Hepatocyte hypertrophy	I	0	++	0+	0+	0+	0+	0+
Kupffer cell hypertrophy	I	0	0	0	0	0	0	0
Sinusoidal dilatation	I	0	0	0	0	0	0	0
Vacuolization in hepatocytes	II	0	+	0	0+	0^+	0	0
Degeneration of hepatocytes	II	0	+	0	0+	0	0	0
Degeneration of central vein	II	0	0	0	0	0	0	0
Necrotic foci	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

Table 9: Liver histopathology of common carp fed on Noni diets exposed to sub-lethal concentration of aluminium

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Hepatocyte hypertrophy	I	0	+	0+	0^+	0+	0+	0+
Kupffer cell hypertrophy	I	0	0	0	0	0	0	0
Sinusoidal dilatation	I	0	0	0	0	0	0	0
Vacuolar degeneration	II	0	+	0	0	0	0	0+
Degeneration of hepatocytes	II	0	+	0	0+	0+	0+	0
Degeneration of central vein	II	0	0	0	0	0	0	0
Necrotic foci	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

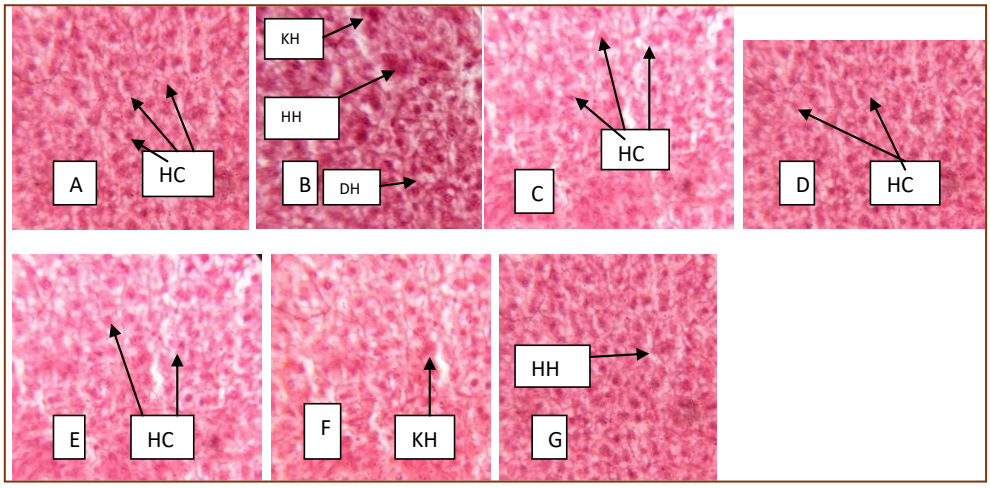

Figure 5 (A-G): T.S. of liver of common carp fed on Spirulina diet and exposed to sub-lethal concentration of aluminium. (A: Control, B: Al, C: SP, D: 0.25%SP+Al, E: 0.50%SP+Al, F: 0.75%SP+ Al, G: 1%SP+Al; HH: Hepatocyte Hypertrophy, DH: Degeneration of Hepatocyte, KH: Kupffer cells hypertrophy; H&E x100)

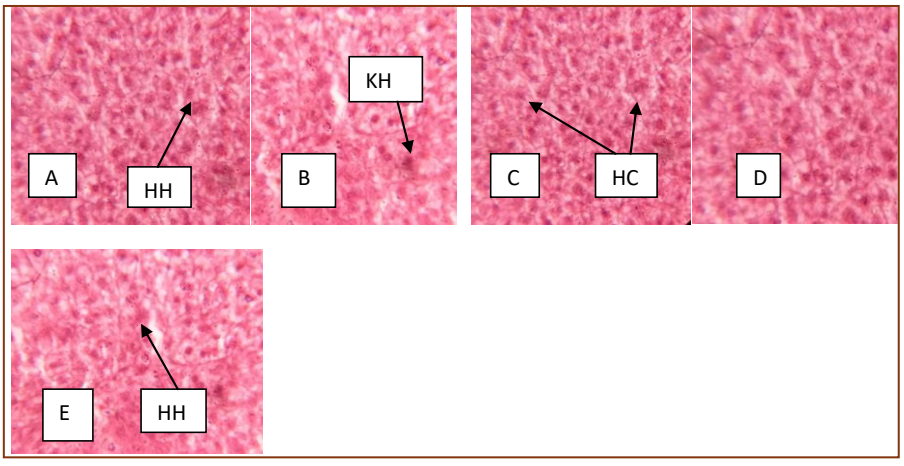

Figure 6 (A-E): T.S. of liver of common carp fed on Noni diet and exposed to sub-lethal concentration of aluminium (A: Noni, B: 0.25%N+Al, C: 0.50%N+Al, D: 0.75%N+Al, E: 1%N+Al) (KH: Kupffer cell hypertrophy, HH: Hepatocyte hypertrophy, HC: Hepatocyte; H&E x100)

The exposure of sub-lethal concentration fluoride caused hypertrophy of the hepatocytes and Kupffer cell, sinusoidal dilatation, degeneration of hepatocytes, central vein and vacuoles (Fig 10 A). However, dietary supplementation of Spirulina and Noni improved the liver histopathology by reducing the occurrence of hypertrophy, pyknotic nuclei in Kufpper cells, vacuolar degeneration as well as degeneration of hepatocytes (Figure 7 & 8)(Table 10 & 11).

Table 10: Liver histopathology of common carp fed on Spirulina diets and exposed to sublethal concentration of fluoride

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Hepatocyte hypertrophy	I	0+	+++	0+	0+	0+	0+	0
Kupffer cell hypertrophy	I	0	++	0	0+	0	0+	0+
Sinusoidal dilatation	I	0	++	0	0	0+	0	0
Vacuolar degeneration	II	0	++	0	0	0	0	0
Degeneration of hepatocytes	II	0	+++	0	0	0+	0	0
Degeneration of central vein	II	0	+	0	0	0	0	0
Necrotic foci	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

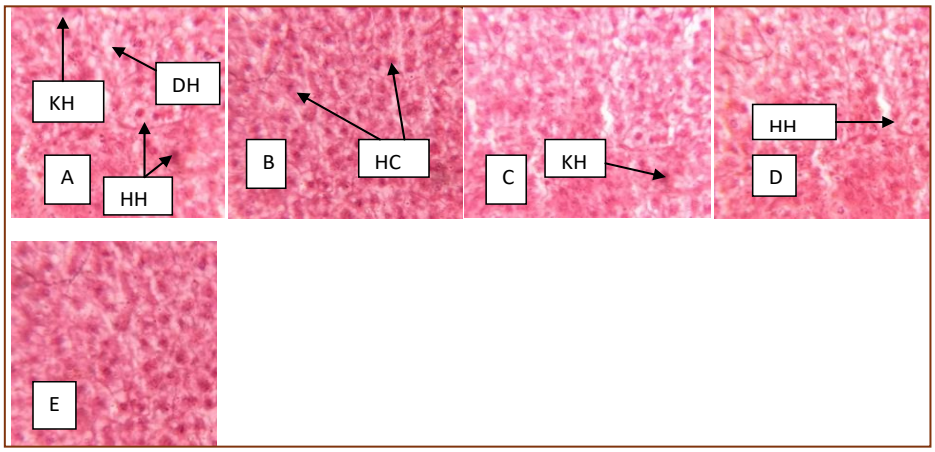

Figure 7 (A-E): T.S. of liver of common carp fed on Spirulina diet and exposed to sub-lethal concentration of fluoride. (A: Fluoride, B: 0.25%SP+F, C: 0.50% SP+F, D: 0.75%SP+ F, E: 1%SP+F) (HH: Hepatocyte Hypertrophy, DH: Degeneration of Hepatocyte, KH: Kupffer cells hypertrophy, HC: Hepatocyte, H&E x100)

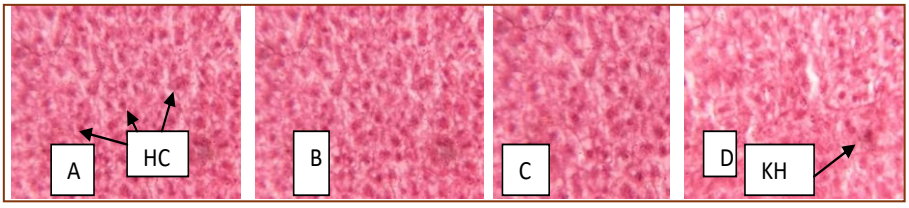

Figure 8 (A-D): T.S. of liver of common carp fed on Noni diet and exposed to sub-lethal concentration of fluoride. (A: 0.25%N+F, B: 0.50%N+F, C: 0.75%N+F, D: 1%N+F) (HC: Hepatocytes, KH: Kupffer cell hypertrophy, H&E x100)

Table 11: Liver histopathology of common carp fed on Noni diets and exposed to sub-lethal concentration of fluoride

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Hepatocyte hypertrophy	I	0+	+++	0+	0+	0+	0+	0+
Kupffer cell hypertrophy	I	0	++	0	0	0	0+	0+
Sinusoidal dilatation	I	0	++	0	0	0	0	0
Vacuolar degeneration	II	0	++	0	0	0	0	0
Degeneration of hepatocytes	II	0	+++	0	0	0	0	0
Degeneration of central vein	II	0	+	0	0	0	0	0
Necrotic foci	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

3.3. Kidney histopathology

The Transverse Section (T.S.) of kidney of common carp (T1) showed the regular occurrence of glomeruli, Bowman's capsules, proximal convoluted tubules and distal convoluted tubules (Fig 12A). The aluminium caused the occurrence of irregular diameter of proximal and distal convoluted tubules. Aluminium also caused the degeneration of renal corpuscles and renal tubules. T2 also showed the shrinkage of glomeruli (12B). However, dietary supplementation by Spirulina and Noni reduces the occurrence of these histopathological changes in T4, T5, T6, T7 compared to the T2 (Table 12,13) (Fig 9,10).

Table 12: Kidney histopathology of common carp fed on Spirulina diets exposed to sub-lethal concentration of aluminium

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Shrinkage of glomeruli (SG)	I	0+	++	0+	0+	0+	0+	0+
Cytoplasmic vacuolation	I	0	0	0	0	0	0	0
Cellular hypertrophy	I	0	0	0	0	0	0	0
obliteration of lumen (OL) of the tuble	II	0	0	0	0	0	0	0
complete absence of glomeruli (CAG)	II	0	0	0	0	0	0	0
Degeneration of renal corpuscle	II	0	+	0	0	0	0	0
Degeneration of renal tubule	III	0	+	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

Table 13: Kidney histopathology of common carp fed on Noni diets exposed to sub-lethal concentration of aluminium

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Shrinkage of glomeruli (SG)	I	0+	++	0	0+	0+	0	0
Cytoplasmic vacuolation	I	0	0	0+	0	0	0+	0
Cellular hypertrophy	I	0	0	0	0	0	0	0
obliteration of lumen (OL) of the tuble	II	0	0	0	0	0	0+	0
complete absence of glomeruli (CAG)	II	0	0	0	0	0	0	0+
Tubular degeneration	II	0	+	0	0	0	0	0+
Degeneration of tuble	III	0	+	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

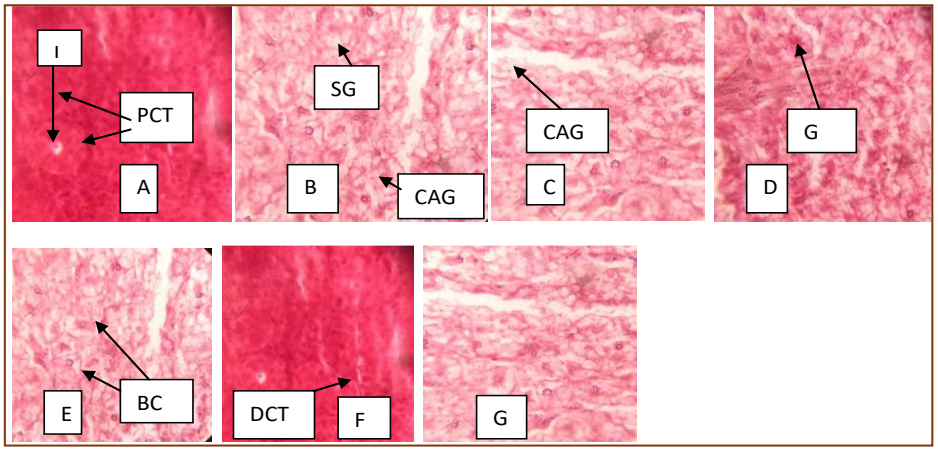

Figure 9 (A-G): T.S of kidney of common carp fed on Spirulina diet and exposed to sub-lethal concentration of aluminium. (A: Control, B: Al, C: SP, D: 0.25% SP+Al, E: 0.50%SP + AL, F: 0.75%SP+ Al, G: 1%SP + Al) (SG: Shrinkage of Glomeruli, CAG: Complete Absence of Glomeruli, PCT: Proximal Convoluted Tuble, DCT: Distal Convoluted Tuble, L: Lumen, G: Glomerulus, BC: Bowmans capsule, H&E x100)

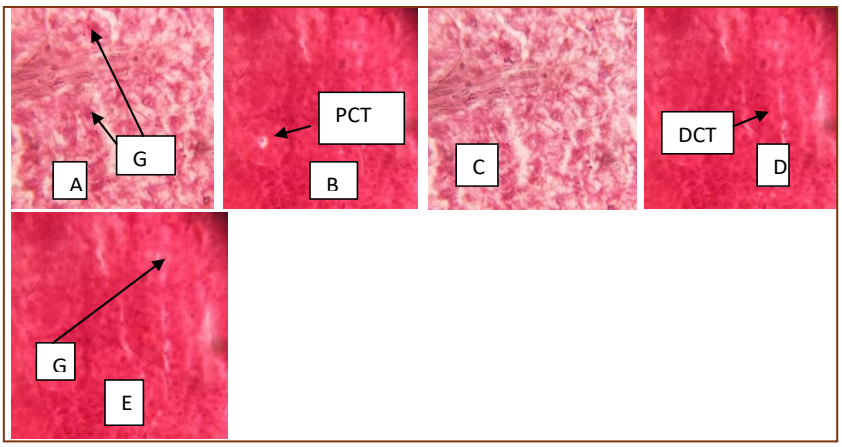

Figure 10 (A-E): T.S. of kidney of common carp fed on Noni diet and exposed to sub-lethal concentration of aluminium. (A: 0.25% Noni, B: 0.25%N+Al, C: 0.50%N+Al, D: 0.75%N+Al, E: 1%N+Al; G: Glomerulus, DCT: Distal Convoluted Tuble, H&E x100)

The fluoride caused the occurrence of shrinkage of glomeruli, cytoplasmic vacuolation, cellular hypertrophy, obliteration of lumen (OL) of the tubule, complete absence of glomeruli, and degeneration of tubules more frequently compared to the control. However, dietary supplementation by Spirulina and Noni reduce the occurrence of these histopathological changes in T4, T5, T6, T7 compared to the T2 (Table 14 & 15, Figure 11 & 12).

Table 14: Kidney histopathology of common carp fed on Spirulina diets and exposed to sublethal concentration of fluoride

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Shrinkage of glomeruli (SG)	I	0+	+++	0+	0+	0+	0+	0+
Cytoplasmic vacuolation	I	0	+	0	0	0+	0	0
Cellular hypertrophy	I	0	++	0	0	0	0+	0
obliteration of lumen (OL) of the tuble	II	0	++	0	0	0	0	0+
complete absence of glomeruli (CAG)	II	0	+++	0	0	0	0+	0
Tubular degeneration	II	0	+	0	0	0+	0	0
Degeneration of tuble	III	0	0	0	0	0	0+	0+

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

Table 15: Kidney histopathology of common carp fed on Noni diets and exposed to sub-lethal concentration of fluoride

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Shrinkage of glomeruli (SG)	I	0	+++	0	0+	0+	0+	0
Cytoplasmic vacuolation	I	0+	+	0+	0	0	0+	0+
Cellular hypertrophy	I	0	++	0	0	0	0+	0
obliteration of lumen (OL) of the tubule	II	0	++	0	0	0	0	0
complete absence of glomeruli (CAG)	II	0	+++	0	0	0	0+	0
Tubular degeneration	II	0	+	0	0	0	0+	0
Degeneration of tuble	III	0	0	0	0	0	+	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

Table 16: Ovary histopathology of common carp fed Spirulina diets exposed to sublethal concentration of aluminium

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Cracks in the oocytes	I	0+	++	0+	0+	0+	0+	0+
Ruptured follicle	II	0	++	0	0+	0+	0+	0+
Nuclear degeneraiton	II	0	+++	0	0	0	0	0
Atretic follicle	II	0	0	0	0	0	0	0
Degeneration of oocytes	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

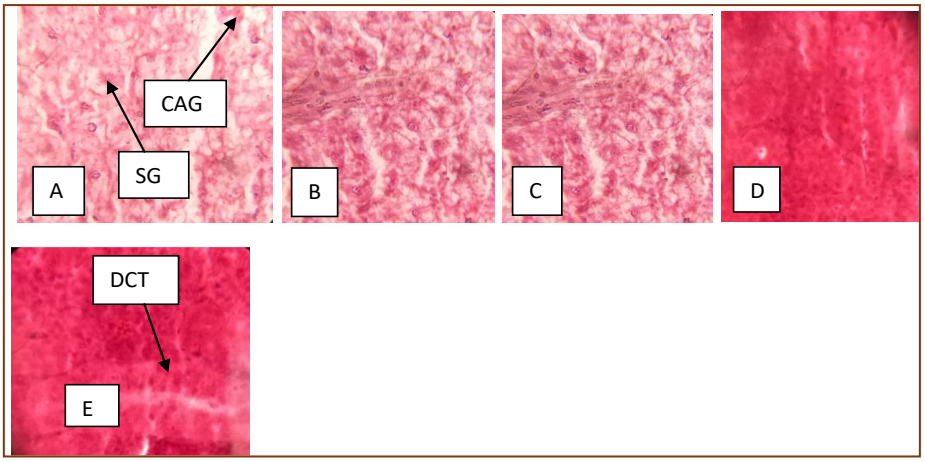

Figure 11(A-E): T.S. of kidney of common carp fed on Spirulina diet and exposed to sub-lethal concentration of fluoride. (A: Fluoride, B: 0.25%SP+F, C: 0.50%SP+F, D: 0.75%SP+ F, E: 1%SP+F) (SG: Shrinkage of Glomeruli, CAG: Complete Absence of Glomeruli, DCT: Distal Convoluted Tubule, H&E x100)

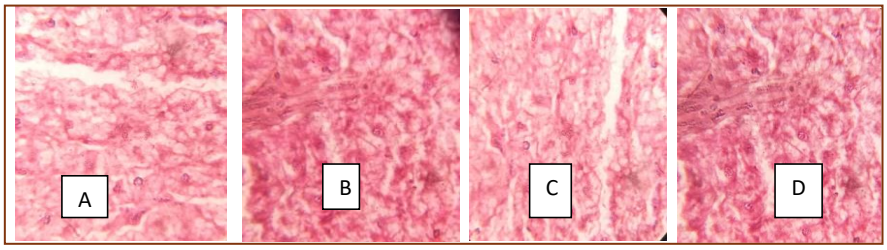

Figure 12(A-D): T.S. kidney of common carp fed on Noni diet and exposed to sub-lethal concentration of fluoride. (A: 0.25%N+F, B: 0.50%N+F, C: 0.75%N+F, D: 1%N+F; H&E x100)

3.4. Ovary histopathology

The Transverse Section (T.S.) of ovary of common carp (T1) showed the normal architecture of ovary with outer layer of thin peritoneum, thick vascularised connective tissue, and germinal epithelium having many oocytes in different stages of development. The oocytes were visible and well formed (Figure 13A). On the other hand, sub-lethal concentration of aluminium caused the occurrence of ruptured follicles, cracks in the oocytes and degeneration of nucleus (Figure 13B). Dietary supplementation by Spirulina improved the histopathology of ovary by reducing the occurrence of these degenerative changes in T4, T5, T6 and T7 compared to T2 (Table 16 & 17) (Figure 13 & 14).

Exposure of sub-lethal concentration of fluoride caused the ruptured follicles, atretic follicles, cracks in the oocytes, thickening of the ovarian wall, degeneration of nucleus, and vascularization of cytoplasm of oocytes. Dietary supplementation by Spirulina improved the histopathology of ovary by reducing the occurrence of these degenerative changes in T4, T5, T6 and T7 compared to T2 (Table 18 & 19, Figure 15 & 16).

Table 17: Ovary histopathology of common carp fed on Noni diets exposed to sub-lethal concentration of aluminium

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Cracks in the oocytes	I	0+	++	0+	0+	0	0+	0+
Ruptured follicle	II	0	++	0	0	0+	0+	0+
Nuclear degeneraiton	II	0	+++	0	0	0	0	0+
Atretic follicle	II	0	0	0	0	0	0+	0
Degeneration of oocytes	III	0	0	0	0	0+	0	0+

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

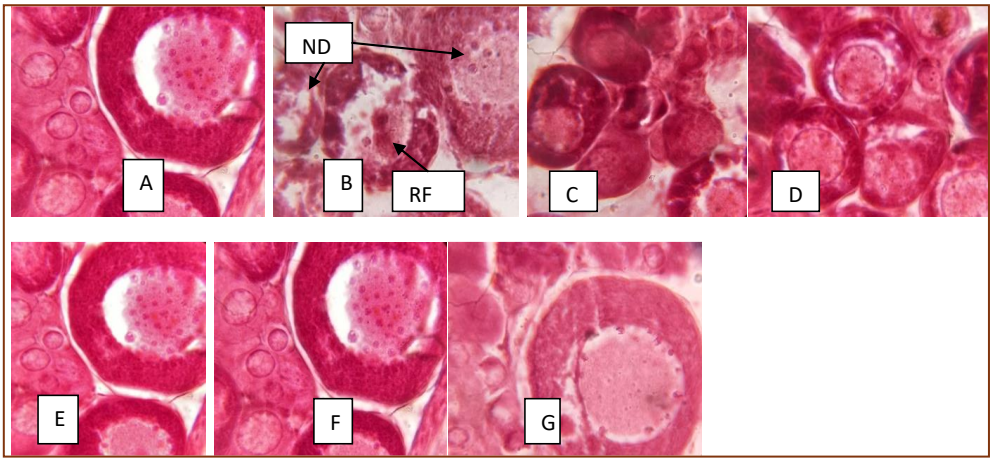

Figure 13(A-G): T.S of ovary of common carp fed on Spirulina diet and exposed to sub-lethal concentration of aluminium. (A: Control, B: Al, C: SP, D: 0.25% SP+Al, E: 0.50%SP + AL, F: 0.75%SP+ Al, G: 1%SP + Al; RF: Ruptured Follicle, CO: Crack in Oocyte, ND: Nuclear Degeneration, H&E x100)

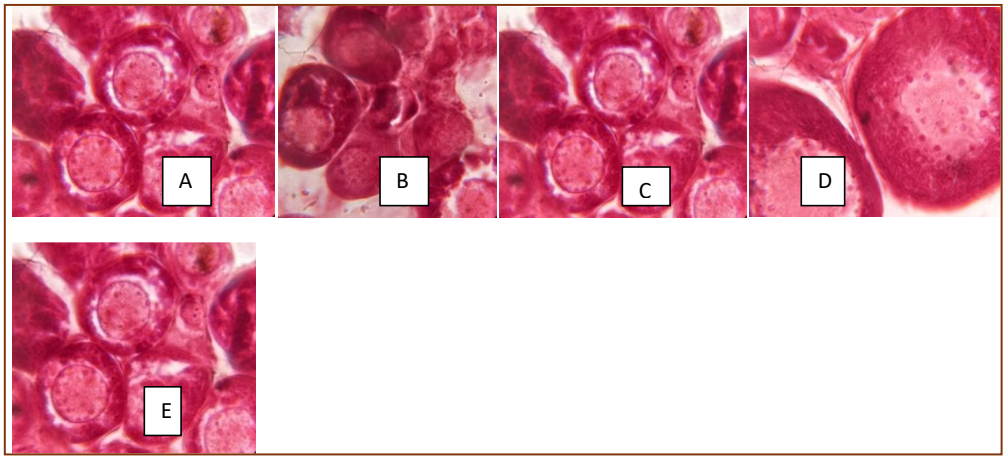

Figure 14 (A-E): T.S. of ovary of common carp fed on Noni diet and exposed to sub-lethal concentration of aluminium. (A: Noni, B: 0.25%N+Al, C: 0.50%N+Al, D: 0.75%N+Al, E: 1%N+Al, H&E X100)

Table 18: Ovary histopathology of common carp fed on Spirulina diets and exposed to sub-lethal concentration of fluoride

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Cracks in the oocytes	I	0+	++	0+	0+	0+	0+	0+
Ruptured follicle	II	0	++	0	0+	0+	0	0
Nuclear degeneraiton	II	0	+++	0	0+	0	0+	0
Atretic follicle	II	0	+	0	0	0	0	0+
Degeneration of oocytes	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

Table 19: Ovary histopathology of common carp fed on Noni diets and exposed to sub-lethal concentration of fluoride

Histopathology	Stages	T1	T2	T3	T4	T5	T6	T7
Cracks in the oocytes	I	0+	++	0+	0+	0	0+	0+
Ruptured follicle	II	0	++	0	0	0+	0+	0+
Nuclear degeneration	II	0	+++	0	0+	0	0+	0
Atretic follicle	II	0	+	0	0+	0	0	0
Degeneration of oocytes	III	0	0	0	0	0	0	0

0=Absent, 0^+ = Rare, +=Low Frequency, ++= Frequent, +++= Very frequent

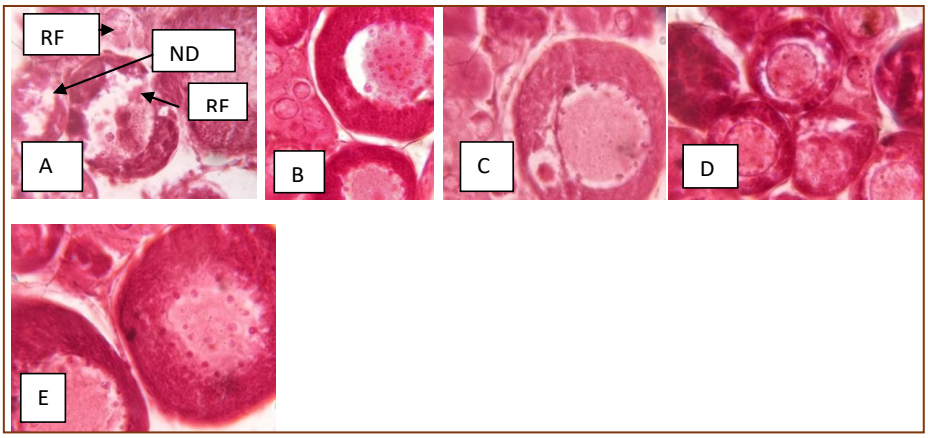

Figure 15(A-E): T.S. of ovary of common carp fed on Spirulina diet and exposed to sub-lethal concentration of fluoride. (A: Fluoride, B: 0.25%SP+F, C: 0.50%SP+F, D: 0.75%SP+ F, E: 1%SP+F; RF: Ruptured Follicle, CO: Crack in Oocyte, ND: Nuclear DegenerationH&E X100)

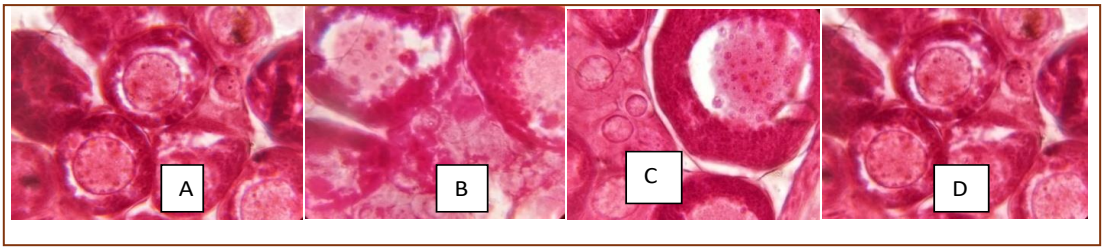

Figure 16 (A-D): T.S. of ovary of common carp fed on Noni diet and exposed to sub-lethal concentration of fluoride. (A: 0.25%N+F, B: 0.50%N+F, C: 0.75%N+F, D: 1%N+F; H&E x100)

4. Discussion

The effect of sub-lethal concentration of aluminium and fluoride on the histopathology of intestine, liver, kidney and ovary of common carp was investigated in the present study. Exposure of sub-lethal concentration of aluminium caused severe degenerative changes in the intestine which includes necrosis in the absorptive epithelium of the villi, and blunting of villi. Aluminium induced alteration of intestinal histopathology was due to oxidative stress via lipid peroxidation [32].

On the other hand, sub-lethal concentration of fluoride caused severe degenerative changes in the intestine which includes necrosis in the absorptive epithelium of the villi, massive fusion and blunting of villi. Present study is corroborated with several previous studies in which fluoride cause degenerative changes in the histopathology of intestine of fish by necrosis in the absorptive epithelium of villi, and massive fusion and blunting of villi [33-35]. Fluoride reacts with the HCl secreted from the parietal cells, form hydrofluoric acid which severely damages the villi of intestine [35].

The teleost liver is the sensitive organ, and also the main organ of all metabolic pathways of body. The hepatotoxic potential of any toxic substances can be evaluated by studying the histopathology of liver [36]. The exposure of sub-lethal concentration aluminium caused hypertrophy and degeneration of hepatocytes. It also causes the occurrence of vacuoles in the hepatocytes. Present study is in consistent with several previous studies in which aluminium induces hypertrophy, vacuolization and necrosis of hepatocytes in fish [14,37]. Hypertrophy is the increase of volume of the cell due to proliferation of endopasmic reticulum membrane [14]. Vacuolization in the liver indicates the intracellular degenerative process due to metabolic disorder. The cellular degeneration and necrosis of hepatocytes may be due to greater accumulation of aluminium in liver [14, 38].

The exposure of sub-lethal concentration of fluoride caused hypertrophy of the hepatocytes and kupffer cell, sinusoidal dilatation, degeneration of hepatocytes, central vein and vacuoles. Present study is in agreement with the previous studies in which fluoride adversely affected the histopathology of liver in common carp [33], *Heteropneustes fossilis* [39-40], *Rasbora daniconius*

[41]. Fluoride causes lipid peroxidation via generation of free radical which in turn damages the hepatocyte of liver [42-43].

The kidney of teleost considered to be an important organ to study the toxic effect of fluoride and aluminium for their accumulation as well excretion from the body [14,43]. The aluminium caused the occurrence of irregular diameter of proximal and distal convoluted tubules. Aluminium also caused the degeneration of renal corpuscles and renal tubules, and shrinkage fo glomeruli. Present study is in agreement with the study of Hadi and Alwan [14] in which aluminium induces histopathological alteration of kidney by damaging renal corpuscle and severe degeneration of renal tubule cells, demonstrates the involvement of kidney in aluminium excretion [14].

On the other hand, fluoride induced histopathological alteration of kidney includes shrinkage of glomeruli, cytoplasmic vacuolation, cellular hypertrophy, obliteration of lumen (OL) of the tubule, complete absence of glomeruli, and degeneration of tubules, is corroborated with the study of [33] in *C. carpio* and Haque *et al*. [34] in *Channa punctatus*. Fluoride induced damages in kidney in the present study probably due to the increased lipid peroxidation through the generation of reactive oxygen species [33-44].

In the present study sub-lethal concentration of aluminium caused the occurrence of ruptured follicles, cracks in the oocytes and degeneration of nucleus of oocytes in the ovary of fish. Aluminium adversely affects the structure and function of ovary through decreasing the mineral contents (Zn, Fe, Cu) [45]. Aluminium also inhibits the secretion of FSH and LH from pituitary results in alteration of histopathology of ovary [45-46]. The result of the present study is in agreement with the study of [45], reported the aluminium induced alteration of histopathology of ovary in rat.

Similarly, sub-lethal concentration of fluoride caused the occurrence of ruptured follicles, atretic follicles, cracks in the oocytes, thickening of the ovarian wall, degeneration of nucleus, and vascularization of cytoplasm of oocytes in the ovary of fish. Fluoride induced different degenerative changes in the ovary is due to the inhibition of the secretion of FSH and LH from the pituitary gland [47]. The result is in par with the study of Yadav and Tripathy [47] in which fluoride causes alteration of the histopathology of ovary in *H. fossilis* [47]. The alteration of the structure of oocyte in the present study is due to the excessive production of reactive oxygen species [48].

Dietary supplementation by spirulina and Noni improved the aluminium and fluoride toxicity induced alteration of histopathology by reducing the occurrences of all the degenerative changes in the intestine, liver, kidney and ovary of both the common carp and mosquitofish. The Spirulina at 1% and Noni at 0.25% showed the best response in Spirulina and Noni dietary supplementation groups respectively. Several studies reported the ameliorative effect of vitamin C against oxidative stress induced tissue damage through neutralizing the reactive oxygen species [49,50, 51,52]. Kaur *et al*. [33] also reported the protective effect of vitamin C against fluoride toxicity induced histopathological changes of intestine, gills, liver, kidney in common carp.

Spirulina as well as Noni fruit extract are rich with ascorbic acid or vitamin C, reduce the oxidative stress throgh scavenging the free radical generated by the exposure of aluminium and fluoride which in turn protect the histopathology of liver, kidney, intestine and ovary [33,47,49]. Raghuvanshi *et al.* [53] also reported the protective effect of Spirulina against beryllium toxicity induced alteration of histopathology in rat.

Spirulina as well as Noni fruit extract are rich with antioxidant compounds, mitigated oxidative stress through scavenging the free radical generated by the exposure of aluminium and fluoride, which in turn protect the histopathology of intestine, liver, kidney, ovary of fish. Thus, Spirulina and Noni fruit extract may be used as dietary supplement in aquafeed at 1% and 0.25% respectively to ameliorate the fluoride and aluminium toxicity in fish.

References

1. Reverter M, Bontemps N, Lecchini D, Banaigs B and Sasal P (2014) Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture 433: 50-61; doi: 10.1016/j.aquaculture.2014.05.048.
2. Zhang F, Man YB, Mo WY and Wong MH (2019) Application of Spirulina in aquaculture: a review on wastewater treatment and fish growth. Reviews in Aquaculture 1-18.
3. Yadav KK and Trivedi SP (2009) Sublethal exposure of heavy metals induces micronuclei in fish, Channa punctata. Chemosphere 77: 1495-1500.
4. Ward DM, Nislow KH, Chen CY & Folt CL (2010). Reduced trace element concentrations in fast growing juvenile Atlantic Salmon in natural streams. Environmental Science and Technology 44: 3245-3251.
5. Authman MM, Zaki MS, Khallaf EA and Abbas HH (2015) Use of fish as bio-indicator of the effects of heavy metal pollution. Journal of Aquaculture Research and Development 6(4):1-13.
6. Hem JD (1985) The study and interpretation of the chemical characteristics of natural water. US Geological Survey Water-Supply, Paper 2254. 3rd ed.
7. Li L. (2003) The biochemistry and physiology of metallic fluoride: action, mechanism and implications. Critical Reviews in Oral Biology and Medicine 14:100-114.
8. Sharma KP, Upreti N, Sharma S and Sharma S (2012) Protective effect of Spirulina and tamarind fruit pulp diet supplement in fish (Gambusia affinis Baird & Girard) exposed to sublethal concentration of fluoride, aluminium and aluminium fluoride. Indian Journal of Experimental Biology 50: 897-903.
9. Naskar R, Sen NS and Ahmad MF (2006) Aluminium toxicity induced poikilocytosis in an air breathing teleost, Clarias batrachus(Linn.). Indian Journal of Experimental Biology 44: 83-85.
10. Fernandez-Devila ML, Razo-Estrada AC, Garcia-Medina S, Gomez-Olivan LM, Pinon-Lopez MJ, Ibarra RG and Galar-Martinez M (2012) Aluminium-induced oxidative stress and neurotoxicity in grass carp (Cyprinidae-Ctenopharyngodon idella). Ecotoxicology & Environmental Safety 76: 87-92.

11. Garcia-Medina S, Razo-Estrada AC, Gomez-Olivan LM, Amaya-Chavez A, Madrigal-Bujaidar E and Galar-Martinez M (2010) Aluminium induced oxidative stress in lymphocytes of Common carp (Cyprinus carpio). Fish Physiology and Biochemistry 36: 875-882.
12. Garcia-Medina S, Nunez-Betancourt JA, Garcia-Medina AL, Galar-Martinez M, Neri-Cruz N, Islas-Flores H and Gomez-Olivan LM (2013) The relationship of cytotoxic and genotoxic damage with blood aluminium levels and oxidative stress by this metal in common carp (Cyprinus carpio) erythrocytes. Ecotoxicology & Environmental Safety 96:191-197.
13. Hadi AA, Shokr AE and Alwan SF (2009) Effects of aluminium on the biochemical parameters of fresh water fish, Tilapia zillii. Journal of Science and its applications 3(1): 33-41.
14. Hadi AA and Alwan SF (2012) Histopathological changes in gills, liver and kidney of fresh water fish, Tilapia zillii, exposed to aluminium. International Journal of Pharmacy and Life Sciences 3(11): 2071-2081.
15. Mosbah A, Guerbej H, Boussetta H, Bouraoui Z and Banni M (2017) Protective Effects of Dietary Garlic Powder Against Cadmium-induced Toxicity in Sea Bass Liver: a Chemical, Biochemical, and Transcriptomic Approach. Biological Trace Elements Research 183(5). Doi 10.1007/s12011-017-1146-4.
16. Fernández-Navarro M, Peragón J, Esteban FJ, de la Higuera M and Lupiáñez JA (2006) Maslinic acid as a feed additive to stimulate growth and hepatic protein-turnover rates in rainbow trout (Onchorhynchus mykiss). Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology 144(2):130–140.
17. Nicoletti M (2016) Microalgae nutraceuticals. Foods 5(54): 1-13.
18. Abdel-Tawwab M, Sharafeldin KM and Ismaiel NEM (2017) Interactive effects of coffee bean supplementation and waterborne zinc toxicity on growth performance, biochemical variables, antioxidant activity and zinc bioaccumulation in whole body of common carp, Cyprinus carpio L. Aquaculture Nutrition 24: 123–130. https://doi.org/10.1111/ anu.12540.
19. Velasquez SF, Chan MA, Abisado RG, Traifalgar RFM, Tayamen MM, Maliwat GCF and Ragaza JA (2016) Dietary Spirulina (Arthrospira platensis) replacement enhances performance of juvenile nile tilapia (Oreochromis niloticus). Journal of Applied Phycology 28:1023-1030.
20. Benatrehina PA, Pan L, Naman CB, Li J and Kinghorn AD (2018) Usage, biological activity, and safety of selected botanical dietary supplements consumed in the United States. Journal of Traditional and Complementary Medicine 8(2): 267-277. doi: 10.1016/j.jtcme.2018.01.006
21. Abdel-Tawwab M and Ahmad MH (2009) Live Spirulina (Arthrospira platensis) as a growth and immunity promoter for nile tilapia, Oreochromis niloticus (L.), challenged with pathogenic Aeromonas hydrophila. Aquaculture Research 40: 1037-1046.
22. Ciferri O (1983) Spirulina, the edible microorganism. Microbiological Reviews 47(4): 551-578.
23. Habib MAB, Parvin M, Huntington TC and Hasan MR (2008) A review on culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish. Food and Agricultural Organization of the United Nations, Rome, FAO Fisheries and Aquaculture Circular No. 1034.
24. Belay A, Ota Y, Miyakawa K and Shimamatsu H (1993) Current knowledge on potential health benefits of Spirulina. Journal of Applied Phycology 5: 235-241.

25. Palu AK, Kim AH, West BJ, Deng S, Jensen J and White L (2008) The effect of Morinda citrifolia L. (Noni) on the immune system: its molecular mechanism of action. Journal of Ethnopharmacology 115:502-506.
26. Chan-Blanco Y, Vaillant F, Perez AM, Reynes M, Brillouet JM and Brat P (2006) The Noni fruit (Morinda citrifolia L.): A review of agricultural research, nutritional and therapeautic properties. Journal of Food Composition and Analysis 19: 645-654.
27. Wang MY and Su C (2001) Cancer preventive effect of Morinda citrifolia(Noni). Annals of the New York Academy of Sciences 952: 161-168.
28. Pawlus AD and Kinghorn AD (2007) Review of the ethnobotany, chemistry, biological activity and safety of the botanical dietary supplement Morinda citrifolia(Noni). Journal of Pharmacy and Pharmocology 59: 1587-1609.
29. AOAC (1990) Official methods of Analyses. In: Arlington VA (ed) Association of Official analytical Chemists. 16th edn. Washington DC.
30. APHA (1992) Standard methods for the examination of water and wastewater. American Public Health Association. 22nd ed. Washington DC
31. Slaoui M and Fiette L (2011). Histopathology procedures: from tissue sampling to histopathological evaluation. Methods in Molecular Biology 691:69-82.
32. Orihuela D, Meichtry V, Pregi N and Pizarro M (2005) Short-term oral exposure to aluminium decreases glutathione intestinal levels and changes enzyme activities involved in its metabolism. Journal of Inorganic Biochemistry 99: 1871-1878.
33. Kaur R, Batra M and Saxena A (2021). Histopathological investigation on the effect of vitamin c on sodium fluoride exposed freshwater Amur carp, cyprinus carpio haematopterus. Fluoride 54(3): 241-256.
34. Haque S, Pal S, Mukherjee AK and Ghosh AR (2012) Histopathological and ultramicroscopic changes induced by fluoride in soft tissue organs of the air breathing teleost, Channa punctatus (Bloch). Fluoride 45:263-273.
35. Bhatnagar C, Bhatnagar M and Regar BC (2007) Fluoride-induced histopathological changes in gill, kidney and intestine of fresh water teleost, Labeo rohita. Fluorid 40(1): 55-61.
36. Roy S and Bhattacharya S (2006) Arsenic-induced histopathology and synthesis of stress proteins in liver and kidney of Channa punctatus. Ecotoxicology and Environmental Safety 65: 218-229.
37. Authman MMN (2011) Environmental and Experimental Studies of Aluminium Toxicity on the Liver of Oreochromis niloticus (Linnaeus, 1757) fish. Life Science Journal 8(4):764-776.
38. Al-Yousuf MH, El-Shahawi MS and Al-Ghais SM (2000) Trace metals in liver, skin and muscle of Lethrinus lentjan fish species in relation to body length and sex. The Science of the Total Environment 256:87-94.
39. Yadav SS, Kumar R and Tripathi M (2014). Effects of fluoride exposure on some enzymatic and histopathogical changes in the liver of Heteropneustes fossilis(Bloch). International Journal of Fauna and Biological Studies 1(5): 80-84.
40. Bajpai S, Tewari S and Tripathi M (2012) Impact of fluoride on structural changes in gills of Indian cat fish, Hetropneustesfossilis (Bloch) after acute exposure. Trend Bioscience 4(2):165-8.

41. Sangve KB (2015) Histological alterations in the liver architecture of freshwater fish, Rasbora daniconius, exposed to sodium fluoride. International Journal of Chemical and Physical Sciences 4 (Special issue):278-83.
42. Pieta BS, Bielec B, Birkner K and Birkner E (2012) The influence of vitamin E and methionine on the activity of enzymes and the morphological picture of liver of rats intoxicated with fluoride. Food and Chemical Toxicology 50: 972–978; doi:10.1016/j.fct.2012.01.014. PMID:22266362.
43. Thangapandiyan S and Miltonprabu S (2013) Molecular mechanism of fluoride induced oxidative stress and its possible reversal by chelation therapy. Research and Reviews: A Journal of Toxicology 3(2).
44. Yang K and Liang X (2011) Fluoride in drinking water: Effect on liver and kidney function. Encyclopedia of Environmental Health 769-75.
45. Fu Y, Jia FB, Wang J, Song M, Liu SM, Li YF, Liu SZ and Bu QW (2014) Effects of sub-chronic aluminum chloride exposure on rat ovaries. Life Sciences 100: 61-66.
46. Wang N, She Y, Zhu YZ, Zhao HS, Shao B, Sun H, Hu CW and Li YF (2011) Effects of subchronic aluminum exposure on the reproductive function in female rats. Biological Trace Element Research 145:382–387.
47. Yadav SS and Tripathy M (2020) Role of vitamin C on hormonal and pathological changes in Heteropneustes fossilis (Bloch) due to exposure to sodium fluoride. Indian Journal of Experimental Biology 58: 706-713.
48. Li M, Cao J, Zhao Y, Wu P, Li X, Khodaei F, Han Y and Wang J (2020) Fluoride impairs ovary development by affecting oogenesis and inducing oxidative stress and apoptosis in female zebrafish (Danio rerio). Chemosphere 256(127105): 1-10.
49. Kurutas EB (2016) The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition Journal 15:71-93.
50. Yilmaz BO and Erkana M (2015) Effects of vitamin C on sodium fluoride-induced oxidative damage in sertoli cells. Fluoride 48(3): 241-51.
51. Guney M, Oral B, Demirin H, Karahan N, Mungan T and Delibas N (2007) Protective effects of vitamins C and E against endometrial damage and oxidative stress in fluoride intoxication. Clinical and Experimental Pharmacology and Physiology 34(5-6):467-74.
52. Choubisa SL, Mishra GV, Sheikh Z, Bhardwaj B, Mali P and Jaroli VJ (2011) Food, fluoride, and fluorosis in domestic ruminants in the Dungarpur district of Rajasthan, India. Fluoride 44(2):70-76.
53. Raghuvanshi S, Agrawal ND, Rawat P, Srivastave S and Shukla S (2020) Hepatorenal protective action of Spirulina platensis against beryllium induced hepatorenal dysfunction and histopathological alterations in rats. Indian Journal of Experimental Biology 58: 23-32.