# Biochemical evaluation of cytoprotective and anti-inflammatory effect of spirulina platensis and melatonin against fluoride induced brain injury and oxidative stress in rats.

**Samy Ali Hussein[1*]; Samir Abdel Latif Abdel Aal[2] and Eman, M. Khafagy[1]**

*[1] Department of Biochemistry, Faculty of Vet. Med., Benha University, Egypt.*
*[2] Department of Animal, Poultry and Environmental Hygiene, Faculty of Vet. Med., Benha University, Egypt.*
*[*]Corresponding author: Samy Ali Hussein; email: samyaziza@yahoo.com*

**A B S T R A C T**

The purpose of this study was to evaluate the protective and anti-inflammatory effect of spirulina platensis (SPP) and or melatonin (MEL) against sodium fluoride (NaF) toxicity induced brain injury and oxidative stress in rats. Seventy male albino rats were divided into five main equal groups. Group I (control): rats administered distilled water. Group II (sodium fluoride exposed group): rats received 1/20 th of LD50 of sodium fluoride orally (2.5 mg/kg b.wt/day) over a period of 8 weeks. Group III (sodium fluoride + SPP treated group): rats received sodium fluoride (2.5 mg/kg b.wt) and treated with SPP (300 mg/kg b.wt/day/orally). Group IV (sodium fluoride + MEL treated group): rats received sodium fluoride (2.5 mg/kg b.wt) and treated with melatonin (10 mg/kg b.wt/day/orally). Group V (sodium fluoride +SPP + MEL treated group): rats received sodium fluoride (2.5 mg/kg b.wt) and treated with SPP (300 mg/kg b.wt) and MEL (10 mg/kg b.wt). The obtained results showed significant increase in serum liver marker enzymes (ALT, AST and ALP) activities, kidney function tests (creatinine and urea), pro-inflammatory cytokines (TNF-α and IL-6) and brain tissue MDA levels in sodium fluoride exposed rats. However, brain tissue antioxidant enzymes (SOD, CAT and GPX) and GSH concentration were markedly decreased. Administration of spirulina and or melatonin with NaF exposed rats caused significant improvement of all previous parameters towards its normal ranges with best results obtained in combined (SPP+MEL) treated group. These results suggested that, SPP or MEL treatment may have a protective effect against sodium fluoride toxicity induced brain injury and oxidative stress in rats via free radical scavenging and anti-inflammatory activity as well as regenerating endogenous antioxidant defense system mechanisms.

**Keywords:** Fluoride, spirulina platensis, melatonin, oxidative stress, pro-inflammatory cytokines.

(http://www.bvmj.bu.edu.eg) (BVMJ-32(2): 31-39, 2017)

## 1. INTRODUCTION

Fluorine ($F^-$) is a chemically reactive electronegative univalent gaseous halogen found in small amount in the water, air, plants and animals. Fluorine is essential for the maintenance and solidification of our bones and to prevent dental decay when it is present at low concentration in drinking water (Fu et al., 2016). Also, fluoride can occur naturally in surface waters as a result of the deposition of particulates from the atmosphere and the weathering of fluoride-containing rocks and soils (Pratusha et al., 2011). Ground water can also contain high concentrations of fluoride owing to leaching from rocks. Chemical manufacturing plants and waste products can contribute fluoride to raw water sources directly through effluents or indirectly through volatilization (ATSDR (Agency for Toxic Substances and Disease Registry), 2003). Natural geological sources and increased industriazation and attendants environmental pollution have contributed greatly to the increasing evidence of fluoride- related human health issue (Susheela, 2007). Being highly soluble in water, environmental fluoride is absorbed easily in the stomach and gut. Although high amount of fluoride in plasma is in bound form, a small fraction of it is in ionic form. Fluorine in solution forms $F^-$ ions. Fluoride passes easily through cell membranes in its ionic form. After about 50% of an ingested fluoride dose has been absorbed, plasma concentrations decline rapidly. This is due to renal excretion and uptake by calcified tissues. However, fluoride is freely filtered through the glomerular capillaries and then undergoes a variable degree of tubular reabsorption. Approximately 99% of fluoride in the body fluids is associated with calcified tissues. The fluoride concentration in bone is not uniform (Kataraki et

al., 2012). High levels of fluoride in drinking water have been considered a potential hazard all over the world (Susheela et al., 2013). Chronic fluorosis is a slow and progressive process causing symptoms related to several systems, particularly musculoskeletal and bone (Izuora et al., 2011). A major cause of fluorosis is the inappropriate use of fluoride containing dental products such as toothpaste and mouth rinses (Dean, 2015). Besides its hyperlipidemic effect (Khudair and Aldabaj, 2014), metabolic, functional and structural damages caused by chronic fluorosis have been reported in many tissues, including kidney, liver (Nabavi et al., 2013) and brain. Chronic fluorosis induces oxidative stress leading to generation of free radicals and alterations in antioxidants or reactive oxygen species (ROS) scavenging enzymes (Bouasla et al., 2014). Fluoride, in fact, can increase the release of cytochrome c from mitochondria and activate the apoptotic pathway (Izquierdo-Vega et al., 2011). Melatonin (MEL) is an indole amine exists in most of mammals and produced by various organs, mainly secreted by the pineal gland. It involved in circadian regulation of physiological and neuroendocrine function. During the last decade, melatonin has been shown to possess potent free radical scavenger properties against reactive oxygen species (ROS). Moreover, by reduction of the activation of pro-oxidant enzymes, melatonin indirectly could protect cells against a variety of free radical-related diseases (Eghbal et al., 2016). Besides, melatonin increased the endogenous antioxidant defense systems (enzymatic and non-enzymatic) as well as inhibiting the lipid peroxidation and protein carbonyl formation. Accordingly, it may be suggested that, melatonin can serve as a potential therapeutic candidate for the liver injury associated with heavy metal induced oxidative stress (Patel and Rao, 2016). Also, spirulina platensis (SPP) is a species of filamentous cyanobacteria that has long been used as a nutraceutical food supplement due to its high protein and vitamin content, although its other potential health benefits have attracted much attention (Wu et al., 2016). Spirulina strongly activates cellular antioxidant enzymes, inhibits lipid peroxidation and DNA damage, and scavenges free radicals, increases the activity of superoxide dismutase and catalase (Abdelkhalek et al., 2015). The promising antioxidant and free radical-scavenging properties of spirulina platensis may be due to its β-carotene and phycocyanin content, which in turn attributes its antioxidant activity to phycocyanobilin. Moreover, spirulina protects against neurotoxicity, hepatonephrotoxicity, and colitis in animals by reducing oxidative stress (Abdel-Daim et al., 2015). Spirulina also exerts a variety of immunomodulatory and anti-inflammatory activities by regulating key cytokines, including interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-10, and TNF-α (Ali et al., 2015).

This study was designed to investigate the possible beneficial effect of some natural antioxidants (spirulina platensis and melatonin) against deleterious effect of sodium fluoride intoxication in adult male rats through investigation of hepato-renal functions, Pro-inflammatory cytokines, biomarkers of oxidative stress and enzymatic antioxidant status.

## 2. MATERIALS AND METHODS

### *2.1. Experimental animals:*

Seventy white male albino rats of 8-10 weeks old and weighing 160 – 200 g were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The rats were fed on constant ration and fresh, clean drinking water was supplied ad-libitum. All rats were acclimatized for minimum period of two weeks prior to the beginning of study.

### *2.2. Chemicals and drugs:*

All chemicals were of analytical grade and obtained from standard commercial suppliers. The drug and chemicals used in the present study were:
a- Sodium fluoride: sodium fluoride was obtained by chemie lab as solid material with high purity 98.5%, it was dissolved in distilled water, freshly prepared and administered orally and daily at a dose of 2.5 mg/kg b.wt (1/20 of L.D.50) (Vani and Reddy, 2000). b-Spirulina platensis: Pure SPP powder was purchased from (Laboratory of Algal Technology at Zagazig University) and given orally and daily at a dose 300 mg/kg b.wt (Simsek et al., 2009). c-Melatonin: MEL was obtained as packs of 120 tablets. Each tablet contains melatonin 5 mg. Melatonin purchased from puritan's pride, inc. (Oakdale, NY 11769 U.S.A.) and administered orally and daily at a dose of (10 mg/kg b.wt) (Bharti et al., 2014).

### *2.3. Experimental design:*

After acclimatization to the laboratory conditions, the animals were randomly divided into five groups (15 rats each) placed in individual cages and classified as follow: Group I (control normal group): Rats received no drugs, served as control non- treated for all experimental groups. Group II (sodium fluoride exposed group): Rats

received sodium fluoride 1/20 of LD50 (2.5 mg/kg b.wt) orally and once per day over a period of 8 weeks. Group III (sodium fluoride + SPP treated group): Rats received sodium fluoride (2.5 mg/kg b.wt) and treated daily with SPP (300 mg/kg body weight/ orally). Group IV (sodium fluoride + MEL treated group): Rats received sodium fluoride (2.5 mg/kg b.wt/ orally) and treated daily with MEL (10 mg/kg b.wt/orally). Group V (sodium fluoride +SPP +MEL treated group): Rats received sodium fluoride (2.5 mg/kg b.wt) and treated daily with SPP (300 mg/kg b.wt) and MEL (10 mg/kg b.wt).

*2.4. Sampling:*

*2.4.1. Blood samples:*

About 7 ml of blood samples were collected by ocular vein puncture from all animal groups two times along the duration of experiment in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minute. The serum was taken by automatic pipptte and received in dry sterile tubes, then kept in deep freeze at -20 °C until use for subsequent biochemical analysis. All sera were analyzed for determination of the following parameters: creatinine, urea, AST, ALT, ALP, TNF-α and IL-6

*2.4.2. Tissue samples:*

About 0.5 g of brain tissue specimen was taken two times from each groups of rats after had been sacrificed at four and eight weeks from the onset of the experiment. The specimens were immediately removed and washed several times with saline and blotted between two damp filter papers, weighed and stored at -20°C for subsequent biochemical analyses. Brain tissue preparation: Briefly, brain tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant was used for the determination of the following parameters: Glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA).

*2.5. Biochemical analysis:*

Serum creatinine, urea, ALT, AST, ALP, TNF-alpha were determined according to the method described by Beyaert and Fiers (1998); Kaplan et al. (2003); Moss (1982); Schumann et al. (2002); Tietz (1995) respectively. Rat IL-6 was determined by method of Chan and Perlstein (1987). Moreover, brain tissue MDA, CAT, SOD, GPx and GSH were determined according to the method described by Esterbauer et al. (1982); Gross et al. (1967); Packer and Glazer (1990); Sinha (1972) Beutler et al. (1963); Necheles et al. (1968), respectively.

*2.6. Statistical analysis:*

The results were expressed as mean ± SE and statistical significance was evaluated by one-way ANOVA using SPSS (version10.0) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when $P<0.05$.

## 3. RESULTS:

The obtained data presented in table (1) revealed that, at four weeks, sodium fluoride intoxicated rats showed significant decrease in brain tissue SOD, CAT, GPx activities and GSH level accompanied with significant increase in brain tissue MDA with marked increase in serum creatinine, urea, ALT, AST, ALP activities, TNF-α and IL-6 concentrations when compared with normal control group. Treatment with SPP alone to fluoride exposed rats showed significant decrease in brain tissue MDA and serum creatinine, urea, ALT, ALP, TNF-α and IL-6. However, serum AST activity was non-significantly decreased and brain tissue GSH and SOD were significantly increased. Also, a non-significant increase in brain tissue GPX and CAT activities were observed in SPP treated rats. Treatment with MEL alone to sodium fluoride intoxicated rats showed significant decrease in brain tissue MDA and serum creatinine and urea levels, AST, ALT and ALP activities, TNF-α and IL-6 concentrations and non-significantly increased brain tissue CAT activity. Also significant increase in brain tissue GPX, SOD activities and GSH concentration were observed in melatonin treated group when compared with sodium fluoride toxic group. A significant decrease in brain tissue MDA and serum creatinine, urea levels, AST, ALT and ALP activities, TNF-α and IL-6 concentrations with marked increase in brain tissue CAT, GPX and SOD activities and GSH concentration were observed in combined group (SPP + MEL + sodium fluoride) when compared with sodium fluoride exposed group. The obtained data presented in table (2) revealed that, at eight weeks, a significant decrease in brain tissue CAT, GPx, SOD activities and GSH concentration with

significant increase in MDA and serum creatinine, urea levels, AST, ALT, ALP activities, TNF-α and IL-6 concentration were observed in sodium fluoride intoxicated rats when compared with control normal group. Spirulina or melatonin treatment alone showed significant decrease in serum creatinine, urea levels, AST, ALT, ALP activities TNF-α and IL-6 concentration and MDA level with significant increase in brain tissue CAT, GPX, SOD activities and GSH concentration when compared with sodium fluoride toxic group. In combined group (SPP + MEL + sodium fluoride) showed significant decrease in serum creatinine, urea levels, AST, ALT, ALP activities, TNF-α and IL-6 concentration and brain tissue MDA concentration with significant increase in brain tissue CAT, GPX, SOD activities and GSH concentration when compared with sodium fluoride exposed group.

Table (1) Protective effects of spirulina platensis and or melatonin on some serum and brain tissue parameters in sodium fluoride intoxicated rats (four weeks).

| parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Serum: | | | | | |
| Urea (mg/dl) | 38.00±1.00$^{b}$ | 45.67±0.88$^{a}$ | 33.33±4.70$^{b}$ | 34.03 ± 0.20$^{b}$ | 37.37 ± 0.88$^{b}$ |
| Creatinine (mg/dl) | 0.94 ± 0.02$^{d}$ | 1.49 ± 0.01$^{a}$ | 1.36 ± 0.03$^{b}$ | 1.16 ± 0.02$^{c}$ | 0.77 ± 0.04$^{e}$ |
| ALT(U/L) | 25.33± 2.03$^{c}$ | 37.63± 0.33$^{a}$ | 31.48± 1.43$^{b}$ | 31.07 ± 0.17$^{b}$ | 28.17± 1.30$^{bc}$ |
| AST(U/L) | 33.13± 1.62$^{c}$ | 41.67± 2.19$^{a}$ | 38.33±0.88$^{ab}$ | 37.17±0.15$^{bc}$ | 35.58± 0.31$^{bc}$ |
| ALP(U/L) | 114.33±1.76$^{d}$ | 227.33±4.18$^{a}$ | 192.00±4.36$^{b}$ | 136.67±2.17$^{c}$ | 117.00±2.08$^{d}$ |
| IL- 6 (pg/ml) | 97.77 ± 2.93$^{c}$ | 127.40±5.54$^{a}$ | 105.53±1.53$^{bc}$ | 112.10±3.59$^{b}$ | 104.17±2.89$^{bc}$ |
| TNF- α (pg/ml) | 45.83 ± 0.88$^{c}$ | 92.77 ± 1.59$^{a}$ | 78.23 ± 3.24$^{b}$ | 73.87 ± 7.62$^{b}$ | 78.80 ± 3.73$^{b}$ |
| Brain tissue: | | | | | |
| MDA(mmole/g. tissue) | 4.16 ± 0.33$^{c}$ | 7.30 ± 0.33$^{a}$ | 5.21 ± 0.36$^{b}$ | 5.63 ± 0.14$^{b}$ | 4.88 ± 0.27$^{bc}$ |
| SOD(u/g. tissue) | 69.15 ± 5.77$^{a}$ | 40.47± 2.28$^{d}$ | 51.04 ± 1.98$^{c}$ | 53.17±2.51$^{bc}$ | 62.95± 1.35$^{ab}$ |
| CAT(mmole/g. tissue) | 1.63 ± 0.25$^{a}$ | 0.55 ± 0.03$^{c}$ | 0.66 ± 0.02$^{c}$ | 0.68 ± 0.02$^{c}$ | 1.15 ± 0.02$^{b}$ |
| GPx(ng/g. tissue) | 7.14 ± 0.13$^{a}$ | 4.54 ± 0.69$^{b}$ | 6.12 ± 0.22$^{ab}$ | 7.46 ± 0.78$^{a}$ | 6.40 ± 0.45$^{a}$ |
| GSH(ng/g. tissue) | 4.37 ± 0.14$^{a}$ | 1.81 ± 0.18$^{d}$ | 2.75 ± 0.04$^{bc}$ | 2.54 ± 0.09$^{c}$ | 3.06 ± 0.12b |

Data are presented as (Mean ± S.E) S.E = Standard error. Mean values with different superscript letters in the same raw are significantly different at (*P≤0.05)*. Group1: control, group 2: sodium fluoride, group 3: sodium fluoride+spirulina, group 4: sodium fluoride+melatonin, group5: sodium fluoride+spirulina+melatonin.

Table (2) Protective effects of spirulina platensis and or melatonin on some serum and brain tissue parameters in sodium fluoride intoxicated rats (eight weeks).

| parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| serum | | | | | |
| Urea (mg/dl) | 35.67± 1.20$^{b}$ | 46.33± 2.33$^{a}$ | 28.00± 3.00$^{c}$ | 36.33± 0.54$^{b}$ | 34.10 ± 0.49$^{b}$ |
| Creatinine (mg/dl) | 0.94 ± 0.02$^{d}$ | 1.39 ± 0.01$^{a}$ | 1.27 ± 0.02$^{b}$ | 1.07 ± 0.03$^{c}$ | 0.66 ± 0.02$^{e}$ |
| ALT(U/L) | 24.67± 1.20$^{d}$ | 38.43± 0.30$^{a}$ | 30.10±0.67$^{b}$ | 30.33± 0.15$^{b}$ | 27.23 ± 0.64$^{c}$ |
| AST(U/L) | 34.87± 0.59$^{b}$ | 44.33± 1.45$^{a}$ | 36.37± 1.49$^{b}$ | 36.23± 0.09$^{b}$ | 34.00 ± 0.58$^{b}$ |
| ALP(U/L) | 106.00±1.53$^{d}$ | 233.67±4.91$^{a}$ | 176.33±6.36$^{b}$ | 126.33±2.96$^{c}$ | 111.67±3.93$^{d}$ |
| IL- 6 (pg/ml) | 104.97±2.13$^{c}$ | 157.20±5.47$^{a}$ | 130.97±1.35$^{b}$ | 130.63±1.44$^{b}$ | 123.73±5.47$^{b}$ |
| TNF- α (pg/ml) | 74.63 ± 2.19$^{c}$ | 105.73±1.24$^{a}$ | 76.63±3.32$^{bc}$ | 83.17 ± 2.94$^{b}$ | 81.80±1.78$^{bc}$ |
| Brain tissue | | | | | |
| MDA (mmol/g. tissue) | 4.13 ± 0.32$^{b}$ | 7.19 ± 0.33$^{a}$ | 5.25 ± 0.38$^{b}$ | 5.28 ± 0.46$^{b}$ | 4.98 ± 0.35$^{b}$ |
| SOD(u/g.tissu) | 72.25 ± 2.59$^{b}$ | 33.80± 1.47$^{d}$ | 54.04 ± 1.68$^{c}$ | 64.02 ± 2.18$^{b}$ | 67.10±1.45$^{ab}$ |
| CAT(mmol/g. tissue) | 1.77 ± 0.02$^{a}$ | 0.44 ± 0.03$^{d}$ | 0.80 ± 0.07$^{c}$ | 0.84 ± 0.07$^{c}$ | 1.31 ± 0.05$^{b}$ |
| GPx(ng/g. tissue) | 7.28 ± 0.08$^{ab}$ | 3.42 ± 0.14$^{c}$ | 6.66 ± 0.92$^{ab}$ | 6.51 ± 0.74$^{b}$ | 8.21 ± 0.57$^{a}$ |
| GSH(ng/g. tissue) | 4.26 ± 0.23$^{a}$ | 1.76 ± 0.16$^{d}$ | 2.41 ± 0.15$^{c}$ | 2.59 ± 0.07$^{c}$ | 3.11 ± 0.07$^{b}$ |

Data are presented as (Mean ± S.E) S.E = Standard error. Mean values with different superscript letters in the same raw are significantly different at (*P≤0.05*). Group1: control, group 2: sodium fluoride, group 3: sodium fluoride+spirulina, group 4: sodium fluoride+melatonin, group5: sodium fluoride+spirulina+melatonin.

## 4. DISCUSSION

Sodium fluoride intoxicated rats showed a significant increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatse (ALP) activities, urea and creatinine concentrations all over the periods of the experiment when compared with normal control group. These results came in accordance with the recorded data of Thangapandiyan and Miltonprabu (2013) who reported that, in rats injected with sodium fluoride, the activities of serum AST, ALT and ALP were significantly increased when compared to those values in control rats. Also, Mohamed (2014) reported that, administration of fluoride to rats induced deterioration in kidney functions as proved by the significant increase in serum urea and creatinine concentrations. Moreover, Oyagbemi et al. (2017) indicated that, a significant increase in blood urea nitrogen and creatinine concentrations was observed in NaF-treated rats when compared to the control. Additionally, Khudair and Aldabaj (2014) documented that, a significant elevation in serum AST and ALP activities were observed in NaF- treated rats after 30 and 60 days of the experiment.

Elevation in AST and ALP activities were found to be related to damage in the liver and the change in hepatic functions. Similarly, Muthumani and Prabu (2012) found that, cell damage correlated well with the enzyme leakage. Hence, cellular damage caused by toxic substances is frequently accompanied by increasing cell membrane permeability. The increased of serum AST, ALT, and ALP activities obviously indicated that, the liver is susceptible to NaF induced toxicity. This increase could be attributed to hepatic damage resulting in either increased release of functional enzymes from biomembranes, or their increased synthesis. On the other hand, Birkner et al. (2006) showed that, disturbance in the function of kidney, creatinine, urea and calcium were observed after fluoride administration. This disturbance may be due to increase protein catabolism resulting from sodium fluoride-induced systemic oxidative damage. This suggests extensive glomerular damage and tubular epithelial cells damage which may also reduce the rate of creatinine clearance from the kidneys and equally its retention in the blood circulation (Seelhammer et al., 2016).

Treatment with SPP and MEL individually or in combination in sodium fluoride intoxicated rats significantly reduced elevated serum ALT, AST and ALP activities, urea and creatinine concentrations when compared with fluoride intoxicated rats. These findings are in harmony with results of Hassanen et al. (2015) who indicated that, spirulina treatment ameliorates liver injuries, which induced by doxorubicin via free radical generation by decreasing the activities of liver marker enzymes such as serum ALT and AST. Also, Bharti and Srivastava (2011) reported that, therapeutic administration of melatonin significantly reduced serum ALT, AST and ALP activities because of its free radical scavenging ability, melatonin can have an important role in ameliorating fluoride toxicity. Moreover, Elshazly et al. (2015) showed that, co-administration with spirulina brought about a significant restorative reduction of the elevated serum urea and creatinine levels versus those measured in chromium treated-rats group. Furthermore, Rao et al. (2009) reported that, melatonin treatment along with NaF reduced the toxic effects of fluoride in kidney, since all the biochemical parameters were not appreciably altered as compared to the controls, confirming the antioxidative properties of melatonin. Thus the results obtained from the study suggest a protective action of melatonin against fluoride induced nephrotoxicity due to its antioxidant properties.

Presented findings showed that, treatment with sodium fluoride in rats exhibited a significant increase in serum IL-6 and TNF-α concentration when compared with control normal group. IL-6 is a cytokine not only involved in inflammation and infection responses but also in the regulation of metabolic, regenerative, and neural processes (Scheller et al., 2011). Similarly, Thangapandiyan and Miltonprabu (2014) showed that, fluoride intoxication in rats significantly elevated the levels of TNF-α, NO, IL-6 and NF-kB in renal tissue. Also, (Afolabi et al., 2013) confirmed the increasing effect of fluoride on IL-2, IL-6, and TNF-α concentrations in rats. Treatment with spirulina and melatonin alone or in combination in sodium fluoride intoxicated rats significantly reduced elevated serum IL-6 and TNF-α concentrations when compared with fluoride intoxicated rats These results came in accordance with the recorded data of Wu et al. (2016) who revealed, the antioxidant,, immunomodulatory, and anti-inflammatory activities of spirulina in both animals and humans by regulating key cytokines, including interleukin IL-1β, IL-2, IL-4, IL-6, IL-10, and TNF-α. Also, Dong et al. (2016) reported that, melatonin administration after brain injury induced by subarachnoid hemorrhage led to a significant reduction in IL-1b and IL-6 concentrations. The obtained results demonstrated that, a significant decrease in brain tissue CAT, SOD, GPx and GSH in fluoride treated rats. These results are nearly similar to those

reported by Banala and Karnati (2015) who documented that, NaF induced oxidative stress through depletion in levels of various anti-oxidants such as glutathione, SOD, with increased levels of lipid peroxidation (LPO). Other mechanisms of action of NaF through which it induced oxidative stress and altered antioxidant defense mechanism have been reported (Samanta et al., 2016; Umarani et al., 2015). Catalase is a major antioxidant enzyme having heme as the prosthetic group which reduces hydrogen peroxide to molecular oxygen and water (Al-Rasheed et al., 2016) and NADH acts as a substrate or cofactor for activation of this enzyme from its inactive form. These antioxidant enzymes are the primary enzymatic defense against toxic oxygen reduction metabolites, and each enzyme has an integral function in free radical modulation. Thus, the accumulated free radical could consume SOD, CAT, and GSH-Px in the kidney and liver. Moreover, if the balance between reactive oxygen species (ROS) production and antioxidant defense was disrupted, the enzyme may be exhausted and its concentration may be depleted (Liu et al., 2010).

The obtained results showed that, treatment with spirulina and melatonin individually or in combination in sodium fluoride intoxicated rats significantly increased the reduced brain tissue CAT, GPx, SOD and GSH levels at the end of the experimental periods. In a similar way Sharoud (2015) reported that, treatment with spirulina significantly restored the enzymatic activities SOD, CAT, GPx and GSH to be approximately near the normal limits in the paracetamol treated animals. Spirulina has a property of reducing heavy metals and nephrotoxic substances from the body (Deepti et al., 2011). Also, Bharti et al. (2014) reported that, CAT, SOD, GPx, and GR activities were increased significantly in heart, liver, and kidney tissues of fluoride exposed rats during melatonin supplementation. Moreover, Abdelkhalek et al. (2015) showed that, supplementation of spirulina platensis significantly increased SOD, CAT, GPx activities and GSH levels in deltamethrin intoxicated fish. As confirmed by Bulan et al. (2015) who suggested that, treatment of rats with melatonin normalized the activities of antioxidant enzymes (CAT, SOD, GPx) activities and GSH levels to their control values in aluminum induced oxidative stress. However, the obtained results showed that, treatment with sodium fluoride caused a significant increase in MDA level at the end of the experiment when compared with control normal group. The elevated MDA level led to the enhanced lipid peroxidation was probably due to the production of superoxide, peroxyl, and hydroxyl radicals (Abdel-Wahhab et al., 2008). Increased peroxidation of membrane lipids is one of principal consequences of oxidative damage produced by NaF exposure. Likewise, Dubey et al. (2013) reported that, NaF increased kidney lipid peroxidation end product MDA. Treatment with spirulina and melatonin alone or in combination in sodium fluoride intoxicated rats significantly reduced elevated MDA level at the end of the 8-week experiment. These findings are in agreement with the results of Karadeniz et al. (2008) who reported that, administration of spirulina reduced the elevation of peroxide levels in mercury intoxicated rats. Spirulina reduced the oxidative stress via its antioxidant components, phycocyanin and β-carotene or via reduction of lipid peroxidation. The potential protective role of spirulina may be associated with its antioxidant constituents such as selenium, chlorophyll, carotene, gamma-linolenic acids, tocopherol, phenolic compounds content, vitamin E and C working individually or in synergy (Garcia-Martinez et al., 2007). Also, Rao and Bhatta (2012) reported that, therapeutic treatment with 10 mg/kg melatonin resulted in significant decrease in MDA levels in NaF intoxicated rats. Similarly, Rao et al. (2010) reported that, supplementation of melatonin to mercury-fed rats significantly diminished the levels of oxidative stress markers, lipid peroxidation and protein carbonyls in brain.

## 5. CONCLUSION

The present study demonstrated that, administration of spirulina platensis and or melatonin alleviated actions and harmful effects caused by exposure to toxic fluoride. Fluoride toxicity affected different organs mainly brain, liver and kidney and these occurred via affected various parameters. Fluoride caused significant increase in serum AST, ALT, ALP, urea, creatinine, TNF-α, IL-6 and brain tissue MDA. While, a marked decrease in brain tissue SOD, CAT, GPX and GSH. Spirulina and or melatonin treatment in fluoride intoxicated rats alleviated all previous parameters towards its normal range with best result in combined treated group. So, these results confirm the strong antioxidant, anti-inflammatory and cytoprotective effects of both spirulina platensis and melatonin in fluoride toxicity.

## 6. REFERENCES

Abdel-Daim, M.M., Farouk, S.M., Madkour, F.F., Azab, S.S., 2015. Anti-inflammatory and immunomodulatory effects of Spirulina platensis in comparison to Dunaliella salina in acetic acid-induced rat experimental colitis.

Immunopharmacol Immunotoxicol 37, 126-139.

Abdel-Wahhab, M.A., Abdel-Azim, S.H., El-Nekeety, A.A., 2008. Inula crithmoides extract protects against ochratoxin A-induced oxidative stress, clastogenic and mutagenic alterations in mal rats. Toxicon. 52, 566–573.

Abdelkhalek, N.K., Ghazy, E.W., Abdel-Daim, M.M., 2015. Pharmacodynamic interaction of Spirulina platensis and deltamethrin in freshwater fish Nile tilapia, Oreochromis niloticus: impact on lipid peroxidation and oxidative stress. Environ Sci Pollut Res Int 22, 3023-3031.

Afolabi, O.K., Oyewo, E.B., Adekunle, A.S., Adedosu, O.T., Adedeji, A.L., 2013. Oxidative indices correlate with dyslipidemia and pro-inflammatory cytokine levels in fluoride-exposed rats. Arh Hig Rada Toksikol 64, 521-529.

Al-Rasheed, N., Faddah, L., Al-Rasheed, N., Bassiouni, Y.A., Hasan, I.H., Mahmoud A.M., Mohamad, R.A., Yacoub, H.I., 2016. Protective Effects of Silymarin, Alone or in Combination with Chlorogenic Acid and/or Melatonin, Against Carbon Tetrachloride-induced Hepatotoxicity. Pharmacogn Mag. 12, 337–345.

Ali, E.A., Barakat, B.M., Hassan, R., 2015. Antioxidant and angiostatic effect of Spirulina platensis suspension in complete Freund's adjuvant-induced arthritis in rats. PLoS One 10, e0121523.

ATSDR (Agency for Toxic Substances and Disease Registry), 2003. Toxicological profile for fluorides, hydrogen fluoride, and fluorine. Division of Toxicology /Toxicology Information Branch. pp: 1-351. 1600 Clifton Road NE, Mailstop E-29, Atlanta Georgia.

Banala, R.R., Karnati, P.R., 2015. Vitamin A deficiency: An oxidative stress marker in sodium fluoride (NaF) induced oxidative damage in developing rat brain. Int J Dev Neurosci 47, 298-303.

Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J Lab Clin Med 61, 882-888.

Beyaert, R., Fiers, W., 1998. Tumor Necrosis Factor and Lymphotoxin. In Cytokines, A.R.M.-S. a. R. Thorpe, eds. Academic Press, San Diego, pp. 335-360.

Bharti, V.K., Srivastava, R.S., 2011. Effect of pineal proteins and melatonin on certain biochemical parameters of rats exposed to high-fluoride drinking water. Fluoride 44, 30-36.

Bharti, V.K., Srivastava, R.S., Kumar, H., Bag, S., Majumdar, A.C., Singh, G., Pandi-Perumal, S.R., Brown, G.M., 2014. Effects of melatonin and epiphyseal proteins on fluoride-induced adverse changes in antioxidant status of heart, liver, and kidney of rats. Adv Pharmacol Sci 2014, 532969.

Birkner, E., Grucka-Mamczar, E., Zwirska-Korczala, K., Zalejska-Fiolka, J., Stawiarska-Pieta, B., Kasperczyk, S., Kasperczyk, A., 2006. Influence of sodium fluoride and caffeine on the kidney function and free-radical processes in that organ in adult rats. Biol Trace Elem Res. 109, 35-48.

Bouasla, A., Bauasla, I., Boumedjel, A., El-Feki, A., Messarahi, M., 2014. Hepatoprotective role of gallic acid on sodium fluoride-induced liver injury in rats. Int. J. Pharm. Sci. Rev. Res. 29, 14-18.

Bulan, N.O., Sarikaya-Ünal, G., Tunali, S., Arda-Pirinççi, P., Yanardag, R., 2015. Melatonin is a potent modulator of antioxidative defense and cellular proliferation against aluminum toxicity in rats. turkish Journal of Biology 39, 911-924.

Chan, D.W., Perlstein, N.T., 1987. Immunoassay: A Practical Guide. Eds, Academic Press: New York, pp. 71.

Dean, J.A., 2015. McDonald and Avery's Dentistryfor the Child.

Deepti, G., Shabad, P., Dua, K.K., 2011. Prevention of cadmium bioaccumulation by herbal adaptogen: Spirulina platensis. Journal of Chemical and Pharmaceutical Research 3, 603-608.

Dong, Y., Fan, C., Hu, W., Jiang, S., Ma, Z., Yan, X., Deng, C., Di, S., Xin, Z., Wu, G., Yang, Y., Reiter, R.J., Liang, G., 2016. Melatonin attenuated early brain injury induced by subarachnoid hemorrhage via regulating NLRP3 inflammasome and apoptosis signaling. J Pineal Res 60, 253-262.

Dubey, N., Khan, A.M., Raina, R., 2013. Sub-acute deltamethrin and fluoride toxicity induced hepatic oxidative stress and biochemical alterations in rats. Bull Environ Contam Toxicol 91, 334-338.

Eghbal, M.A., Eftekhari, A., Ahmadian, E., Azarmi, Y., Parvizpur, A., 2016. A Review of Biological and Pharmacological Actions of Melatonin: Oxidant and Prooxidant Properties. J Pharma Reports. 1, 106.

Elshazly, M.O., Abd El-Rahman, S.S., Morgan, A.M., Ali, M.E., 2015. The remedial efficacy of Spirulina platensis versus

chromiuminduced nephrotoxicity in male sprague-dawley rats. PLoS ONE 10, e0126780.

Esterbauer, H., Cheeseman, K.H., Danzani, M.U., Poli, G., Slater, T.F., 1982. Separation and characterization of the aldehyde products of ADP/Fe2+C stimulated lipid peroxidation in rate liver microsomes. Biochem. J. 208, 129-140.

Fu, X., Xie, F.N., Dong, P., Li, Q.C., Yu, G.Y., Xiao, R., 2016. High-Dose Fluoride Impairs the Properties of Human Embryonic Stem Cells via JNK Signaling. PLoS One 11, e0148819.

Garcia-Martinez, D., Rupe´ rez, F.J., Ugarte, P., Barbas, C., 2007. Tocopherol fate in plasma and liver of streptozotocin-treated rats that orally received antioxidants and Spirulina extracts. Int. J. Vitam. Nutr. Res. 77, 263–271.

Gross, R.T., Bracci, R., Rudolph, N., Schroeder, E., Kochen, J.A., 1967. Hydrogen peroxide toxicity and detoxification in the erythrocytes of newborn infants. Blood 29, 481-493.

Hassanen, M.R., Mahfouz, M.K., Farid, A.S., Fadlullah, A.H., 2015. Biochemical effects of spirulina platensis against oxidative stress caused by doxorubicin. BVMJ 28, 147-154.

Izquierdo-Vega, J.A., Sanchez-Gutierrez, M., Del Razo, L.M., 2011. NADPH oxidase participates in the oxidative damage caused by fluoride in rat spermatozoa. Protective role of alpha-tocopherol. J Appl Toxicol 31, 579-588.

Izuora, K., Twombly, J.G., Whitford, G.M., Demertzis, J., Pacifici, R., Whyte, M.P., 2011. Skeletal fluorosis from brewed tea. J Clin Endocrinol Metab 96, 2318-2324.

Kaplan, L.A., Pesce, A.J., Kazmerczak, S.C., 2003. First MR: Renal Function. In: Clinical Chemistry: Theory, Analysis and Correlation, Kaplan, L.A. and A. Pesce (Eds.). 4th Edn., Mosby Inc., St. Louis, MO., USA.

Karadeniz, A., Yildirim, A., Simsek, N., Kalkan, Y., Celebi, F., 2008. Spirulina platensis protects against gentamicin-induced nephrotoxicity in rats. Phytother Res 22, 1506-1510.

Kataraki, P., Rao, P., Dileep Rathi, D., 2012. Study of oxidative stress, bone mineral status and fluoride levels in postmenopausal women in endemic fluorotic area of Andhra Pradesh, India. 2, 77-83.

Khudair, K.K., Aldabaj, A.M.A., 2014. Effect of high concentration of sodium fluoride on serum lipid profile of male rabbits: Hypolipidemic effect of grape seed oil. Online Int. Interdisciplin. Res. J. 5, 17-24.

Liu, C.M., Ma, J.Q., Sun, Y.Z., 2010. Quercetin protects the rat kidney against oxidative stress-mediated DNA damage and apoptosis induced by lead. Environ Toxicol Pharmacol 30, 264-271.

Mohamed, N.E., 2014. The Role of Calcium in Ameliorating the Oxidative Stress of Fluoride in Rats. Biochemistry & Analytical Biochemistry 03, 152.

Moss, D.W., 1982. Alkaline phosphatase isoenzymes. Clin Chem 28, 2007-2016.

Muthumani, M., Prabu, S.M., 2012. Silibinin potentially protects arsenic-induced oxidative hepatic dysfunction in rats. Toxicol Mech Methods 22, 277-288.

Nabavi, S.F., Nabavi, S.M., Habtemariam, S., Moghaddam, A.H., Sureda, A., Jafari, M., Latifi, A.M., 2013. Hepatoprotective effect of gallic acid isolated from Peltiphyllumpeltatum against sodium fluoride-induced oxidative stress. Industrial Crops and Products 44, 50–55.

Necheles, T.F., Boles, T., Allen, D.M., 1968. Erythrocyte glutathione peroxidase deficiency and hemolytic diseases of the newborn infant. J. Ped. 72, 319-324.

Oyagbemi, A.A., Omobowale, T.O., Asenuga, E.R., Adejumobi, A.O., Ajibade, T.O., Ige, T.M., Ogunpolu, B.S., Adedapo, A.A., Yakubu, M.A., 2017. Sodium fluoride induces hypertension and cardiac complications through generation of reactive oxygen species and activation of nuclear factor kappa beta. Environ Toxicol 32, 1089-1101.

Packer, L., Glazer, A.N., 1990. Oxygen Radicals in Biological Systems Part B: Oxygen Radicals and Antioxidants (Methods in Enzymology, Volume 186). Academic Press Inc., New York, USA. ISBN: 978-0-12-182087-9, pp: 251.

Patel, T.A., Rao, M.V., 2016. Melatonin modulates mercury induced oxidative stress in rat liver. World Journal of Pharmaceutical Research 5, 1198-1211.

Pratusha, G., Banji, F., Bangi, D., Ragina, M., Pavani, B., 2011. Fluoride Toxicity- A Harsh Reality. Intern. Res. J. Pharmacol. 2, 79-85.

Rao, M.V., Bhatta, R.N., 2012. Melatonin protection against F-induced oxidative stress and testicular dysfunction in rats. Fluoride 45, 116–124.

Rao, M.V., Chawla, S.L., Patel, N., 2009. Melatonin reduction of fluoride-induced nephrotoxicity in mice. Research report Fluoride 42, 110–116l.

Rao, M.V., Purohit, A., Patel, T., 2010. Melatonin protection on mercury-exerted brain toxicity in the rat. Drug Chem Toxicol 33, 209-216.

Samanta, A., Chanda, S., Bandyopadhyay, B., Das, N., 2016. Establishment of drug delivery system nanocapsulated with an antioxidant (+)-catechin hydrate and sodium meta borate chelator against sodium fluoride induced oxidative stress in rats. J Trace Elem Med Biol 33, 54-67.

Scheller, J., Chalaris, A., Schmidt-Arras, D., Rose-John, S., 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 1813, 878-888.

Schumann, G., Bonora, R., Ceriotti, F., Férard, G., Ferrero, C.A., Franck, P.F., Gella, F.J., Hoelzel, W., Jørgensen, P.J., Kanno, T., Kessner, A., Klauke, R., Kristiansen, N., Lessinger, J.M., Linsinger, T.P., Misaki, H., Panteghini, M., Pauwels, J., Schiele, F., Schimmel, H.G., Weidemann, G., Siekmann, L., 2002. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 degrees C. International Federation of Clinical Chemistry and Laboratory Medicine. Part 6. Reference procedure for the measurement of catalytic concentration of gamma-glutamyltransferase. Clin Chem Lab Med. 40, 734-738.

Seelhammer, T.G., Maile, M.D., Heung, M., Haft, J.W., Jewell, E.S., Engoren, M., 2016. Kinetic estimated glomerular filtration rate and acute kidney injury in cardiac surgery patients. J Crit Care 31, 249-254.

Sharoud, M.N.M., 2015. Protective effect of Spirulina against paracetamol-induced hepatic injury in rats. Journal of Experimental Biology and Agricultural Sciences 3.

Simsek, N., Karadeniz, A., Kalkan, Y., Keles, O.N., Unal, B., 2009. Spirulina platensis feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. J Hazard Mater 164, 1304-1309.

Sinha, A.K., 1972. Calorometric assay of catalase. Analytical biochemistry 47, 389.

Susheela, A.K., 2007. Industrial fluorosis. In: Treatise on fluorosis. 3rd edition. Fluorosis Research and Rural Development Foundation in India, Delhi., pp. 68-76.

Susheela, A.K., Mondal, N.K., Singh, A., 2013. Exposure to fluoride in smelter workers in a primary aluminum industry in India. Int J Occup Environ Med 4, 61-72.

Thangapandiyan, S., Miltonprabu, S., 2013. Epigallocatechin gallate effectively ameliorates fluoride-induced oxidative stress and DNA damage in the liver of rats. Can J Physiol Pharmacol 91, 528-537.

Thangapandiyan, S., Miltonprabu, S., 2014. Epigallocatechin gallate effectively ameliorates fluoride-induced oxidative stress and DNA damage in the liver of rats. Can. J. Physiol. Pharmacol. 91, 1–10.

Tietz, N.W., 1995. Clinical Guide to Laboratory Tests. 3rd edn., W.B. Sauders, Philadolphia, USA.

Umarani, V., Muvvala, S., Ramesh, A., Lakshmi, B.V., Sravanthi, N., 2015. Rutin potentially attenuates fluoride-induced oxidative stress-mediated cardiotoxicity, blood toxicity and dyslipidemia in rats. Toxicol Mech Methods 25, 143-149.

Vani, M.L., Reddy, K.P., 2000. Effects of fluoride accumulation on some enzymes of brain and gastronemius muscle of mice. Fluoride 33, 17-26.

Wu, Q., Liu, L., Miron, A., Klimova, B., Wan, D., Kuca, K., 2016. The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: an overview. Arch Toxicol 90, 1817-1840.