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Protective Efficacy of *Spirulina platensis* Against Cadmium Induced Neurotoxicity in Rats

[1]Rasha T.M. Alam and [2]M.Y. Hendawi

[1]Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, Egypt
[2]Department of Plant Protection, Faculty of Agriculture, Zagazig University, Egypt

Abstract: Cadmium (Cd) is a potent neurotoxic heavy metal, which induces oxidative stress and membrane disturbances in brain. The present study investigate the possible neuroprotective efficacy of *Spirulina platensis* (Sp) against $CdCl_2$ induced changes in the plasma neurochemistry of catecholamine (adrenaline, nor adrenaline, dopamine), serotonin, gamma amino-butyric acid (GABA), activity of acetyl cholinesterase (AChE), DNA fragmentation of the brain cells and the histopathology of the brain tissue in rats. Animals were divided into five groups. Gp.(1) Control, gp.(2) $CdCl_2$ (2mg/kg bwt) S/C injection, gp.(3) *Spirulina* (150 mg/kg bwt) orally, gp(4) Co-treated with both $CdCl_2$ and Sp and gp.(5) Administered $CdCl_2$(2mg/kg bwt), in all gps the treatment is day after day for 30 days except gp.(5) post- treated by Sp for another 30 days. The results indicated that cadmium induce oxidative stress in the brain leading to alterations in the plasma neurochemistry and increase the DNA fragmentation of the brain tissue, *Spirulina platensis* given incombination and post-treatment with Cd significantly reduces pathological alterations in plasma neurochemistry, decrease the degree of DNA fragmentation of the brain cells and capable of reducing the pathological damage of the brain tissue. We concluded that *S.platensis* can significantly modify the brain damages against cadmium chloride induced toxicity.

Key words: Cadmium, *Spirulina* · Neurotoxicity · DNA Fragmentation · Catecholamine · GABA

INTRODUCTION

Cadmium (Cd) is a toxic metal which still attracts the attention of researchers and the public health because its level in the food products may be exceeds the maximum allowable limits. The harmful effects of cadmium in many organs and its exposure associated with nephrotoxicity, osteoporosis, neurotoxicity, carcinogenicity, genotoxicity, teratogenicity, endocrine and reproductive side effects [1, 2]. Exposure to Cd severely affects the function of the nervous system [3,4].

Moreover, exposure to cadmium (0.25&1 mg/kg/day) for 45 days modifies the CNS regional content of acetylcholine (ACh), serotonin (5-HT) and dopamine [5]. Rats daily s/c injected with $CdCl_2$ (0.3 mg/100g bwt) for two weeks in normal and protein restricted (5% casein) groups shows significant decrease in 5-HT concentration in different brain areas [6]. Also cadmium exposed rats (0.5 and 1mg/kgm bwt) decrease dopamine level in the different brain areas [7]. Cadmium increases the serotonin sensibility in the CNS [8]. Associated changes in norepinephrine, dopamine and serotonin content and/or its metabolism in the different brain regions were observed in cadmium exposed rats [9], [10,11]. While GABA content in different brain regions decreased in rats given 50 ppm of cadmium chloride for 1 month [12]. Some studies on Cd toxicity found an association with behavioral disturbances and cholinergic neurotransmission since an increase or a decrease in the acetylcholinesterase activity was verified in both animal models and human that showed behavioral impairments after exposure to Cd [13]. AChE activity in the brain increased in rats administered cadmium (1 mg/kg/day for 14 days/ ip) [14]. Rats exposed to Cd (3mg/kg/day sc) for 3 weeks, a significant increase in the activities of AChE were observed in brain tissue [13]. Cadmium intoxicated

Corresponding Author: Mohamed Yossif Ibrahim Hendawi, Department of plant protection, Faculty of Agriculture, Zagazig University, Egypt. E-mail: Hendawi.m@zu.edu.eg.

rats (5mg/kg/day by gavage) for 4 weeks significantly reduced the AChE levels in the plasma and brain tissue [15]. Also the AChE activity increased in the hippocampus, hypothalamus and cerebellum of chronic Cd-intoxicated rats (25mg/kg) [16].

Spirulina platensis (SP) is a cyanobacterium being used as nutritional supplement for human and animal consumption. *Spirulina platensis* has been labeled as a powerful food, rich in proteins, carbohydrates, polyunsaturated fatty acids, sterols and some vital elements like calcium, iron, zinc, magnesium, manganese and selenium. It is a natural source of vitamin B12, vitamin E, ascorbic acid and β carotene and xanthophyll phytopigments [17,18]. *Spirulina* has been evaluated for various activities including neuroprotection in rodents [19,20]. In addition, several studies have shown that *Spirulina* species exhibit various biological activities such as antioxidant, antibacterial and antiparasitic properties and for several medical conditions such as allergies, ulcers, anemia, heavy-metal poisoning and radiation poisoning [21,22]. Moreover, combined treatment of *Spirulina* with mercuric chloride lead to a significant decrease in MDA content and elevation in LDH and ALP activity, it modify the renal damages against mercuric chloride induced toxicity [23]. Also treatment with *Spirulina platensis* elevate the levels of FT3, FT4 and TSH, *Spirulina* facilitated antioxidant formation, improved behavioral changes and thyroid dysfunction in fluoride toxicity in rats [24].

Since SP has many therapeutic roles including neuroprotective properties, the present study aimed to identify the protection exerted by *Spirulina platensis* against cadmium induced neurotoxicity in the rats.

MATERIALS AND METHODS

Animals: A total of 25 healthy adult male white albino rats (180-200 gm body weight). They were obtained from the Laboratory Animal Housing Unit, Research, Institute, Dokki, Cairo, Egypt. Animals kept under hygienic conditions. They were maintained on basal ration, given water *ad-libitum* and exposed to 12h light-darkness cycle for two weeks of acclimatization before use.

Tested Compounds

Cadmium Chloride ($CdCl_2$): Purchased from El-Faraana Co. for trading, Egypt in the form of white and colorless powder.

Spirulina Platensis *(Sp)* Powder: Was purchased from El Hellowa for Biological products Egypt.

Experimental Design: The rats were assigned into five equal groups each containing 5 rats. Gp.(1) kept as control, gp.(2) was given $CdCl_2$ (2mg/kg bwt) S/C injection, gp.(3) administered Spirulina powder (150 mg/kg bwt) in drinking water, gp.(4) rats co-treated with both Cd and Sp at the same mentioned routes and doses day after day for 30 days in the mentioned groups and gp.(5) administered $CdCl_2$ (2mg/kg bwt) day after day for 30 days with post- treatment by Sp (150 mg/kg bwt) for another 30 days.

Samples Collection and Preparation: At the end of the experiment the animals were sacrificed, followed by collection of blood without anticoagulant then centrifuged at 3000 rpm for 10 min for separation of serum which stored at -20°C until used for biochemical analysis. Brains were dissected out and washed in saline. Brain tissues were homogenized in double distilled water and the homogenates were stored at -80°C until used. Other samples from brain were used for histopathological examination.

Biochemical Analysis: Dopamine, adrenaline, noradrenaline, Serotonin, Acetylcholinesterase activity (mybiosource.co) and GABA (EIAab.co) were measured using an ELISA kit. A diphenylamine colorimetric assay (Rouch.co) was used to quantify DNA fragmentation, the prepared brain homogenate were centrifuged at 300 g for 10 min to separate supernatant from cell pellets. One hundred µl aliquots of supernatants were used for DNA fragmentation assay.

Histopathological Investigation: Specimens from the brain of different groups were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (H&E) dyes [25] and then examined microscopically.

Statistical Analysis: Data of the current study expressed as mean ± standard error was statistically analyzed using the computer program SPSS/PC (2001). The statistical method was one way ANOVA test.

RESULTS

Table (1) depicts the effects of Cd and SP on the plasma catecholamine (adrenaline, nor adrenaline and dopamine) levels, serotonin, GABA, AChE activity in control and experimental rats. In Cd treated rats (gp.2), the catecholamine (adrenaline, nor adrenaline and dopamine) levels significantly (p<0.05) increase when compared with control rats. Administration of *Spirulina* alone (gp.3) produce non significant changes in the catecholamine, while treatment with Sp (gps.4 and 5) effectively attenuated the Cd-induced alterations in levels of catecholamine. A significantly (p<0.05) decrease in the plasma serotonin and GABA levels in gp.(2), while gp.(3) showed non significant changes when compared with control. Treatment with Sp (gps.4 and 5) showed significant increase in the levels of serotonin and GABA when compared with gp.(2). The activities of AChE in the plasma was significantly (p<0.05) increased in gp.(2) and gp.(3) showed non significant changes when compared with control rats, while gps.(4&5) significantly (p<0.05) decrease the activities of AChE to near normal levels when compared with gp.(2).

Figure (1) represents the quantitative measurement DNA fragmentation. In cadmium treated group, indicating significant (p<0.05) increase in the DNA fragmentation, while administration of Sp alone showed non significant changes when compared with control rats. Treatment with Sp (gps. 4 and 5) showed significant (p<0.05) decrease in the DNA fragmentation when compared with gp.(2).

Histological Changes in Brain: Figures (2, 3,4,5,6 &7) illustrate the histopathological assessment of brain tissue of control and experimental animals. Cd intoxicated rats exhibited cerebral hemorrhage (Fig. 3), depletion of granular cell layer (arrowhead) and necrotic changes in the Purkinje cells (Fig. 4) and dilated lateral ventricle, subventricular spongiosis and gliosis (Fig. 5). Administration of Sp alone the brain tissue showing normal neurons and neuronoglias (Fig. 2). Treatment with Sp (gps.4 and 5) the brain tissue showing few degenerated neurons, satellitosis and neuronophagia (Fig. 6) and moderate degenerated neurons, satellitosis and neuronophagia (Fig. 7) respectively.

Table 1: Effect of oral administration of *Spirulina platensis* (150mg/kg) on the plasma levels of some neurological parameters in Cd treated rats (2mg/kg) (M ±SE) (n=5)

Groups	ADPg/ml	NADPg/ml	Dopamineng/ml	Serotonineng/ml	GABAng/ml	AChEu/L
Control (Gp1)	18.10±0.13^{d}	106.88±0.09^{d}	0.47±0.05^{d}	25.96±0.17^{a}	3.06±0.09^{a}	20.68±1.23^{d}
Cd (Gp2)	28.94±0.07^{a}	119.55±0.11^{a}	0.91±0.06^{a}	22.26±0.15^{d}	2.01±0.05^{d}	28.69±1.33^{a}
Spirulina (Gp3)	18.59±0.66^{d}	106.44±0.59^{d}	0.46±0.04^{d}	25.72±0.14^{a}	3.06±0.08^{a}	20.70±0.56^{d}
Treated A (Gp4)	23.25±0.05^{c}	111.00±0.11^{c}	0.68±0.02^{c}	24.43±0.10^{b}	2.66±0.07^{b}	23.89±0.20^{c}
Treated B (Gp5)	26.03±0.11^{b}	115.88±0.38^{b}	0.76±0.01^{b}	22.95±0.08^{c}	2.23±0.06^{c}	25.78±0.27^{b}

Values are mean ± SD for five rats in each group. Values not sharing a common superscript letters (a, b and c) differ significantly at p<0.05

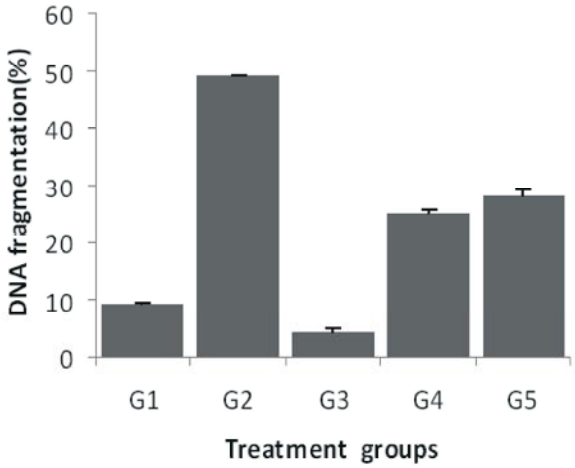

Fig. 1: Effect of oral administration of *Spirulina platensis* on DNA fragmentation in brain tissue of Cd treated rats

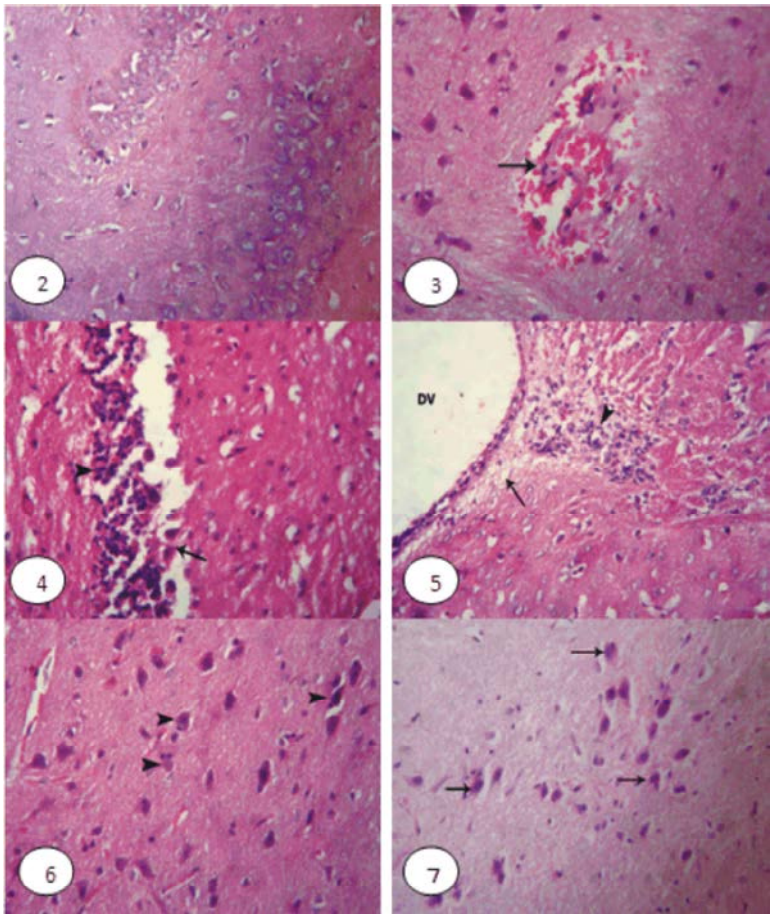

Fig. 2: Brain section of gp. (3) showing normal neurons and neuronoglias
Fig. 3: Brain section of gp. (2) showing cerebral hemorrhage (arrow)
Fig. 4: Brain section of gp. (2) showing depletion of granular cell layer (arrowhead) and necrotic changes in the Purkinje cells (arrow)
Fig. 5: Brain section of gp.(2) showing dilated lateral ventricle, subventricular spongiosis (arrow) and gliosis (arrowhead)
Fig. 6: Brain section of gp.(4) showing few degenerated neurons, satellitosis and neuronophagia (arrows)
Fig. 7: Brain section of gp.(5) showing moderate degenerated neurons, satellitosis and neuronophagia (arrows)

DISCUSSION

Cadmium (Cd) is an important environmental contaminant due to its extensive use in phosphate fertilizers, electrochemical industry, aircraft electroplating, nuclear fusion, laboratory uses and in everyday life products [26], Cd is able to induce neurotoxicity including neurological disturbances with changes in the normal neurochemistry of the brain [27]. Cadmium exert toxic effects on the central nervous system (CNS) [28].

In the present study, the plasma catecholamine (adrenaline, nor adrenaline and dopamine) levels increased in rats treated with cadmium (gp.2), it has been suggested that cadmium produce alteration in the plasma neurochemistry due to oxidative damage to the brain tissue which may be responsible for its toxic action [29,30]. Also Cd induce lipid peroxidation by stimulating the production of superoxide anions [31] which act as oxidative stress, this process can damage cells directly by destroying their membranes and indirectly by producing reactive carbonyl products [32]. The higher lipid peroxidation induced by Cd indicates the membrane damage resulting in neuronal dysfunction leading to decrease the uptake of catecholamine in brain synaptosomes [33] and increase it in the plasma. Our results are partially agreement with [5] who reported that chronic treatment with low doses of cadmium significantly increased the level of striatal dopamine in rats; also [34]

found that diabetes enhances the effect of cadmium elevating the levels of dopamine. Discrepancies with other authors [10,33] could be due to differences in age of animals during cadmium exposure, dose, route and treatment duration.

On the other hand, serotonin (5-HT) is chiefly present in the hypothalamus, midbrain and limbic system of the brain [35,36], along with a parallel distribution of tryptophan hydroxylase [37]. The plasma serotonin levels significantly decreased in gp.(2) which may be attributed to the non uniform accumulation of Cd in the brain leading to inhibition of a number of sulfhydryl (-SH) groups containing enzymes [38], that affect the central monoaminergic neurotransmitter system [39], Since the permeability of the blood brain barrier of the median eminence region alters rapidly, it allows more Cd to enter, which greatly decreases hypothalamic 5-HT level [40]. Also the cadmium treatment significantly decreases the concentration of 5-HT in most of the discrete brain areas, due to interferes with the function of tryptophan hydroxylase which plays an important role during conversion of tryptophan to 5-HT utilizing oxygen by inhibiting the oxidative metabolism of the serotonergic neurons [41]. Our results are partially agreement with Das *et al*. [6] who reported that rats treated with $CdCl_2$ shows significant decrease in 5-HT concentration in different brain areas which lead to decrease the plasma level of serotonin. The present results are disagreement with Herba *et al*. [8] who reported that cadmium increase the serotonin level in the brain, the difference may be due to change in dose or duration and behavior response of the animal models.

GABA is considered as the main inhibitory neurotransmitter in the central nervous system [42]. GABA has been associated with the development of neurodegenerative diseases [43]. This results show that rats treated with cadmium significantly decrease the GABA level in the plasma which may be attributed to decrease the GABA content in the different brain areas (the anterior and mediobasal hypothalamus and median eminence [12]. Our data partially agree with Lafuente *et al*. [44] who reported reduction in the GABA content in posterior hypothalamus and prefrontal cortex in rats exposed to cadmium. Also Soreq and Seidman [45] reported that rats exposed to Cd significantly decrease the GABA content in the anterior and mediobasal hypothalamus.

The study of brain enzyme activities, such as AChE is a key enzyme in detecting the neurotoxic effect of certain heavy metals. AChE activity is often considered a biomarker for the assessment of Cd-induced neurotoxicity [16]. This enzyme hydrolyses the neurotransmitter acetylcholine (ACh) in the synaptic cleft of cholinergic synapses and neuromuscular junctions [46]. Cadmium significantly increase the AChE activity, which may be attributed to the mechanism of action of Cd on AChE enzyme which has been hypothesized to be either the displacement of metal cofactors from the active site or the direct deactivation of the enzyme site [47]. Moreover, Cd can leads to alteration of the structural integrity of lipids and affects membrane-bound enzymes such as AChE, thus, alterations in the lipid membrane by oxidative stress could be a decisive factor in the modification of the AChE molecule, which would explain the changes in their activity [48,49]. However, results are controversial and the activation as well as the inhibition of the AChE activity has been reported. Our results are partially agreement with Carageorgiou et al.,[50] who reported that the activity of AChE in rat brain synaptosomal plasma membranes showed a considerable decrease after 6 h of Cd exposure, followed by a progressive increase up to 24 h. However, an increase in the AChE activity was found in brain of rats exposed to 1 mg/kg Cd i.p for 14 days or i.m for 4 months [15, 51]. While our results are disagreement with [52] who reported that decreased AChE activity was found in hippocampus, cerebellum and hypothalamus of rats intoxicated with 2 mg/kg Cd by gavage, the difference may be due to the change in the route of administration. Shagirtha et al., [15] also reported decrease the level of AChE in plasma and brain in Cd exposed rats (5mg/kg /orally), the difference may be due to large dose of Cd or different route of administration leading to different response of the rats AChE activity.

Recent studies have shown that cadmium produces ROS, resulting in an increased lipid peroxidation, depletion of sulphydryls, altered calcium homeostasis, impairment of antioxidant defenses and finally DNA damage [53]. Under the present experimental conditions, it has been observed that cadmium toxicity significantly increase the DNA fragmentation of the brain tissue, which may attributed to the ability of accumulated Cd in the brain to damage the brain cells due to increased lipid peroxidation [55], also cadmium toxicity increased the extent of DNA fragmentation which may be regarded as an indicator of increased ROS production during toxin exposure period [16]. The previous results are confirmed with histopathological examination of the brain tissue of cadmium-treated rats (gp.2). Cadmium induced neurotoxicity which characterized by cerebral hemorrhages scattered on the brain tissue (Fig. 3).

Depletion of the granular cells in the cerebellum, besides some necrotic changes in the Purkinje cells (Fig. 4). Encephalomalacia, degenerated neurons, satellitosis and neuronophagia, sever perivascular and perineural edema besides dilated ventricles were visualized in addition to subventricular spongiosis and intense gliosis (Fig. 5). Our results are agreement with Marles *et al*. [56] who reported nuclear condensation of chromatin, shrunken Purkinje cells and interstitial edema in molecular layer in rats treated with cadmium. Also Goncalves *et al*. [16] who reported that the brain tissue reveals that cadmium intoxication caused abnormal ultra structural changes in the brain tissue including spongiform necrosis, nuclear vacuolization pyknosis and lymphocytic inflammatory changes.

In the present study, *Spirulina Platensis* alone did not cause any side effects or brain toxicity. Rats treated with SP (gp.3) showed non significant changes in the plasma neurochemistry (adrenaline, noradrenalin, dopamine, serotonin, GABA and AChE activity), it also protect the normal cells from damage by decreasing the degree of DNA fragmentation in the brain cells when compared with the control. The protective and improving effects of *Spirulina Platensis* on brain tissue may be due to it has various components, such as B-complex vitamins, chlorophyll, carotene, vitamin E, superoxide dismutase and numerous minerals, which involved in tissue protection when ingested [57]. Our results are confirmed by the histopathological examination of the brain tissue which revealed no significant difference has been observed between control and Spirulina treated groups, (gp.3) which showed nearly normal histoarchitecture (normal neurons and neuronoglias, Fig. 2. *Spirulina Platensis* was remarkably effective in reducing the incidence of neurotoxicity and DNA fragmentation in the brain caused by oxidative stress induced by Cd which disrupts the integrity of the neuronal cell membrane leading to alteration in the plasma neurochemistry suggesting its potential therapeutic effect in our model. Our research team has already reported that rats treated with Cd in combination and post treated with *Spirulina Platensis* (gps.4 and 5) showed significant decrease in the plasma catecholamine (adrenaline, noradrenalin and dopamine) when compared with Cd treated group (gp.2). This may be attributed to the SP prevented cadmium from reaching a sufficient concentration in the brain, so decrease its damage and decrease its toxic effect. The mechanism of this protection seems to be based on the complex molecular structure which contains many antioxidants acting on the oxidative stress. β-carotene of *Spirulina* may scavenge free radicals generated by Cd and reduces the lipid peroxidation. The antioxidant mechanism of β-carotene has been suggested to be singlet oxygen quenching, free radical scavenging and chain breaking during lipid peroxidation [58,59].

Treatment with SP (gps.4 and 5) significantly improved the plasma levels of serotonin and GABA and restored the activity of AChE when compared with gp.(2). An important aspect of *Spirulena*'s role in neuroprotection is due to the inhibition of radical formation through its anti-inflammatory effects. Selenium present in *Spirulina*-induced selenium containing enzyme GSH peroxidase, proteins or compounds such as selenodiglutathione, selenocysteine and dimethylselenide, which are known to modulate the toxic effects of heavy metals [23]. The cyanobacteria Sp is an excellent source of phycocyanin [60] capable of exerting strong antioxidant action, which may be the cause of reducing the lipid peroxidation and reduces the neurotoxicity in rats by efficiently interacts with various reactive oxygen and nitrogen species. *Spirulina* may be counteracted the effects of Cd on the brain tissue by its powerful antioxidant nature and by facilitating the displacement of Cd, it reducing their accumulation in the body. The ability of *Spirulina* to prevent peroxidation of lipids could be linked to the presence of antioxidants (β carotene and vitamin C, E). Chlorophyll and its derivatives scavenge free radicals [61], so the presence of chlorophyll in *Spirulina* can contribute to the antioxidant action. Moreover *Spirulina platensis* contains a-lipoic acid, riboflavin, xanthophyll phytopigments, SOD enzyme, selenium, magnesium and manganese, which can contribute to the antioxidant effect [62, 63].

Spirulina platensis in combination and post treatment in Cd toxicated rats (gps.4&5) decrease the degree of the DNA fragmentation when compared with gp.(2) may be due to decreasing the cell damage and protecting the cells by its powerful antioxidant activity. The present results are agreement with [64] who reported that β carotene of *Spirulina* may reduce cell damage, especially the DNA molecules. Such an inhibitory effect may be attributed to the repair of carcinogen- damaged DNA and SP has been suggested as an efficient radical scavenger [65,66]. Other studies have reported that the unique polysaccharides of SP enhance cell nucleus enzyme activity and potentiate the process of DNA repair [67, 68].

The previous results of the treated groups with *spirulina platensis* were confirmed with histopathological examination of the brain cells which showed that the lesions caused by Cd in the brain in gp.(2) were generally alleviated in gps.(4&5). Gp. `(4) showed few degenerated neurons, satellitosis and neuronophagia (Fig. 6). While gp.(5) revealed slight improvement with moderate degenerated neurons, satellitosis and neuronophagia (Fig 7). Our results are partially agreement with Banji *et al*. [24] who reported that Co-supplementation with *Spirulina* restored the structural features of the cerebellar neurons (improvement in the Purkinje layer and showing restoration in the structure of Purkinje cells and a dense granule layer) in rats exposed to fluoride.

CONCLUSION

The results of the present study demonstrates that *Spirulina platensis* exhibited a significant protective effect against cadmium chloride induced neurotoxicity in rats by increasing the endogenous antioxidant defense systems in plasma and brain with subsequent restoration of catecholamine, serotonin, GABA, AChE and DNA fragmentation. Furthermore, *Spirulina platensis* restored the normal histological architecture of the brain. Accordingly it may be suggested that *Spirulina platensis* can serve as a potential therapeutic candidate for the brain injury associated with $CdCl_2$ induced oxidative stress in the brain.

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