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Effect of Some Nutritional Supplement on the Cerebellum against Cyclophosphamide Toxicity

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ABSTRACT

The cerebellum is the largest part of the hindbrain. As an important part of nervous system, it can be affected by oxidative stress. Dietary supplements had been proved that they have antioxidants properties that may protect our bodies against the effects of free radicals. One of these supplements is Aphanizomenon flos-aquae (AFA) that has health-improving effects especially on the nervous system. Cyclophosphamide (CP) is a widely used medication in chemotherapy and can cause oxidative stress. This study was conducted to investigate the role of AFA in preventing cyclophosphamide-induced adverse effects on the cerebellum of treated rats with CP. It is an experimental study carried out in the period from January 2021 to August 2021. It was carried out on 36 rats with body weights of 270-300 g. The animals were divided into three groups. The first is control group, the second one is CP treated group, received one dose of CP at 100 mg/kg-1 BW), and last one is CP +AFA group, received orally extract of AFA after CP injection. The structures of the cerebellum were compared in the different groups histologically. Examined sections showed significant cellular injury in the second group in comparison to the control groups. The third group showed marked improvement in the changes that occurred compared to the second group. These results provide evidence that AFA has a protective effect as they reduced the cellular injuries in the cerebellum induced by cyclophosphamide.

Key words: Cerebellum, Antioxidant, Cyclophosphamide, Rat, AFA, Food supplement

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INTRODUCTION

The cerebellum is responsible for coordinating skilled voluntary movements. Also, it is active precision and motor learning, as well as in timing of movements. It is different from other parts of the brain. It undergoes its major developing time from the 3rd trimester to infant period. [1].

As a result, the cerebellum is highly susceptible to injury especially in young children [2]. The cerebellar cells have capability to generate depends on food consumption and subsequently the amino acids concentration in the bloodstream [3,4].

The cerebellum is important for making postural adjustments in order to maintain balance. One major function of the cerebellum is to coordinate the timing and force of these different muscle groups to produce fluid limb or body movements [5].

AFA (Aphanizomenon flos-aquae) is an algal species gathered every summer in Oregon area one of the United States. It was sold as a food supplement for about 20 years as its nutritional benefits of AFA have been liked by many people [6,7].

Aphanizomenon flos-aquae (AFA) metabolize directly molecular nitrogen in the air and synthesize several

Peptide Groups of Low Molecular Weight. These peptides are considered as neurotransmitters precursors that are used via various brain regions and body to release other substances and influence metabolic functions [6,7]. The AFA had been confirmed to be a good origin of Omega-3 and 6 that build neural fibres in the brain [8].

Cyclophosphamide (CP) is one of the common chemotherapeutic drugs that ceases the malignant cell growth and inhibits the immune system. It has a good application in a variety of malignant and non-malignant tumors [9]. It is used for the treatment of many types of cancers, multiple sclerosis, and other benign tumors. Also, it is used in the treatment of nephrotic syndrome and following an organ transplant [10].

Our aim is to study the protective effect of AFA on the cerebellum of albino rats against the cyclophosphamide-induced hazards effects.

MATERIALS AND METHODS

CP was purchased from Germany (Frankfurt, Baxter Oncology). AFA-Klamath capsules (350 mg) have been bought from Egypt (German Egyptian Pharmaceutical Company) were dissolved in distilled water. It was given orally by gastric tube. The dose was 94.5 mg/kg /B W/day for 30 days.

This study is an experimental study carried out in the period from January 2021 to August 2021.

In this study, 36 healthy, aged 9 weeks, male albino rats (270-300 g.) were used. They were obtained from an animal house at PSA University. They were kept under standard animal housing conditions at the Animal Care Facility. The rats were stayed under supervision for about 2 weeks before the start of the study for adaptation.

The animals were divided into three groups of ten as follows: the first one is a control group. The second group received intraperitoneal one dose of CP (100 mg/kg BW). Before starting our study 100 milligrams of CP injected into four rats to ensure significant histological changes in the cerebellum. The dose was chosen based on previous studies. The last group received AFA extract orally 500 mg/kg per day for 30 days after a cyclophosphamide injection intraperitoneal.

One month later, the rats were sacrificed and after dissection, small pieces from the cerebellum were taken for the histological. Specimens were prepared for fixation in formalin solution. These sections stained with Harris haematoxylin and eosin (Hx&E). After that, some sections stained to detect polysaccharides. For detection of Nissel granules, we used Toluidine blue stain. Programmed cell death and apoptotic changes were detected in the brain of all groups by Caspase-9 immunostaining.

The image J 1.4 analyser was used to take out the morphometric data. For example, carbohydrate content of the neuronal cells using PAS-stained and Caspase-9 immnuostained sections were used.

PAST 3.0 Version of statistical analyses was done via statistical software. The obtained data were expressed as mean ± standard deviation (SD). The $p<0.05$ was significant.

RESULTS

The H&E stain examination of the control group: the cerebellum showed its three layers the molecular layer, the Purkinje cell layer, and the granular layer (Figures 1 and 2).

In the cerebellar white matter, few neurons were found with their nerve fibers and capillaries. Periodic Acid Chief (PAS) showed efficient PAS response on normal neurons.

Toluidine blue stain showed a strong blue stain of Nissel granules in Purkinje cells in the cerebellar cortex. In addition, Immunohistochemical studies of brain sections showed a mild expression of Caspase-9 immunostaining for neuronal cell bodies and glial cells.

The second group treated with cyclophosphamide (CP) stained with Hx. & E. showed distorted Purkinje cells of different shapes.

Either degenerated or with karyolytic nuclei, some purkinje cells appeared the shrinkage of their cytoplasm and nucleus pyknosis, degenerated purkinje cells emerged.

The PAS reactions of the cerebellar cells of this group showed mild PAS reactions in degenerated neurons and moderate reactions in some normal neurons. Toluidine blue stained sections of cerebellar cortex parts displayed a weak reaction to Nissel granules.

Immunohistochemical investigations showed a significantly increased Caspase-9 expression in degenerating neuronal cell bodies, degenerating neurons in comparison to the control groups (Figure 3 and Table 1).

The third group treated with AFA extract stained with Hx. & E. showed an effective preventing of degenerative changes as most of cerebellar cells are almost similar to the first group. The Purkinje cell layer appear with similar to control group.

Most cerebellar medulla neurons have maintained their normal appearance, when the same group stained with Toluidine blue showed marked increase in Toluidine blue stain intensity, showed marked increase in Toluidine blue stain intensity.

With Periodic Acid Schiff stain showed marked increase in carbohydrates content. And, with Caspase-9 immunostaining showed a marked increase in the expression of Caspase-9 (Figure 4 and Figure 5A, 5B)(Table 1).

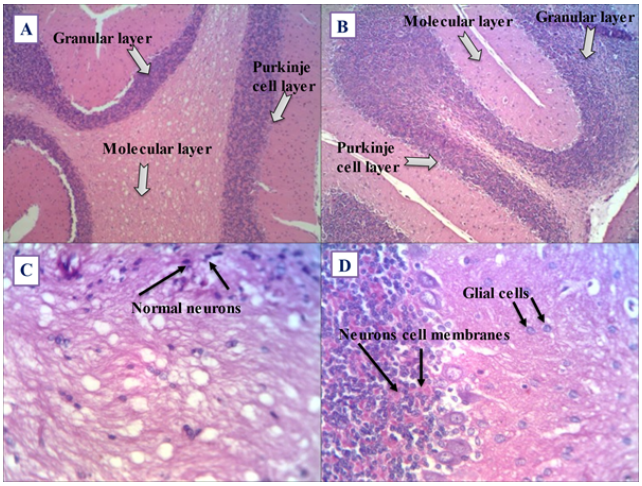

Figure 1: A, B) Hx. &E. of the cerebellum of control group showing all layers (X200). B, C) showing strong PAS +ve reaction in the cerebellar cortex cells and in glial cells(X400).

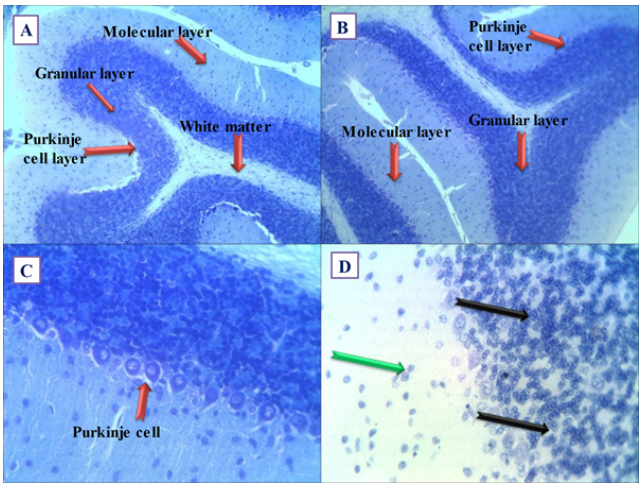

Figure 2: A, B) Toluidine blue stain of the cerebellum of control group showing all layers (X200). C) Toluidine Blue stain of cerebellum of control group showing high cellular content of Nissel granules in the Purkinje cells (X400). D) Caspase 9 immunostaining in neuronal cell bodies of the cerebellar cortexshowing normal neuronal cell bodies (black arrows) in glial cells (green arrow) (X400).

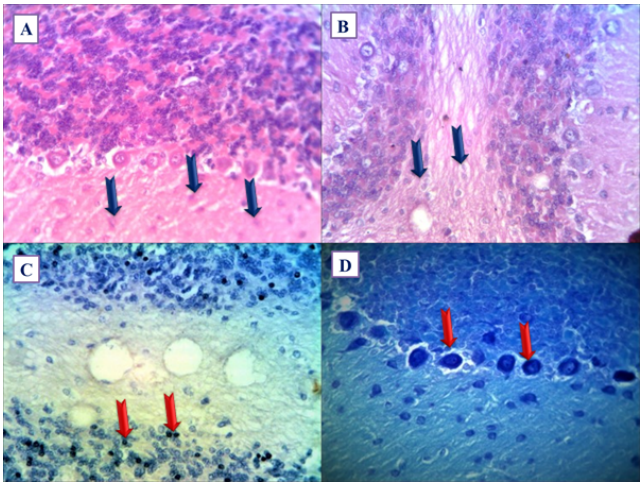

Figure 3: A, B) PAS reaction of some cerebellar cells (blue arrows) and a mild PAS reaction in degenerated neurons of adult rat cerebellar medulla treated with cyclophosphamide (CP). (PAS, X400). C) Toluidine Blue stain of the treated group with CP showing decrease in cellular content of Nissel granules in Purkinje cells as well as degenerated cells (red arrows). D) Caspase-9 immunostaining in degenerating cerebellar cell bodies (red arrows) (X400).

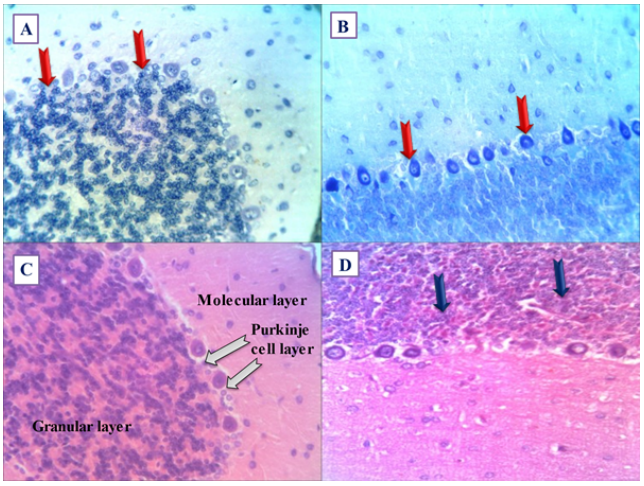

Figure 4: Cerebellar tissue of group treated with AFA showing A) Mild expression of Caspase-9 immunostaining in the cerebllar cortex cells (red arrows) B) Strong basophilic stained cytoplasm of Nissel granules in Purkinje cells (red arrows) with Toluidine blue stain (X400). C) Hx. &E. of the cerebellum of group treated with AFA almost near to control group cells. D) Strong PAS reaction of almost normal neurons (blue arrows) (X400).

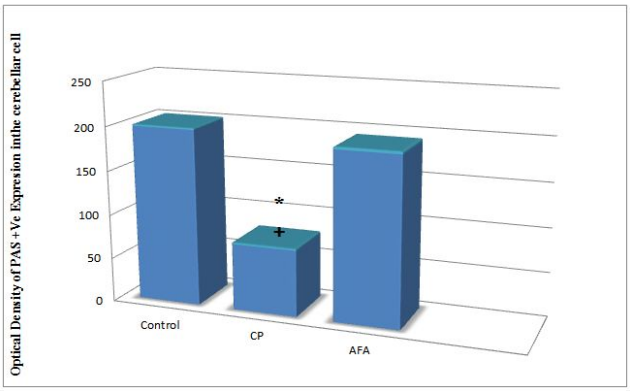

Figure 5A: PAS +ve expression of Optical Density Caspase-9+ve expression.

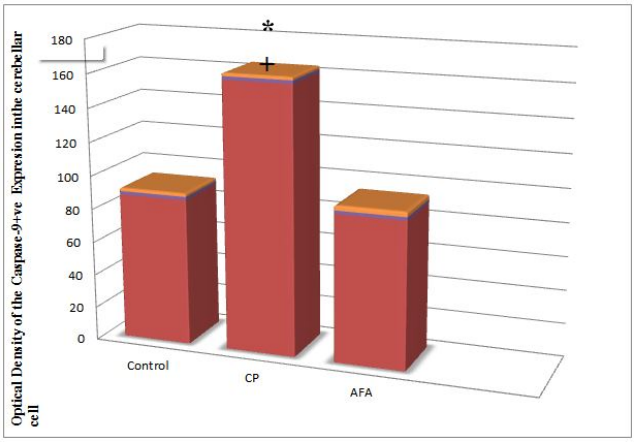

Figure 5B: Cerebellar cells for all groups.

Table 1: PAS+ve and Caspase-9+ve expressions optical density of the cerebellar cells for all groups expressed as mean ± SD. *Significant increase in the parameters levels (P<0.05).

Study Groups	Optical Density of the PAS +ve expression in Cerebellar cells	Optical Density of the Caspase-9 +ve expression in Cerebellar cells
Control group	199.10 ± 43.62	87.6 ± 11.15
CP group	75.30 ± 14.03	159.6 ± 26.67*
AFA group	189.50 ± 24.51	88.00 ± 27.48

DISCUSSION

Cerebellar cells are particularly at risk of destruction by induced free radicals due to high amount of iron. On the other hand; the brain has a relatively weak antioxidant defence mechanisms [11].

The aim of our research was to evaluate the protective effect of AFA on the cerebellum of albino rats against the cyclophosphamide-induced hazards effects. Our study demonstrated that normal overall structure of the cerebellum of control rats. It was harmonic with previous study [12].

Some laboratory studies attributed this effect to the increased ROS production associated with C.N.S complications such as cerebrovascular complications, reduced cerebral blood flow, blood-brain barrier disturbance and cerebral oedema [13,14]. Because neurons need relatively large amounts of oxygen due to their high metabolic rate [15].

Oxygen depletion may contribute to the degenerative changes found in the group treated with CP. Additionally, AFA can protect Cerebellum via production of peptides of low molecular weight that initiate secretion of other substances (such as hormones) and influence metabolic functions [16].

In our study, the medulla of cerebellum of rats treated with CP showed a weak PAS reaction, indicating a decrease in the quantity of mucopolysaccharides in their cytoplasm. Some studies proved this before the glycogen content in the whole brain was proved to decline after lithium administration [17].

Vulnerability of the brain to oxidative stress produced by ROS is due to that it utilizes about one fifth of the total oxygen demand of the body and its relatively poor in antioxidant enzymes content [18].

Some previous studies proved this effect to the increased ROS production accompanied by C.N.S problems like cerebrovascular hazards, decreased cerebral blood flow and brain oedema [19]. In another study on the cerebellum disclosed a weak reaction of PAS, suggesting a reduce in the amount of mucopolysaccharides in their cytoplasm [17].

Some other studies proved that the antioxidant activity of AFA extract due to the synergic effect of all its various components. They also found that the AFA extract has a beneficial effect on neurons in which toxicity was induced by neurodegenerative agents [20,21].

CONCLUSION

Our study results support these pervious researches as it showed remarkable effects of AFA extract against the degenerative adverse effects of CP on cerebellum. Consequently, these results give indicator that AFA supplement may be used as a protective effective method against hazards of anticancer drugs as cyclophosphamide.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A. Ali, Abdulrahman M. Alkassar Alanazi and Shaban Ragab Ibrahim participated in the study design and wrote the first draft of the manuscript. Hamad Mesfer H. Alatif, Abdulrahman M. Almalki, and and Bandar Suliman S Alsultan collected and processed the samples. Bakheet Mulfi S Alrashdi and Yousef K. Alhuzaimi participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

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CONFLICTS OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AVAILABILITY OF DATA AND MATERIALS

The data are available upon request from the authors.

ETHICS APPROVAL

All series of steps that were implemented in this study that included animal models were in compliance with Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (PSAU-2020 ANT 4/42PI).

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