

Effect Of Astaxanthin On Rat Brains Against Oxidative Stress Induced By Cadmium:Biochemical, Histopathological Evaluation

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ABSTRACT: Aim of this study is to evaluate protective impact of Astaxanthin (AST) on rats with experimental brain injury induced with Cadmium (Cd). 32 male Wistar albino rats were divided into four groups as Control, Cadmium, Astaxanthin (AST), Cadmium (Cd)+Astaxanthin (AST). Rat brain tissues were obtained at the end of 30th day. Malondialdehyde (MDA), glutathione (GSH) levels and superoxide dismutase (SOD) enzyme activities were measured in brain homogenates and histopathological examination was performed. MDA levels were improvement in cadmium administered group ($p<0.01$) as well as Cd+AST administered group ($p<0.05$) compared to control group. In addition a substantial reduction Cd+AST group was observed compared to cadmium administered group ($p<0.01$). GSH level shows a decrease in Cd and Cd+AST groups compared to control ($p<0.05$). SOD enzyme activity was found significantly lower in Cd and Cd+AST groups compared to control ($p<0.01$). In addition, increase of SOD in Cd+AST group compared to cadmium group was also found significant ($p<0.05$). Histopathological findings in the cerebral cortex and hippocampus were different between groups. In the control and AST administered groups, normal histological structure was observed in the brain, while severe lesions were seen in the Cd administered group and in the Cd+AST group only mild degenerative lesions were observed.

As a result, elevated MDA level due to Cd administration was attenuated with AST administration. Decreased GSH level and SOD enzyme activity due to Cd administration was increased with AST administration. In addition, AST administration decreased histopathological lesions. Consequently, it is thought that AST may be used for protection against brain oxidative damage due to Cd.

Keywords: Astaxanthin, cadmium, oxidative stress.

Kadmiyum ile Oksidatif Strese Maruz Kalan Sıçanların Beyin Dokusunda Astaksantin Etkisi;Biyokimyasal, Histopatolojik Değerlendirme

ÖZET: Bu çalışmada, ratlarda kadmiyum (Cd) ile meydana gelen beyin hasarını önlemede astaksantin (AST), koruyucu etkisinin değerlendirilmesi amaçlandı. 32 adet Wistar albino cinsi rat kontrol, Kadmiyum (Cd), Astaksantin (AST), Kadmiyum (Cd)+Astaksantin (AST), olmak üzere dört gruba ayrıldı. 30. Günün sonunda rat beyin dokuları alındı. Beyin doku homojenatında malondialdehit (MDA), Glutasyon (GSH) seviyeleri ve süperoksitdismutaz (SOD) enzim aktivitesi ölçülerek, histopatolojik inceleme yapıldı. Beyin dokusu MDA, düzeyleri değerlendirildiğinde kontrol grubu ile kıyaslandığında Cd uygulaması yapılan grupta ($p<0,01$) ve yine Cd+AST uygulanan grupta artış ($p<0,05$) olduğu belirlendi. Cd uygulaması yapılan gruba oranla Cd+AST grubunda görülen azalmanın istatistiksel olarak önemli olduğu belirlenmiştir ($p<0,01$). GSH düzeyleri değerlendirildiğinde, Cd ve Cd+AST grupları, kontrol grubu ile kıyaslandığında gruplar arasında istatistiksel olarak azalışın olduğu belirlendi ($p<0,05$). SOD enzim aktivitesi değerlendirildiğinde ise Cd ve Cd+AST gruplarında, kontrol grubuna kıyasla görülen azalışın ($p<0,01$) istatistiksel olarak önemli olduğu, Cd grubuna oranla, Cd+AST uygulaması yapılan grupta SOD düzeyinde görülen artışın önemli olduğu belirlendi ($p<0,05$). Histopatolojik bulgular değerlendirildiğinde, Serebral korteks ve hipokampusta histopatolojik bulgular gruplar arasında farklılık gösterdi. Kontrol ve AST uygulanan gruplarda beyinde normal histolojik yapı gözlenirken, Cd uygulanan grupta ciddi lezyonlar görüldü. Cd+AST grubunda hafif dejeneratif lezyonlar gözlemlendi. Sonuç olarak, Cd uygulamasına bağlı olarak artan MDA düzeyi, AST uygulaması ile azaldı. Cd uygulaması ile azalan GSH düzeyi ve SOD enzim aktivitesi AST uygulaması ile arttı. Aynı zamanda AST uygulamasının histopatolojik lezyonları azalttığı gözlemlendi. Bundan dolayı, AST'nin Cd kaynaklı beyin oksidatif hasara karşı koruyucu olarak kullanılabileceği düşünülmektedir.

Anahtar Kelimeler: Astaksantin, Kadmiyum, Oksidatif stres.

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INTRODUCTION

Cadmium(Cd) is a serious environmental pollutant that is very toxic to humans and animals, even in very small quantities (Singh et al., 2013). This metal is mainly used in agriculture, plastic stabilization, pigments and manufacturing of nickel-Cd batteries (Alaee et al., 2014). Cd can be found in certain drinks, fish, meat, milk, eggs, grains as well as in cigarette. Therefore, important sources of Cd intake are via food or cigarette (Yadav et al., 2010). Prolonged exposure to Cd causes toxic effects on cardiovascular, immune/hematopoietic, skeletal, endocrine and reproductive systems and also kidneys, liver, brain and lungs (Fowler, 2009; Satarug et al., 2010). Cd accumulation causes serious toxic effects in brain (Karoui-Kharrat et al., 2017) as well as other neurological disorders such as hyperactivity, memory loss and learning toughness (Hart et al., 1989; Ciesielski et al., 2012; Szkup et al., 2012). Since brain has lower activity in enzymatic defense systems match other organs it is indefensible oxidative stress (Shagirtha et al., 2016). Astaxanthin (3,30-dihydroxy- β , β -carotene-4,40-dion) is one of the xanthophyll carotenoids found mostly in microalgae, fungi, complex plants, seafood, flamingos and quails (Hwang et al., 2017). Astaxanthin has some a wide range of biological activities such as including antioxidant, anti-inflammatory, anticancer and cardioprotective activities (Wu et al., 2012). Astaxanthin has a strong antioxidant property (Zuluaga et al., 2017). Therefore, In this study, it was aimed to investigate the protective effect of astaxanthin against rat brain injury exposed to cadmium in rats.

MATERIAL AND METHODS

Experimental Animals

32 (250 -300 gr) Male Wistar albino rat was obtained from Bingöl University Experimental Research Facility. Study was performed after acceptance of protocols by **BUHADYEK** (Date:04.04.2017/2017/04, Decision:04/02). 32 rats were divided into 4 groups including 8 animals per group. Control group (n=8): Oral isotonic saline was administered these animals. Cd group (n=8):40 mg/L CdCl₂ was administered as orally (Amara et al., 2011). AST group (n=8):AST was given at 20mg/kg dose

with gastric gauge (Otton et al., 2010). Cd+AST(n=8): Rats were administered with CdCl₂:40 mg/L day, orally and AST 20 mg/kg/day. At the end of 30th day animals were anesthetized with i.p.60 mg/kg ketamine hydrochloride, i.p.10 mg/kg xylazine and brain tissues were obtained. Brain tissues were washed with saline and after removing excessive water they were kept in deep freezer (-80°C) until analysis. Tissue sections were obtained in 10 % formaldehyde prepared for histopathological analysis.

Preparation of Tissue Extract and Biochemical Assays

Tissue extract was prepared according to method used by Xia (Xia et al., 1994). MDA levels was done according to method used by Ohkava (Ohkava et al., 1979). GSH concentration was measured according to Tietz (Tietz, 1969) and Fairbans et al., (1999). SOD enzyme activity is detected according to modified method by Sun et al. (Sun et al., 1988). The total protein concentration in the tissue homogenate was according to Lowry (1951).

Histopathological Analysis of The Brain

After treatment, eight rats in each group were anaesthetized with 60 mg/kg ketamine i.p.10 mg/kg Xylazine, euthanized by decapitation under deep anesthesia and necropsied. After that the cerebrum were taken out and isolated immediately. Also, brain tissue samples were fixed in 10 % neutral-buffered formalin for 48h. The tissue samples were processed as routine protocol of dehydrated with alcohol series (50 %, 70 %, 96 % and 100 %) cleared in xylene and then embedded in paraffin. And then paraffin embedded tissue samples sectioned at 5 μ m thickness by rotary microtome (Leica, RM2125) (Anderson et al., 2002). The slides were stained with hematoxylin and eosin for histological examination (Lilli, 2008). Finally the slides were examined and photographed by using light microscope with imaging system (Leica, DM2500/DFC295).

Statistical Analysis

Results of statistical analysis regarding biochemical values (MDA, GSH, SOD) are presented as $X \pm SEM$. Statistical significance of difference between means was evaluated with one-way Analysis of variance. Analysis of data was performed with SPSS for Windows version 11.5.

RESULTS AND DISCUSSION

Brain MDA levels show that increase in Cd administered group ($p<0.01$) as well as increase in Cd+AST administered group ($p<0.05$) was found significant compared to control. In addition decrease in Cd+AST groups compared to Cd groups was also

found significant ($p<0.01$). GSH levels shows a decrease in Cd and Cd+AST groups match control ($p<0.05$). SOD enzyme activity was found significantly lower in cadmium and Cd+AST groups match control ($p<0.01$). In addition increase of SOD in Cd+AST groups match Cd groups was also found significant ($p<0.05$) (Table 1).

Table 1. Results of MDA, GSH levels and SOD enzyme activity

Groups	MDA(nmol/mg protein)	GSH(μ mol/mg protein)	SOD(IU/ mg protein)
Cont	3.45 \pm 0.13* [#]	4.96 \pm 0.12 ^{###}	8.04 \pm 0.63* ^{**}
Cd	8.15 \pm 0.60* ^{**}	1.18 \pm 0.04 [#]	5.06 \pm 0.38* [#]
AST	3.80 \pm 0.16	3.88 \pm 0.19	7.56 \pm 0.74
Cd + AST	6.20 \pm 0.90 ^{#**}	2.85 \pm 0.20 ^{##}	6.79 \pm 0.59* ^{**#}

Values are expressed as mean \pm S.E.M *^{*},** $p<0,01$, ^{###} $p<0,05$

In control groups, no significant histopathological differences were noted and the cerebral cortex and hippocampus of the brain tissues were in normal histological structure (Figure 1 –A, E). In the Cd (Cadmium) administered group in brain, perineural vacuolation, neuronophagia, severe congestion of blood vessels and satellitosis were found most frequently in cerebral cortex (Figure 1-B) and severe pyramidal neuron loss was observed in the hippocampus (Figure 1-F). Common lesion in the cerebral cortex are as follows; necrosis of some of pyramidal neurons with pyknotic nuclei, neuronal shrinkage and mild microgliosis (Figure 1-B). Mild congestion of blood vessels, slightly neuronal shrinkage and mild microgliosis findings were observed in the Cd+AST administered group in cerebral cortex of brain tissues (Figure 1-C). It is also identified that there was mild pyramidal neuron loss in the hippocampus (Figure 1-G). In addition, no

significant histopathological evidences are observed in AST in cerebral cortex and hippocampus in the section of rat brain, which is similar to control groups (Figure 1 – D, H).

Control groups (Group-1), normal histological structure of cerebral cortex (A), and hippocampus (star) (E). Cd administered group (Groups-2), perineural vacuolation, neuronophagia, satellitosis, and neuronal shrinkage (arrow heads); severe congestion of blood vessels (arrows) in cerebral cortex (B), and severe pyramidal neuron loss (star) in hippocampus (F). Cd + AST administered group (Groups-3), slightly neuronal shrinkage and mild microgliosis (arrow heads); mild congestion of blood vessel (arrow) in cerebral cortex (C), and mild pyramidal neuron loss (star) in hippocampus (G). AST administered groups (Groups-4), normal histological structure of cerebral cortex (D), and hippocampus (star) (H).

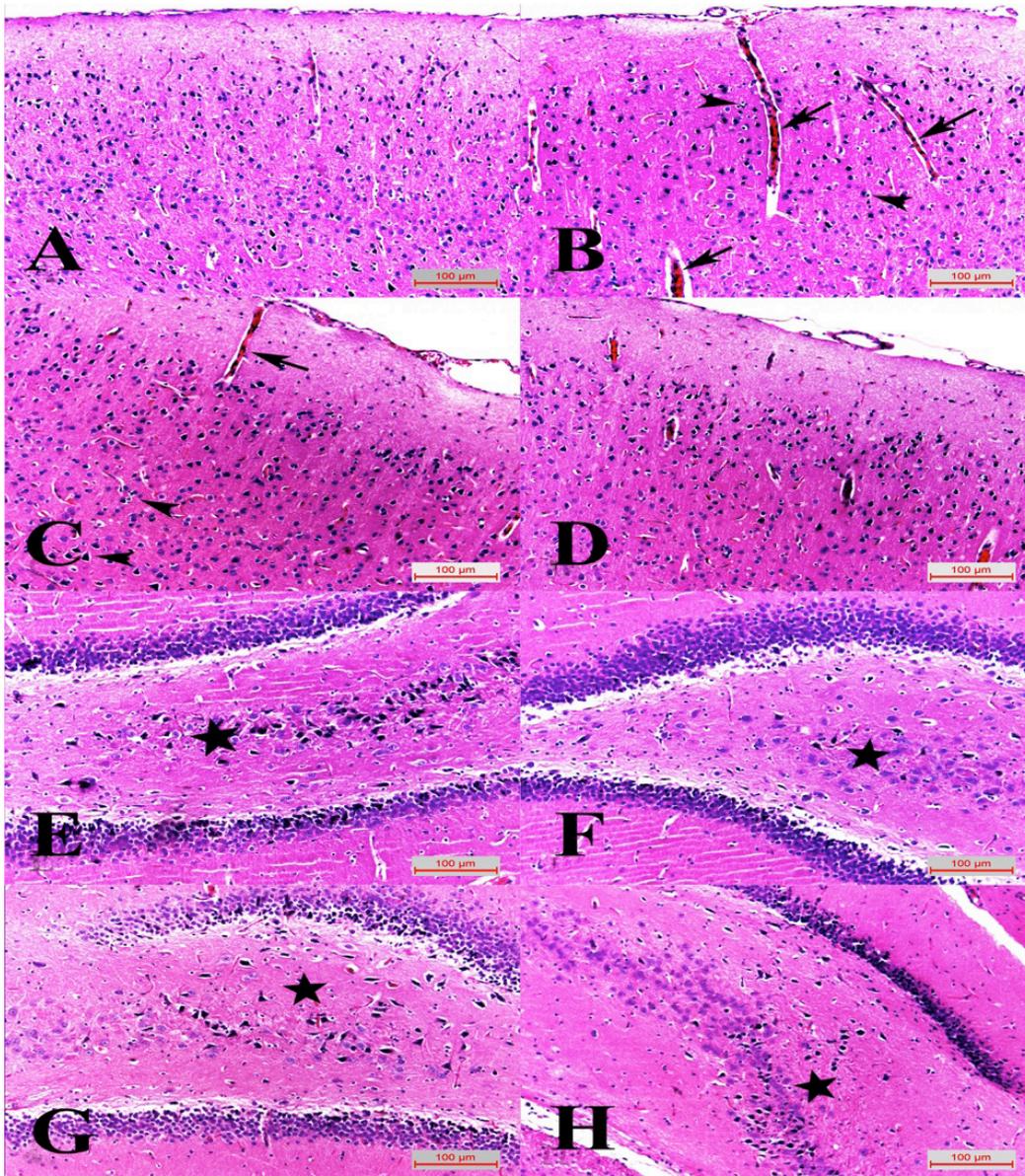


Figure 1. Histopathology of cortex and hippocampus of brain tissues; magnification x10, H&E staining

AST is a xanthophyll carotene which is found in certain microorganisms and algae and sea animals mainly crustaceans (Choi et al., 2011). It has antioxidant, anti-inflammatory, anticancer and immune modulatory properties (Zhang et al., 2014). One of the biggest heavy metal effects is oxidative stress (Görka et al., 2015). Oxidative stress can be explained as the alterations due to irrationality between oxidant and antioxidant agents found in cell. Free oxygen radicals in cells increase with presence of oxidative stress (Evans, 1993). Cd which is a heavy metal, is found in soil, air and water. It is a member of IIb in periodical table (Stoepler et

al., 1991). It is known that Cd which is a highly toxic environmental pollutant causes generation of ROS such as hydroxyl radical, superoxide anions, nitric oxide and hydrogen peroxide (Stohs et al., 2001). Tissue detriment caused by generation of ROS may trigger some defense mechanisms. Initial defense mechanism contain antioxidants such as SOD and GSH (Cetin et al., 2001; Gürlek et al., 2006). GSH and SOD which are among the most significant antioxidants against free radicals prevent lipid peroxidation (Seyhan and Canseven., 2006; Suzer et al., 2000). In this study lipid peroxidation was done with MDA which arises after

detriment of lipid structures found in cell membranes. In our study brain MDA level rise significantly in Cd group compared to control as expected. In this experimental setup AST administration decreased MDA level which is evident Cd+AST group compared to Cd group. In a study oxidative stress triggered with Cd, protective role of α -tocopherol blood, liver and brain tissue was investigated. They found an rise in brain MDA level in Cd group compared to control whereas a reduce in Cd + α -tocopherol group compared to Cd group (Nemliche et al., 2007). In another study, brain damage caused by Cd was tried to be alleviated with ferulic acid. They found a significantly increase in MDA levels in Cd group compared to control (Adefegha et al., 2016). Rise in MDA level is an indicator of augmented lipid peroxidation and oxidative stress. Our findings are in parallel with results of other studies in this subject. GSH levels shows a decrease in Cd group compared to control and an amelioration in Cd+AST group compared to Cd group. In a study protective impact of melatonin on neurotoxicity and oxidative stress induced by Cd was evaluated. GSH level was found lower in Cd compared to control and an amelioration of this level in Cd+melatonin group was found (Shagirtha et al., 2011). In another study investigating oxidative stress by Cd in rat brain and testis tissue and protective effect of b-carotene, GSH levels were found lower in Cd group (El-Missiry and Shalaby, 2000). SOD enzyme activity was attenuated in Cd and Cd+AST groups compared to control significantly. In addition SOD level rise significantly in Cd+AST groups compared to cadmium administrated group significantly.

For GSH and SOD parameters our results are in accordance with existing literature. Experimental studies in the central nervous system of newborn rats and rabbits subject to high doses of Cd, have shown several hemorrhages in the cerebral cortex, pyramidal cells with neuroglial cells with cytolysis (Gabbiani et al., 1967). It has been found in a study that central nervous system detriment in newborn rats generation by low doses of cadmium; brain cortex, cerebellum showed extensive necrosis and hemorrhage (Wong and Klaassen, 1982). It is reported that in the necrotic areas of the brain cortex (pyknosis and interstitial edema), slightly damaged vascular endothelium in cerebral cortex were shown

after cadmium administration (Mendez et al., 2001; Mendez and Rios, 2007). Yoshida (Yoshida, 2001) has indicated that implement of Cd primarily impact the integrity and permeability of the vascular endothelium and necrotic vary in nerve cells occur secondarily to this impact. It is reported that, Cd can rise permeability of the blood brain barrier in rats (Shuka and Chandra, 1987) penetrate and collect in the brain of improve and grown rats (Mendez and Rios, 2007; Goncalves et al., 2010) leading to brain intracellular collection, cellular dysfunction and cerebral edema. One report of a 2-year-old, boy with abruptly death exhibition the exist of Cd and histological proof of distinct cerebral edema with perivascular protein infiltrate, indicating blood-brain barrier disruption (Provias et al., 1994). It has been shown that histopathologically, the structure in the cortex was obviously disorganized and Cd may have caused brain detriment, and the cellular distribution across cortex, and infarction in hippocampus caused the abnormal appearance in mice (Chen et al., 2014). In our study, in the Cd administered group in brain, perineural vacuolation, neuronophagia, severe congestion of blood vessels and satellitosis were found most frequently in cerebral cortex (Figure1-B) and severe pyramidal neuron loss was observed in the hippocampus (Figure1-F). Common lesion in the cerebral cortex are as the following; necrosis of some of pyramidal neurons with pyknotic nuclei, neuronal shrinkage and mild microgliosis (Figure1-B). These findings are similar to most of Cd neurotoxicity studies. AST can be transported by fat molecules directly to the organs and tissues most needed, such as the brain. (Higuera et al., 2006). In experimental models of neurological disorders, AST-mediated neuroprotection contain anti-oxidative, anti-inflammatory and anti-apoptotic mechanisms. (Shen et al., 2009; Yamagishi et al., 2014). It is also showed that AST improve cell and mitochondrial membrane stability. This study propose that AST may have preventive impression on neurodegenerative diseases, addicted its antioxidant potential and mitochondria preventive, and might be a promising neuroprotective medicinal agent for oxidative stress-related neurodegenerations (Chan et al., 2009; Ikeda et al., 2008; Liu et al., 2009). In our experiment, mild congestion of blood vessels,

slightly neuronal shrinkage and mild microgliosis are the findings that were observed in the Cd+AST administered group in cerebral cortex of brain tissues (Figure1-C). It is also identified that there was mild pyramidal neuron loss in the hippocampus (Figure1-G). In our study, difference observed in histopathological findings in the cerebral cortex and hippocampus with the aid of microscopic examination shows that in the control and AST administered groups, no microscopic findings were recognize, in the brain, while severe lesions were seen in the Cd administered group and only mild degenerative lesions were seen in the Cd+AST group.

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CONCLUSION

As a result elevated MDA level due to Cd administration was attenuated with AST administration. Decreased GSH level and SOD enzyme activity due to Cd administration was increased with AST administration. In addition, AST administration decreased histopathological lesions. Consequently, it is thought that AST may be used for protection against brain oxidative damage due to Cd.

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