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The antioxidant effect of astaxanthin is higher in young mice than aged: a region specific study on brain

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Abstract Astaxanthin is a potential antioxidant which shows neuroprotective property. We aimed to investigate the age-dependent and region-specific antioxidant effects of astaxanthin in mice brain. Animals were divided into 4 groups; treatment young (3 months, $n = 6$) (AY), treatment old (16 months, $n = 6$) (AO), placebo young (3 months, $n = 6$) (PY) and placebo old (16 months, $n = 6$) (PO) groups. Treatment group was given astaxanthin (2 mg/kg/day, body weight), and placebo group was given 100 µl of 0.9 % normal saline orally to the healthy *Swiss albino* mice for 4 weeks. The level of non-enzymatic oxidative markers namely malondialdehyde (MDA); nitric oxide (NO); advanced protein oxidation product (APOP); glutathione (GSH) and the activity of enzymatic antioxidants i.e.; catalase (CAT) and superoxide dismutase (SOD) were determined from the isolated brain regions. Treatment with astaxanthin significantly ($p < 0.05$) reduces the level of MDA, APOP, NO in the cortex, striatum, hypothalamus, hippocampus and cerebellum in both age groups. Astaxanthin markedly ($p < 0.05$) enhances the activity of CAT and SOD enzymes while improves the level of GSH in the brain. Overall, improvement of oxidative markers was significantly greater in the young group than the aged animal. In conclusion, we report that the activity of astaxanthin is age-dependent, higher in young in compared to the aged brain.

Introduction

Aging is an undeniable and unstoppable process. Regular functioning capacity gradually decreases in aging processes. It is well established that oxidative stress increases with aging and makes the brain more vulnerable to neurodegenerative disease (Floyd and Hensley 2002; Mariani et al. 2005). Redox homeostasis theory suggests that cellular redox maintenance system keeps up a balance between oxidant and antioxidant factors in aging cells (Cakatay et al. 2010). Aging activates glial cells and increases the number of abnormal astrocyte cells in the cases of neurodegenerative disease (Mrak and Griffin 2005). Increased level of oxidative stress has been reported in Alzheimer's disease (Padurariu et al. 2013; Wang et al. 2014b), Parkinson's disease (Dias et al. 2013) and Multiple sclerosis (Wang et al. 2014a). Aging-associated oxidative stress causes deficits in memory formation in older individuals since oxidative stress affects synaptic plasticity in neural networks in the hippocampus (Haxaire et al. 2012). Hippocampal neurogeneration is associated with learning and memory processes (Deng et al. 2010; Gould et al. 1997; Kempermann et al. 1997). Age-dependent hippocampal oxidative stress is reported by a recent study in mice (Al-Amin et al. 2014). But stress factors decrease the formation of granule cells in the dentate gyrus of hippocampus (Tanapat et al. 1998).

Antioxidant defense mechanism is weakened in the aged brain and in the cases of neurodegenerative disorders which makes the brain more susceptible to oxidative stress. Antioxidants i.e. superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) are depleted and the level of lipid peroxidation is raised in the Alzheimer's

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brain (Marcus et al. 1998). Reactive Oxygen Species (ROS) from mitochondrial origin are the key components which damages mitochondrial DNA (mtDNA). Previous study reports the deletions of mtDNA in the cortex of Alzheimer's (Horton et al. 1995) and striatum of Parkinson's disease (Ikebe et al. 1990).

Numerous antioxidant defense mechanisms exist in the brain to protect neural cell from oxidative stress. SOD, CAT, GSH, Vitamin- C, Vitamin- E, and heat-shock proteins are the major antioxidants that play vibrant role to counteract ROS and Reactive Nitrogen Species (RNS) (Koutsilieri et al. 2002). SOD neutralizes superoxide anions to H_2O_2 while CAT neutralizes H_2O_2 to water and molecular oxygen.

Application of antioxidant treatment in the study of age-associated changes has received much attention in contemporary aging research. In this context, a powerful and potential antioxidant astaxanthin (CAS number 472-61-7) has shown promising neuroprotective activity. Astaxanthin may cross the blood–brain barrier and it possibly protects neuronal cells from damage (Liu and Osawa 2009). Astaxanthin shows neuroprotective effect (Barros et al. 2014), improves oxidative stress markers by the restoration of GSH, catalase activities, and the reduction MDA and ROS productions (Chan et al. 2009) in the H_2O_2 and 1-methyl-4-phenylpyridinium ion (MPP(+)) – induced oxidative stress model, and improve oxidative stress in neurodegenerative disease model (Chan et al. 2009; Chang et al. 2010), relieves brain aging by reducing oxidative stress in rats (Wu et al. 2014).

Aim of the study

Oxidative stress is a major player in declining age-dependent neurodegenerative diseases. Young animals fight aging in a different way than the elderly at the molecular, structural, functional and cognitive levels (Villeda et al. 2014) and elderly animals are less capable to counteract oxidative stress compared to the young (Villeda et al. 2014).

The aim of the present study was to investigate the effect of astaxanthin on age-linked oxidative stress in the frontal and parietal cortex, striatum, hypothalamus, hippocampus, and cerebellum. These brain areas are selected because they plays vital role in brain functioning. For example, hippocampus plays a critical role in memory formation that is generally affected during aging process. Two age groups (young and old) were selected to compare the age-dependent effect.

Astaxanthin was chosen since it is (i) 100 times more potent antioxidant than α-tocopherol (Miki 1991), (ii) able to cross the blood brain barrier (Liu and Osawa 2009) and (iii) possesses neuroprotective property (Chan et al. 2009). Thus astaxanthin might relieve age-dependent oxidative stress via its neuroprotective properties in various brain regions.

Materials and methods

Animals

Swiss albino male mice weighing 30–35 g were purchased from the Department of Pharmacy, Jahangirnagar University, and were kept in the animal house of Department of Pharmaceutical Sciences, North South University with maintaining a light/dark cycle of 12 h (6.00 a.m.−6.00 p.m.) and a persistent temperature 23 ± 1 °C. The mice were allowed to fed ad libitum and given free access to drinking water. Animals were grouped in four (i) placebo young (3 months) (PY); (ii) astaxanthin treated young (3 months) (AY); (iii) placebo old (16 months) (PO) and (iv) astaxanthin treated old (16 months) (AO) mice. The experimental procedure was reviewed and approved by the local ethical committee at the North South University, Dhaka, Bangladesh. Animals were handled in accordance with the international principles guiding the usage and handling of experimental animals (United States National Institute for Health Publication, 1985).

Preparation of astaxanthin and placebo

Astaxanthin powder was collected from Pharmaraw Bangladesh. It was dissolved in distilled water at a concentration of 2 mg/20 ml and administered orally at a dose of 2 mg/kg; (Bhuvaneswari et al. 2014). The test sample was administered at single doses (2 mg/kg; per oral) for 4 weeks. The control animals received vehicle 100 μl of 0.9 % saline.

Tissue processing

Mice were anesthetized with ketamine (100 mg/kg, body weight, 0.1 ml) and perfused through the heart with cold 0.9 % Sodium Chloride to wash blood from the brain tissue. Then animals were killed by decapitation. The entire brain was rapidly removed cautiously and kept in a petridish placed over ice. The brain regions were dissected in the following order; frontal cortex, striatum, hypothalamus, parietal cortex, cerebellum, and hippocampus. Homogenate of various brain regions, 10 % (w/v) were prepared in phosphate buffer saline (PBS) (10 mmol/l, pH 7.0) using Ultra-Turrax T25 (USA) homogenizer. Homogenized tissue samples were sonicated at 5 s cycle for 150 s using an ultrasonic processor and centrifuged at 10,000 RPM (7960 g) for 10 min. Then, the upper clear supernatants were collected for the biochemical analysis.

Oxidative stress measurement

The level of lipid peroxidation

Lipid peroxidation was evaluated colorimetrically measuring thiobarbituric acid reactive substances (TBARS)

followed by previously described method (Niehaus and Samuelsson 1968). Briefly, 0.1 ml of tissue homogenate (PBS buffer, pH 7.5) was treated with 2 ml of (1:1:1 ratio) TBA-TCA-HCl reagent (2-thiobarbituric acid 0.37 %, 0.25 N HCl and 15 % TCA) and placed in water bath for 15 min and cooled. The absorbance of clear supernatant was measured against reference blank at a wavelength of 535 nm.The level of MDAwas measured by using standard curve and expressed as nmol/mg of tissue.

The level of nitric oxide (NO)

Nitric Oxide (NO) was assayed according to the method described by (Tracey et al. 1995). In this study, Griess-Illosvoy reagent was modified by using naphthyl ethylene diaminedihydrochloride (0.1 % w/v) instead of 1-napthylamine (5 %). The reaction mixture (3 ml) containing brain homogenates (2 ml) and phosphate buffer saline (0.5 ml) was incubated at 25 °C for 15 min. Rest of process was followed as described in previous experiment(Al-Amin et al. 2015). A pink colored chromophore was formed in diffused light. The absorbance was measured at a wavelength of 540 nm against the corresponding blank solutions. NO level was measured by using standard curve and expressed as μmol/mg of tissue.

Activity of catalase (CAT)

The activity of catalase enzyme was assayed colorimetrically at a wavelength of 240 nm (Sinha 1972). The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate (supernatant) and 0.4 ml of 2 M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5 % potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).The equation for measuring the activity of catalase was = [(Initial absorption - subsequent absorption at 1 min interval)/Initial absorption] X100.

The level of advanced protein oxidation products (APOP)

Determination of APOPs was based on spectrophotometric detection according to (Witko-Sarsat et al. 1996). Concisely, 50 μl of sample (diluted 1:2 with phosphate-buffered saline (PBS), chloramin T (0–100 mmol/l) were used for the preparation of calibration curve and PBS was used as blank. One hundred microliter of 1.16 M potassium iodide and 50 μl of acetic acid were added to each well and absorbance at 340 nm was measured immediately. Concentration of APOP was expressed in (μmol/mg).

Activity of superoxide dismutase (SOD)

The activity of SOD was assayed by a modified procedure done by (Ma et al. 2010). Briefly, each 300 μl reaction mixture contained 50 mM sodium phosphate (pH 7.8), 13 mM methionine, 75 mMnitrobluetetrazolium (NBT), 2 mM riboflavin, 100 mM EDTA, and 2 ml of sample. The change in absorbance of each sample was then recorded at 560 nm followed the production of blue formazan. Results were expressed as percentage activity of SOD enzyme. The equation for measuring the activity of SOD was = [(Initial absorption-subsequent absorption at 30 s interval)/Initial absorption] X 100.

The level of glutathione (GSH)

Glutathione in the brain was evaluated based on the previous method (Ellman 1959). Briefly, 1 ml of sample, 2.7 ml of PBS (0.1 mol/l, pH 8) and 0.2 ml of 5, 5-dithio-bis (2-nitrobenzoic acid) were added. The color developed was determined instantly at 412 nm. Results of glutathione tests were expressed as mmol/mg protein.

Data analysis

A one-way between subjects ANOVA was conducted to compare the effect of astaxanthin on dependent variables in placebo young (PY), astaxanthin young (AY), placebo old (PO) and astaxanthin old (AO) groups. Post-hoc test namely, "Newman-Keuls" was used to compare between groups. All analyses were carried out in GraphPad prism (version 6.0, GraphPad Software, Inc.). The difference was considered significant when p value was less than 0.05. Data were represented as mean ± S.E.M (Standard Error of the Mean).The ratios of treated versus untreated tissues between young versus aged was further analyzed using the odds ratio [(AY/PY)/(AO/PO)] for young and [(AO/PO)/(AY/PY)] equation for aged groups.

Results

Effect of astaxanthin on the level of lipid peroxidation (MDA)

Treatment with astaxanthin significantly ($p < 0.05$) decreases the level of lipid peroxidation in frontal cortex, parietal cortex, striatum, hippocampus and cerebellum compared to the control group (Fig. 1). Both young and old groups are benefited with the treatment of astaxanthin. There was no significant difference in between young and aged treatment group.
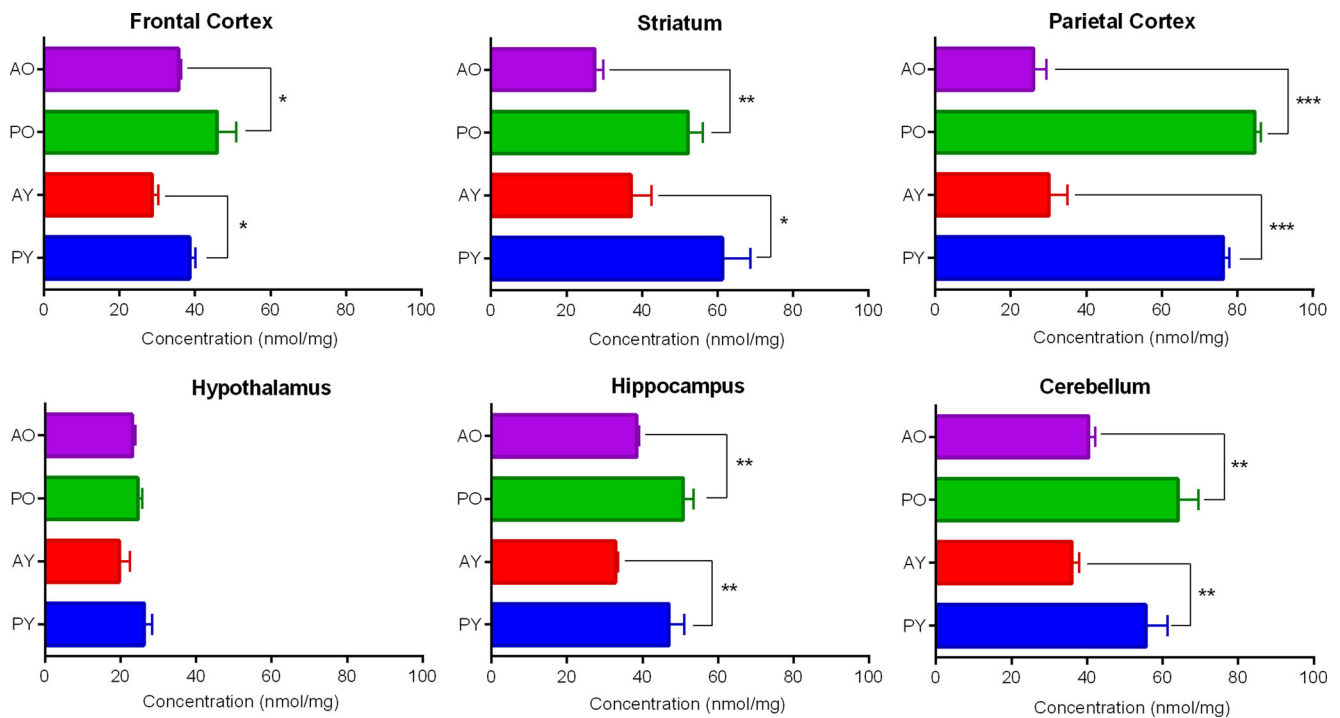
Fig. 1 Effect of astaxanthin (AST) treatment on the level of lipid peroxidation (MDA) in the frontal cortex, striatum, parietal cortex, hypothalamus, hippocampus and cerebellum. Horizontal axis represents the concentration of MDA in nano mole per mg (nmol/mg) and vertical axis represents groups such as Astaxanthin Old (AO); Placebo Old (PO); Astaxanthin Young (AY) and Placebo Young (PY). Values are represented as mean ± SEM. Newman-Keuls multiple comparisons test was used to compare between groups.*, ** and *** indicates p value at the level of 0.05, 0.01 and 0.001 respectively

Effect of astaxanthin on the level of nitric oxide (NO)

Treatment with astaxanthin appreciably ($p < 0.05$) reduces the level of nitric oxide in all brain regions except hippocampus (Fig. 2). The level of NO was noticeably ($p < 0.05$) reduced in the young treatment group in the frontal cortex, hypothalamus and cerebellum than the aged treatment group.

Effect of astaxanthin on the activity of catalase (CAT)

Astaxanthin considerably augmented ($p < 0.05$) the activity of catalase enzyme in the six segmented regions of the brain in both young and old group (Fig. 3). In addition, treatment with astaxanthin resulted in substantial ($p < 0.05$) improvement of catalase activity in young treatment group in striatum, hippocampus and cerebellum in comparison to the aged treatment group.

Effect of astaxanthin on the level of advanced protein oxidation product (APOP)

Astaxanthin treated young and aged group showed a noteworthy ($p < 0.05$) reduction of APOP level in six brain regions (Fig. 4). Astaxanthin treated young group showed a prominent ($p < 0.05$) reduction of APOP level in striatum, hypothalamus, hippocampus than the aged treatment group.

Effect of astaxanthin on the activity of superoxide dismutase (SOD)

Astaxanthin ominously rises ($p < 0.05$) the activity of SOD enzyme in six brain regions in both aged group (Fig. 5). Besides, treatment with astaxanthin appreciably ($p < 0.05$) boosts the activity of SOD enzyme in the parietal cortex, hippocampus, hypothalamus and cerebellum while declinein the striatum in the young group than their corresponding old treatment group.

Effect of astaxanthin on the level of glutathione (GSH)

The results showed that treatment with astaxanthin pointedly ($p < 0.05$) upsurges the level of glutathione in the parietal cortex and hypothalamus in both age groups (Fig. 6). On the contrary, treatment with astaxanthin markedly ($p < 0.05$) reduces the level of glutathione in striatum and hypothalamus in aged animals compared to the young treatment group.

Effect of astaxanthin on the oxidative markers between young and old groups

Effect of astaxanthin on the oxidative markers MDA, APOP, CAT, SOD, NO and GSH between young and old age groups are summarized in Table 1. In case of parietal cortex, young
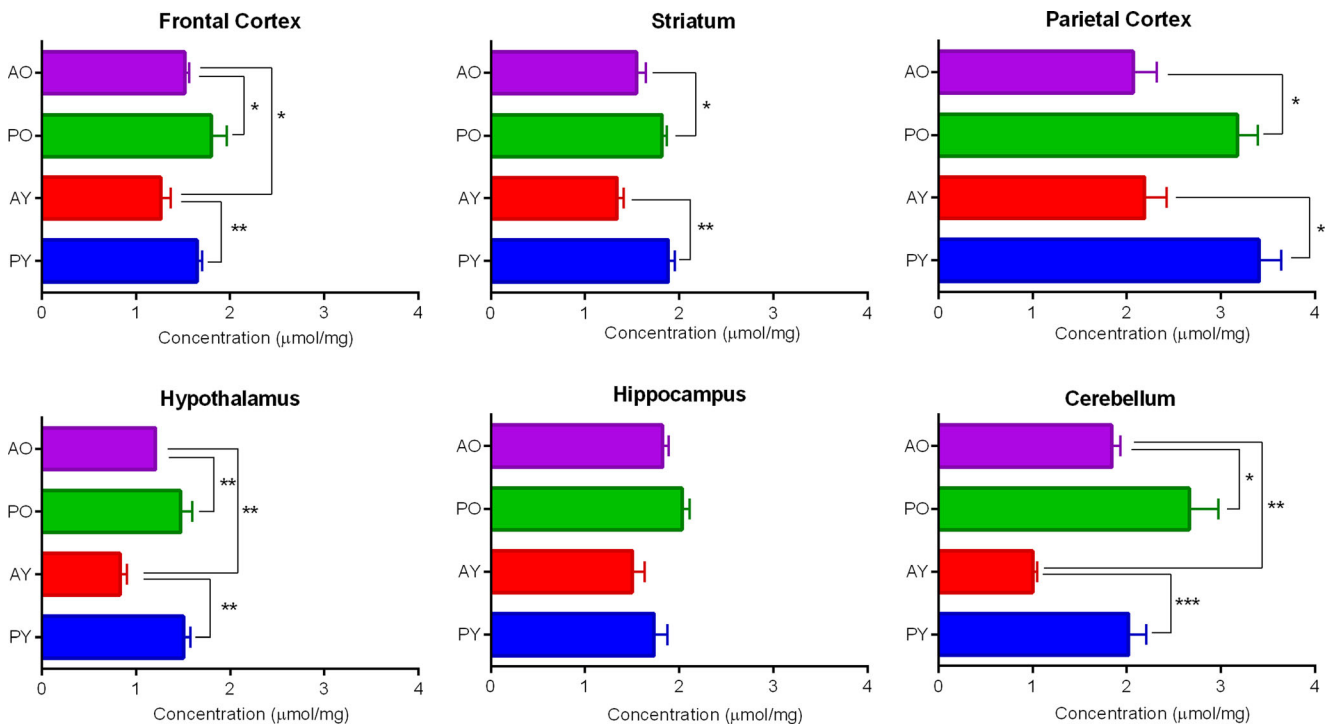
Fig. 2 Effect of astaxanthin (AST) treatment on the level of nitric oxide (NO) in the frontal cortex, striatum, parietal cortex, hypothalamus, hippocampus and cerebellum. Horizontal axis represents the concentration of NO in micro mole per mg (μmol/mg) and vertical axis represents groups such as Astaxanthin Old (AO); Placebo Old (PO); Astaxanthin Young (AY) and Placebo Young (PY). Values are represented as mean ± SEM. Newman-Keuls multiple comparisons test was used to compare between groups. *, ** and *** indicates p value at the level of 0.05, 0.01 and 0.001 respectively

age group showed higher improvement in APOP, CAT, SOD and GSH than the aged group, while old group showed greater improvement in MDA Table 1. Cerebellum data demonstrated that young group showed better improvement in MDA, CAT,
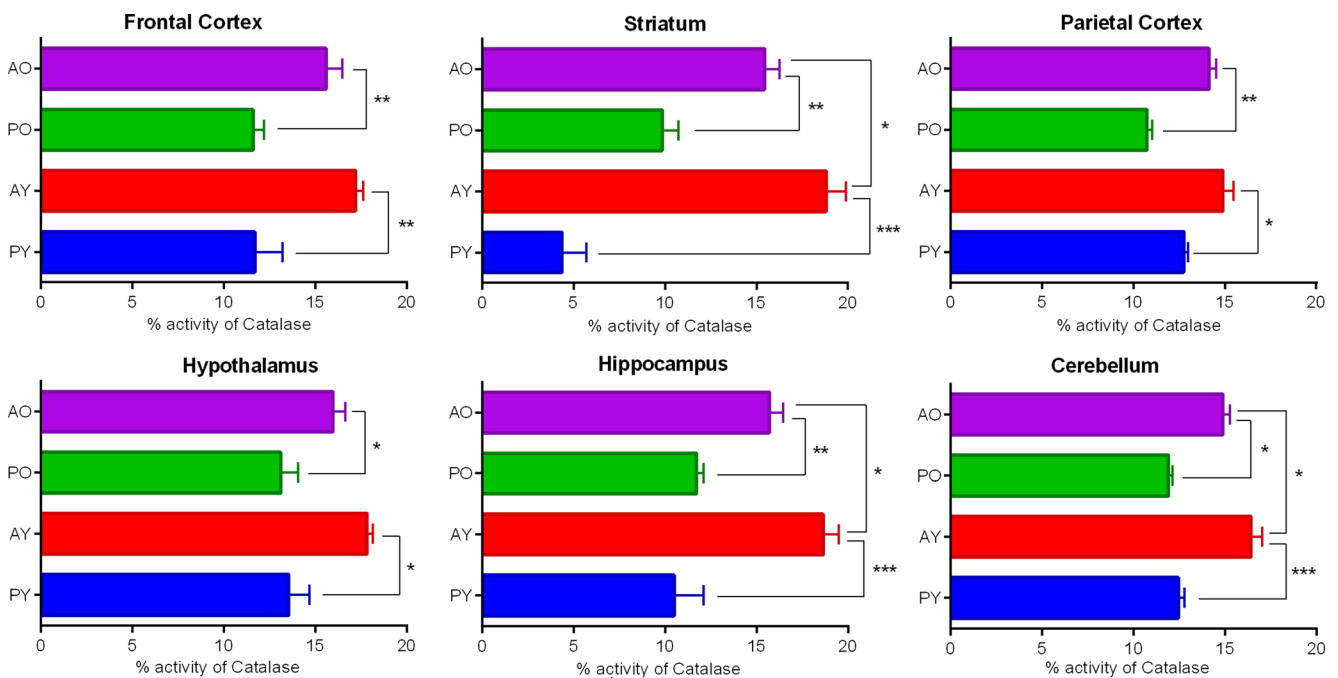

Fig. 3 Effect of astaxanthin (AST) treatment on the activity of catalase in the frontal cortex, striatum, parietal cortex, hypothalamus, hippocampus and cerebellum. Horizontal axis represents the activity of catalase in percentage and vertical axis represents groups such as Astaxanthin Old (AO); Placebo Old (PO); Astaxanthin Young (AY) and Placebo Young (PY). Values are represented as mean ± SEM. Newman-Keuls multiple comparisons test was used to compare between groups. *, ** and *** indicates p value at the level of 0.05, 0.01 and 0.001 respectively
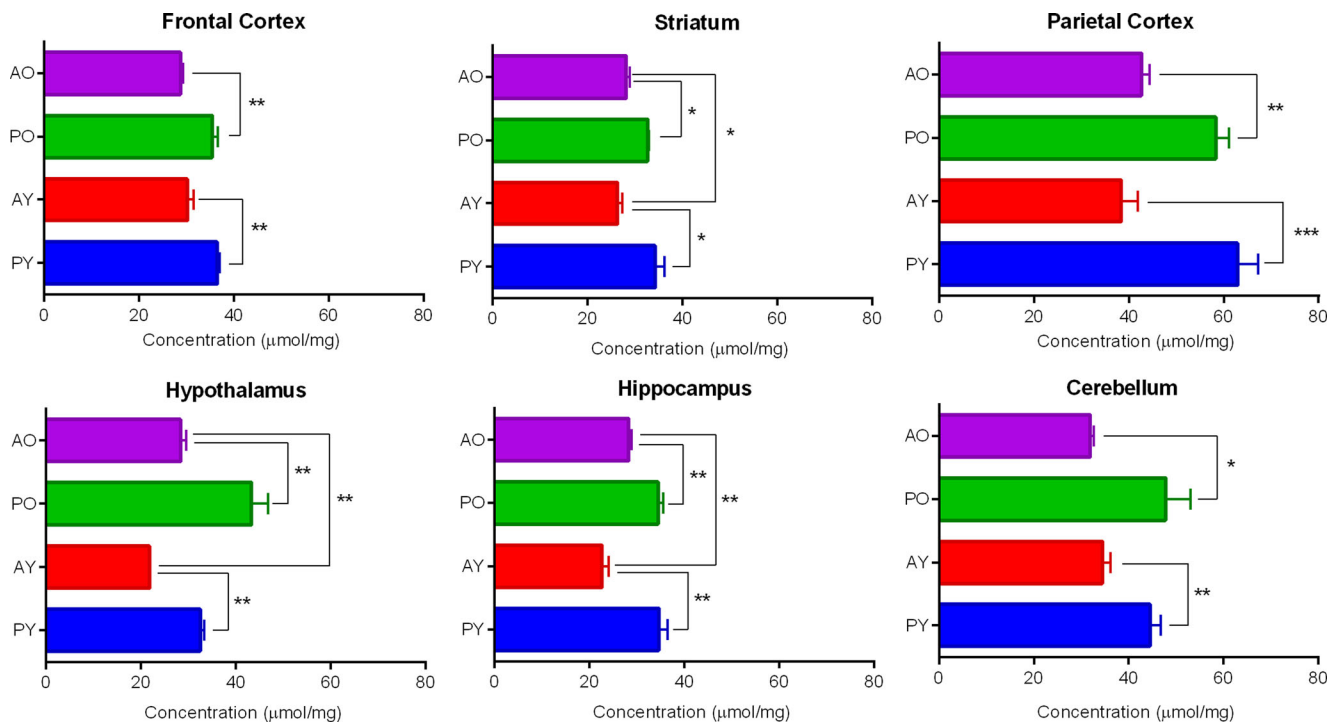
Fig. 4 Effect of astaxanthin (AST) treatment on the level of advanced protein oxidation product (APOP) the in frontal cortex, striatum, parietal cortex, hypothalamus, hippocampus and cerebellum. Horizontal axis represents the concentration of APOP in micro mole per mg (μmol/mg) and vertical axis represents groups such as Astaxanthin Old (AO); Placebo Old (PO); Astaxanthin Young (AY) and Placebo Young (PY). Values are represented as mean ± SEM. Newman-Keuls multiple comparisons test was used to compare between groups. *, ** and *** indicates p value at the level of 0.05, 0.01 and 0.001 respectively
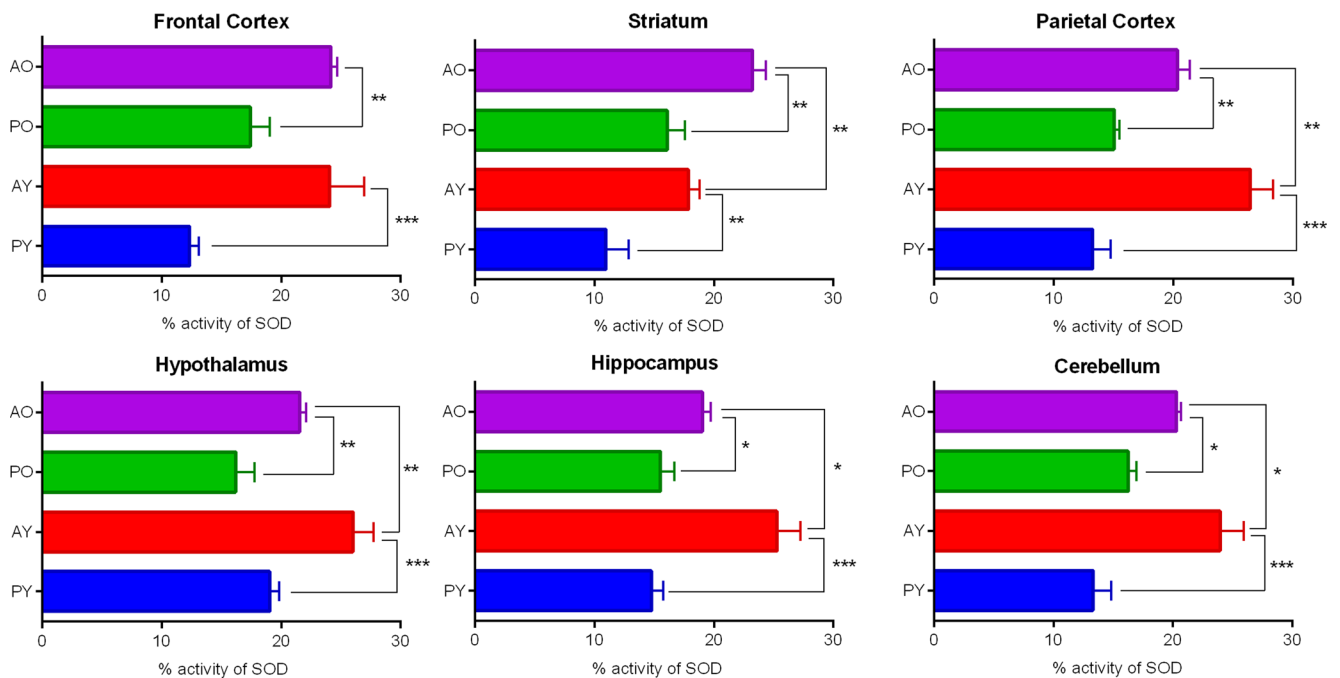

Fig. 5 Effect of astaxanthin (AST) treatment on the activity of superoxide dismutase (SOD) in the frontal cortex, striatum, parietal cortex, hypothalamus, hippocampus and cerebellum. Horizontal axis represents the activity of SOD in percentage and vertical axis represents groups such as Astaxanthin Old (AO); Placebo Old (PO); Astaxanthin Young (AY) and Placebo Young (PY). Values are represented as mean ± SEM. Newman-Keuls multiple comparisons test was used to compare between groups. *, ** and *** indicates p value at the level of 0.05, 0.01 and 0.001 respectively
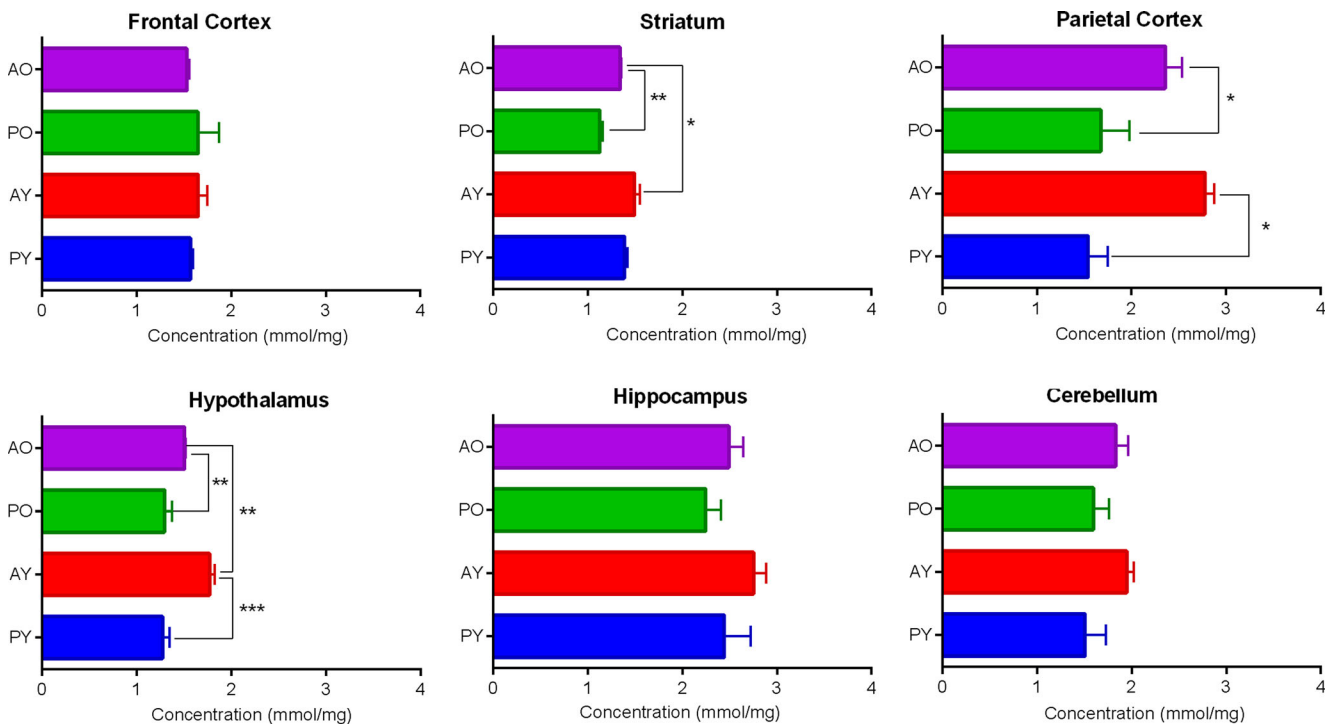
Fig. 6 Effect of astaxanthin (AST) treatment on the level of glutathione (GSH) in the frontal cortex, striatum, parietal cortex, hypothalamus, hippocampus and cerebellum. Horizontal axis represents the concentration of GSH in mili mole per mg (mmol/mg) and vertical axis represents groups such as Astaxanthin Old (AO); Placebo Old (PO); Astaxanthin Young (AY) and Placebo Young (PY). Values are represented as mean ± SEM. Newman-Keuls multiple comparisons test was used to compare between groups. *, ** and *** indicates p value at the level of 0.05, 0.01 and 0.001 respectively

SOD, NO & GSH while aged group showed greater improvement in APOP level. Frontal Cortex data showed better improvement in young group in MDA, CAT, SOD, NO & GSH while aged group showed better in APOP level. Hippocampus data revealed that young group showed better improvement in MDA, APOP, CAT, SOD and GSH. Hypothalamus data revealed that young group showed marked improvement in MDA, CAT, NO, APOP, SOD and GSH. Striatum data stated that young group showed better improvement in APOP, CAT, NO and GSH markers while aged group showed better improvement in MDA & SOD level.

Discussion

This study was designed to investigate the antioxidant effect of astaxanthin in young and aged mice. Our results showed that the treatment with astaxanthin improves enzymatic and non-enzymatic oxidative markers in the brain of both young and aged animals.

MDA is considered as one of the key intermediates of free radical induced damages. Increased level of MDA has been reported in the cortex (Dei et al. 2002), hippocampus, cerebellum (Gemma et al. 2002) in the neurons and astrocyte of aged rodents. MDA interferes with the brain homeostasis between inhibitory and excitatory neurons (Li et al. 2010), impairs the

function of the brain mitochondria (Vaishnav et al. 2010) which results in the disturbances of brain function. Our results showed the increment of MDA level in the placebo treated old than the young group, while treatment with astaxanthin improves the level of MDA i.e. higher decrement in the parietal cortex and striatum in old animals. Effect of astaxanthin treatment is also shown in a cell culture study which reports that astaxanthin treatment reduces the level of MDA (Chan et al. 2009).

Increased level of APOP might be associated with the reduction of signal transduction in aging process. Elevated level of protein carbonyl group has been shown in the hippocampus (Nicolle et al. 2001), cortex of human (Smith et al. 1991) and rats (Cakatay et al. 2001). Placebo treatment raises the level of APOP in the hypothalamus and cerebellum in the old animals while treatment with astaxanthin reduces the level of APOP in both age groups. Young treatment group showed lower level of APOP in the striatum, and hippocampus. Therefore, reduced protein oxidation would help the young animals to maintain intact signal transduction in these brain regions.

Increased level of NOs activity has been shown in previous study in aged (11 months) in comparison to the young (3 months) (Inada et al. 1996). Our results demonstrated that astaxanthin treatment reduces the level of NO in the frontal and parietal cortex, striatum, hypothalamus and cerebellum in both age groups. Reduction of the level of NO was higher in

Table 1 Ratios of astaxanthin treated versus saline treated tissues between young versus aged [(AO/PO)/(AY/PY)] animals. AO: astaxanthin old, PO: placebo old, AY: astaxanthin young, PY: placebo young. * indicates the levels of significance at $p < 0.05$

Brain Tissue	Age group	MDA	APOP	CAT	SOD	NO	GSH
Frontal Cortex	(AY/PY)/(AO/PO)	0.54*	1.00	1.06*	1.41*	0.50*	1.20*
	(AO/PO)/(AY/PY)	1.83	0.99	0.94	0.70	2.00	0.83
Striatum	(AY/PY)/(AO/PO)	1.12	0.90	1.66*	0.87	0.50*	1.10*
	(AO/PO)/(AY/PY)	0.88*	1.11	0.60	1.13*	2.00	0.90
Parietal Cortex	(AY/PY)/(AO/PO)	1.29	0.83*	1.01	1.50*	1.00	1.32*
	(AO/PO)/(AY/PY)	0.78*	1.20	0.98	0.66	1.00	0.75
Hypothalamus	(AY/PY)/(AO/PO)	0.83*	0.97	1.04	1.00	0.50*	1.20*
	(AO/PO)/(AY/PY)	1.19	1.02	0.95	0.99	2.00	0.83
Hippocampus	(AY/PY)/(AO/PO)	0.91	0.78*	1.18*	1.40*	1.00	1.02*
	(AO/PO)/(AY/PY)	1.08	1.27	0.84	0.71	1.00	0.97
Cerebellum	(AY/PY)/(AO/PO)	0.97	1.16	1.06	1.47*	0.75*	1.18*
	(AO/PO)/(AY/PY)	1.02	0.85	0.93	0.67	1.33	0.84

the frontal cortex, hypothalamus and cerebellum in young treatment group compared to the aged group. Previous results are consistent with us suggesting that astaxanthin reduces the level of NO production by suppressing NF-kappaB activation (Lee et al. 2003). NO plays vital role in learning and memory formations as a neurotransmitter in the brain. NO is released only during training (Harooni et al. 2009). We did not measure the level of NO during training. This result is possibly not linked with the signal transmission. However, the reduction of NO that is coupled with free radical production is beneficial.

GSH possess key cellular functions including astrocyte dependent detoxification of xenobiotics and endogenous compounds (Dringen et al. 2014). Age-dependent reduction of glutathione (GSH) level in all brain regions was shown in the past (Zhu et al. 2006) and in our present study, the same was seen in the striatum and hippocampus of placebo group. Astrocyte cell provides essential precursors for the production of GSH in the neurons, and regulates (Sagara et al. 1993) the level of GSH (Bolanos et al. 1996; Dringen et al. 1999). Our results showed that treatment of astaxanthin raised the level of GSH in the parietal cortex, striatum and hypothalamus. Greater improvement of GSH level was observed in young treatment group. The result of a cell culture study is consistent with our results. Cell culture study reports that astaxanthin treatment showed neuroprotective effect via the restoration of glutathione level and reduction of ROS formation (Chan et al. 2009).

Age-dependent decreased enzymatic antioxidant activity of catalase and SOD were reported in the past (Alper et al. 1998). SOD activity is increased with age until 12 months and decreased after 12 months in the rat brain (Tsay et al. 2000). Cell culture study (Chan et al. 2009) and our results are consistent in this regard revealing that treatment with astaxanthin restores the activity of CAT. Our findings are promising since an enhanced activity

of SOD and CAT are beneficial in fighting against the oxidative stress in the brain.

We have observed that placebo treated old animals showed reduced level of oxidative markers in several brain regions. However, oxidative stress is usually expected to be raised with aging process (Matsuo et al. 2004). But, it has been shown that aged cells are more capable to prevent oxidative stress compared to the young cells (Kolosova et al. 2006). Treatment with astaxanthin improves oxidative markers in both age groups but preferentially in young group. It is possible due to the differential bioavailability, the level of dose and total antioxidant capacity of the brain. Nevertheless, the result is beneficial in a sense that astaxanthin treatment improves oxidative stress markers in the brain.

This study focused on specific brain regions that are distinguishable and responsible for vital brain functions. Our study revealed that the treatment with astaxanthin improves oxidative markers in most of the brain regions. From the results, we may expect that antioxidant capacity helps to provide neuroprotective effect resulting in improved cognitive functions and behaviors. The effect of astaxanthin on the frontal and parietal cortex was observed in our study which is consistent with the previous findings (Lin et al. 2010). In addition, the result of finding in hippocampus also supports the finding of Wu et.al (Wu et al. 2014). Moreover, our study showed significant effect of astaxanthin on oxidative markers in hypothalamus which is in line with a study carried out earlier (Shinagawa et al. 2013). To our knowledge, there was no study which investigated the effect of astaxanthin on oxidative markers in age-dependent manner. The analysis of region-specific antioxidant effect of astaxanthin showed greater improvement of oxidative markers in hippocampus and hypothalamus in young animals compared to aged animals.

Recent study showed that astaxanthin reverses D-galactose induced brain aging in rats (Wu et al. 2014). The authors report that astaxanthin treatment improves total anti-oxidant capacity (TAOC), GSH, SOD and GSH-PX, MDA in the

brain of aging rodent (Wu et al. 2014). The study of Wu et al. (2014) and the findings of present study are in same line suggesting that this antioxidant is useful in improving age-dependent oxidative stress.

We have several limitations, histological study was not conducted, unable to determine several important brain neurotransmitters, higher dose of astaxanthin was not investigated as higher dose might be associated with the improvement of oxidative stress in the aged group, ROS, GSH/GSSG ratio were also not determined. In our lab settings, these methods are not established because of the limited financial and technical resources. Future study may include the aforesaid issues. Despite the limitations, the present study is novel in many respects. First, to our knowledge, this study first addresses the effect of astaxanthin on two different age groups. Second, this study focuses on various brain tissues that are associated with vital brain functions. Third, brain tissues are highly susceptible to oxidative stress therefore, the measurement of oxidative stress in two different groups were needed to be investigated.

Conclusion

The effect of astaxanthin treatment on brain oxidative markers in between young and aged animals was investigated. Six brain regions namely frontal cortex, striatum, parietal cortex, hypothalamus, hippocampus, cerebellum were focused for analysis. Four non-enzymatic oxidative and two enzymatic antioxidant markers were evaluated. The result demonstrated that the treatment with astaxanthin improves all types of oxidative markers in both young and aged animal in specific brain regions. The result of the present study could be useful in the development of pharmacological strategy in combating the age-dependent cognitive function declining.

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