ARTÍCULO ORIGINAL

Gac Méd Caracas 2021;129(Supl 2):S436-S441
DOI: 10.47307/GMC.2021.129.s2.23

Effect of astaxanthin on malondialdehyde level in damaged cerebral cortex tissue in male rat (*Rattus Norvegicus*) induced by formaldehyde orally

Efecto de la astaxantina sobre el nivel de malondialdehído en el tejido de la corteza cerebral dañada en ratas macho (*Rattus norvegicus*) inducido por formaldehído por vía oral

Erik Ahmad Hasyim[1*•], Virhan Novianry[2*], Mistika Zakiah[3*], Andriani[4*], Dyan Roshinta Laksmi Dewi[5**]

SUMMARY

Background*: Malondialdehyde (MDA) is a dialdehyde substance that is the final product of lipids peroxidation in the human body, and it can be used as a biomarker of oxidative stress. One of the most potent antioxidants known nowadays is astaxanthin. This study aims to investigate the effect of astaxanthin on the MDA level in cerebral cortex tissue of Rattus norvegicus, which was given oral formaldehyde.*
Methods*: This study used 25 Wistar rat model (Rattus norvegicus) which were divided into five groups: negative control, normal control groups, astaxanthin 12 mg/day, 24 mg/day, and 48 mg/day groups.*
Results*: The mean tissue MDA levels in normal control group was 11.10 ± 5.11 nmol/mL, negative control group was 9.74 ± 5.19 nmol/mL, astaxanthin 12 mg/day group was 10.71 ± 4.92 nmol/mL, 24 mg/day group was 13.14 ± 3.34 nmol/mL, and 48 mg/day groups was 6.10 ± 1.83 nmol/mL. Compared with the normal control group and negative control group, there was no significant difference (p>0.05), respectively, with the treatment-3 group of astaxanthin.*
Conclusion: *There were not effective doses of astaxanthin (12 mg/day, 24 mg/day, and 48 mg/day) in this study that significantly reduced MDA levels in cerebral cortex tissue, in oral formaldehyde induced male Rattus norvegicus.*

Keywords*: Antioxidant, astaxanthin, cerebral cortex, formaldehyde, malondialdehyde, oxidative stress.*

DOI: https://doi.org/10.47307/GMC.2021.129.s2.23

ORCID: 0000-0002-7231-0070[1]
ORCID: 0000-0001-5044-1216[2]
ORCID: 0000-0003-0139-3590[3]
ORCID: 0000-0003-2029-1880[4]
ORCID: 0000-0002-5876-579X[5]

Recibido: 8 de junio 2021
Aceptado: 22 de junio 2021

[*]*Faculty of Medicine, University of Tanjungpura, Pontianak 78115, Indonesia*
[**]*Regional Public Hospital of Doctor Soedarso, Pontianak 78115, Indonesia*

• Corresponding Author: Erik Ahmad Hasyim
Faculty of Medicine, University of Tanjungpura, Pontianak 78115, Indonesia. Jalan Prof. Hadari Nawawi, Pontianak 78115, Indonesia.
Tel: +62-0561-765342; Fax: +62-0561-765342
E-mail: erikahmadhasyim@yahoo.com

RESUMEN

Antecedentes: *El malondialdehído (MDA) es una sustancia dialdehído que es el producto final de la peroxidación de los lípidos en el cuerpo humano y se puede utilizar como biomarcador de estrés oxidativo. Uno de los antioxidantes más potentes que se conocen hoy en día es la astaxantina. Este estudio tiene como objetivo investigar el efecto de la astaxantina en el nivel de MDA en el tejido de la corteza cerebral de Rattus norvegicus, al que se le administró formaldehído oral.*
Métodos: *Este estudio utilizó 25 modelos de ratas Wistar (Rattus norvegicus) que se dividieron en cinco grupos: control negativo, grupos de control normal, grupos de astaxantina 12 mg/día, 24 mg / día y 48 mg / día.*
Resultados: *Los niveles medios de MDA en tejido en el grupo de control normal fue de 11,10 ± 5,11 nmol/ mL, el grupo de control negativo fue de 9,74 ± 5,19 nmol / mL, el grupo de astaxantina 12 mg / día fue de 10,71 ± 4,92 nmol / mL, el grupo de 24 mg / día fue 13,14 ± 3,34 nmol / mL, y los grupos de 48 mg / día fueron 6,10 ± 1,83 nmol / mL. En comparación con el grupo de control normal y el grupo de control negativo, hubo una diferencia insignificante (p> 0.05), respectivamente, con el grupo de tratamiento-3 de astaxantina.*
Conclusión: *No hubo dosis efectivas de astaxantina (12 mg / día, 24 mg / día y 48 mg / día) en este estudio que redujeran significativamente los niveles de MDA en el tejido de la corteza cerebral, en Rattus norvegicus macho inducido por formaldehído oral.*

Palabras clave: *Antioxidante, astaxantina, corteza cerebral, formaldehído, malondialdehído, estrés oxidativo.*

INTRODUCTION

The cerebral cortex is the neural tissue in cerebral hemispheres containing mostly grey matter. Memory, attention, perceptual awareness, mental language, and consciousness are all facilitated by the cerebral cortex. The cerebral cortex of adult mammals comprises six layers with nerve cell bodies, dendritic arborizations, and synaptic interconnections (1). In humans, the cerebral cortex consists of approximately 80 % of the whole brain mass. It is estimated that there are 10 to 16 billion neurons in the cortex of the adult human brain with an estimated average mass of 1 233 g, and the number of neurons above is only an estimate, meaning that the number can be less or more (2). The cerebral cortex has a variety of roles in maintaining the essential functions of life. This does not make the cerebral cortex invulnerable to various metabolic, toxic, microbial, and circulatory disorders. Various diseases related to damage or disorders in the cerebral cortex are among the highest in Indonesia (3). Indonesia is the fourth most populous country in the world, located in Southeast Asia, with a total population of ~260 million in 2017, which is composed of various ethnic groups (4). Based on data from the Indonesian Hospital Association in 2009, diseases related to damage of the cerebral cortex area cause up to 65 % of disabilities (3).

Formaldehyde is a molecule that degrades rapidly in the environment and the human body has been related to neurodegenerative disease. Neurodegenerative disease is a disorder of the nervous system which may result in neuron degeneration or atrophy (5-7). Cerebral cortex tissue damage caused by free radicals can derive from formaldehyde metabolism (8,9). A free radical is an atom with an unpaired electron that becomes unstable and reactive as a result. Free radicals could harm the body's critical macromolecules resulting in cell damage and disruption of homeostasis (10). Based on a study (11), formaldehyde exposure can cause oxidative stress by increasing the production of Reactive Oxygen Species (ROS) compounds in the body (11). Oxidative stress can lead to degenerative diseases. According to the World Health Organization (WHO), approximately 17 million people die each year from degenerative disease (10). This oxidative damage often causes severe problems in susceptible neurons such as the frontal cortex, entorhinal cortex, and the substantia nigra in general, which are the most significant constituents of the cerebral cortex, causing cell damage, death, and leading to neurodegenerative processes, such as Alzheimer's disease, Parkinson's disease, and lateral amyotrophic sclerosis (12).

Malondialdehyde (MDA) is a dialdehyde molecule that is formed by free radicals during ionization events in the body (13). The quantity of malondialdehyde (MDA) in the blood may indicate oxidative stress levels in the organism. MDA levels in the blood increase in correlation with the body's oxidative stress (14).

Antioxidants are necessary chemicals compound for the body to neutralize free radicals and prevent free radicals' damage. Antioxidants stabilize free radicals by compensating for the electron deficiency of free radicals and preventing chain events caused by free radical generation that might result in oxidative stress (15). The human body is producing endogenous antioxidants, such as superoxide dismutase (SOD). The huge amount of ROS produced by body cells creates an imbalance condition. This imbalance could reduce the level of endogenous antioxidants. So, it is important to increase the number of exogenous antioxidants to avoid the harmful effects of free radicals (16). One of the antioxidants that are known to have a reasonably strong effect is astaxanthin (17,18). Astaxanthin, produced from *Haematococcus pluvalis*, is the most powerful and safest carotenoid without pro-oxidant effects, comparable to beta-carotene, lycopene, zeaxanthin, and lutein. It has three critical triple properties: antioxidant, anti-inflammatory, and immunomodulator. Astaxanthin reduces the production of inflammatory genes by inhibiting NF-kB activation and protects cells from oxidative damage, therefore astaxanthin also has anti neurodegenerative properties (19). A study conducted by Tso and Lam (1996) showed that compared to beta-carotene, astaxanthin was easier to cross the blood-brain barrier so that it can function as a good antioxidant for the brain (20).

The objective of this study was to examine the effect of astaxanthin on the MDA level in the cerebral cortex tissue of Wistar strain *Rattus norvegicus* that had been exposed to oral formaldehyde and to identify the optimal dose of astaxanthin as an antioxidant based on the MDA level in the damaged cerebral cortex tissue.

METHODS

Materials to be used in this research are stored biological materials, astaxanthin, formaldehyde 37 %, distilled water, standard food, wood powder, phosphate-buffered saline (PBS) 0.1 M Ph 7.4, thiobarbituric acid (TBA) 0.67 %, tetra ethoxy propane (TEP), and trichloroacetic acid (TCA) 20 %. The tools used in this research were rat cage, oral syringe, gastric syringe, spectrophotometer, centrifuge, analytical scale, animal scale, TissueLyser II (QIAGEN), blender, micropipette, 1 000 mL measuring cup, 100 mL measuring cup, 10 mL measuring flask, shaker, test tube, handscoon, microtube, vortex, UV-sterile, hot plate, iron stirrer, and PH-meter.

The sample was taken by simple random sampling technique, sampling is done by selecting available members of the population, randomly. In this study, the sample was obtained from *Rattus norvegicus* Wistar strains with several criteria: three months old, 200-gram rat body weight, and good general health. The sample size was 25 white rats, which were divided into five groups randomly.

Then tested animals were divided into five groups, with five rats in each group. The negative control group was given formaldehyde 0.01 mL; the normal control group was given distilled water and standard diet; treatment-1, treatment-2, and treatment-3 groups were given astaxanthin 12 mg/day, 24 mg/day, and 48 mg/day, respectively, for 14 days after being given formaldehyde for 14 days.

Measurement of MDA levels was carried out by measuring the absorbance of each experimental group. The measurement method used was the TBA method. TBA has a high sensitivity value to free radicals. TBA will react with carboxylate groups from MDA through the addition of nucleophilic to form a TBA-MDA complex in an acidic atmosphere and form a pink color so that it can be quantified by spectrophotometry. The results of the data obtained were processed with IBM SPSS Statistics v22.0 for Windows. The data were tested using the One-Way ANOVA hypothesis test followed by the Post Hoc LSD test.

RESULTS

Standard curve determination was carried out before measuring the MDA level of the sample tissue. From the determination of this standard curve, the sample wavelength and the standard curve equation will be obtained and be used to calculate the MDA level of the sample. The standard curve used is TEP. TEP, when reacted with TBA, will form MDA and methanol as byproducts. Measurements were made at six standard TEP concentration points

with a concentration of 0.15625 nmol/mL, 0.3125 nmol/mL, 0.625 nmol/mL, 1.25 nmol/mL, 2.5 nmol/mL, and 5 nmol/mL. The standard curve equation obtained is:

$Y = 0.058\ X + 0.010$

With $a = 0.058$

$b = 0.010$

Relation coefficient (r) = 0.999

From this formula, the linear regression curve between the MDA standard solutions on the absorbance can be seen in Figure 1.

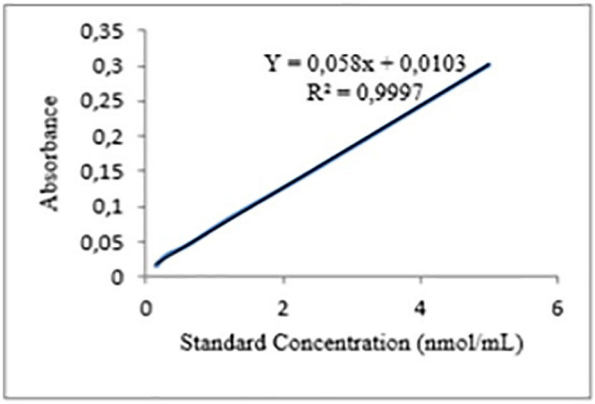

Figure 1. Linear regression curve of the MDA standard.

All absorbance obtained in the treatment group was calculated using the formula for MDA levels, comparing the absorbance with a standard curve, using the standard curve equation as follows:

MDA level = $df \times (x$ / sample volume)

With df = diluting factor

The mean tissue MDA levels are shown in Table 1. The MDA levels of the cerebral cortex were statistically analyzed using the One-Way ANOVA test and the Post Hoc test. MDA levels from the five groups were analyzed using the One-Way ANOVA test. The differences in MDA levels between each group were analyzed using the Post Hoc test.

Table 1

Comparison of MDA Level between Groups

Tested Groups	Mean ± SD MDA Levels (nmol/mL)
Normal control group	11.10 ± 5.11
Negative control group (K-)	9.74 ± 5.19
Treatment-1 group (K1)	10.71 ± 4.92
Treatment-2 group (K2)	13.14 ± 3.34
Treatment-3 group (K3)	6.10 ± 1.83

Note. K1=astaxanthin dosage of 12 mg/day, K2=astaxanthin dosage of 24 mg/day, K3=astaxanthin dosage of 48 mg/day.

A comparison diagram of MDA levels in the cerebral cortex tissue of each treatment group can be seen in Figure 2.

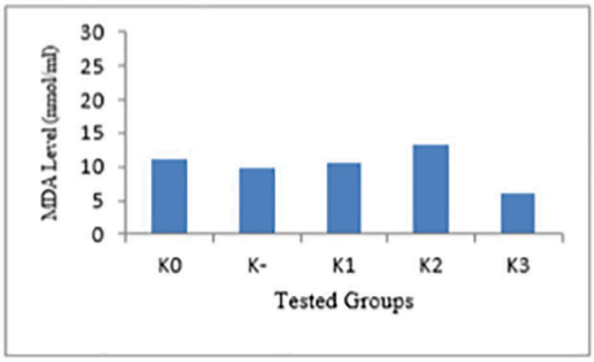

Figure 2. Mean MDA levels in cerebral cortex tissue.

DISCUSSION

The Post Hoc test results showed the results of the comparative analysis of MDA levels between each treatment group. Measurement of MDA levels in the normal group is used compared to the treatment groups. Normal group had insignificant different levels of MDA, compared to the negative group. The negative control group was the only group that given oral formaldehyde exposure without given astaxanthin. MDA

levels in the normal group were higher than in the negative control group, this difference was not significant in statistical tests. This is because the formaldehyde levels that reach the cerebral cortex are less than the initial exposure dose of 0.01 mL/day, thus showing the result was found. Formaldehyde can combine with cellular constituents easily in all exposed tissues throughout its route. This process occurs mainly in the liver and to a lesser extent in erythrocytes, kidneys, muscles, and the brain. This is also supported by the evidence that the half-life of formaldehyde so that it is converted to formic acid is very fast, which is about 1.5 minutes (21).

The following comparison is between the normal group and the treatment groups. The treatment groups were given oral formaldehyde and astaxanthin exposure. There were three different doses used, that was 12 mg/day (K1), 24 mg/day (K2), and 48 mg/day (K3). Although the 12 mg/day and 48 mg/day dose groups had different levels of MDA compared to normal control group, this cannot yet be said to be an effect of astaxanthin in that group. These conclusions were drawn for two reasons. The first is that the statistical test showed that the difference was not significant, and the second is that MDA levels in the negative control group were also relatively insignificant lower than the normal control group. This is because the astaxanthin doses of 12 mg/day and 24 mg/day have not significantly inhibited lipid peroxidation in the cerebral cortex tissue after exposure to formaldehyde in these two groups. The third treatment group was analyzed, only the 48 mg/day dose group had lower MDA levels than the negative control group, although the Post Hoc test showed that this difference was not significant.

Among the numerous natural carotenoids, astaxanthin is regarded as one of the most effective at protecting membrane cells, lipids, and lipoproteins from oxidative damage (22). The polyene chain in the chemical structure of astaxanthin can trap free radicals in the cell membrane while the terminal ring tames free radicals on the inner and outer sides of the cell membrane, promote the preservation of cell membrane structure thus inhibiting lipid peroxidation, which leads to increased MDA level (23).

The analysis of MDA levels obtained from the three doses of astaxanthin showed that the most effective dose was 48 mg/day if compared with the two other doses. The dose of 48 mg/day reduced MDA levels so that it was not significantly different from the negative control group and the normal control group.

CONCLUSION

These results indicate no effective dose of 12 mg, 24 mg, and 48 mg of astaxanthin which significantly reduced the MDA levels of the cerebral cortex tissue in the male *Rattus norvegicus* Wistar strain that was induced by formaldehyde orally. Future studies are needed to assess the therapeutic effect of astaxanthin by looking at the histopathological features of the cerebral cortex.

Acknowledgments

All praise and gratitude are to Allah SWT. We deeply appreciate both our parents, the supervisors of this paper, and all those who have helped us in completing this research.

REFERENCES

1. Evayanti LG, Kalanjati VP, Abdurachman. The effect of vitamin C on the cerebral cortex neurons of rats exposed by prenatal noise stress. Asian J Microbiol Biotechnol Environ Sci. 2018;20:S34-37.
2. Kalanjati VP, Hendrata MW, Ardana FN. Cellular composition in the aging cerebral cortex of humans. Neurophysiology. 2019;51(6):424-429.
3. Kemenkes RI. Menkes Lakukan Soft Opening Rumah Sakit Pusat Otak Nasional. 2013.
4. Miftahussurur M, Waskito LA, Syam AF, Nusi IA, Siregar G, Richardo M, et al. Alternative eradication regimens for helicobacter pylori infection in Indonesian regions with high metronidazole and levofloxacin resistance. Infect Drug Resist. 2019;12:345-358.
5. Genuis SJ, Kelln KL. Toxicant exposure and bioaccumulation: A common and potentially reversible cause of cognitive dysfunction and dementia. Behav Neurol. 2015;2015:620143.
6. Kundu S, De A, Mitra S. Formaldehyde: Fact sheet

reflecting uses, exposure hazards, health effects and toxicological profile-A broad overview for medical professionals and embalmers. J Clin Exp Res. 2015;3(2):183-196.

7. Williams LS, Hopper PD. Understanding medical surgical nursing. FA Davis; 2015.
8. Sakr HF, Abbas AM, El Samanoudy AZ. Effect of vitamin E on cerebral cortical oxidative stress and brain-derived neurotrophic factor gene expression induced by hypoxia and exercise in rats. J Physiol Pharmacol an Off J Polish Physiol Soc. 2015;66(2):191-202.
9. Zhang Y, Liu X, McHale C, Li R, Zhang L, Wu Y, et al. Bone marrow injury-induced via oxidative stress in mice by inhalation exposure to formaldehyde. PLoS One. 2013;8(9):e74974.
10. Kusumaningsih T, Firdausi A, Diyatri I, Ridwan RD, Arundina I, Yuliati. Antioxidant effects of graptophyllum pictum leaf extract on malondialdehyde (MDA) levels of mice induced by a toxic dose of paracetamol. J Krishna Inst Med Sci Univ. 2018;7(3):59-64.
11. Poljšak B, Fink R. The Protective Role of Antioxidants in the Defence against ROS/RNS-Mediated Environmental Pollution. In: Dal-Pizzol F, editor. Oxid Med Cell Longev. 2014;2014:671539.
12. Wang X, Michaelis E. Selective neuronal vulnerability to oxidative stress in the brain. Frontiers in Aging Neuroscience. 2010;2:12.
13. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. In: Ramana K V, editor. Oxid Med Cell Longev. 2014.p.360438.
14. Gunawan VA, Soetjipto H, Mustika A. Hypoglycemic and antioxidant activity of Petiveria alliacea in diabetic rat models. Biomol Heal Sci J. 2020;3(1):19.
15. Ashari FY, Yudiwati R, Aswin RH. The effect of antioxidant vitamin C and E administration on Spermatozoa Quality of White Rat (Rattus Norvegicus) Strain Wistar Male Against Allethrin Exposure. Biomol Heal Sci J. 2018;1(2):113.
16. Husen SA, Winarni D, Wahyuningsih SPA, Ansori ANM, Hayaza S, Susilo RJK, et al. Antioxidant potency of various fractions of okra pods extract to ameliorate liver structure and function in diabetic mice. Ann Biol. 2020;36(2):154-158.
17. Biswal S. Oxidative stress and astaxanthin: The novel supernutrient carotenoid. Int J Heal Allied Sci. 2014;3(3):147.
18. Régnier P, Bastias J, Rodriguez-Ruiz V, Caballero-Casero N, Caballo C, Sicilia D, et al. Astaxanthin from Haematococcus pluvialis prevents oxidative stress on human endothelial cells without toxicity. Mar Drugs. 2015;13(5):2857-2874.
19. Tjokroprawiro A. Astaxanthin – oxidative stress – diabetes mellitus from basics to clinics and from general to specific. Folia Medica Indones. 2008;44(4):293-299.
20. Tso MOM, Lam T-T. Method of retarding and ameliorating central nervous system and eye damage. Univ Pennsylvania; 1994.
21. Sullivan JB, Krieger GR. Clinical environmental health and toxic exposures. Lippincott Williams & Wilkins; 2001.
22. Karppi J, Rissanen TH, Nyyssönen K, Kaikkonen J, Olsson AG, Voutilainen S, et al. Effects of astaxanthin supplementation on lipid peroxidation. Int J Vitam Nutr Res Int Zeitschrift fur Vitamin- und Ernahrungsforschung J Int Vitaminol Nutr. 2007;77(1):3-11.
23. Fassett RG, Coombes JS. Astaxanthin: A potential therapeutic agent in cardiovascular disease. Mar Drugs. 2011;9(3):447-465.