

Conference Paper

The Effect of Astaxanthin on Glutathione Levels in Damaged Liver Tissues of Male Wistar Rats Induced By Oral Formaldehyde

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Abstract

Formaldehyde is an aldehyde derivative which is illegally used as a food preservative. An impaired liver function could result from exposure to formaldehyde through the process of oxidative stress. Astaxanthin is expected to increase the levels of glutathione (GSH), which is a natural antioxidant in the human body. An antioxidant can be used to inhibit formaldehyde-induced free radicals. This study aimed to determine the effect of astaxanthin on GSH levels in damaged liver tissues of male Wistar rats induced by oral formaldehyde. This study was an experimental study with a posttest-only control group design. Thirty rats were divided into normal control group; the negative control group which was given only formaldehyde; Group 1, 2, and 3 which was given a 12, 24, and 48 mg/day dose of astaxanthin. GSH levels of each group were measured using the Ellman Method and the data were analyzed statistically using SPSS 23.00. The value of GSH levels in treatment group 1 was 4.492 ± 0.29 $\mu\text{g/ml}$, treatment group 2 was 6.075 ± 0.96 $\mu\text{g/ml}$, and treatment group 3 was 5.132 ± 0.52 $\mu\text{g/ml}$. GSH levels in group 2 and 3 were significantly different compared with the negative control group (LSD, $p < 0.05$). However, GSH levels in group 1 were not significantly different compared with the normal control group and negative control group (LSD, $p > 0.05$). Astaxanthin could increase GSH levels in damaged liver tissues of male Wistar rats induced by oral formaldehyde.

Keywords: astaxanthin, formaldehyde, glutathione

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1. Background

The liver is the largest and most important metabolic organ in the human body, with a very complex function for maintaining body homeostasis [1, 2]. Liver damage can be caused by various metabolic, toxic, microbial, and circulatory disturbances. In most cases, the disease process occurs mainly in the liver. The hepatic disease itself is a cause of large burden disease across Europe [3]. World Health Organization (WHO) states that

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disease caused by a hepatic impairment is the cause of high mortalities in Indonesia [4]. Liver damage may be caused by free radicals from formaldehyde metabolism. Formaldehyde (CH_2O) is a derivative of aldehydes, which are highly reactive, flammable and can explode in the air. Formaldehyde is present in many types of building construction materials and is also used in hospitals, research, and laboratories as agents of sterilization, antibacterial, and cadaver preservation [5-7]. However, in Indonesia formaldehyde has been widely abused as a preservative in foods [8]. Based on the data from the Food and Drug Supervisory Agency (BPOM), there are still many food traders using formaldehyde as a preservative in various regions of Indonesia from 2011 to 2014. The Indonesian government has established some laws that formaldehyde use is prohibited for food preservative (References). Law No. 7/1996 about Food, Law No. 8/1999 about Consumer Protection and Government Regulation No. 28 of 2004 about Food Safety. In West Kalimantan, a variety of foods such as tofu, yellow noodles, sweets, fish bloated, red cherries, and corn vermicelli contain formaldehyde, which is found in some traditional markets [9, 10].

Formaldehyde exists in our body with a normal level of approximately 2.6 mg / L in the blood [11]. Excess formaldehyde will bind into natural antioxidant compounds in the body; for example, reduced glutathione (GSH), and will conjugate to form S-hydroxymethyl glutathione, causing a drastic reduction in the amount of GSH [12]. Formaldehyde may also increase the production of Reactive Oxygen Species (ROS) compounds in the body, causing oxidative stress. Oxidative stress is an unbalanced state between the antioxidants and the prooxidant present in the body that can further lead to the hepatic cell damage and death [13]. Under conditions of oxidative stress, there is a decrease in GSH levels in the body [14]. GSH is an endogenous antioxidant that has several functions in tissue protection from oxidative damage and maintains the stability of the intracellular environment. GSH prevents free radical formation by functioning as a substrate in the decomposition reaction of a non-radical compound H_2O_2 into H_2O and O_2 molecules catalyzed by glutathione peroxidase (GPx) enzyme. GSH itself will turn into oxidized glutathione (GSSG) in this reaction [15].

The use of antioxidants has begun to develop recently as the understanding of its role in inhibiting degenerative diseases caused by the accumulation of ROS, such as heart disease, atherosclerosis, cancer, and the symptoms of aging [16]. Antioxidants are chemical compounds that can contribute one or more electrons to free radicals so that free radicals can be inhibited [17]. The human body doesn't have excessive amounts of antioxidants, so if there is excessive radical exposure, then the body needs exogenous

antioxidants [18]. One of the known exogenous antioxidants that have very strong effects is astaxanthin [19].

Astaxanthin is a carotenoid pigment that naturally found in foods such as shrimp, crabs, lobsters, freshwater fish, marine fish, salmon, as well as several types of bacteria and fungi [20, 21]. *Haemotococcus pluvialis*, one species of green algae, is the largest natural source of astaxanthin [22]. The antioxidant capability of astaxanthin is primarily caused by its unique chemical structure, allowing astaxanthin to capture a single ROS. Biswal reported that showed that the effect of astaxanthin as an antioxidant is 10 times stronger than other carotenoids such as lutein, zeaxanthin and β -carotene and 100 times stronger than α -tocopherol [19]. Chan et al reported astaxanthin supplementation significantly improved the production of depleted GSH and reduced ROS production [23]. Otton et al reported that endogenous antioxidants such as GSH, GPx, and Superoxide Dismutase (SOD) will increase after 45 days of astaxanthin administration [24]. Based on the background above, the aim of this study was to investigate the effect of astaxanthin on glutathione levels in damaged liver tissues of male Wistar rats induced by oral formaldehyde.

2. Materials and Methods

This was an experimental research study with the post-test only control group design. Pure astaxanthin powder derived from *Haematococcus pluvialis* extract was purchased from Futamed Industries. The sample used was liver tissues of Wistar rats. A total of 30 rats, which aged two to three months, were divided into 5 groups: control (normal and negative) groups and treatment groups (group 1, 2 and 3). Rats were adapted for 14 days and treatment was administered for the next 28 days. The normal control group is given only standard feed and drink. The negative control group was given oral formaldehyde induction for 14 days. Treatment group 1, 2 and 3 were given oral formaldehyde induction for 14 days, followed by administration of astaxanthin dose 12, 24 and 48 mg/day.

GSH level assay of hepatic tissue was tested by using Ellman Method Measurements [25] and was performed after 4 weeks of treatment. The study period was from September to November 2015 at the research laboratory in Tanjungpura University. All data are reported as mean \pm SD. Statistical tests were performed using One-way Anova test, followed by Post Hoc Test. A p value of <0.05 was considered to be statistically significant.

3. Results

From the research that has been done, the mean value of GSH levels of hepatic tissue can be seen in Figure1.

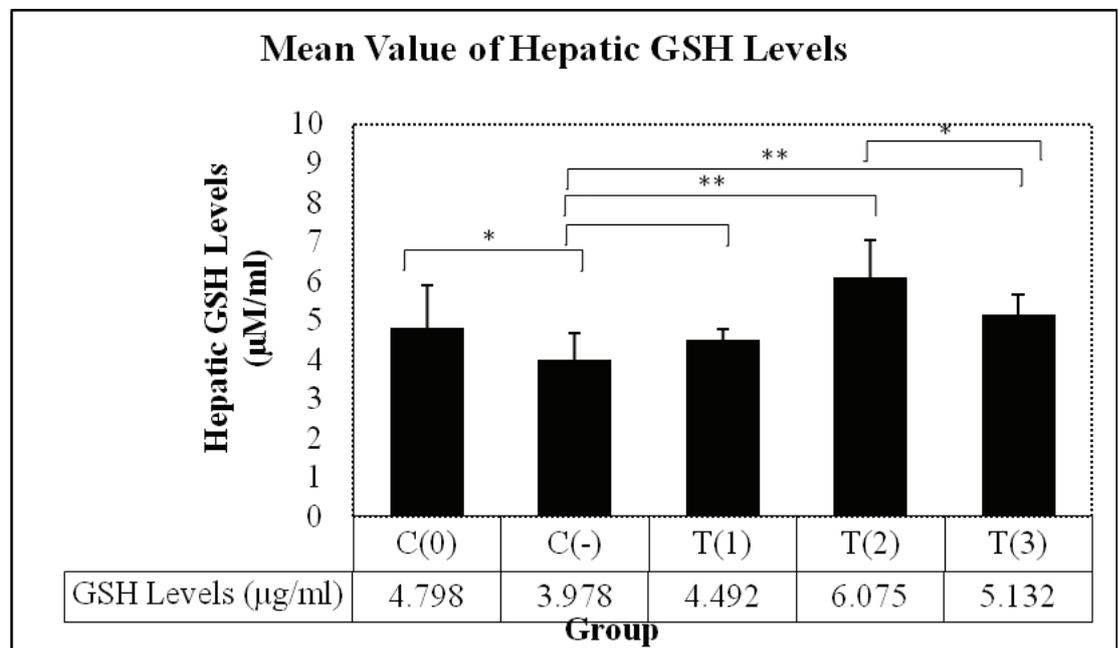


Figure 1: Effect of Astaxanthin on Hepatic GSH Levels. Each value represents mean value \pm SD. * $p > 0.05$ by One-way Anova test followed by Post Hoc test; ** $p < 0.05$. C(0), normal control; C(-), negative control; T(1), treatment of astaxanthin dose 12 mg/day; T(2), treatment of astaxanthin dose 24 mg/day; T(3), treatment of astaxanthin dose 48 mg/day.

We have demonstrated the effect of astaxanthin on hepatic GSH levels in rat liver damage induced by oral formaldehyde. Figure 1 showed that the lowest mean value of GSH levels was in the negative control group and the highest was in the treatment group 2. Furthermore, GSH levels in the treatment group 1 were not significantly different from the normal control group. However, there was a significant improvement of GSH levels in the treatment group 2 compared with the normal control group and the negative control group. In the treatment group 3, there was a significant difference with the negative control group but was not different significantly with the treatment group 3.

4. Discussion

We conducted *in vivo* studies to investigate the effects of astaxanthin, a natural antioxidant, on hepatic GSH levels. We demonstrated that post-treatment with astaxanthin could improve hepatic GSH levels. The three treatment groups tested had higher GSH levels when compared with the negative control group, although the treatment group 1

was not statistically significant compared to the negative control group. A previous study by Kang et al (2001) [26] and Wang et al (2014) [27] also indicated that administration of astaxanthin could increase GSH levels of tissue, in hepatic and renal tissue. These results collaborated those of previous studies.

The effect of elevated GSH levels on a hepatic tissue by astaxanthin may be caused by several mechanisms, such as increased GSH biosynthesis through activation of the NRF2 pathway and decreased ROS through its work as a scavenger, thus assisting GSH in eliminating ROS and inhibiting lipid peroxidation in hepatic cells due to exposure to formaldehyde [28, 29]. Astaxanthin has the ability to trigger NRF2 to mediate endogenous antioxidant systems in the body, by increasing the expression of various antioxidant enzymes that play a role in biosynthesis and GSH function. It should be noted that there are two GSH biosynthesis pathways, among others through the de novo synthesis pathway and the resynthesis pathway. GSH biosynthesis via the de novo pathway takes place by utilizing the amino acids available in the body as its constituent materials, including glutamate, cysteine, and glycine, already present in the cell or transported from the extracellular. The resynthesis pathway occurs by reducing GSSG to GSH with the help of Glutathione Reductase (GR) enzyme and requires NADPH [30].

In addition to activating the NRF2 system, astaxanthin as an antioxidant scavenger could be effective in counteracting various ROS in the cells. Astaxanthin has a wide antioxidant capacity, due to its unique chemical structure, distinct from other antioxidants. It has polar and nonpolar clusters, so it can enter all parts of the cell membrane in a linear fashion. These structures and positions cause astaxanthin to counteract ROS both outside and inside the cell [31, 32].

The results showed that the treatment group 2 had higher levels of GSH than the treatment group 3. In other words, although the dose of treatment group 3 was greater than the treatment group 2, the effect on GSH was lower instead. In the body, there is a mechanism for setting up homeostasis for GSH levels in various tissues, one of which is liver. GSH is an important and required tripeptide by all cells, with normal levels of about 1-10mM in all cell types, including hepatocyte cells. GSH deficiency can cause oxidative stress, which causes aging, as well as various diseases in humans that include neurodegenerative diseases, liver disease, diabetes, cystic fibrosis, AIDS, heart disease and cancer. However, when intracellular GSH levels meet the needs of the cell, both in the prevention of free radicals and other functions, there will be a GSH degradation mechanism, using the γ -Glutamyl-transpeptidase (γ -GT) enzyme that will break GSH into cysteine glycine and glutamate residues which will be transported to other amino acid acceptors. Furthermore, cysteine glycine will be broken down by dipeptidase enzyme

to produce amino acids cysteine and glycine which are then allocated for other needs [33].

The effective dose in this study was treatment group 2, with a dose of 24 mg/day. This was due to a statistically significant increase in GSH levels when compared with the normal control group and negative control group.

Although this research has reached its aims, there was an unavoidable limitation. For animal research outcome, there were some intrinsic and extrinsic factors that could affect the result, such as genetics, nutritional and immune status, endocrine factors, cage design, temperature, humidity, ventilation, noise, etc. In addition, due to a limited fund, this research was conducted to see the effect of astaxanthin on hepatic GSH levels only. Therefore, further research are needed to see the effect of astaxanthin on another organ, different biomolecular indicator or method.

5. Conclusion

Astaxanthin could increase GSH levels in damaged liver tissues of male Wistar rats induced by oral formaldehyde

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