Original Article

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Protective Effects of Chlorella Vulgaris in Alcohol Intoxication

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Abstract

Objectives: The aim of the study, to investigate the effect of *Chlorella vulgaris* on the liver, kidney and heart MAPK (Mitogen-activated protein kinase), lipid peroxidation antioxidant enzyme activity with ethyl alcohol toxification.

Materials and Methods: 10-12 monthly, weighing 200-250 gr, 24 adult male Sprague Dawley rats were used. Rats were divided into 3 (n=8) groups which 2 experiments and a control. 5mg/kg of isocaloric maltose was given to the control group by gavage. 15 g/kg ethyl alcohol diluted with 50% water was given to the alcohol group and 300 mg/kg *C. vulgaris* and then 15 g/kg ethyl alcohol diluted with 50% water were given to *C. vulgaris* group. At the end of the experiment tissue samples were taken. Blood samples were collected into EDTA tubes and the tissues were kept at -20°C. The blood and tissue samples were used to investigate the GSH/GSH-Px, MAPK activity and MDA levels.

Results: MAPK activities in liver and lung tissue were increased with *C. vulgaris* which decrease with ethyl alcohol while MAPK activities in kidney and heart tissue decreased with *C. vulgaris*. The reduction in tissue GSH-Px levels with alcohol was increased significantly with *C. vulgaris* application ($p<0.05$). The declining GSH levels of liver and kidney tissue with alcohol were found to significantly increase with *C. vulgaris* ($p<0.05$). When tissue MDA levels of groups were compared, increasing MDA levels with alcohol in liver and heart tissues were determined to significantly decrease with *C. vulgaris* ($p<0.001$, $p<0.05$ respectively).

Conclusions: As a conclusion, *C. vulgaris* increased antioxidant enzyme activity especially in liver tissues and decreased lipid peroxidation in tissue which arises with ethyl alcohol that was evaluated this effect has tissue protective activity of *C. vulgaris*. Also, increasing in liver tissue MAPK activities as a result of alcohol toxication was fixed with *C. vulgaris*. This situation may be associated with a protective effect on the intracellular enzyme system activity of *C. vulgaris*.

Keywords: Ethyl alcohol, Chlorella vulgaris, GSH, GSH-Px, MAPK, MDA.

Introduction

Ethyl alcohol, which is used in the production of alcoholic beverages, is easily absorbed into the blood from all parts of the digestive system. The level of alcohol in the blood usually reaches its highest level in 45-60 minutes, depending on the fullness of the stomach and the rate of alcohol intake[1]. Behavioral disorders, manifested by decreased reflexes and muscle dissonance, occur due to toxication due to excessive alcohol consumption. Depending on the severity of the toxicity, liver enzyme dysfunction and death due to respiratory failure occur[2,3]. 90% of alcohol is metabolized in the liver and 10% in the lungs at 15 mg/dl per hour. Alcohol creates oxidative stress in tissues and affects a wide variety of cellular targets rather than a specific tissue area[4,5,6], causing many adverse metabolic disorders, especially liver damage[7,8]. About 20% of people diagnosed with alcoholism develop irreversible liver damage and severe liver disease[9,10,11].

As a result of the deterioration of the oxidative balance, "oxidative stress" occurs. Oxidative stress resulting in cellular destruction is accepted as an indicator of the deterioration of metabolic balance. The main elements that play a role in the detoxification mechanisms required by metabolism to prevent cell and tissue damage due to oxidative stress are enzymes, proteins, vitamins, plant polyphenols and derivatives that are activated to reduce or eliminate the damage caused by oxidant molecules[12,13]. Alcohol causes oxidative stress and therefore many metabolic problems[14,15]. Oxidative stress damages lipid, protein and DNA cell components, causing cell destruction and death. Superoxide dismutase, catalase and glutathione peroxidase play an active role in cell repair mechanisms[16,17]. Free radicals cause lipid peroxidation by the effect of oxidizing agents on fatty acids in the structure of membranes and lipoproteins. Malondialdehyde (MDA), a reactive aldehyde derivative, is formed as a result of lipid peroxidation caused by free radicals. The amino groups of MDA proteins react with phospholipids or nucleic acids to damage membrane structures and cause cellular damage. Changes in cellular damage are also an indicator of oxidative damage[18,19].

Antioxidants can be endogenous or exogenous. Superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GSH-Px) and catalase are antioxidants in enzyme structure. GSH helps transport amino acids across cell membranes, and the GSH sulfhydryl

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group is used to reduce peroxides formed during oxygen transport. In addition, GSH acts as a very important defense mechanism against peroxides formed as a result of ethyl alcohol metabolism reactions. Oxidative stress is effective in the progression of alcoholic liver disease, and antioxidants fight this stress. Antioxidant mechanisms such as GSH and GSH-Px have high activity to prevent damage[20,21].

Chlorella is the most important member of the *Chlorellaceae* family from the *Chlorophyta* group. *C. vulgaris* is rich in vitamins, proteins, minerals, amino acids, nucleic acids, essential fatty acids, enzymes and carotenoids, alpha-beta carotene, alpha-tocopherol, lycopene, lutein and zeaxanthin. Due to its rich content of *Chlorella vulgaris*, it has become a popular food supplement in oxidative stress studies in terms of preventing cell damage and death[22,23].

Mitogen-activated protein kinase (MAPK) is the familiar name for a set of receptor-mediated proteins that play a vital role in signal transduction in all eukaryotic cells from yeast to humans. It plays an important role in transporting the information sent from the receptors to the cell to the nucleus. MAPKs are proteins with apoptotic function, responsible for signal transduction and involved in the regulation of gene expressions and cell functions[24]. Alcohol use is associated with liver disease, neurotoxicity, hypertension, cardiomyopathy, immune response patterns, and increased cancer risk. Available data suggest that the MAPK family plays a central role in these alcohol-affected processes. The role of MAPK may differ depending on the cell type and the type of chronic or acute administration. For example; Acute alcohol administration causes increased MAPK activation in hepatocytes, astrocytes, and vascular smooth muscle cells. In the studies carried out, the effect processes of alcohol in such pathological conditions are not fully understood[37].

Materials and Methods

The study was carried out by the principles of Kafkas University KAU Animal Experiments Local Ethics Committee (KAU-HADYEK Decision No: 2014-31). In this study, 24 adult male Sprague Dawley rats, 10-12 months old, weighing 200-250 g, were used. The rats were kept at 25°C under standard light and 12 hours a day/12 hours in the dark and were fed ad libitum with water and food.

Experimental Groups

Rats were divided into 3 groups and each group consisted of 8 animals, 2 experimental and 1 control group. The control group was given 5 mg/kg of isocaloric maltose every 12 hours by gavage. Ethyl alcohol was given to the rats in the alcohol group (n=8) at 15g/kg/day and diluted with 50% water, and 300 mg/kg of *Chlorella* and then 50% was given to the rats in the *Chlorella* group (n=8). It was diluted with water and given 15g/kg of ethyl alcohol. The study was repeated for 20 days.

The groups used in the study were formed as follows.

Control Group (n=8, male): 5 mg/kg isocaloric maltose by gavage

Alcohol Group (n=8, male): 15 g/kg/day Ethyl alcohol + 50% water

Chlorella Group (n=8, male): First 300 mg/kg *Chlorella*, then 15 g/kg/day Ethyl alcohol + 50% water

At the end of the experiment, the anterior abdominal wall of the rats was opened with an incision under ether anesthesia. The rats, whose blood was taken by puncture by reaching the heart from the diaphragm, were sacrificed. Blood samples taken intracardially into EDTA tubes were centrifuged at + 4°C, 3000 rpm for 5 minutes and plasmas and homogenates prepared by the methods were placed in polyethylene tubes and stored at -20°C until laboratory procedures. The blood samples taken were used to measure MDA, MAPK, GSH and GSH-Px values.

Biochemical Analysis

MAPK levels were determined using the RAT MAPK14 (Mitogen-activating protein kinase 14) ELISA Kit (Elabscience Biotechnology Co. Ltd., P.R.C).

Malondialdehyde (MDA), a secondary product of lipid peroxidation (LPO), is formed by incubation of plasma with thiobarbituric acid (TBA) at 100°C. The resulting MDA forms a pink complex with TBA. By measuring the pink color at 532 nm in the spectrophotometer, LPO is determined as nmol/ml[25]. 1,1,3,3 tetra ethoxy propane was used for drawing the standard curve.

The measurement of the thiol group by enzymatic or chemical processes by dissolving the sulfhydryl group of GSH in acid forms the basis of the quantification of this compound. The absorbance values determined at 412 nm are measured as μmol/ml[26].

Glutathione peroxidase activity was measured spectrophotometrically at 412 nm in Ellman's reagent, which is based on the principle of GSH reduction in enzymatic reactions, in which cumene hydroperoxide and reduced GSH are used as co-substrates[27].

Statistical Analysis

In statistical calculations, the ONE-WAY ANOVA test was used to compare the changes in the experimental groups compared to the control groups. Results were determined as mean ± standard deviation (X ± SD) and $p<0.05$ showed the statistical difference. All calculations were made using the SPSS (16.0) package program.

Results

Statistical analysis of the experimental work of all groups is shown in the figures.

***Changes Determined in MAPK Levels of Kidney, Heart and Liver Tissues of the* Groups**

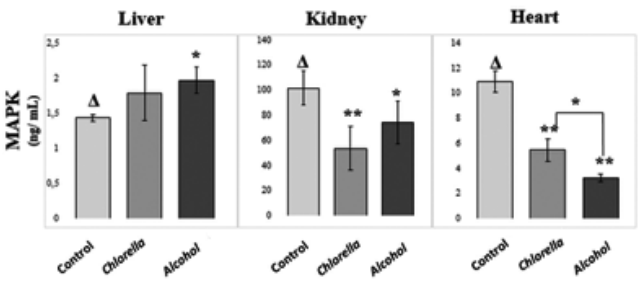

Figure 1: MAPK Levels ($^{\Delta}$* = p<0.05, $^{\Delta}$** = p<0.001)

It was determined that the values of the experimental groups were statistically significantly higher than the control group (p<0.05).

Changes in liver MAPK levels when compared between the control group and the alcohol group, it was determined that the liver MAPK levels were statistically significantly higher in the alcohol group (p<0.05). In the comparison made between the control group and the *Chlorella* group, it was determined that the liver MAPK levels did not show a statistical difference.

In the comparison between the control group and the *Chlorella* and Alcohol groups, it was determined that the kidney MAPK levels were statistically lower in the *Chlorella* and Alcohol groups (Respectively p<0.001, p<0.05).

In the statistical comparison between the control group and the *Chlorella* and Alcohol groups, a significant decrease was found in the Cardiac MAPK levels of the *Chlorella* and Alcohol groups (p<0.001). When *Chlorella* and Alcohol groups were compared, it was determined that there was a statistically significant decrease in the Alcohol group compared to the *Chlorella* group (p<0.001).

Changes Determined in MDA Levels of Kidney, Heart and Liver Tissues of the Groups

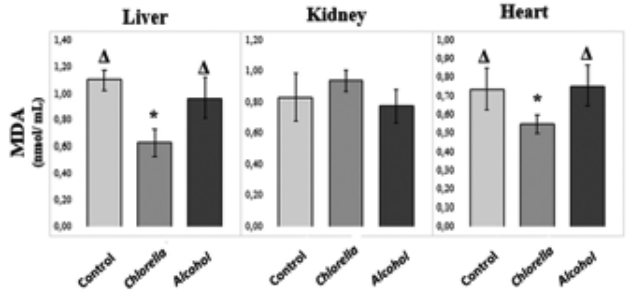

Figure 2: MDA Levels ($^{\Delta}$* = p<0.001)

A statistically significant difference was found between the Control and Alcohol groups and the *Chlorella* groups in liver MDA levels (p<0.001). It was determined that the MDA levels of the *Chlorella* group were statistically lower than the alcohol group (p<0.001).

There was no statistically significant difference in kidney tissue MDA levels.

A statistically significant difference was found between the Control and Alcohol groups and the *Chlorella* groups in the heart MDA levels (p<0.05). It was determined that the heart MDA levels of the *Chlorella* group were statistically lower than the alcohol group (p<0.05).

Changes Determined in GSH Levels of Kidney, Heart and Liver Tissues of the Groups

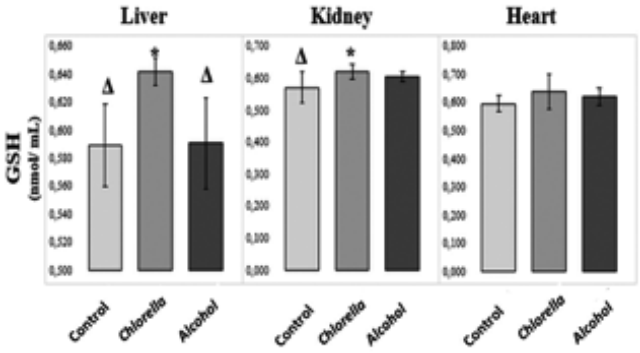

Figure 3: GSH Levels ($^{\Delta}$* = p<0.05)

When examined in terms of GSH levels in the liver tissue, it was determined that there was a statistically significant increase in the *Chlorella* Group compared to the Control and Alcohol groups (p<0.05).

When the kidney tissue GSH levels were compared, a statistically significant increase was found in the GSH enzyme levels in the *Chlorella* group compared to the control group (p<0.05).

No statistically significant difference was found when the changes in heart tissue GSH levels were compared.

Changes Determined in GSH-Px Levels of Kidney, Heart and Liver Tissues of the Groups

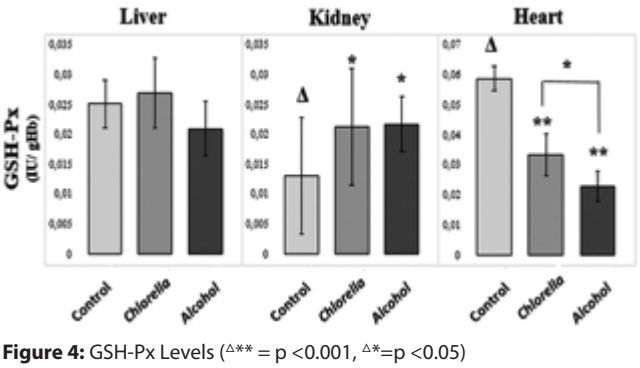

Figure 4: GSH-Px Levels ($^{\Delta}$** = p <0.001, $^{\Delta}$*=p <0.05)

In the comparison between the groups, it was determined that the changes in liver GSH-Px levels did not show a statistically significant difference.

When the changes in kidney GSH-Px levels were compared, no statistically significant difference was observed between the *Chlorella* and Alcohol groups. In the comparison between the kidney GSH-Px levels of the control group and the *Chlorella* and Alcohol groups, it was found that the GSH-Px levels of the *Chlorella* and Alcohol groups were statistically high (p<0.05).

In the comparison between the control group and the *Chlorella* and Alcohol groups, it was determined that the heart GSH-Px levels were statistically significantly lower in the *Chlorella* and Alcohol groups ($p<0.001$). In the comparison between the *Chlorella* group and the Alcohol group, it was found that the heart GSH-Px values were statistically higher in the *Chlorella* group than in the Alcohol group ($p<0.05$).

Discussion

Alcohol toxicity is caused by other metabolic products of ethanol, especially reactive oxygen species (ROS) produced during the biotransformation of ethanol. Alcohol is a strong oxidant; the Mitochondrial respiratory chain activates intracellular ROS production pathways including xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathways. As a result, there is an increase in the production of superoxide (O_2-•), hydrogen peroxide (H_2O_2) and hydroxyl (•OH) radicals in the cell. High ethanol consumption reduces intracellular antioxidant capacity. In the damage caused by alcohol, antioxidant systems protect hepatocytes and are activated by supporting the cell membrane against the increase of radicals, and the imbalance in the cell results in the oxidation of protein, lipid and DNA. In rats, MDA, hydroperoxide and conjugated diene levels were increased due to alcohol. However, ascorbic acid application prevented the increase in free radical levels, and GSH and GSH-Px levels that decreased as a result of ethyl alcohol applications increased again after ascorbic acid application28. The P-450 enzyme system, which plays an active role in alcohol metabolism, causes ROS production in hepatocytes and causes damage[29]. As a result of the increase in the alcohol level in the blood, the cytochrome C level decreased and accordingly, it caused the formation of superoxide radicals in the liver[30]. Toxic doses of ethyl alcohol combine with superoxides in liver cells to initiate lipid peroxidation and the ROS products formed trigger cellular deterioration. DNA breaks caused by free radicals can be prevented by applying metallothionein, which is a rich protein source[31].

In our study, we determined that alcohol application increased lipid peroxidation in liver and heart tissue, while *Chlorella* application decreased this increase. However, we did not observe any difference in terms of MDA levels in kidney tissue. We think that the results we obtained in the liver and heart tissue may be due to the effect of antioxidant membrane components, especially in *Chlorella*. The lack of difference in kidney tissue may be due to the longer time alcohol reaches these regions.

Thiol and GSH constitute the most important of the non-enzymatic intracellular antioxidants group among the advanced defense mechanisms to prevent cellular damage in the organism due to reactive oxygen species. The glutathione peroxidase system, one of the intracellular enzymatic antioxidants, includes the use of glutathione peroxidase enzymes, glutathione reductase (GR), reduced glutathione (GSH) and reduced NADPH as cofactors. Glutathione peroxidase; It reduces hydrogen peroxide and other organic peroxides by using GSH that is oxidized to glutathione disulfide forms (GSSG). They show activity either by inhibiting the formation of ROS or by scavenging free radicals and their precursors. In individuals with alcohol dependence, oxidant and antioxidant balances deteriorate and increase ROS[32,33].

Thanks to the antioxidants it contains, it is known that the protective effect of *Chlorella vulgaris* plays an active and preventive role in cases such as cell damage and cell death caused by oxidative stress-induced substances[34,35].

Chlorella vulgaris reduced the oxidative damage of lead, which is a heavy metal and has a very high toxic effect, in brain cells. In addition, hepatotoxicity caused by cadmium, which is a heavy metal and has a toxic effect, has been shown to have an inhibitory effect on tissue damage due to its antioxidant property[36].

In our study, we determined that ethyl alcohol caused a decrease in GSH-Px levels in the heart tissue, but this decrease was improved by the application of *Chlorella vulgaris*. We determined that *Chlorella vulgaris* did not change GSH-Px levels in liver and kidney tissues. We think that these changes may be due to the intracellular enzymatic system supportive activity of *Chlorella vulgaris* and the high antioxidant compounds it contains. In our literature review, we could not find any study showing the effect of *Chlorella vulgaris* on ethyl alcohol toxicity.

Alcohol use is associated with liver disease, neurotoxicity, hypertension, cardiomyopathy, immune response modulations, and an increased risk of cancer. In the studies, the effect processes of alcohol in such pathological conditions are not fully understood. The available data point out that the MAPK family plays a central role in these processes affected by alcohol[37,38,39].

In our study, it was determined that MAPK was increased in the liver in animals in the alcohol-administered group, but this alcohol-induced increase was suppressed in the *Chlorella*-treated group. However, in the literature review, no study was found on the effects of *Chlorella* on MAPK in alcohol toxicity. In our study, it can be thought that *Chlorella* has a corrective activity against liver damage caused by alcohol, with this aspect of liver MAPK levels increased by alcohol administration and decreased by *Chlorella* administration. In addition, the reasons for the alcohol-related decrease in MAPK activities in kidney and heart tissue require molecular evaluation.

As a result, it is evaluated that *Chlorella vulgaris* increases antioxidant enzyme levels especially in liver tissues against lipid peroxidation that occurs in tissues with the effect of alcohol, and has tissue protective activity with

this effect. In addition, increased MAPK activities in liver tissues as a result of alcohol intoxication improved with the effect of *Chlorella*, and it is thought that this situation may be associated with the protective effect of *Chlorella* on intracellular enzyme systems. New studies are still needed to determine the molecular pathway by which *Chlorella* displays its tissue protective activity in alcohol intoxication.

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