

RESEARCH ARTICLE

Hepatoprotective Effect of Sun Chlorella as an Antioxidant in *Rattus norvegicus* Induced Carbon Tetrachloride**Wahyu Siswandari,¹ Fitranto Arjadi,² Hernayanti,³ Vitasari Indriani¹**¹Department of Clinical Pathology, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia,²Department of Anatomy, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, Indonesia,³Department of Ecotoxicology, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Indonesia**Abstract**

Carbon tetrachloride (CCl₄) is a chemical that can cause damage to liver cells. One of the natural ingredients developed to reduce liver damage due to chemicals and infections is *Chlorella* sp. The research aimed to explore the hepatoprotective effect of the antioxidant superoxide dismutase (SOD) by administering Sun Chlorella in *Rattus norvegicus* CCl₄ induced rats. The study design used a post-test with a control group design with a completely randomized design trial on 30 male rats of Wistar strains, aged 2–3 months, 200–250 grams in weight. The research was conducted in November 2016–January 2017 in Purwokerto. Rats were divided into five groups and treated for four weeks as follows: K1 group was given aquades; K2 was given CCl₄; K3, K4, K5 were given CCl₄ and Sun Chlorella 3.6 mg/200 gBW, 7.2 mg/200 gBW, and 14.4 mg/200 gBW respectively, by the gastric probe. Statistical analysis with correlation test and one way ANOVA multivariate test showed that Sun Chlorella 7.2 and 14.4 mg/200 gBW significantly increased SOD levels in rats induced CCl₄ ($p=0.004$, $p=0.009$). SOD rates were significantly strong associated with aspartate aminotransferase/AST ($r=-0.685$, $p=0.000$) and alanine aminotransferase/ALT ($r=-0.659$, $p=0.000$). The conclusion is Sun Chlorella increases SOD levels in CCl₄-induced rats. Increased SOD levels may decrease AST and ALT levels.

Key words: Antioxidant, CCl₄, *Chlorella*, SOD**Efek Hepatoprotektif *Sun Chlorella* sebagai Antioksidan pada *Rattus norvegicus* yang Diinduksi Karbon Tetraklorida****Abstrak**

Karbon tetraklorida (CCl₄) merupakan bahan kimia yang dapat menyebabkan kerusakan sel hati. Berbagai bahan alami telah dikembangkan untuk mengurangi kerusakan hati baik akibat bahan kimia maupun infeksi, salah satunya adalah *Chlorella* sp. Penelitian ini bertujuan melihat efek hepatoprotektif dengan pemberian *Sun Chlorella* pada tikus *Rattus norvegicus* yang diinduksi CCl₄. Desain penelitian menggunakan *post-test with a control group* dengan rancangan percobaan rancangan acak lengkap pada 30 tikus jantan galur Wistar, usia 2–3 bulan, dan berat 200–250 gram. Penelitian ini dilakukan di Purwokerto pada periode November 2016–Januari 2017. Tikus dibagi menjadi lima kelompok dan mendapatkan perlakuan per oral dengan sonde lambung selama 4 minggu sebagai berikut: kelompok K1 sebagai kontrol negatif diberikan aquades; kelompok kontrol positif K2 diberikan CCl₄; kelompok perlakuan K3, K4, dan K5 diberikan CCl₄ dan *Sun Chlorella* 3,6 mg/200 gBB; 7,2 mg/200 gBB; 14,4 mg/200 gBB tikus berurutan. Uji statistik dengan menggunakan uji korelasi dan ANOVA satu arah menunjukkan bahwa pemberian *Sun Chlorella* 7,2 mg dan 14,4 mg meningkatkan kadar SOD pada tikus yang diinduksi CCl₄ secara bermakna ($p=0,004$; $p=0,009$). Kadar SOD berhubungan kuat dengan kadar aspartat aminotransferase/AST ($r=-0,685$; $p=0,000$) dan alanin aminotransferase/ALT ($r=-0,659$; $p=0,000$). Kesimpulan penelitian ini adalah pemberian *Sun Chlorella* meningkatkan kadar SOD pada tikus yang diinduksi CCl₄. Peningkatan kadar SOD menyebabkan penurunan kadar AST dan ALT.

Kata kunci: Antioksidan, CCl₄, *Chlorella*, SOD

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Introduction

The liver is an organ essential in the metabolism and detoxification of foreign substances in the body, such as carcinogens, chemicals, drugs, and insecticides.^{1,2} These substances can cause damage to hepatocyte cells in the liver.³ One of the chemicals that can impair the liver is carbon tetrachloride (CCl₄), which increases lipid peroxidation and hepatocyte death marked by swelling, vacuolization, and hepatocyte in rats.^{4,5} It also promotes cirrhotic change.⁶ Therefore, induction of CCl₄ in experimental animals is often used in research.^{6,7}

Various drugs, natural ingredients, or organisms have been developed to reduce liver damage due to chemicals and infections. One of the organisms used to resolve liver damage is *Chlorella* sp.⁸ *Chlorella* is a unicellular green alga that contains various antioxidants such as chlorophyll, essential amino acids, protein, minerals, vitamins, dietary fiber.⁹ It also contains phytochemical composition such as alkaloids, flavonoids, triterpenes, glycosides, tannins, and phenols.¹⁰ Carotenoid is one of the substances in *Chlorella* sp., which plays a role as an antioxidant.¹¹

Many studies of *Chlorella* sp. have been extensively researched. Lee et al.⁷ (2010) showed that the administration of *Chlorella* supplements to smokers has antioxidant effects. Azocar and Diaz¹² (2013) found that replenishment of *Chlorella* can reduce aspartate aminotransferase levels in adult chronic hepatitis C patients. Cai et al.¹³ (2015) showed that *Chlorella vulgaris* extract led to a significant decrease in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rats, followed by an increased antioxidant activity such as superoxide dismutase (SOD), catalase, and glutathione (GSH), which compared to the CCl₄ induced rats.^{12,13}

This study aims to explore hepatoprotective effects of Sun Chlorella (CNI Sun Chlorella; PT Citra Nusa Insan Cemerlang [PT CNI], Jakarta, Indonesia) based on antioxidant status, specifically in levels of superoxide dismutase (SOD) in *Rattus norvegicus* carbon tetrachloride (CCl₄) induced rats. This study differs from Cai et al.¹³ in model animals for CCl₄ and *Chlorella* dosage and treatment duration.

Methods

This research was conducted in Animal House and Research Laboratory, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto, from November 2016–January 2017.

Thirty male white rat Wistar strain with 2–3 months of age and 200–250 g of weight from LPPT IV Universitas Gadjah Mada Yogyakarta was used in this study. Ethics approval for this research was issued by the Research Ethics Committee, Faculty of Medicine, Universitas Jenderal Soedirman, Purwokerto No. 176/KEPK/X/2016.

The *Chlorella* preparation used in this study was the Sun Chlorella tablet produced by PT CNI (SD 081534191), which contained 170 mg of *Chlorella* powder extracted from *Chlorella pyrenoidosa*. This Sun Chlorella was chosen because it is widely available and has been commonly used as a health supplement.

This research was conducted experimentally with a completely randomized and post-test only with a control group design. The rats were divided into five groups, namely K1, K2, K3, K4, K5, and were acclimated within seven days. The negative control group (K1) were given aquades, the positive control group (K2) were given CCl₄ (Merck, Sigma-Aldrich 289116); CCl₄ + *Chlorella* 3.6 mg (K3 group); CCl₄ + *Chlorella* 7.2 mg (K4 group); CCl₄ + *Chlorella* 14.4 mg (K5 group). Aquades or CCl₄ were given orally with a gastric probe at a dose of 0.2 mL/100 g of body weight (BW) two times a week for two weeks, followed by 0.1 mL/100 gBW two times a week for two weeks. Sun Chlorella (CNI Sun Chlorella) was given orally with a gastric probe at a dose of 3.6 mg or 7.2 mg or 14.4 mg/200 gBW for four weeks.¹⁴ *Chlorella* dose of 7.2 mg/200 gBW was obtained from converting an adult human dose to 400 mg/70 kg. Next, the dose was divided by two (3.6 mg) and multiply by two (14.4 mg).¹⁵

Blood collection was done through the orbitalis vein of the rat one day after the treatment. Furthermore, the blood was centrifuged at a speed of 4.000 rpm. The blood serum was then separated from erythrocytes, and then AST, ALT, and SOD were examined.

AST (DiaSys 126019910920), ALT (DiaSys 127019910026), and SOD (Randox SD126) levels were examined using a spectrophotometer (Shimadzu UV 1800). The data obtained were

analyzed using a correlation test and one way ANOVA multivariate test. One data from the K3 group was excluded because it was an outlier.

Results

The mean levels of AST, ALT, and SOD in various treatment groups are presented in Table 1. AST, ALT, and SOD levels have normal data distribution ($p > 0.05$), but the homogeneity of

AST and ALT variance showed different variants. The highest mean levels of AST (3,210.00 U/L) and ALT (2,616.66 U/L) were acquired in the K2 group, the group with CCl₄ administration, while the highest mean levels of SOD (73.00 U/mL) was in the negative control group (K1).

The results showed that administration of CCl₄ increased AST levels from 267.33 U/L to 3,210.00 U/L ($p = 0.027$). Sun Chlorella at either dose of 3.6 mg, 7.2 mg, or 14.4 mg/200 gBW did

Table 1 AST, ALT, and SOD Levels in Experimental Animals

Groups	AST (U/L) Mean ($\pm$ SD)	ALT (U/L) Mean ($\pm$ SD)	SOD (U/mL) Mean ($\pm$ SD)
K1	267.33 (99.32)	233.83 (135.24)	73.00 (4.42)
K2	3,210.00 (154.07)	2,616.66 (1,105.72)	37.83 (4.95)
K3	821.20 (477.84)	886.00 (716.34)	40.00 (3.74)
K4	1,385.00 (643.60)	1,110.00 (503.65)	48.50 (2.94)
K5	947.50 (646.85)	795.83 (621.38)	66.00 (5.58)

Table 2 Correlation between SOD Levels with AST and ALT Levels

AST and ALT Levels	r^*	Significance (p)
AST	-0.685	0.000
ALT	-0.659	0.000

Note: *Spearman's correlation

not significantly reduce AST compared to the positive control group (respectively $p = 0.058$, $p = 0.160$, and $p = 0.073$).

CCl₄ induced an increase in ALT from 233.83 U/L to 2,616.66 U/L ($p = 0.016$). Sun Chlorella dose of 14.4 mg/200 gBW significantly reduced ALT levels to 795.83 U/L compared to the positive control group (K2) with $p = 0.047$.

Table 3 ANOVA Test and Post Hoc Analysis of AST, ALT, and SOD Levels among Groups

Groups	AST		ALT		SOD	
	ANOVA	Post Hoc	ANOVA	Post Hoc	ANOVA	Post Hoc
	0.000*		0.000*		0.000*	
K1 - K2		0.027*		0.016*		0.000*
K1 - K3		0.235		0.392		0.000*
K1 - K4		0.039		0.037*		0.000*
K1 - K5		0.212		0.313		0.119
K2 - K3		0.058		0.074		1.000
K2 - K4		0.160		0.099		0.004*
K2 - K5		0.073		0.047*		0.009*
K3 - K4		0.498		0.973		0.043*
K3 - K5		0.995		0.999		0.000*
K4 - K5		0.765		0.866		0.000*

Note: *the mean difference is significant at the 0.05 level

SOD levels in experimental animals decreased from 73.00 U/mL to 37.83 U/mL due to CCl₄ administration ($p=0.000$). Sun Chlorella tablet given at a dose of 7.2 mg and 14.4 mg/200 gBW significantly increased SOD levels ($p=0.004$ and $p=0.009$) compared to the positive control group. The increase in SOD levels by Sun Chlorella administration dosed of 14.4 mg/200 gBW approached the SOD levels of the healthy control group ($p=0.119$). This study found a significant strong relationship between SOD levels with AST ($r=-0.685$, $p=0.000$) and ALT levels ($r=-0.659$, $p=0.000$).

Discussion

CCl₄ is proven to cause damage to liver cells, which is observed from increased AST and ALT levels. The highest AST and ALT levels were found in the positive control group that received CCl₄ only. Adipocytokine adiponectin¹⁶ mediates liver injury by CCl₄ through bioactivation of endoplasmic reticulum and mitochondrial centrilobular hepatocytes, which contain many cytochrome P450 2E1 (CYP2E1). This mechanism cause trichloromethyl (CCl₃) and trichloromethyl peroxy (CCl₃OO) free radicals to activate haloalkylation of cell macromolecules. Cell destruction due to CCl₃ can occur anaerobically or aerobically. The anaerobic process is in the form of dimerization of CCl₃ to form hexachloroethane. CCl₃ can also bind directly to lipids and microsomal proteins and the heme part of CYP450. The aerobic process causes CCl₃ to bind to oxygen to form trichloromethyl peroxy (CCl₃OO). CCl₃OO can bind directly to tissue proteins or break down to form phosgene (COCl₂) and the electrophilic form of chlorine. CCl₃ peroxy radical is the main initiator of lipid peroxidation that is formed from carbon tetrachloride exposure. This mechanism causes lipid peroxidation, which harms membrane integrity, decreased organelle function, and cell death.¹⁶⁻¹⁸

Hepatocyte cell death causes the release of enzymes, including AST and ALT enzymes. AST (around 80%) comes from mitochondria and 20% from the cytoplasm of liver cells. ALT is an indicator of liver cell damage enzymes, mainly produced by the cytoplasm of liver cells. Increased ALT and AST are found in liver damage.¹⁹ The results of this study indicate that liver cell damage occurs in experimental animals due to CCl₄ induction based on an increase in AST and ALT

levels. The result of this study is consistent with some previous studies. AbouGabal et al.²⁰ found a significant increase in AST and ALT levels in the group given only CCl₄ (1 mL/kgBW mice/day for three weeks) compared to the control and olive oil groups. Likewise, the research of Fortea et al.²¹ showed an increase in AST and ALT levels in the group given CCl₄ twice a week for 12 weeks compared with the control group. Ito et al.,²² Song et al.,²³ El-Bialy et al.,²⁴ El-Dakhly et al.²⁵ also reported a significant difference in AST and ALT level between CCl₄ and control group. CCl₄ induction treatment for four weeks is likely to cause chronic liver damage, which is observed from an increase in AST levels higher than ALT.¹⁹

This study shows that *Chlorella* can increase SOD antioxidant and decrease AST and ALT levels in *Rattus norvegicus* rats administered by carbon tetrachloride (CCl₄). These results are similar to the previous studies. Cai et al.¹³ reported that pigment-protein complex (PPC) was isolated from *Chlorella vulgaris* effectively restored SOD level. Research by Sikiru et al.²⁶ in the white rabbit showed the highest levels of SOD found in the group given *Chlorella vulgaris* supplements at a dose of 200 mg/gBW. Another study showed that the administration of 5 mg/100 gBW of *Chlorella vulgaris* extract could increase SOD activity to protect liver cells' damage caused by CCl₄ exposure.²⁷ Decreased AST and ALT levels are similar with research results from Cai et al.¹³ and Ito et al.²² Another study proved that *Chlorella* could reduce AST and ALT level in acetaminophen-induced liver damage rat model.²⁸

The carotenoid content in *Chlorella* probably causes an increase in SOD levels.¹¹ Carotenoids are known to have antioxidant functions that significantly reduce free radicals and activate oxidative compounds. Carotenoid content in the form of β -carotene or astaxanthin prevents the oxidation of cells by free radicals through scavenging free radicals to prevent and stop the oxidative reaction chain. The protective mechanism by β -carotene in cells against oxidative reactions is by preventing the formation of oxygen singlets. Singlet oxygen is a non-electrophilic radical ROS that is easily bonded with organic molecules, affecting the oxidation process by directly attacking electron-rich compounds without the involvement of free radicals. β -carotene is also efficient as an oxygen scavenger, reducing ROS release, which decreases in microsomal and cytochrome p450,

prevents chain reactions, or damages the lipid peroxidation reaction.^{27,29,30}

This study found a significant relationship between SOD levels with AST and ALT. The increase in SOD levels would reduce AST and ALT levels. It proves that *Chlorella* has a hepatoprotective effect. In vivo research by Cai et al.,¹³ the administration of protein pigment complexes taken from *Chlorella* showed a hepatoprotective effect. This hepatoprotective effect is expected from the carotenoid content in *Chlorella*, which can inhibit and bind ROS.^{11,30}

Conclusions

CCl₄ induction cause liver damage, as shown by increased levels of AST and ALT. *Chlorella* administration has a hepatoprotective effect in CCl₄-induced *Rattus norvegicus* rats based on increased SOD levels, decreased AST and ALT levels.

Conflict of Interest

The authors state that there is no conflict of interest.

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