International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.2, pp 45-55, 2017

Antioxidant and Hepatoprotective Effect of Black Cincau (*Mesona palustris BL*) Supplement Againts Oxidative Stress in Rats

Tri Dewanti. Widyaningsih[1], Betty Teodora F. Sari[2*]

[1]Food Science and Technology Department , Faculty of Agricultural Technology, Brawijaya University, Malang 65145, East Java-Indonesia
[2]Master Degree Student of Food Science and Technology Department , Faculty of Agricultural Technology, Brawijaya University, Malang 65145, East Java-Indonesia

Abstract : The purpose of this study was to determine the antioxidant activity and hepatoprotective effect of black cincau supplement againts ethanol induced oxidative stress in Rats liver. The research was divided into five treatment groups, namely negative group, positive group, black cincau dose 1, black cincau dose 2, and commercial group. The parameters observed were levels of MDA, SOD, SGPT, and SGOT. Wistar rats induced by alcohol 20% as much as 10 ml/kg/BW (Body Weight) for 2 weeks, and they were made in a state of oxidative stress.
The results showed that treatment with black cincau supplement at a dose of 135 mg/kg/BW per day per mouse had higher hepatoprotective effect than the dose of 90 mg/kg/BW per day per mouse. Histopathology and the percentage of liver cell damage, black cincau supplement group dose of 135 mg/kg/BW and commercial supplement of acai of 135 mg/kg/BW show a protective effect against liver cell damage induced by ethanol.
Keywords : Black Cincau (*Mesona palustris* BL), Supplement, MDA, SOD, SGPT, SGOT.

Introduction

Alcohol is a psychoactive substance that causes dependence on users[1]. Abuse of alcohol is now a major problem often faced by developed countries and developing countries. Based on WHO data, in 2012 about 3.3 million people died or 5.9% of all deaths in the world was due to excessive alcohol consumption[1]. Excessive alcohol consumption can cause various health problems, including disorders of the liver function.

Ethanol cause auto-oxidation in the liver happen through two ways, namely acting as pro-oxidants in liver cells that cause lipid peroxidation process and reducing the amount of antioxidants causing damage to liver cells [2]. An indicator of liver damage is an increase in transaminase enzymes in the liver such as SGPT and SGOT [3].

SOD and MDA is a parameter for measuring antioxidant activity. Lipid peroxidation will lead to increased levels of MDA and decreased SOD enzyme activity in the body. In the oxidative stress condition, in which the amount of endogenous antioxidants is insufficient to address the free radical attack, additional exogenous antioxidants are required, one of them is by taking antioxidant supplement. The source of natural antioxidants are found in all parts of the plants such as fruits, vegetables, nuts, seeds, leaves, roots and barks[4-5].

Black cincau leaves and red ginger are plants that contain bioactive compounds that can be used as an antioxidant supplement.

Black cincau (*Mesona palustris BL*) is traditional Indonesian food produced from *janggelan* plant that produces solid black gel [6]. In China, black cincau is known as *Hsian tsao* and is typically used as a dessert, as well as herbal drinks for the treatment of hypertension, diabetes, and liver[7]. *Hsian tsao* contains bioactive components including polyphenols (ursolic acid, protocatechuic acid, p-hydroxybenzoic acid, vanilic acid, caffeic acid, syringic acid stigmasterol, β-sitosterol, oleanolic acid, and ursolic acid), flavonoids, alkaloids, tannins , saponins, sterols, terpenoids, chlorophyll, ascorbic acid, tocopherols (α-, β-, γ-, δ-) and β-carotene [7,8]. Black *cincau* or *hsian tsao* can function as an antioxidant and antibacterial, hepatoprotective, anti-diarrhea, anti-diabetic, anti-hypertensive, anti-cancer and immunomodulators [9-18].

Red ginger (Zingiber officinale var rubrum) is one of the Indonesian spices generally used as a mixture of herbal remedies, food supplement, beverages, and food [19]. The bioactive components of red ginger are phenols (gingerol and shogaol), flavonoids, alkaloids, tannins, saponins and terpenoids [20]. The main component of ginger have antioxidant effect and can scavenge superoxide anion and hydroxyl radicals[21]. Antioxidant activity of ginger extracts as measured by TPC, FRAP and DPPH which were given as 136.82 GAE/100g DW, 192.52 TE/100g DW, 76.21%[22]. The pharmacological effects of ginger, among others, are as antioxidants, anti-inflammatory, analgesic, anti-carcinogenic, non-toxic and non-mutagenic even though at high concentrations [23,24,25,26]. The research by[21] ginger has protective effect againts liver damage to improve histological parameters in liver tissue.

This study used black cincau and red ginger processed into supplement subsequently given to rat experienced oxidative stress due to ethanol induction. Measurement of antioxidant activity was from the increased activity of SOD and decrease activity of MDA (malondialdehyde), while hepatoprotective activity is assessed from SGOT and SGPT blood serum. Microscopic changes in the liver tissue are observed on histopathological picture of liver cells.

Materials and Method

Materials

Black cincau supplement were derived from previous studies [27]. Acai supplement were purchased from PT. Kimia Farma. The tools used in this study were spectrophotometers, vortex, analytical balance, test tubes, measuring pipettes, flask, cuvette, pipette, stir bars, magnetic stirrer, water bath, a tube Eppendorf, Centrifuge, microtomes, microscopy electrical, and appliance for the maintenance of rat and surgery.

Materials for Analysis

Materials for supplement analysis, such as distilled water, ethanol 96%, Na_2CO_3, Folin Ciocalteau, DPPH reagent (1,1-diphenyl-2-pyorilhidrazil) were purchased from PT. BRATACO Chemistry. Materials for MDA and SOD analysis were obtained from the Laboratory of Physiology Sciences Division of Molecular Physiology. Materials for SGPT and SGOT analysis were obtained from the Laboratory of Clinical Pathology. Materials for liver tissue histology were obtained from the Laboratory of Pathology, University of Brawijaya.

Animal for the Experiment

Male Wistar rats were obtained from Singosari, Malang, East Java. Rats used in the study were male Wistar rats, aged 2-3 months, weighing 200-250 grams, were healthy, and did not show any defective anatomy. Rats were adapted in the cage for ± one week before they were used for research. During experiments, rats were housed in groups, fed with Comfeed pars, and drank in *ad libitum* manner. All experiment procedures were approved by the Ethical Clearance Committe, Brawijaya University, Malang, Indonesia.

Testing on Antioxidant and Hepatoprotective Effects

Rats were grouped into five groups and each group consisted of four. To induce oxidative stress in them, except for the negative control treatment, four groups of rats were induced by treatment of ethanol 20% [28]

with the administration of 10 ml/kg/BW for 14 days. After 14 days, the groups of KC I, KC II, and KA II were treated with supplement. The details of each treatment group are presented in Table 1.

Table 1. Types of Treatment Group

Treatment Group		Note
Group 1 (KN)	:	Healthy rats (induced with distilled water)
Group 2 (KP)	:	Rats were induced with ethanol 20% as much as 2ml/200 gr per BW for 14 days
Group 3 (KC I)	:	Ethanol induction + black cincau supplement dose I (90 mg/kg per BW per day) for 14 days
Group 4 (KC II)	:	Ethanol induction + black cincau supplement dose II (135 mg/kg per BW per day) for 14 days
Group 5 (KA II)	:	Ethanol induction + acai supplement dose II (135 mg/kg per BW per day) for 14 days

On day 29, all rats were dissected to take blood from the heart and organs for examination of the levels of MDA, SOD, SGPT, SGOT and liver histopathology.

Measurement of Oxidative Stress

The amount of MDA detected illustrates the number of lipid peroxidation. The methods for lipid peroxidation by [29]

SOD Enzyme Activity Measurements

SOD measurement method is described by [30] and can be used to determine the antioxidant activity of a sample [31].

Evaluation of Liver Function

The levels of SGPT and SGOT measurement in serum was done using colorimetry at 505 nm according to the method adapted from [32].

Liver Histopathology

Liver taken was soaked in formalin, processed routinely, and then stained with hematoxylin-eosin (HE) [33]. Histopathological staining results were observed under a light microscope to determine differences in histopathology between treatments.

Total Fenol Testing (A modified method of [34])

1. A total of 10 mg of sample was added to 10 ml of distilled water, then divortex.
2. Further samples were taken of 200 µl.
3. As much as 1 ml of Folin reagent Ciocalteau 10% and 800 µl 7.5% saturated Na_2CO_3 solution was added.
4. Then the mixture was divortex and allowed to stand for 30 minutes in an enclosed space (dark).
5. The sample was then introduced into the cuvette and measured using a spectrophotometer at $\lambda = 765$ nm.
6. Total phenol was measured by absorbance values and the linear regression equation using a standard curve. Results obtained in the form of units of ppm were converted into units of mg/g. The analysis was repeated three times.

Antioxidant Activity Measurement using DPPH Method (A modified method of [35]).

1. A sample of 0.5 g was dissolved in 10 ml of ethanol (w/v).
2. A total of 1 ml of 0.2 mM solution of 1,1-diphenyl-2-pyorihidrazil (DPPH) in ethanol was prepared, and then 1 ml and this solution was added to 4 ml of antioxidant extract.
3. It was settled for 30 minutes, then measured absorbance at $\lambda = 517$ nm

4. The free radical scavenging activity was calculated as a percentage reduction of DPPH color using the equation [36,37].

$$\text{Free radical scavenging (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

The value of IC_{50} sample is a sample concentration required to inhibit 50% of DPPH free radicals, calculated by linear regression plot where the abscissa represents the concentration of plant extracts tested, and the ordinate is the average percentage of the antioxidant activity.

Total moisture/total dry weight

Total moisture content/dry weight was determined using air oven method according to AOAC method 950.46 [38]. The dry matter/moisture content was obtained as follows :

$$\text{Dry matter content (\%)} = \frac{\text{Weight of dried sample}}{\text{Original wet weight}} \times 100$$

Statistical Analysis

The results of hepatoprotective and antioxidant activities are expressed as mean ± SD. Results were statistically analyzed using one way ANOVA, followed by the Tukey's HSD (honest significant difference) test. The $p<0.05$ was considered to be significant. Data were statistically analyzed using SPSS 17.0.

Results

Black Cincau Supplement (BCS)

The results of chemical analysis supplements are used to determine and compare the components contained in the supplement and the effect on in vivo studies. Antioxidant activity, IC_{50}, and total phenol of acai supplement is higher when compared with black cincau supplement. However, the water content of the black cincau supplements is smaller than acai supplements. This can be due to differences in the raw materials and methods of manufacture of both supplements. The characteristics of black cincau supplement and acai are presented in Table 2.

Table 2. Characteristics of Black cincau and Acai Supplement

Test Parameter	Black cincau Supplement	Acai Supplement
Antioxidant activity (%)	74.54±0.63	81.88±0.51
IC_{50} (ppm)	75.18±0.03	52.44±3.27
Total Phenol (mg CAE/g)	66.38±1.45	84.67±0.71
Water Content (%)	4.99±0.11	5.36±0.22

Effect BCS on Antioxidant Activity

The antioxidant activity assessed by measuring the levels of MDA and SOD. MDA and SOD levels in the blood serum of curative treatment on rats are shown in Figure 1 and Figure 2.

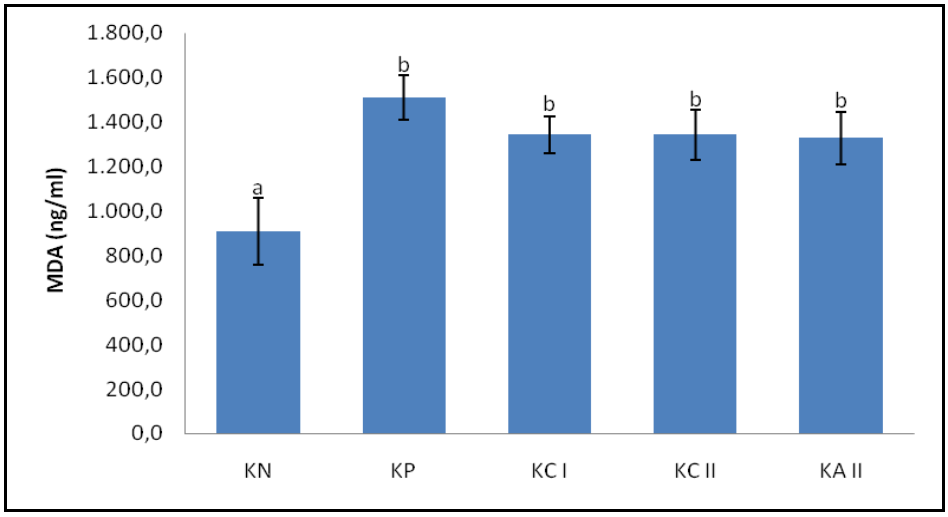


Figure 1. MDA levels of blood serum. KN = Normal; KP = stress; KC I = Black cincau Supplement Dose 90 mg/kg BW; KC II = Black cincau Supplement Dose 135 mg/kg BW; KA II = Acai Supplement Dose 135 mg/kg BW

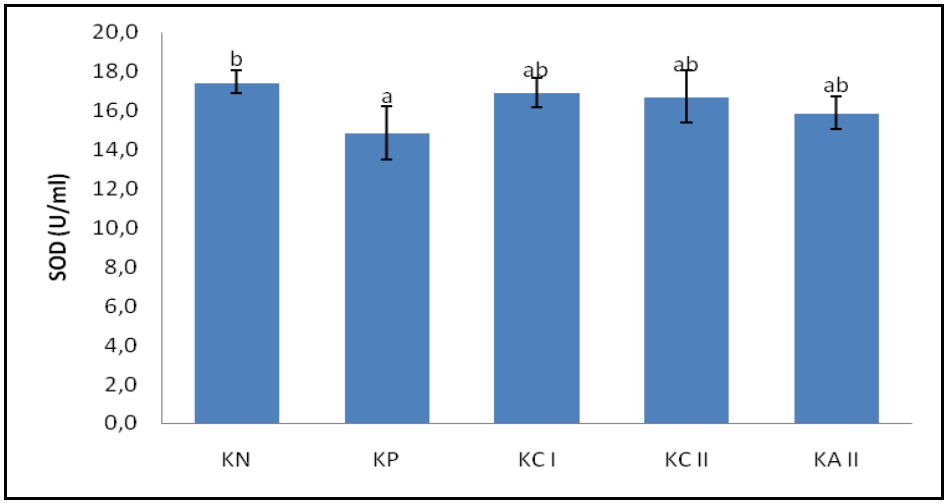


Figure 2. SOD levels of blood serum. KN = Normal; KP = stress; KC I = Black cincau Supplement Dose 90 mg/kg BW; KC II = Black cincau Supplement Dose 135 mg/kg BW; KA II = Acai Supplement Dose 135 mg/kg BW

Results of analysis of variance show that the type of treatment given to rats brings significant effect on MDA and SOD levels of blood serum ($p < 0.05$). Further test results show the increased of MDA and decreased of SOD in rats induced by ethanol. At curative treatment, black cincau supplement and acai supplement dose I and II for 2 weeks have not been able to lower the value of MDA and increased the levels of SOD significantly ($p < 0.05$).

MDA levels did not drop significantly, as rats were made sick with induction of ethanol for 2 weeks, causing the accumulation of increased levels of MDA and decreased levels of SOD enzyme. Period of curative supplementation was two weeks, suspected to be less effective in lowering MDA levels close to normal. Aside from the length of time of supplementation, the dose of the supplement also affects the decreased levels of MDA. The condition of rats required a higher intake of supplements to counteract the production of free radicals. The dose that may be used for curative treatment is 1500 mg and 2000 mg, equivalent to the consumption of 3-4 capsules per day for human beings.

Effect BCS on Hepatoprotective Effect

The results of measurements of SGPT and SGOT is presented in Figure 3 and Figure 4.

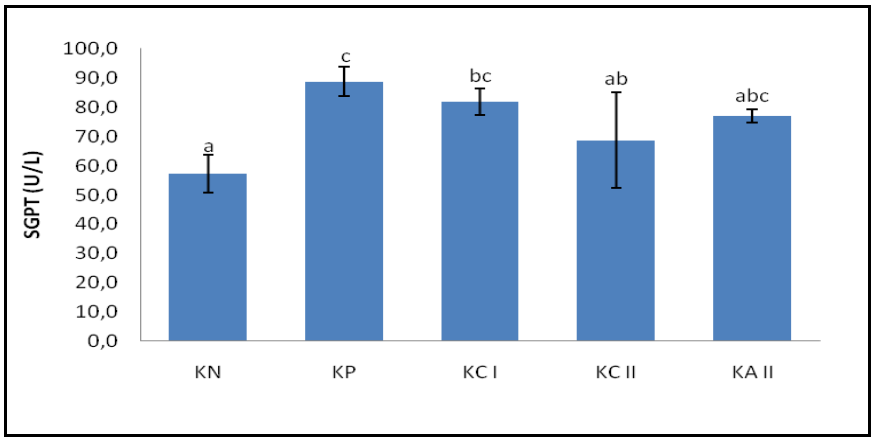


Figure 3. SGPT levels. KN = Normal; KP = stress; KC I = Black cincau Supplement Dose 90 mg/kg BW; KC II = Black cincau Supplement Dose 135 mg/kg BW; KA II = Acai Supplement Dose 135 mg/kg BW

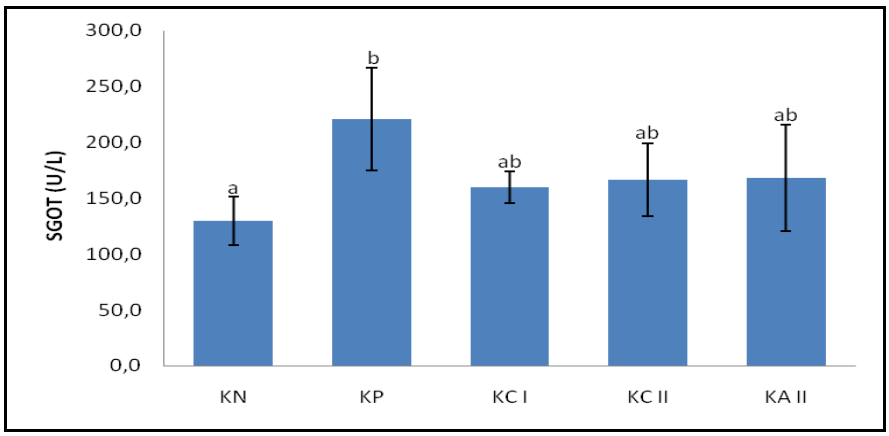


Figure 4. SGOT levels. KN = Normal; KP = stress; KC I = Black cincau Supplement Dose 90 mg/kg BW; KC II = Black cincau Supplement Dose 135 mg/kg BW; KA II = Acai Supplement Dose 135 mg/kg BW

Results of analysis of variance show that the type of treatment given to rats bring significant effect on the levels of SGPT and SGOT ($p < 0.05$). Further test results show increased levels of SGPT and SGOT in rats induced by ethanol. Curative treatment of black cincau supplement dose II (KC II) is the most significant ($p < 0.05$) in lowering the value of SGPT compared to acai supplement. But for SGOT, suplementation of black cincau and acai dose I and II was not able to lower the value of SGOT significantly ($p < 0.05$).

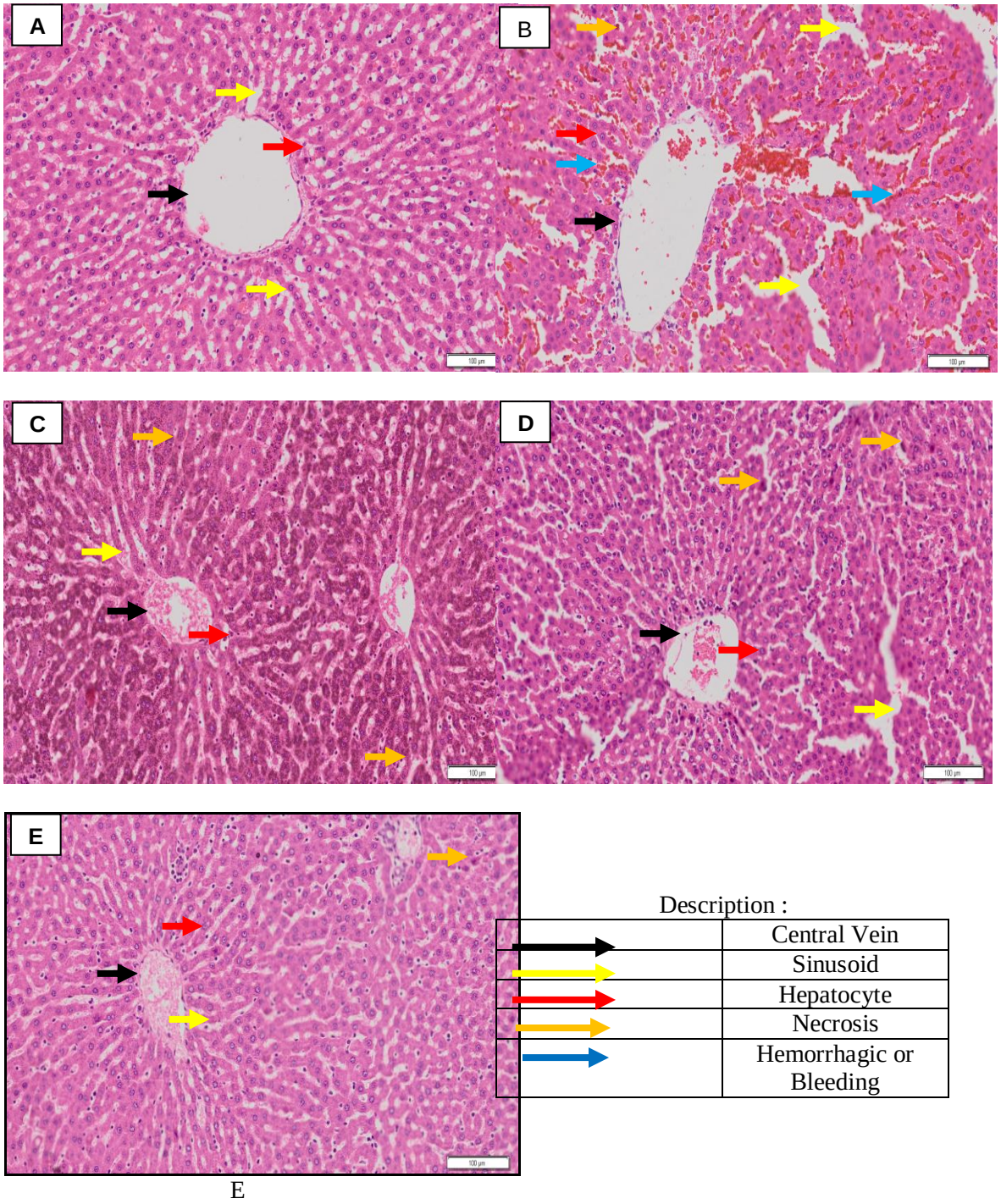
The SGOT levels did not decrease significantly in curative treatment and this may be due to the period of supplementation, which was only two weeks. This is in contrast to the SGPT levels showing a significant decrease during the two weeks of supplementation. The use of a higher dose (1500 mg) did also not significantly influence the levels of SGOT.

Effect of BCS on liver histopathology

Liver histopathology was done qualitatively and quantitatively. Qualitative observation used to describe the liver cells among the treatment group (Figure 5), whereas quantitative by calculating the percentage of liver cell damage (Table 3). Figure 5 shows histopathology differences among the treatment groups. Figure 5A, for the normal control, shows that liver tissue is composed of hepatocytes red cells with nucleus round purple. Groups of hepatocyte cells are separated by sinusoid. Hepatocyte cell structure is dense and sinusoid shows regularity that leads toward the central vein. The negative control did not reveal any characteristics of cell death

or necrosis. Figure 5B shows that sinusoid is widening or dilate, there is hemorrhagic or bleeding, and cell necrosis.

Treatment with black cincau supplement in Figure 5C shows that liver lobule can still be observed, cell hepatocytes appears purplish red and the flecks are purple-black, sinusoid indicates regularity that leads toward the central vein, and cell necrosis. Figure 5D shows that hepatocyte cells are pink and are arranged regularly and radial, but there is a sinusoid which is widening or dilate. Cell necrosis appear. Acai treatment (Figure 5E) shows pink hepatocytes with rounded cell nuclei, hepatocyte plates are clearly visible and organized, sinusoidal dilation does not appear, and there is visible presence of cell necrosis.



E

Figure 5. Liver Histopathology. A : Liver tissue of normal rat, B : Liver tissue of ethanol-treated rat, C : Liver tissue of rats treated with ethanol and black cincau supplement (90 mg/kg), D : Liver tissue of rats treated with ethanol and black cincau supplement (135 mg/kg), E : Liver tissue of rats treated with ethanol and acai supplement (135 mg/kg). Staning H&E, 20X

Percentage of Damaged Liver Cells

Table 3. **Percentage of Damaged Liver Cells**

Histological Observation	KN	KP	KC I	KC II	KA II
1	0	86.67	56.1	38.18	36.16
2	0	75.62	31.43	45.42	34.12
3	0	81.67	43.41	37.06	29.41
4	0	76.24	38.42	32.05	48.52
Mean	0	80.05	42.34	38.18	37.05

KN: Negative Control; KP: Positive Control; KCI: Black cincau Dose 90 mg/kg BW; KC II : Black cincau Dose 135 mg/kg BW; KA II: Acai Dose 135 mg/kg BW

Based on Table 3, it is known that under normal conditions (negative control), rat liver cells does not experience damage (0% of damaged cells). On the positive control, the level of liver cell damage is 80.05%. As for the curative treatment, the highest liver cell damage is on black cincau supplement highest dose of 90 mg/kg/body weight of 42.34% and the lowest is on acai supplement dose of 135 mg/kg/body weight of 37.05%.

From the description of rat liver histopathology between treatments, black cincau supplement dose 135 mg/kg and acai supplements dose 135 mg/kg showed a protective effect against liver cell damage induced by ethanol. The result is marked with fewer numbers of cell necrosis and cell degeneration.

Discussion

This study aims to determine antioxidant activity and hepatoprotective effects of black cincau supplement (BCS) againts oxidative stress in rats with ethanol-induced. Provision of ethanol can caused auto-oxidation liver cells either by acting as a pro-oxidant or reducing antioxidant activity resulted in hepatotoxicity [2]. In this study, measurement of MDA and SOD as a marker of lipid peroxidation and decreased antioxidant enzyme activity.

Ethanol causes increased lipid peroxidation and decreased antioxidant enzyme activities that reflect the occurrence of oxidative stress in the liver [39]. Administration of ethanol causes the accumulation of ROS such as superoxide, hydroxyl radicals, and hydrogen peroxide [40]. The research by [r41] show several mechanisms of ethanol that can cause oxidative stress including increased generation of ROS at microsomal level, especially through the involvement of cytochrome P450 2E1, which is caused by high doses of ethanol or consumption of chronic ethanol [42,43]. ROS in turn has the ability to initiate lipid peroxidation in cell membranes and affect the antioxidant systems [40].

Ethanol is first converted into acetaldehyde primarily by alcohol dehydrogenase (ADH) although cytochrome P4502E1 (CYP2E1) and catalase can also contribute to the formation of aldehydes. Acetaldehyde is subsequently metabolized to acetate by aldehyde dehydrogenase (ALDH) [44]. Pathologic analysis reveals that administration of alcohol causes damage to the liver that causes histological changes, such as micro and macro-vesicular fatty infiltration, hyperplasia of Kupffer cells and sinusoidal dilatation in the liver. Ethanol administration affects the liver biochemical function such as increased levels of SGPT (ALT) and SGOT (AST). Increased serum ALT, AST, HA (hyaluronic acid), LN (laminin) and PCIII (type III collagen terminal peptide) also confirms liver damage and fibro genesis in rats [45].

The content of bioactive compounds in the black cincau supplement coupled with red ginger seem to have the hepatoprotective ability so as to protect the liver from free radicals induced by ethanol. The content of bioactive compounds in black cincau among others are polyphenols (ursolic acid, protocatechuic acid, p-hydroxybenzoic acid, vanilic acid, caffeic acid, syringic acid stigmasterol, β-sitosterol, oleanolic acid, and ursolic acid), flavonoids, alkaloids, tannins, saponins, sterols, terpenoids, chlorophyll, ascorbic acid, tocopherols (α-, β-, γ-, δ-) and β-carotene[7,8]. The bioactive components in red ginger include phenol (gingerol and shogaol), flavonoids, alkaloids, tannins, saponins and terpenoids [20]. Phytochemicals of plants such as

phenol and flavonoids shown have pharmacological effect that are associated with lower occurrence and lower mortality rates of several human diseases [5,46,47].

Phytochemicals of plants such as polyphenols, flavonoids, terpenoids, steroids, and so forth have received attention in recent years due to the diverse pharmacological properties including hepatoprotective and antioxidant activity [48]. Caffeic acid (CA) is a highest phenolic compound in extracts of black cincau having antioxidant and hepatoprotective activity[7,49-52]. Ursolic acid also has a powerful hepatoprotective effect [53]. Oleanic acid is also reported to enhance the antioxidant components in the liver. Both also improve glutathione enzyme, which plays an important role in protecting the liver against induction of acetaminophen [53].

Black cincau and acai supplement could be expected to restore the function of liver cells so that an increasing number of cell necrosis or irreversible cell can be prevented. The improvement of rat liver cells is suspected due to the presence of antioxidant compounds in supplements. Increase in the production of free radicals in the body can also lower the effectiveness of intracellular antioxidant enzymes. Therefore, administration of exogenous antioxidants is essential to help the intracellular antioxidant enzymes to work better to prevent cell damage[54].

Conclusions

The treatment with black cincau supplement at a dose of 135 mg/kg/BW per day has higher hepatoprotective effect than the dose of 90 mg/kg/BW per day.

Acknowledgements

The authors gratefully acknowledge the financial support for this research by the Faculty of Agricultural Technology, Brawijaya University, Indonesia.

References

1. WHO, (2014), *Global status report on alcohol an d health 2014*, www.who.int/iris/.../9789240692763_eng.pd.. accessed on 25 Februari 2015
2. Crawford, D.W. and Balakenhoan, D.H., (2001), *Arterial Wall Oxygenation Oxy Radicals and Atheroslerosis,* Atherosclerosis, 89 : 97-108
3. Sherlock, S., (2002), *Disease of The Liver and Biliary System 7th Edition*, Blackwell Scientific Publishing, London.
4. N.Syamala Devi , T. Hari Prasad, K.Ramesh, Ramchander Merugu., (2015), *Antioxidant Properties of Coconut Sap and its Sugars*, International Journal of PharmTech Research, 8(1): 160-162.
5. Rezaeifar, M and Maryam Rezaeifar., (2016), *Antioxidant properties of the methanolic extract of the shell root of Amygdalus eburnean*, International Journal Of PharmTech Research, 9(9): 514-518.
6. Ruhnayat A., (2002), Cincau Hitam Tanaman Obat Penyembuh, Penebar Swadaya, Jakarta.
7. Hung C.Y. and Yen G.C., (2002), *Antioxidant Activity of Phenolic Compounds Isolated from Mesona procumbens Hemsl*, J. Agric. Food Chem, 50: 2993-2997.
8. Hung, C.Y. and Yen G.C., (2001), *Extraction and Identification of Antioxidative Components of Hsian tsao (Mesona procumbens Hemsl.)*. Lebensm – Wiss u. – Technol 34 : 306 – 311.
9. Widyaningsih T.D., (2007), *Olahan Cincau Hitam*, Trubus Agrisarana, Surabaya.
10. Widyaningsih, T.D. and Adilaras P., (2013), *Hepatoprotective Effect of Extract of Black Cincau (Mesona palustris BL) on Paracetamol Induced Liver Toxicity in Rats*, Advance Journal of Food Science and Technology 5 (10) : 1390 – 1394.
11. Widyaningsih, T.D., (2011), *Immunomodulatory and Chemopreventive Effect of the Water Extract of Black Cincau (Mesona palustris BL) Againts Interferon Gamma Expression, Immunosurveillance Activation, and Apoptosis on Benzo (a) pyrene Induced Fibrosarcoma Carsinogenesis in Rat*, Unpublished Dissertation, Postgraduate Studies, Airlangga University.
12. Widyaningsih, T.D., (2012), *Cytotoxic Effect of Water, Ethanol, and Ethyl Acetate Extract of Black Cincau (Mesona palustris BL) againts HeLa Cell Culture*, APCBEE Procedia 2 : 110 – 114.

13. Widyaningsih, T.D., Sukardiman, Djoko Agus P. and Darmanto W., (2012), *Efek Imunomodulator Ekstrak Air Cincau Hitam (Mesona palustris BL) terhadap Karsinogenesis Mencit*, Jurnal Teknologi dan Industri Pangan 23 (1) : 31 – 37.
14. Widyaningsih, T.D., Mahar J., Wijayanti, N., Rahmani, and Najmuddin, M., (2013), *Immuno modulatory Effect of Instant Tea Powder from Black Cincau (Mesona palustris BL) in the Treatment of Salmonella typhimurium Induced Infection in Balb Rat*, Food Public Health 3 (3) : 142 – 146.
15. Yen, G.C., Duh, P.D., and Hung Y.L., (2001), *Contribution of Major Components to the Antimutagenic Effect of Hsian-tsao (Mesona procumbens Hemsl.)*. J. Agric. Food Chem. 49 : 5000 – 5004.
16. Yen, G.C., Yeh T.C., and Yen C.J., (2004), *Protective Effect of Mesona procumbens Against Tert-Butyl Hydroperoxide-induced Acute Hepatic Damage in Rats*, Journal Agric and Food Chem 52:4121-4127.
17. Yeh, C T.., Huang W.H., and Yen, G.C., (2008), *Antyhypertensive Effect of Hsian Tsao and Its Active Compound Inspontaneously Hepertensive Rats*, Science Direct Journal of Biochemistry, 001-010.
18. Yang, M., Xu, Z.P., Xu, C.J., Meng, J., Ding, G.Q., Zhang X.M., and Weng, Y., (2008), *Renal Protective Activity of Hsian tsao Extracts in Diabetic Rats.* Biomedical and Enviromental Science 21 : 222 – 227.
19. Rafi, M., Lim L.W,, Takeuchi T., and Darusman L.K., (2013), *Simultaneous Determination of Gingerols and Shogaol using Capillary Liquid Chromatography and Its Application in Discrimination of Three Gingers Varieties from Indonesian*, Talanta 103 : 28 – 32.
20. Hernani and Hayani E., (2001). *Identification of chemical components on red ginger (Zingiber officinale var. Rubrum) by GC-MS*. Proc. International Seminar on natural products chemistry and utilization of natural resources. UI-Unesco, Jakarta : 501-505.
21. Ahmed. G. Moh.Jabran., Setyawati Soeharto., Hidayat Sujuti., (2015), *The Effect of Ginger (Zingiber officinale Roscoe) Extract on Liver Histopathology and Alanine Aminotransferase Serum Level in Carbofuran-Induced Rats*, International Journal of PharmTech Research, Vol.8, No.5, pp 889-897.
22. Malik, S. N., (2014-2015), *Evaluation of Antibacterial and Antioxidant Activity of Ginger Rhizome and Ziziphus Leaves* , International Journal of PharmTech Research, Vol.7, No.4, pp 554-559.
23. Surh, Y.J., Park K.K., Chun K.S., Lee L., Lee E., and Lee S., (1999), *Antitumor promoting activities of selected pungent phenolic substances present in ginger*, J. Environ. ` Pathol.Toxicol. Oncol.18:131-139.
24. Masuda, T., Jitoe A., and Mabry T.J., (1995), *Isolation and structure determination of cassumunarins A, B, C: new anti-inflammatory antioxidants from a tropical ginger, Zingible cassumunar*, J Am Oil Chem Soc. 72: 1053-1057.
25. Manju, V. dan Nalini N., (2005), *Chemopreventive efficacy of ginger, a naturally occurring anticarcinogen during the initiation, post initiation stages of 1, 2 dimethyl hydrazine-induced colon cancer*, Clin Chim Acta. 358: 60-67.
26. Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P., Gargova, S., (2007), *Antioxidant activity of a ginger extract (Zingiber officinale)*, Food Chemistry 102, 764–770.
27. Widyaningsih, T.D., Handayani, D., Wijayanti, N., (2014), Minuman dan Suplemen Berbasis Cincau Hitam Sebagai Multifungsional untuk anti hiperglikemik, anti hiperkolesterolemia, dan antihipertensi tahun ke-2, Unpublished Research Report, LPPM Universitas Brawijaya.
28. Sudha, A., Srinivasan, P., Manikandaselvi, S., and Thinagargabu, R., (2012), *Protective Effect And Antioxidant Role Of Achyranthus Aspera L. Againts Ethanol-Induced Oxidative Stress In Rats*, International Journal of Pharmacy and Pharmaceutical Sciences, ISSN- 0975-1491 Vol 4, Suppl 3.
29. Ohkawa H., Ohishi W., Yagi K., (1979), *Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction*, Analytical Biochemistry 95, 351–358.
30. Beauchamp, C. and Fridovich, I, (1971), *Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels,* Analytical Biochemistry, 44 : 276-287.
31. Alam, N., Bristi J.N., Rafiquzzaman, (2013), *Review on In Vivo and In Vitro Methods Evaluation of Antioxidant Activity,* Saudi Pharmaceutical Journal 21, 143–152.
32. Reitman, S., Frankel, S., (1957), *A colourimetric method of the determination of plasma glutamic oxaloacetic and glutamic pyruvic transaminases*, American Journal of Clinical Pathology 28, 56–63.
33. Kiernan J.A., (1990), *Histological & Histochemical Methods: Theory and Practice*. 2nd ed. Canada, Pergamon Pr. Pp. 90-97.
34. George, S., Brat, P., Alter, P., Amiot, M.J., (2005), *Rapid Determination of Polyphenols and Vitamin C in Plant-Derived Products*. Journal of Agricultural and Food Chemistry 53 (5), 1370–1373.
35. Manzocco L, Anese M, and Nicoli M.C., (1998), *Antioxidant properties of tea extracts as affected by processing*, LWT 31:694-698.

36. Sembiring HB, Barus T, Marpaung L, Simanjuntak P., (2015), *Antioxidant and Antibacterial Activity of Some Leaves Extracts (Methanol, Ethyl Acetate and N-Hexane) of Scurrula fusca G.Don*, International Journal of PharmTech Research, 8(9): 24-30.
37. Mallick M , Bose A, Mukhi B., (2016), Comparative Evaluation of the Antioxidant Activity of Some commonly used Spices. International Journal of PharmTech Research, 9(1): 1-8.
38. AOAC, (1995), *Official Methods of Analysis of AOAC International*. p. 42-1-42-2. In T.R.Mulvaney (ed.) AOAC International, Arlington, VA.
39. Grissa, A.K., Bessem M., Ezzedine A., Mohamed H., Michelle E.M., Najoua G., Abdelaziz K., Saloua E.F., (2007), *Resveratrol, a red wine polyphenol, attenuates ethanol-induced oxidative stress in rat liver*. Life Sciences 80, 1033–1039.
40. Nordmann, R., Riviere, C., and Rouach, H., (1992), *Implication of Free Radical Mechanisms in Ethanol Induced Cellular Injury*, Free Radical Biology and Medicine, 12:219–240.
41. Lieber C.S., (2004), *Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis*, Alcohol 34: 9-19.
42. Nordmann, R., (1994). *Alcohol and antioxidant systems*, Alcohol and Alcoholism 29, 513–522.
43. Navasumrit P., Ward T.H., Dodd N.J.F., and O'Conner P.J., (2000), *Ethanolinduced free radicals and hepatic DNA strand breaks are prevented in vivo by antioxidants: effects of acute and chronic ethanol exposure*, Carcinogenesis 21, 93–99.
44. Oyama, T., Isse, T., Kagawa, N., (2005), *Tissue distribution of aldehyde dehydrogenase 2 and effects of the ALDH2 gene disruption on the expression of enzymes involved in alcohol metabolism*, Frontiers in Bioscience 10:951–960.
45. Huang, Q., Renbin H., Shijun Z., Jun L., Ling W., Min H.Z., Xing L, (2013), *Protective effect of genistein isolated from Hydrocotyle sibthorpioides on hepatic injury and fibrosis induced by chronic alcohol in rats*. Toxicology Letters 217, 102– 110.
46. Sepideh Nourian, Ali Mohammadi Sani, Ebrahim Golmakani, Peyman Feizi, Katayoun Roghani, (2016), *Determination of Antioxidant activity by High Performance Liquid Chromatography, Phenolic and Flavonoid contents of Vincetoxicum nigrum*, International Journal of PharmTech Research, Vol.9, No.3, pp 150-157.
47. Rubila. S, Ranganathan T.V., (2016), *Effect of Allium sativum paste against Antimicrobial, Antioxidant and Cytotoxicity activity*, International Journal of PharmTech Research, Vol 9, No.3, pp 328-332.
48. Giri, R.K., Anindya B., and Subrat K.M,. (2011), *Hepatoprotective Activity Of Tagets Erecta Againts Carbon Tetrachloride-Induced Hepatic Damage In Rats*. Acta Poloniae Pharmaceutica n Drug Research, Vol. 68 No. 6 pp. 999-1003.
49. Yang, S.Y., Hong, C.O., Lee, G.P., Kim, C.T., Lee, K.W., (2013), *The hepatoprotection of caffeic acid and rosmarinic acid, major compounds of Perilla frutescens, against t-BHP-induced oxidative liver damage*, Food and Chemical Toxicology 55, 92–99.
50. Jayanthi R. and Subash P., (2010), *Antioxidant Effect of Caffeic Acid on Oxytetracycline Induced Lipid Peroxidation in Albino Rats*, Ind J Clin Biochem 25(4):371–375.
51. Coban S., Fahrettin Y., Alpaslan T., Behcet A., Dincer O., Cengiz A., Alaattin P., and Mukaddes E., (2010), *The Effect of Caffeic Acid Phenethyl Ester (CAPE) Against Cholestatic Liver Injury in Rats*, Journal of Surgical Research 159, 674–679.
52. Lee, K.J., Choi J.H., Hwang Y.P., Chung Y.C., and Jeong H.G., (2008), *Protective effect of caffeic acid phenethyl ester on tert-butyl hydroperoxide-induced oxidative hepatotoxicity and DNA damage*, Food and Chemical Toxicology 46, 2445–2450.
53. Sadasivan, S., Latha P.G., Sasikumar J.M., Rajashekaran S., Shyamal S., Shine V.J., (2006), *Hepatoprotective studies on Hedyotis corymbosa (L.) Lam,* Journal of Ethnopharmacology 106, 245–249.
54. Suarsana I.N., Wresdiyati T., Suprayogi A., (2013), *Response of Oxidative Stress and Isoflavone Treatment on Superoxide Dismutase Enzyme Activities and Lipid Peroxidation in Rat's Liver*, JITV 18(2): 146-152.

*****