HEPATOPROTECTOR ACTIVITY TEST OF GREEN TEA (Camellia sinensis, L) GAMBUNG VARIETY ETHANOL EXTRACT IN BALB/C MOUSE INDUCED WITH CCL4

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Abstract, Background: Green Tea Leaves (Camellia sinensis, L) are widely used for various daily needs and have been widely researched and developed. Green tea contains active compounds of flavonoids, alkaloids, terpenoids, polyphenols and saponins. Extraction of tea leaves was carried out by maceration using ethanol as a solvent, after phytochemical tests were found to contain alkaloids, saponins and flavonoids. Flavonoids are the largest group of phenolic compounds which have effective properties to inhibit the growth of viruses and bacteria. Tea leaves are known to contain polyphenols which have the potential as hepatoprotectors. **Methods:** The research method used is a laboratory experiment. This study aims to determine the hepatoprotective activity of the ethanolic extract of green tea leaves against Balb/C mice with CCl4 induction and by observing the activity of the enzymes AST, ALT, ALP, GGT, and MDA levels.
Result: The results showed that there was significant hepatoprotective activity tested by the one-way Anova test with a 95% confidence level (α = 0.05) with a dose of green tea leaf ethanol extract starting at 50 mg/100 mL.
Conclusions: Green tea leaf ethanol extract starting at 50 mg/kg BW gave good results as a hepatoprotector in CCl4-induced Balb/c mice. by analyzing the enzyme activity of ALT, AST, ALP, GGT and MDA levels

Keyword: ethanol extract, green tea leaf, hepatoprotector

Background

Tea leaves (*Camellia sinensis*, L) have been used as an ingredient in traditional Indonesian drinks for a long time. Tea is a beverage ingredient that is universally consumed in many countries and various levels of society. Tea has many functions and benefits for health, including as an antibacterial, antioxidant and free radical inhibiting activity (Rohdiana, et al, 2013). There are two types of tea that are commonly consumed, namely green tea and black tea, the difference between the two lies in the processing of the tea. Green tea is processed only through a heating process and without fermentation, while black tea is processed through a fermentation process (Park., et al., 2012). Tea leaves are rich in phenolic compounds and flavonoids (Rohdiana and Widiantara, 2005). The high polyphenol content in green tea is used to kill the bacteria *Streptococcus mutans* and *Lactobacillus acidophilus* (Fajriani, et al. 2012; Sharkawi, S.M., et al, 2012). The content of flavonoids in tea has an antioxidant effect and can lower cholesterol in humans and animals (Sriyono & Proboningsih, J.

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2012; Zufarisky Sarel, Kristina Simanjuntak, (2020). In addition, tea also has free radical inhibitory activity so that it can protect the liver (hepatoprotector) (Mehri, N. et al., 2016).

Liver damage can be caused by an infectious disease, virus or exposure to chemical compounds in the form of free radicals that enter the body. This can be prevented by giving compounds that have hepatoprotective effects which can be obtained from plants containing compounds that are antioxidants such as tea leaves (Camelia sinensis, L) (Naji, K, M. et al, 2017; Abolpathi, AA, et al, 2012).

The liver plays a role in metabolism and various other physiological functions. Detoxification is carried out by the liver through two phases, namely the first phase includes oxidation, reduction and hydrolysis and the second phase is conjugation with sulfuric acid, glucuronic acid, glutathione, acetic acid and glycine and in these phases The liver will convert toxic materials into harmless metabolites which are then excreted out of the body.

Liver disease is a serious disease, it can be caused by toxic compounds, drugs, bacteria and viruses that enter the body through food or infection. Liver function disorders can also be caused by pre-hepatic, intra-hepatic and post-hepatic disorders. Prehepatic disorders such as hemolytic anemia, intrahepatic or hepatocellular disorders such as hepatitis, cirrhosis and hepatic carcinoma. While post-hepatic abnormalities due to tumor

Toxins that cause liver disease can induce the production of reactive oxygen species (ROS), which can attack and damage liver tissue and cause serious damage (Sarma et al, 2010; Li, S, et al, 2015). Carbon tetrachloride / Carbon tetrachloride (CCl4), is a chemical compound that has the potential as a poison, and it can be used as a model in animals by inducing damage to liver cells. CCl4 can be used to evaluate compounds that have the potential as drugs or food and beverages that can protect the liver from poisoning (hepatotoxicity). Cytochrome P450 will metabolize CCl4 into a reactive compound trichloromethyl radical (CCl3), which can react with oxygen to form trichloromethyl peroxyl radical (CCl3OO) which can then attack fats and proteins. This reaction can initiate lipid peroxidation and cause liver damage. Antioxidants can function as protective compounds against oxidative stress associated with liver pathology, by blocking or cutting the chain of fat peroxidation that occurs due to the induction of liver damage by CCl4 (Naji, K.M. et al., 2017).

In this study, we wanted to investigate the hepatoprotective effect of the ethanolic extract of green tea (Camellia sinensis, L) against acute liver damage in Balb/c strain mice induced by CCl4. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) activities will be tested to evaluate impaired liver function. In addition, histopathological examination of liver cell damage was also carried
and to test the level of proinflammatory cytokines, cytokines were examined, including malondialdehyde (MDA) (Sari, SK., 2012, Tung, BT., et al., 2017). As a positive control used quercetin or ascorbic acid which has antioxidant properties. The aim of the study was to determine the hepatoprotective activity of the ethanolic extract of green tea leaves (Camellia sinensis, L) of the gambang variety against CCl4-induced balb/c mice."

Materials and Methods

The type of research is a laboratory experiment with a completely randomized design (CRD) using male Balb/c mice, aged 6-8 weeks, body weight between 20-30 grams, obtained from PT. Biofarma, as many as 30 heads were taken from the available 50 animals, which were randomly divided into 6 groups @ 5 individuals. Before the study, mice were adapted for 7 days in cages.

1. Group I, the normal control group, were given food and drink ad libitum for 7 days.
2. Group II, positive control group, was given food and drink ad libitum per day for 7 days, fasted a day and then 0.1 mL CCl4 was injected intra-peritoneally.
3. Group III, IV and V treatment group, mice were fed and drank ad libitum and given green tea ethanol extract at a dose of 50, 100 and 150 mg/100mL, orally at a dose of 0.1 mL per day for 7 days, then satisfied. for 1 day and then injected intra-peritoneally with CCl4 at a dose of 0.1 mL.
4. Group VI, standard control, mice were fed and drank ad libitum and given ascorbic acid at a dose of 100 mg/kg body weight, orally at a dose of 0.1 mL per day for 7 days, then

fasted a day and then injected intra-peritoneally with CCl4 at a dose of 0.1 mL.

a. Tools used

Photometer Mikrolab 300 and Kenza Max, Olympus Microscope, Microphotograph, Microtome, water bath, ice block plate, Micropipette 10, 20, 50, 100, 200, 500, 1000 L, Tips, Analytical balance, injector syringe, oral syringe, blender, macerators, evaporators, surgical instruments, glassware, object and dec glass, cages for mice.

b. Materials used

Green tea (Camellia sinensis) from PT Gambung, West Java, olive oil, liquid paraffin, absolute ethanol, CCl4, Hematoxylin Eosin stain, AST enzyme reagent kit, ALT, ALP, GGT, SDS, BHT, MDA Standard, Na2EDTA, acetic acid 20%, quercetin, silymarin, Formaldehyde buffer (Formaldehyde, NAH2PO4, Na2HPO4, alcohol 70, 96 and 100%, benxol, xylol, Canada balsam, physiological NaCl, ether, NaOH, ice cubes, etc.)

c. Procedure

1) Preparation of mice
   Mice were treated according to their groups, blood was taken from cardiac puncture and examined for enzyme activity of AST, ALT, ALP, GGT, MDA activity and the liver was taken from mice and made histological preparations, stained and examined microscopically.
2) AST enzyme activity examination (IFCC method)
   Serum was pipetted into a 100 L tube, then 1000 L of AST reagent was added, mixed until homogeneous, incubated for 1 minute, and read on a photometer with a kinetic program at a wavelength of 340 nm.
3) Examination of ALT enzyme activity (IFCC method)
   Serum was pipetted into a 100 L tube, then 1000 L of ALT reagent was added, mixed until homogeneous, incubated for 1 minute, and read on a photometer with a kinetic program at a wavelength of 340 nm.
4) ALP enzyme activity examination (Kinetic Diethanol amine/DEA method)
   Serum was pipetted into a 10 L tube, then 1000 L of ALP reagent was added, mixed until homogeneous, incubated for 1 minute, and read on a photometer with a kinetic program at a wavelength of 405 nm.
5) Examination of GGT enzyme activity (Kinetic Gamma Glutamil p-nitroanilide / GPNA method)
   Serum was pipetted into a 50 L tube, then 1000 L of GGT reagent solution was added, mixed until homogeneous, incubated for 1 minute, and read on a photometer with a kinetic program at a wavelength of 405 nm.
6) Examination of Malonaldehyde (MDA) Level
   The prepared ingredients were mixed in the following order: serum 700 L, SDS solution 200 L, BHT solution 50 L, Na2EDTA solution 50 L, 1500 L 20% acetic acid solution and 1500 L TBA solution. Mix until homogeneous, then heated in a 100oC water bath for 60 minutes. Removed from the water bath, then immediately put in a place containing ice cubes. The mixture was then centrifuged at 2325 rpm for 10 minutes. The pink supernatant was measured at a wavelength of 532 nm on a spectrophotometer.
7) Data analyzed
8) Data were analyzed by One Way Anova Test and Tukey HSD Advanced Test with 95% confidence level.

Result and Discussion

AST enzyme activity test results

The results of the AST enzyme test are shown in Figure 1 below:

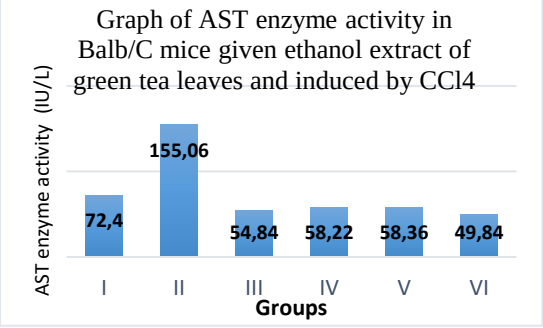

Figure 1. Graph of AST enzyme activity in Balb/C mice given ethanol extract of green tea leaves and induced by CCl4

Notes:
Group I : Negative control group (Normal mice, without treatment)
Group II : Positive control group with CCl4. injection
Group III: Treatment group with 50 mg/kg BW green tea leaf extract
Group IV: Treatment group with 100 mg/kg BW green tea extract
Group V : Treatment group with 150 mg/kg BW green tea extract
Group VI: Standard treatment group ascorbic acid 100 mg/kg BW

AST enzyme activity in group I (normal mice, without treatment was 72.4 IU/L, group II positive control with CCl4 induction was 155.06 IU/L, while groups III, IV and V were treated with green tea extract 50, 100, respectively. and 150 mg/kg BW, the AST activity was 54.84; 58.22 and 58.36 IU/L, and group VI (standard) had AST activity 49.84 IU/L. There was no difference between the treatment group and the treatment group. standard but there is a significant difference with the positive control group.

ALT enzyme activity test results

The results of the ALT enzyme test are shown in Figure 1 below:

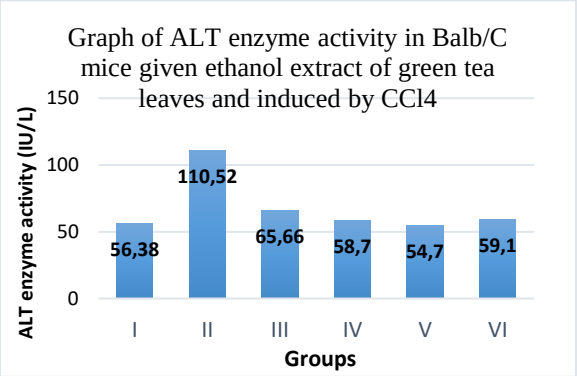

Figure 2. Graph of ALT enzyme activity in Balb/C mice given ethanol extract of green tea leaves and induced by CCl4.
Notes:
Group I : Negative control group (Normal mice, without treatment)
Group II: positive control group with CCl4 injection
Group III : Treatment group with 50 mg/kg BW green tea leaf extract
Group IV : Treatment group with 100 mg/kg BW green tea extract
Group V : Treatment group with 150 mg/kg BW green tea extract
Group VI: Standard treatment group ascorbic acid 100 mg/kg BW

ALT enzyme activity in group I (normal mice, without treatment was 56.38 IU/L, group II positive control with CCl4 induction got 110.52 IU/L while groups III, IV and V were treated with green tea extract 50, 100, respectively). and 150 mg/kg BW obtained ALT activity of 65.66; 58.70 and 54.7 IU/L, and group VI (standard) obtained ALT activity of 59.10 IU/L. There was no difference between the treatment group and the treatment group. standard but there is a significant difference with the positive control group.

ALP enzyme activity test results

The results of the ALP enzyme test are shown in Figure 3 below:

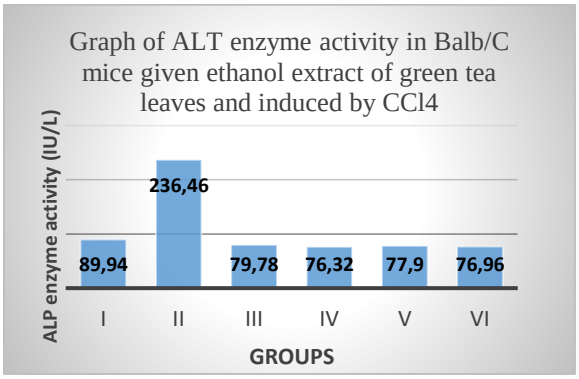

Figure 3 Graph of ALP enzyme activity in Balb/C mice given ethanol extract of green tea leaves and induced by CCl4.
Notes:
Group I : Negative control group (Normal mice, without treatment)
Group II : positive control group with CCl4 . injection

Group III : Treatment group with 50 mg/kg BW green tea leaf extract
Group IV : Treatment group with 100 mg/kg BW green tea extract
Group V : Treatment group with 150 mg/kg BW green tea extract
Group VI : Standard treatment group ascorbic acid 100 mg/kg BW

GGT enzyme activity test results

The results of the GGT enzyme test are shown in Figure 4 below:

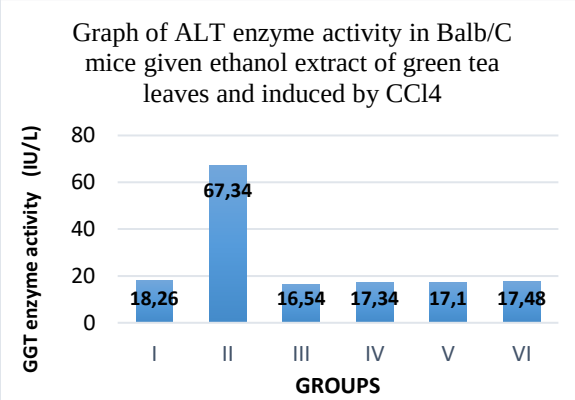

Figure 4. Graph of GGT enzyme activity in Balb/C mice given ethanol extract of green tea leaves and induced by CCl4.

Notes:
Group I : Negative control group (Normal mice, without treatment)
Group II : positive control group with CCl4 . injection
Group III : Treatment group with 50 mg/kg BW green tea leaf extract
Group IV : Treatment group with 100 mg/kg BW green tea extract
Group V : Treatment group with 150 mg/kg BW green tea extract
Group VI : Standard treatment group ascorbic acid 100 mg/kg BW

GGT enzyme activity in group I (normal mice, without treatment was 18.26 IU/L, group II positive control with CCl4 induction got 67.34 IU/L while groups III, IV and V were treated with green tea extract 50, 100 respectively. and 150 mg/kg BW got GGT activity 16.54: 17.34 and 17.10 IU/L, and group VI (standard) got GGT activity 17.48 IU/L. There was no difference between the treatment group and the treatment group. standard but there is a significant difference with the positive control group.

MDA level examination results

The results of the examination of MDA enzyme levels can be seen in Figure 5 below.

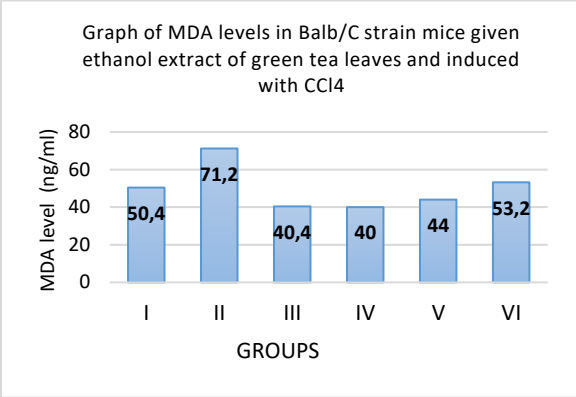

Figure 5. Graph of MDA Level in Balb/C mice given ethanol extract of green tea leaves and induced by CCl4.
Notes:
Group I : Negative control group (Normal mice, without treatment)
Group II : positive control group with CCl4 . injection
Group III : Treatment group with 50 mg/kg BW green tea leaf extract
Group IV : Treatment group with 100 mg/kg BW green tea extract
Group V : Treatment group with 150 mg/kg BW green tea extract
Group VI : Standard treatment group ascorbic acid 100 mg/kg BW

The results of the examination of MDA levels in group I, namely the normal group was 50.4 ng/mL in group II, positive control obtained 71.20 ng/mL and in treatment groups III, IV and V obtained MDA levels were 40.40, respectively; 40.00 and 44.00 ng/mL. In the standard group, the MDA level was 53.20 ng/mL. For MDA levels, the results of the data homogeneity test were 0.111. The data was said to be homogeneous and the ANOVA test showed a significant difference between all groups I – VI with a sig

value of 0.001. The LSD further test showed a significant difference between all groups with group II with a sig value of 0.005 and there was no significant difference between group I, negative control and treatment groups III, IV and V with sig values of 0.154; 0.138; 0.355 and also the standard group (VI) with a sig value of 0.683, so it can be concluded that all groups have a hepatoprotective effect starting from a concentration of 50 mg/100 mL.

Conclusion

Green tea leaf ethanol extract starting at 50 mg/kg BW gave good results as a hepatoprotector in CCl4-induced Balb/c mice. by analyzing the enzyme activity of ALT, AST, ALP, GGT and MDA levels

Acknowledgements:

Director and Head of the Center for Research and Community Service Units, Health Polytechnic, Ministry of Health, Bandung

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