Journal of Innovations in Applied Pharmaceutical Science (JIAPS)
e-ISSN: 2455-5177
www.jiapsonline.com

Research article

Hepatoprotective effect of mengkudu (*Morinda citrifolia*) on rats induced by doxorubicin

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Key words: Hepatoprotective, Mengkudu, AST, ALT.

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Vol. 5(2), 01-06, Apr-Jun, 2020.

Abstract

The liver is the largest solid organ, the largest gland and one of the most vital organs that functions as a center for nutrient metabolism and excretion of waste metabolites. In general, the biological effects caused by the use of anthracycline doxorubicin are the occurrence of apoptosis, necrosis, and autophagy. The purpose of this study was to determine the hepatoprotective activity of mengkudu fruit ethanol extract against the rats induced by doxorubicin. Mengkudu fruit ethanol extract was obtained by maceration. Hepatoprotective activity test is done by measuring aspartate transaminase (AST) and alanine transaminase (ALT). Animals were induced with DOX 5 mg/kgbw on day 1, 7, 14 and 20th. Administration of mengkudu extract 100 mg/kgbw, 300 mg/kgbw, and 500 mg/kgbw given from day 1 to day 20 and on the 21st day cardiac serum levels of AST and ALT were determined. Mengkudu dose of 100 mg/kgbw, 300 mg/kgbw and 500 mg/kgbw have hepatoprotective activity against male rats induced by doxorubicin. Mengkudu shows activity as a hepatoprotection. Mengkudu doses of 100, 300 and 500 mg/kgbw can inhibit the increase in ALT and AST activity. Mengkudu dose 100 mg/kgbw with AST 334.33 U/L and ALT 152 U/L, mengkudu dose 300 mg/kgbw with AST 304.33 U/L and ALT 117.33 U/L, and mengkudu dose 500 mg/kgbw with AST 192.67 U/L and ALT 103.67 U/L showed significant differences ($p < 0.05$) against the negative control group. Mengkudu doses of 100, 300, and 500 mg/kgbw can reduce ALT and AST in doxorubicin-induced male rats.

Introduction

The liver is the largest solid organ, the largest gland and one of the most vital organs that functions as a center for nutrient metabolism and excretion of waste metabolites. Its main function is to control the flow and safety of substances absorbed from the digestive system before the distribution of these substances to the systemic circulation system. Total loss of liver function can cause death within minutes, showing how important the heart is, considering this, this study was conducted to review the physiology of the liver to maintain liver function at its optimum and maintain good health to avoid liver damage such as fatty liver, liver fibrosis, cirrhosis of the liver and other effects arising from anti-cancer drugs [1].

The liver carries out most of the metabolic reactions and goes into detoxification by removing a lot of exogenous and endogenous substances from the body which will be dangerous if they accumulate. Detoxification reactions are divided into phase I (oxidation, hydroxylation, and other reactions mediated by cytochrome P450 and phase II (esterification). Liver function as the center of drug metabolism causes the liver most at risk of toxicity. Based on FDA report (Food and drug administration) in America Unions, there are more than 900 types of drugs, toxins and herbal preparations that have the potential to injure the liver and 20-40% of cases of liver failure are caused by drugs. Drug-induced liver disease can be intrinsic and idiosyncratic. Intrinsic reactions occur if the drug or its metabolites damage the liver predictable, reproducible and dose-dependent, while idiosyncratic reactions are unpredictable and cannot be reproduced, and have a low incidence rate for individuals using drugs. Idiosyncratic reactions can originate from metabolic idiosyncratic or immune allergic reactions [2-4].

The utilization of natural ingredients as traditional medicines has long been developed. This is because people are aware of the side effects caused by the use of synthetic drugs, which are greater than traditional medicines, are quick to make and easily obtain those [5]. Therefore, traditional medicine is a field that is still much in demand to be studied. This is based on several things such as the need for compounds to overcome various diseases such as AIDS (Acquired immunodeficiency syndrome), cancer, including hepatoprotective [6]. Some medicinal plants that have been studied and are recognized as being hepatoprotective are turmeric, bitter, ginger, white Intersection, mengkudu, rosella petals, and yellow turmeric. All of these plants are known to contain high antioxidants because antioxidants are needed to ward

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off free radicals which are one of the causes of liver damage [7].

In general, the biological effects caused by the use of anthracycline doxorubicin are the occurrence of apoptosis, necrosis, and autophagy [8]. The mechanism of action of Doxorubicin can be explained by the use of doxorubicin distributed intravenously in the body within 3-5 minutes and can circulate up to 24-36 hours in the bloodstream. Doxorubicin and the main metabolites of doxorubicin are bound by plasma proteins, then enter cells through passive diffusion with high affinity to bind to cytoplasmic proteasomes. Doxorubicin has a high affinity for DNA nuclei (Deoxyribonucleic acid) when attached to proteasomes [9]. This allows Doxorubicin to separate from the proteasome and bind to DNA. Doxorubicin then intercalates/is stable and stable on DNA replication. The ability of Doxorubicin to insert not only in DNA nuclei but also in mitochondrial DNA, Doxorubicin which interacts with DNA causes inhibition of macromolecular biosynthesis [10].

Based on the antioxidant content in mengkudu fruit that can increase glutathione activity so that it encourages researchers to test the hepatoprotective activity of mengkudu fruit extract on experimental animals by measuring the biochemical parameters of ALT (Aspartate transaminase) and AST (Alanine transaminase).

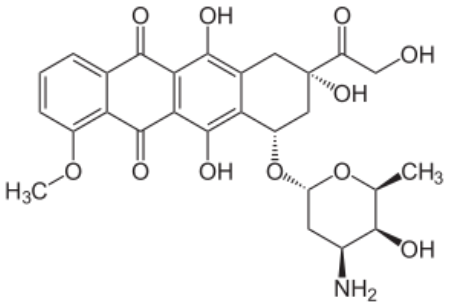

Figure 1. Structure of doxorubicin.

Materials and method

Material

Surgical instruments, laboratory glassware, aluminum foil, blender, porcelain cup, desiccator, incubator, slide glass, cover glass, porcelain crucible, drying cabinet, microtube, light microscope, analytical balance, oral sonde, electric oven, bathwater, tube clamps, test tube racks, rotary evaporators, centrifugation, a set of moisture determination devices, UV spectrophotometers, injection syringes, furnaces, test tubes, animal scales, Spectrophotometers are able to read absorbance numbers at 340 nm, Accurate plumbing devices, Interval Timer, Cuvettes and / or Test Tubes, Mixers (Vortex type), constant temperature Bath, or heating block set at 37°C or temperature controlled, Doxorubicin, NaCl, 10% formalin, chloroform, CMC-Na, Vitamin E, AST reagent, ALT reagent, liquid paraffin, toluene, and acetone.

Animals

Animals used in research are rat (*Rattus norvegicus*) wistar male 150 – 200 g. Before the study began, animal test adjusted for one week with the condition of the room temperature (22-25°C), under the cycle of 12 hours light/ dark, given the food and the drinking water. Ethics Commission from health and science commission, University of Sumatera Utara. Animal ethical committee approval number is 0524/KEPH-FMIPA/2019.

Preparation of ethanol mengkudu

Mengkudu fruit is separated from the seeds, the flesh and the skin are taken. Then chopped and dried in a drying cabinet for 3 days. The making of mengkudu fruit ethanol extract was done by maceration with 96% ethanol solvent. A total of 500 grams of mengkudu powder was put into a glass container, 96% of ethanol was added as much as 3.75 L, cover, leave for 5 days protected from light while stirring frequently, squeeze, wash the dregs with enough liquid to obtain 4 L. Transfer to a closed vessel, leave in a cool place, protected from light for 2 days. Encapsulated or filtered. The results obtained are concentrated with the rotary evaporator until most of the solvent is evaporated and the evaporation process is continued on the water bath until a thick extract is obtained (mengkudu fruit ethanol extract).

Phytochemical screening of ethanol mengkudu

Phytochemical screening of mengkudu ethanol extract method consisted of identification of phenol, steroids/terpenoids, saponins, flavonoids, tannin and alkaloids.

In vivo test hepatoprotective effect of mengkudu

In vivo tested in an experiment by using 25 wistar rats (*Rattus norvegicus*) male and weight 150 g - 200 g, as many as 24 and divided into 6 groups and each group consisted of 4 rats :

Normal : Suspension Sodium-Carboxymethylcellulose (Na-CMC).

Negative control : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw.

Positive control : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + Vitamin E 1%.

Group 1 : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw + 100 mg/kgbw.

Group II : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw+ 300 mg/kgbw.

Group III : Wistar rats (*Rattus norvegicus*) male induced by doxorubicin 15 mg/kgbw+ 500 mg/kgbw.

Induction of liver damage was done using doxorubicin with an accumulative dose of 15 mg/kg for 21 days, with 5 mg/kg once a week. Before the treatment of male wistar rats (*Rattus norvegicus*) was adapted for 14 days then continued with doxorubicin induction and treatment of experimental animals for 21 days with extract of

mengkudu administration with 2 ml, 4 ml, and 6 ml dose. Then on the last day, the treatment of male wistar rats (*Rattus norvegicus*) was fasted for 18 hours, performed surgery on the test animals. Wistar rats (*Rattus norvegicus*) male fasted for about 18 hours (not given food, but still given a drink). Male Wistar rats (*Rattus norvegicus*) were anesthetized with ketamine at a dose of 70 mg/kgbw i.v. Male wistar rats (*Rattus norvegicus*) are then tethered to the board on all four limbs. The chest cavity was dissected and 3 ml of blood from the heart was taken using a 5 ml syringe. The blood is then transferred in a blood tube. Then the blood is centrifuged for 10 minutes at a speed of 3000-4000 rpm to produce 2 layers, namely serum/supernatant and its sediment. The serum layer is then taken using a 1 ml syringe, stored in a microtube and stored in a refrigerator at -4°C. Blood serum is used for the examination of total ALT and AST [11].

Determination of AST and ALT

Measurement of the levels of AST and ALT is performed by following the method). As many as 50 µl samples and 500 µl of AST reagent/ALT mixed in a test tube. Then the initial absorbance read after 1 minute at a wavelength of 340 nm. Next, the absorbance was measured again after 1, 2, and 3 minutes [12].

Statistical analysis

Test analysis was carried out by using one-way analysis of variance (ANOVA) followed by Post Hoc Test using the Tukey HSD test. $P<0.05$ was considered as statistical significance and also use IBM SPSS 20.

Result and discussion

Authentication of plant

The results of the identification of plants carried out by Rozana (2019) at the Medanese Herbarium (MEDA) the University of North Sumatra, the fruit used in this study was Mengkudu (*Morinda citrifolia*).

Phytochemical result

The results of phytochemical screening qualitatively in Mengkudu extract are shown in table 1.

Table 1. Results of Phytochemical Screening of Mengkudu Extract

Chemical Component	Result
Tanin	+
Saponin	+
Flavonoid	+
Steroid	+
Glikosida	+
Alkoloid	+

Phytochemical screening of ethanol mengkudu showed the positive result of flavonoids, tannins, saponins, glycosides, alkaloid, and steroids.

According to the book of Malaysian medicinal plants, the chemical constituents of *Morinda citrifolia* are: 5,7-Acacetin-7-Ob-D (+) - glycopranoside, isomeric ajmalisate, alizarin, asperuloside, asperulosidic acid, chrysophanol (1,8-dihydroxy -3-3- methylanthraquinone), damnacanthol, digoxin, 5,6-dihydroxylucidin, 5,6-dihydroxylucidin-3-b-primeveroside, 5,7-dimethyl apigenin-40-ObD (+) - galactopyranoside, lucidin, lucidin-3-b-primeveroside, 5,7-dimethylapigenin-40-ObD (+) - galactopyranoside, lucidin, lucidin-3-b-primevero side, 5,7-dimethylapigenin-40-ObD (+) – galactopyrano side, lucidin, lucidin-3-b-primeveroside, 5,7-dimethyl apigenin-40-ObD (+), 2-methyl-3,5,6-trihydroxyanthra quinone, 3-hydroxymorindone, 3-hydroxymorindone-6-b-primereroside, a-methoxyalizarin, 2-methyl-3,5,6-trihydroxyanthraquinone-6-b-primeveroside, monoethoxy rubiadine, morindadiol, morindin, morindone 1, 5, 6-trihydroxy-2- methylanthraquinone), morindone-6-b-primeveroside, nordamnacanthal, quinoline, rubiadine, rubiadine 1-methyl ether, saronjidiol, alkuroid, anthraquinone and glycosides, caproic acid, caprylic acid, caprylic acid, caprylic acid fatty acids and alcohol (C5-9), flavone glycosides, flavonoids, glucose (b-Dglucopyranose), indol, purine, and b-sitosterol [13]. Until now, 51 volatile compounds were identified in M. citrifolia ripe fruit, without clear specifications of fruit harvest location and stage conditions. These compounds include organic acids such as octanoic and hexanoic acids, alcohols including 3-methyl-3-butene-1-ol, and esters such as methyl octanoate, and methyl decanoate, and ketones as 2-heptanone, and lactone (E) -6-dodeceno-lactone (Assi et al, 2017). Lyophilized Tahitian Morinda juice contains trace elements including manganese (6.11 ± 0.21 g), copper (2.22 ± 0.31 g), molybdenum (molybdenum) 0.160 ± 0.004 g), and cobalt (0.0474 ± 0.0006 g [14].

AST level

AST activity measurements were carried out on the 21st day, 24 hours after the normal group, administration of 0.5% CMC Na, administration of vitamin E1% body weight, and mengkudu doses of 100, 300, 500 mg/kgbw. The results of serum AST obtained can be seen in table 2.

Table 2. AST Level

No.	Doses	Mean AST ± SD (U/L)
1.	Normal	113.67 ± 1.527
2.	Negative control	402.67 ± 10.785
3.	Positive control	121.67 ± 1.527
4.	Group I	334.33 ± 15.044
5.	Group II	304.33 ± 6.027
6.	Group III	192.67 ± 7.371

The data presented in the form of Mean ± SD. Data obtained results based on the results of statistical tests, serum AST levels in the negative control group CMC Na 0.5% had a significant difference ($p < 0.05$) with other treatment groups. The serum AST levels in the positive control group Vitamin E were not significantly different ($p > 0.05$) from the normal group, and were significantly different ($p < 0.05$) with mengkudu 100, 300, 500 mg/kg BW. The serum AST levels in the mengkudu treatment group 100 mg/kgbw did not have a significant difference ($p > 0.05$) to the mengkudu 300 and 500 mg/kgbw treatment groups and were significantly different ($p < 0.05$) in the normal treatment group, negative groups, and positive groups. The serum AST levels in the mengkudu 300 mg/kgbw group had a significant difference ($p < 0.05$) in the normal group, the negative group, and the positive group. The serum AST levels of the mengkudu treatment group 100 mg/kgbw and mengkudu 300 mg/kgbw have a significant difference ($p < 0.05$) with the normal and positive control groups. The serum AST levels in the mengkudu 500 mg/kgbw group had a significant difference ($p < 0.05$) in the negative group, and the positive group.

Based on table 2 shows the negative control group with AST 402.67 U/L significantly different ($p < 0.05$) with the normal group with AST 113.33 U/L. The positive control group with AST 121.67 U/L did not differ significantly ($p > 0.05$) with the normal group. Mengkudu group dose 100 mg/kg body weight with AST value 334.33 U/L was significantly different ($p < 0.05$) with the normal group. mengkudu group dose 300 mg/kg body weight with AST 304.33 U/L was significantly different ($p < 0.05$) from the normal group. The mengkudu 500 mg/kg BW group with AST value of 192.67 U/L was significantly different ($p < 0.05$) from the normal group. The graph of AST measurement results can be seen in figure 2.

The AST activity value in the negative control is 402.67 U L, where the AST activity value of normal male rats is 70-400 U/L [15]. This AST enzyme can also be found in the heart, skeletal muscles, and liver. If the tissue is acutely damaged then the levels in the serum increase. Based on observations of the average AST activity, there was a decrease in AST activity levels after the administration of mengkudu 500 mg/kg body weight when compared with 2 other dose groups with a value of 192.67 U/L. The results of statistical testing with Tukey One Way Anova analysis showed that mengkudu doses of 100, 300, 500 mg/kgbw had a significant effect on decreasing AST activity ($p < 0.05$).

The results of doxorubicin induction given on days 1, 7, 14, and 20 intraperitoneally caused the majority of hepatocyte cells to undergo hydropic degeneration. Interaction between Doxorubicin with various apoptotic pathways in hepatocytes, namely Doxorubicin produces ROS (Reactive oxygen species) and dissociation of Enos into monomers, the release of cytochrome C oxidase in mitochondria, opening of calcium channels in sarcoplasmic reticulum that activates calcineurin. Oxidative stress caused by Doxorubicin activates apoptotic signals in hepatocyte cells, through their extrinsic or intrinsic apoptotic pathways. All the apoptotic pathways involved in Doxorubicin-induced hepatotoxicity can be seen in the figure which states that doxorubicin can induce apoptotic mechanisms that occur indirectly involving the production of ROS and oxidative stress, and apoptosis itself also produces free radicals

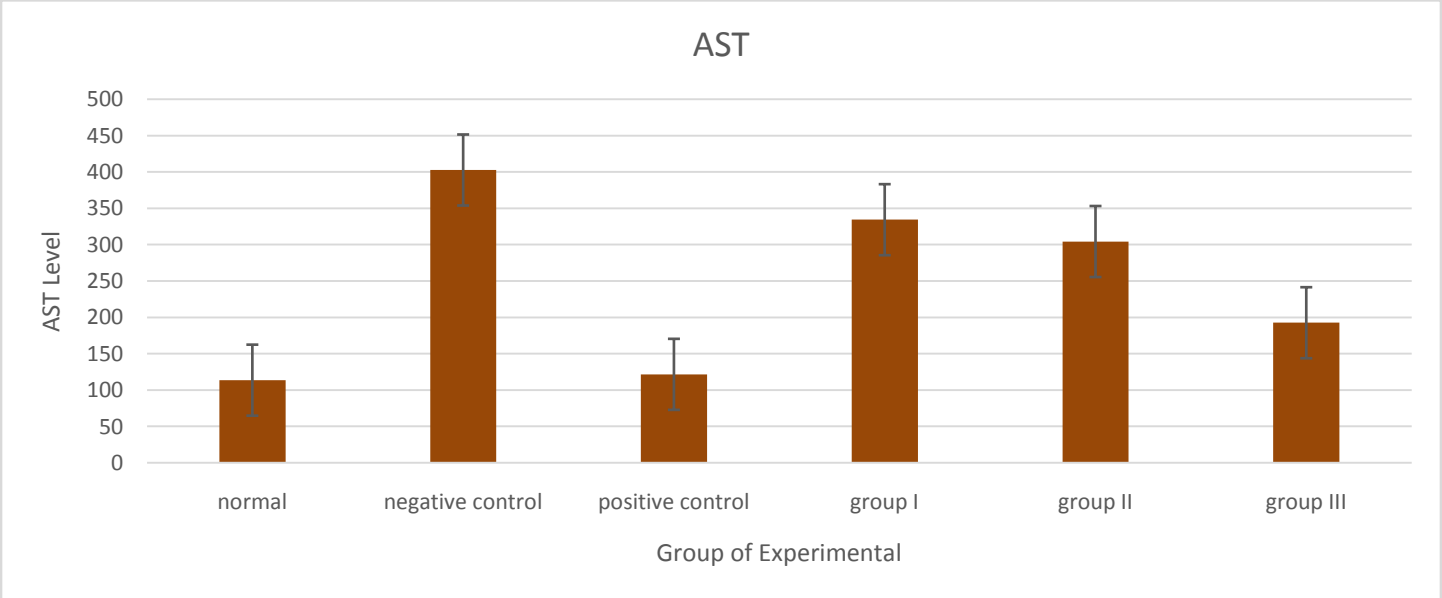

Figure 2. AST level.

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The decrease in AST and ALT is caused by the content of flavonoids and saponins found in mengkudu fruit. Flavonoids act as natural antioxidants because in the flavonoids there is quercetin which works to inhibit lipid peroxidase by blocking the xanthine enzyme. Besides, increasing the absorption of vitamin C, it can protect the antioxidant defense mechanism. The saponin content in mengkudu fruit might also have an impact on decreasing AST and ALT levels [16]. Because this is in line with the results of research conducted by Mohammad *et al.* [17] which mentions the content contained in saponins, namely bisdesmosylsaponin in which oleanolic-glucuronic acid and oleanolic-glucoside acid are showing an effective level of hepatoprotection which is characterized by liver improvement namely decreased ALT AST levels.

The liver is able to secrete transaminase enzymes when the cells repair the disorder. Transaminase is a sensitive indicator of liver cell damage. These enzymes are ALT (alanine aminotransferase). This enzyme catalyzes the transfer of an amino group including alanine and α-ketoglutarate acid to glutamic and pyruvate which is reversible. There are many in hepatocytes and their concentration is relatively low in other tissues. ALT is more sensitive than AST. AST (aspartate aminotransferase) this enzyme acts as a catalyst for the reaction between aspartic acid and α-ketoglutarate becomes glutamate and oxalacetate which are reversible. There are more ASTs in the heart than in the liver, but this enzyme is also found in skeletal muscle, brain, and kidney. Increases sharply when myocardial infarction occurs. This enzyme is less specific for liver disease. When liver cells are damaged by doxorubicin, the enzyme transaminase is in the blood, so that its activity can be measured. This is due to damage to the structure and function of liver cell membranes, ALT activity is earlier and faster than AST activity [17].

ALT level

The results of serum ALT obtained can be seen in table 3.

Table 3. ALT level

No.	Doses	Mean ALT ± SD (U/L)
1.	Normal	41.33 ± 4.932
2.	Negative control	210 ± 5.291
3.	Positive control	46.67 ± 1.527
4.	Group I	152 ± 7.211
5.	Group II	117.33 ± 3.055
6.	Group III	103.67 ± 7.094

The data presented in the form of Mean ± SD. Data obtained based on the results of statistical tests, the serum ALT level of the negative control group CMC Na 0.5% had a significant difference ($p < 0.05$) with other treatment groups. The serum ALT level of the positive control group Vitamin E did not differ significantly ($p > 0.05$) from the normal group, and it was significantly different ($p < 0.05$) with the negative group, mengkudu 100, 300 and 500 mg/kg BW. The serum ALT level of the mengkudu treatment group 100 mg/kg BW did not have a significant difference ($p > 0.05$) to the mengkudu 300 and 500 mg/kg BW treatment groups and was significantly different ($p < 0.05$) to the normal group, negative, and positive groups. The serum ALT level of the mengkudu 100 mg/kg BW group had a significant difference ($p < 0.05$) in the normal group, the negative group, and the positive group. The serum ALT level of the mengkudu treatment group 100 mg/kg BW, 300 mg/kg BW and mengkudu 500 mg/kgbw had significant differences ($p < 0.05$) with the normal, negative and positive control groups.

Based on table 3 it was observed that a negative control group with ALT 210.00 U/L significantly different ($p < 0.05$) from the normal group with ALT 41.33 U/L. The positive control group with ALT 46.66667 U/L did not differ significantly ($p > 0.05$) from the normal group. mengkudu group dose 100 mg/kg BW with ALT value 152.00 U/L significantly different ($p < 0.05$) with a negative control group. mengkudu group dose 300 mg / kg body weight with ALT 117.33 U/L was significantly different ($p < 0.05$) with a negative control group and not significantly different ($p > 0.05$) with the mengkudu group giving a dose of 500 mg/kg body weight. mengkudu group dose 500 mg/kg BW with ALT value 103.66667 U/L significantly different ($p < 0.05$) with normal and significantly different ($p < 0.05$) with mengkudu group dose 300 mg/kgbw. The average bar chart of the measurement of serum ALT in male rats can be seen in figure 3.

The results of doxorubicin induction given on days 1, 7, 14, and 20 intraperitoneally caused the majority of hepatocyte cells to undergo hydropic degeneration. Hydropic degeneration that occurs is caused by the hydration of sodium ions due to permeability in the cell wall which is disturbed by the mechanism of toxicity [18]. The accumulation of sodium ions results in increased plasma cell osmosis values, this condition causes water around the hepatocytes to enter the hepatocytes, causing swelling of cells and cell organelles. If this condition occurs it will result in structural damage and decreased the function of the organelle [19].

The value of ALT activity in the negative control group was 210 U/L, where the normal value of ALT activity in male rats was 25-200 U/L. Based on observations of the average ALT activity, there was a decrease in ALT activity levels after the administration of mengkudu 500 mg/kgbw with a value of 103.67 U/L. The results of statistical testing with Tukey One Way Anova analysis showed that mengkudu doses of 100, 300, 500 mg/kgbw did not have a significant effect on reducing ALT [20].

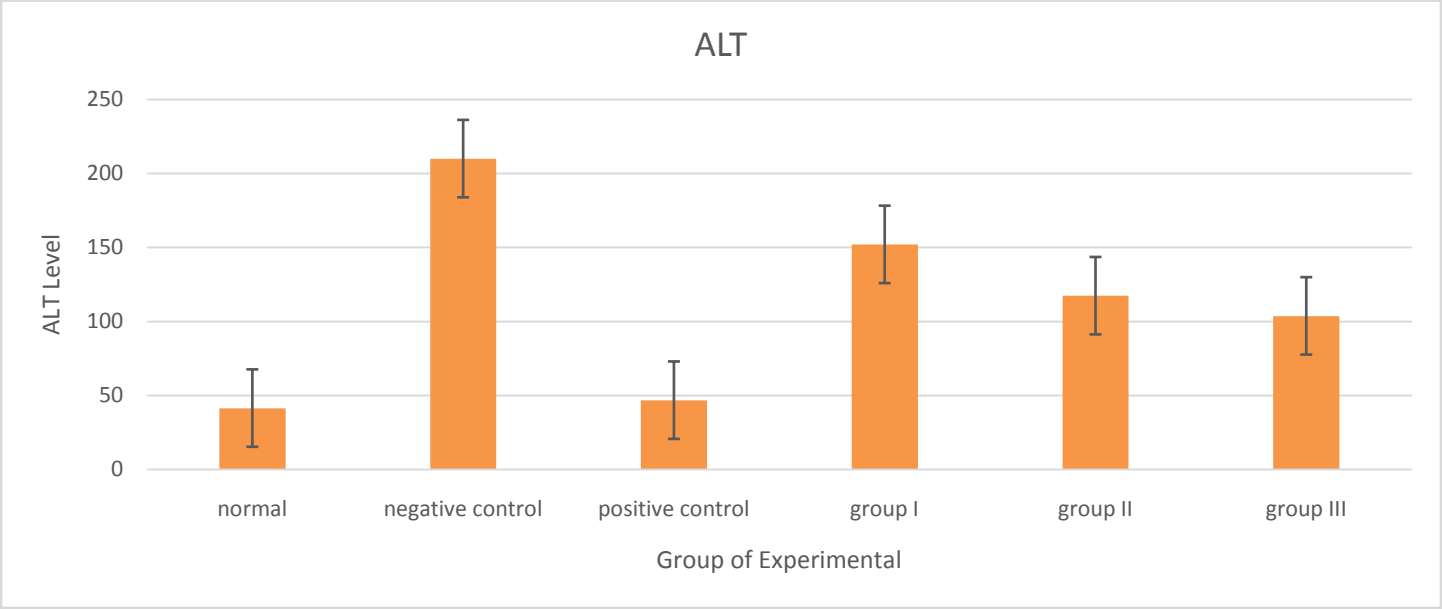

Figure 3. ALT level.

Conclusions

Mengkudu shows activity as a hepatoprotection. Mengkududoses of 100, 300 and 500 mg/kgbw can inhibit the increase in ALT and AST activity. Mengkudu dose 100 mg/kgbw with AST 334.33 U/L and ALT 152 U/L, mengkudu dose 300 mg/kgbw with AST 304.33 U/L and ALT 117.33 U/L, and mengkudu dose 500 mg/kgbw with AST 192.67 U/L and ALT 103.67 U/L showed significant differences ($p < 0.05$) against the negative control group. Mengkudu doses of 100, 300, and 500 mg/kgbw can reduce ALT and AST in doxorubicin-induced male rats.

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