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Research Article

The Effect of Pomegranate Extraction (*Punica granatum* L.) on Albumin Levels and Liver Histology of Male Mice Induced by Paracetamol.

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ABSTRACT

Pomegranates had antioxidants such as flavonoids, thus it was assumed to have a hepatoprotective effect. This study aimed to investigate the effect of pomegranate extract on serum albumin levels and histopathological features of male rat liver induced by paracetamol. Pomegranate extract was carried out by the percolation method using ethyl acetate solvent. Some 25 male sprague dawley rats were randomly divided into 5 groups. Pomegranate extract (200 mg / kg body weight and 400 mg / kg body weight) and sylimarin (100 mg / kg body weight) were carried out every day for 15 days, paracetamol was induced 2 hours after administration of pomegranate extract. The parameters measured were serum albumin level and liver histopathology to assess the effect of pomegranate extract on liver damage caused by paracetamol. The results showed that pomegranate extract (200 mg/kg BB dan 400 mg/kg BB) showed that the activity of serum albumin levels was statistically significant ($p < 0.05$) to negative controls and inhibit the damage of liver tissue histopathology of male rats induced by paracetamol. Pomegranate extract showed the effect of hepatoprotector on liver induced by paracetamol.

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INTRODUCTION

The liver is one of the organs of the body playing an important role in processing the metabolism of foreign materials in the body. The liver is the main organ responsible for many functions. Liver disease in Indonesia has a fairly high prevalence. Riskesdas, the prevalence of hepatitis in Indonesia in 2007 was 0.6%, a

double increase when compared in 2013 by 1.2% (Kemenkes RI, 2017).

Hepatotoxins are compounds that can cause tissue damage in the liver (Robbins and Kumar, 2007). Hepatotoxins can cause acute, sub-chronic, and chronic liver damage (Zimmerman, 1978), 50% of patients with acute hepatitis occur as a result of drug reactions to the liver (PPHI, 2013). Other drugs are metabolized in the liver with long-term use or in excessive doses (Zulizar, 2013).

The use of paracetamol as an analgesic and antipyretic has been known by the common public and is widely sold freely in the market, this causes people to easily consume it without having to use a doctor's prescription and public knowledge about this drug is still very low, especially about its toxicity when used in excessive doses (Hartono et al. al., 2005).

Natural ingredients used as treatment and prevention of hepatitis, including Mangrove roots (Purnobasuki, 2019), honey (Avesina, 2015), Ceplukan leaves (Phisalis angulata L) (Amalia, 2008), Temulawak rhizome (Curcuma xanthorrhiza Roxb) (Hadinata, 2008) 2016), Meniran leaves (Sumardi, 2010), and the others.

Pomegranate fruit is also known as Pomegranate fruit, having several active compounds, such as alkaloids, flavonoids, saponins, tannins, and triterpenoids (Malik et al., 2005). The highest antioxidant activity was found in pomegranate (*Punica granatum* L.), three times more polyphenols than green tea (Emma, 2007). Polyphenols act as antioxidants, such as terminators of free radicals and as redox active metal ion chelators which allow catalyzing lipid peroxidation (Nzaramba, 2008).

Cell membrane lipid peroxidation can actually be captured by antioxidants because the human body has various enzymatic and non-enzymatic antioxidant compounds (Stockham and Scott, 2008). If the accumulation of free radicals increases, the body needs an intake of antioxidant compounds from the outside which are able to protect and repair liver tissue. These compounds are called hepatoprotectors (Dalimartha, 2005).

Liver damage occurred has the characteristics of enlarged liver cells when viewed with a microscope, dark red liver cells, enlarged liver size, and fatty liver (Ulfa, 2008). As a result of liver failure, is necessary to check serum bilirubin, SGOT, SGPT, gamma GT, total protein, especially serum albumin, alkaline phosphatase. Routine blood tests checked are hemoglobin (Hb), leukocytes, prothrombin, bleeding time and clotting (Hadi, 2013). Other parameters of liver damage can be seen from the histopathological picture of the liver, cells that experienced fat degeneration, hydropic degeneration, congestion, apoptosis and cells that underwent necrosis.

MATERIALS AND METHODS

Materials

The tools used in this study were analytical balance, scales, embedding cassette, microtome, surgical board, object glass, light microscope, scalpel knife, microtome knife, cover glass, special staining rack, 60oC oven, percolator, 50 ml erlenmayer, test tube, 1 cc syringe, plain microhematocrit, eppendorf tube, rotary evaporator, ointment pot, red cap vacutainer tube and glassware.

The materials used include adult male Sprague Dawley rats with healthy conditions aged 2 - 3 months, standard feed, pomegranates used are about 3 - 4 months old ripe, ethyl acetate, paracetamol, Na-CMC 0.5 %, sylimarin, alcohol swab, ether, 10% neutral formalin (BNF) buffer, hematoxyilin, absolute ethanol, xylol, paraffin, eosin solution, chloroform and injection of Ketamine HCl.

Methods

Sample Preparation

In this study, the pomegranate was taken from the flesh and seeds. Pomegranate in the oven at a temperature of 25 – 30^0C for 10 days, after drying and then powdered by blended it.

Making extraction

he extraction method used was the percolation method. Pomegranate dry powder was weighed into 340 grams with 5 liters of ethyl acetate as solvent. Extraction was preceded by immersing the sample for at least 1 hour in a closed vessel, then the extraction process was continued in the percolator for 2 days, until the liquid that dripped from the percolator became clear

solution, a liquid extract was obtained. This liquid extract was vacuum distilled and then by using a rotary evaporator at a temperature of 35 - 40^{o}C a thick extract was obtained (Hayouni et al. 2007).

Experimental to Animal Treatment

There were 25 experimental animals in groups into five groups and each group consisted of five animals. The rats were caged in groups on the husk. The standard feed given to rats was 20 g/head/day with drinking water ad libitum.

Prior to the treatment of experimental animals, adaptation was carried out for 7 days to unify the pattern of life. Experimental animals were divided into 5 treatment groups. Group 1 of rats were normal group (K0) given standard feed only during the study and were given 0.5% Na CMC.

Group 2 of rats were a negative control group (KN) given 0.5% Na CMC. Group 3 of rats were a positive control group (KP) given Silymarin at a dose of 100 mg/kg BW. Group 3 of rats were test group 1 (KU1) given pomegranate extract 200 mg/kg BW. Group 5 of rats were test group 2 (KU2) given pomegranate extract 400 mg/kg BW. The treatment was carried out for 15 days and given paracetamol at a dose of 2000 mg/kg BW 2 hours later.

Taking the Blood and Liver Organs

The blood was taken through the orbital sinus of the test animal's eye on day 15 and accommodated in an Eppendorf tube and allowed to stand for 15 minutes, then centrifuged for 15 minutes at 4000 rpm, then separated the supernatant. The supernatant portion obtained was centrifuged again at 4000 rpm for 10 minutes (Roswiyem et al., 2014). The dislocated rats on the same day were removed and their livers were placed in a pot that already contained formalin buffer (Mills, 2007). Parameters of liver damage using light microscopy with 400 times magnification in the entire field of view on each preparation (Rohmani, 2015) which were observed included: cell degeneration consisting of hydropic degeneration, fat degeneration, necrosis, and congestion.

Data analysis

The data analysis used SPSS program. The data were analyzed using the Kolmogorov Smirnov method to determine normality. Then proceed to use the One Way ANOVA method to determine the average difference between groups. If there was a difference, then it was continued by using the Post Hoc LSD test to see the real difference between the treatments.

RESULTS AND DISCUSSION

Marinating

The solvent used in this extraction is ethyl acetate. Ethyl acetate is a good solvent used for extraction because it can be easily vaporized, not hygroscopic and has low toxicity (USP, 2007; Rowe et al, 2009; Wardhani and Sulistyani, 2012). Ethyl acetate is semi-polar therefore it is able to attract both polar and non-polar compounds such as alkaloids, flavonoids, tannins and steroids and the terpenoids contained in pomegranate can be attracted into the solvent. According to research conducted by Jamshidzadeh et al (2012), ethyl acetate solvent attracted more polyphenolic compounds than methanol and N-hexane solvents. The yield obtained from the pomegranate extract used in this study was 10.29%.

Identification of Chemical Ingredients

The result of the identification of pomegranate extraction indicates the presence of chemical compounds in the form of flavonoids, alkaloids, tannins, steroids/triterpenoids.

Table 1: Albumin Examination

Group of Rats	Average Albumin mg/dl ± SD
K0	3,17 ± 0,13
KN	2,75 ± 0,12
KP	3,27 ± 0,15
KU1	3,07 ± 0,19
KU2	3,14 ± 0,17

The measurement of serum albumin level aims to determine the effect of giving pomegranate extract as a hepatoprotector in white rats. Albumin is a plasma protein produced by the liver to help hold intravascular spaces within the vascular space. Decreased albumin (hypoalbuminemia) can cause edema (Horne, 2001).

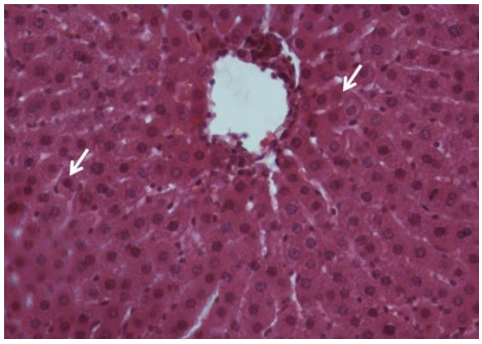

Fig.1: Histopathological picture of K0 shows the presence of necrotic cells (white arrows) with 400x magnification HE staining.

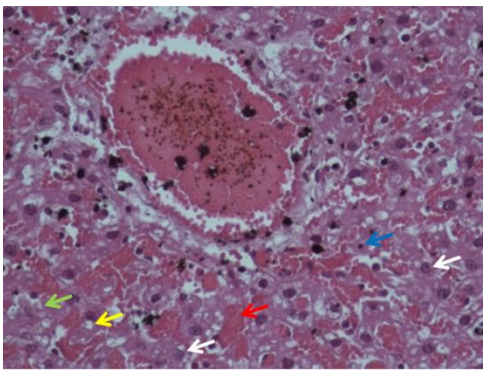

Fig.2: The histopathological description of KN shows fatty degeneration (yellow arrows), hydropic degeneration (green arrows), cells undergoing apoptosis (blue arrows), cells undergoing necrosis (white arrows) and congestion (red arrows) with 400x magnification HE staining.

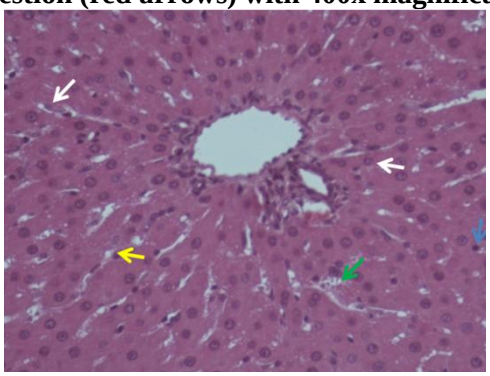

Fig.3: The histopathological description of KP shows hydropic degeneration (green arrows), fat degeneration (yellow arrows), cells undergoing apoptosis (blue arrows), and cells undergoing necrosis (white arrows) with 400x magnification HE staining.

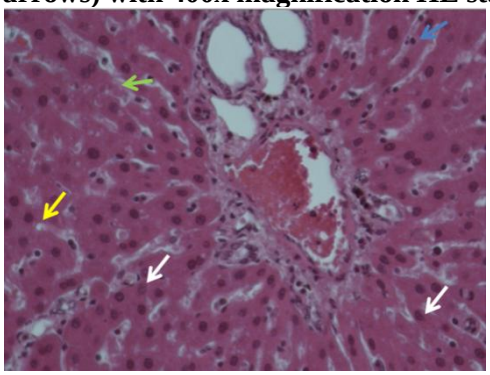

Fig.4: Histopathological description of KU1 shows hydropic degeneration (green arrow), fat degeneration (yellow arrow), cells undergoing apoptosis (blue arrow), and cells undergoing necrosis (white arrow) with 400x magnification HE staining.

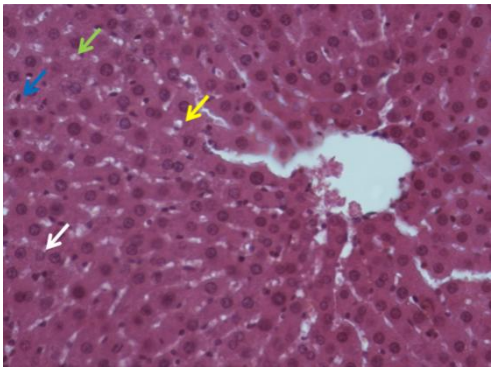

Fig.5: Histopathological description of KU2 shows hydropic degeneration (green arrow), fat degeneration (yellow arrow), cells undergoing apoptosis (blue arrow), and cells undergoing necrosis (white arrow) with 400x magnification HE staining.

In this study, samples of pomegranate (*Punica granatum* L.) are used because they contain high antioxidant compounds and function as hepatoprotectors. In this study, the fruit and seeds (juice) of pomegranate have antioxidant properties because they contain high vitamin C, these antioxidant properties are able to capture free radicals. According to research by Oci (2014) pomegranate fruit and seeds (juice) contain higher polyphenol or flavonoid compounds than green tea.

Inducing agent as hepatotoxic, paracetamol was given after administration of pomegranate extraction. Wibowo (2017) stated that paracetamol at a dose of 2000 mg/kg BW caused an increase in SGPT and SGOT activity. The hepatotoxic process of paracetamol is due to the formation of metabolites during reaction with cytochrome P450. Paracetamol in toxic doses can cause saturation therefore the toxic products are formed during the metabolism of xenobiotic paracetamol (NAPQI) in large quantities, resulting in GSH depression. NAPQI will react with macromolecules causing damage and cell death in the liver.

In this study, the histopathological examination of the liver of rats was observed under light microscopic at 400x magnification to determine the prevalence of liver tissue damage. Parameters of liver damage seen include fat degeneration, hydropic degeneration, necrosis, and congestion.

n the light microscope observation with 400x magnification, was found that there was fat degeneration. Changes in the state of fatness describe the abnormal accumulation of triglycerides in the parenchyma due to the administration of hepatoxin substances such as paracetamol. According to Donatus (2001) disturbances in the balance between triglycerides occurred because the removal and synthesis of fat in liver cells is reduced therefore the number of liver cells increases. Fat degeneration (yellow arrow) in the figure occurs in KN, KP, KU1 and KU2. Each individual animal experiment has a different metabolism, thus it is possible that a lot of fat degeneration can come up even though the dosage is the same. The mechanism of fat degeneration is that paracetamol interferes with hepatic lipoprotein synthesis due to the interaction between paracetamol metabolites in the form of free radicals and lipidal elements of the endoplasmic reticulum as a site of protein synthesis, resulting in changes in endoplasmic endoplasmic morphology, so that the activity of enzymes responsible for drug biotransformation is reduced or even lost (Yuwono, 2010). The presence of fat deposits can be seen in the cytoplasm of liver cells, cytoplasmic fat is seen as fat granules (clear round cavities). Accumulation of fat will follow blood circulation, when blood reaches the central vein usually has run out of oxygen and nutrients.

Hydropic degeneration is a condition in which there is accumulation of intracellular fluid or swelling of cells, there are small to large volumes in the cytoplasm. This observation can be seen in the KN, KP, KU1 and KU2 groups. According to Mills (2007), hydropic

degeneration is a common change in hepatocytes due to several conditions, ranging from mild intoxication to hypoxia. This situation come up because the endoplasmic reticulum takes up a large volume of fluid. Cytoplasm of hepatocytes that undergo hydropic degeneration will see empty spaces with cytoplasmic remnants. Hydropic degeneration is not as clear as fat degeneration, this hydropic degeneration is reversible.

Rat liver cells induced by paracetamol in high doses will trigger apoptosis, which is the process of cell death by eliminating cells which are damaged or dead to maintain osmosis of cell proliferation and limit unnecessary cell proliferation (Peter et al. 1997). Cells undergoing apoptosis have a darker color and the membrane wall looks damaged and the cell nucleus is not visible, as shown in the KN, KP, KU1 and KU2 groups. Cells that is going to undergo apoptosis when the cell is severely damaged and the immune system in the body cannot neutralize it, therefore the cell kills itself (Peter et al, 2007).

The histopathological features of necrosis come up in all groups K0, KN, KP, KU1 and KU2. This condition illustrates that there is same thing as the normal group, it is a result of each individual having a different response to the included xenobiotics. Necrosis is cell death as a result of acute cell damage or trauma. Cell death come up uncontrollably characterized by cell rupture. The stages of necrosis include pyknosis, karyolysis and kariorexis. The characteristics of the occurrence of pyknosis are decreased cell size, signs of karyolysis, cells will become pale and out of shape, a sign of karyorexia, rupture of the cell nucleus and rupture of chromatin, it can be caused by bacterial attack. It is possible that the normal group was exposed to bacteria from the genus Leptospira while in the cage during the treatment.

The leptospira bacteria can enter through wounds on the skin or penetrate mucous tissues such as the conjunctiva, nasopharynx, then enter the blood, multiply, and spread to body tissues. Leptospires can also penetrate tissues such as the vestibule of the eye and the subarachnoid space without causing an inflammatory reaction. Organs that are affected by leptospira are the eyes, blood vessels and liver. Leptospira grows well in aerobic conditions at a temperature of 28^oC - 30^oC and is found in wet or humid environments ranging from surface water, moist soil, and tap water (Rampongan, 2016). When in the cage, the rats are possible to be exposed to this leptospira bacteria when the day rains at night, the rat cage is exposed to rainwater seepage causing the rat cage to get wet.

Furthermore, the histopathological picture of congestion (blood damming) is a state of abnormal or excessive accumulation of body fluids or an abundance of blood in blood vessels in certain regions. Seen under a microscope the hypermic tissue looks dilated and filled with blood, this picture is mostly seen in the negative group and at the some in KU1. These changes come up as a result of the effects of paracetamol free radicals bound to liver cells. Excessive consumption of paracetamol for a long time can cause the formation of free radicals in liver cells (Utami, 2017). Toxic reactive metabolites and free radicals can disrupt the integrity of cell membranes and lead to liver damage (Ikawati, 2010).

CONCLUSION

Based on this study can be concluded that there is an effect in giving pomegranate extract at a dose of 200 mg/kg BW and a dose of 400 mg/kg BW for 15 days of treatment functions as a hepatoprotector in increasing serum albumin levels and inhibiting liver histopathological tissue damage in rats induced by paracetamol dose 2000 mg/kg BW. The giving of pomegranate extract for 15 days at a dose of 200 mg/kg BW and 400 mg/kg BW showed that there was a statistically significant difference in albumin activity ($p \leq 0.05$) against the negative control.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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