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Hepatoprotective Potentials of Dates Extract (*Phoenix dactylifera*) in Acetaminophen-Induced Mice

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Abstract

Dates (Phoenix dactylifera) are considered as a well-known fruit consumed by many people in various countries. This study aimed to examine potential effects of dates as a hepatoprotective agent in mice. This study was conducted at the Iratco Group's eLRosa Laboratory Research Facility, Indonesia, starting from June 2022 to July 2022. Mice from ddY strain were randomly divided into 5 groups (n=5 per group) of positive and negative control groups, and treatment groups 1, 2, and 3. The negative control group as the normal baseline did not receive acetaminophen and date extract. In treatment groups, 30 µL/30 grBW, 60 µL/30 grBW, and 100 µL/30 grBW extract was given per oral to Treatment Group 1, Group 2, and Group 3, respectively for 20 days. On day 21, all treatment groups were induced with 300mg/kgBW acetaminophen for 3 days via the intraperitoneal route. Blood tests were performed on day 24 to measure the serum transaminase level as the parameter of liver damage. The lowest level of transaminase serum was found in group 3 with the highest volume of dates extract, which was 100 µL, followed by group 2 (60 µL), and group 1 (30 µL). There was a significant difference between the positive control group and treatment groups with no significant difference was seen between negative and the treatment groups. This study concludes that dates extract has the potential of being a hepatoprotective agent.

Keywords: Acetaminophen, dates, hepatoprotection, mice

Introduction

The liver is an organ with complex and diverse roles for each individual. In particular, the function of the liver is to filter blood from the intestines through the venous port, then store it and convert food materials received by the venous port. The liver also protects other organs in the body, especially the brain, against toxic substances that are absorbed through the intestine (detoxification), such as ammonia. Kupffer cells are in the liver and work with phagocytizing bacteria and proteins that enter the venous port system through the intestinal wall.[1] Liver damage will cause disturbance to liver functions, and ultimately, resulting in health and body homeostasis. Liver damage or liver disease is a life-threatening disease. Liver damage causes two million deaths annually, with a percentage of one million deaths due to cirrhosis and one million deaths due to viral hepatitis and hepatocellular carcinoma.[2]

One of the most common causes of liver damage is long-term exposure to hepatotoxic drugs (Drug-induced liver injury) and chemicals. This exposure will cause changes in liver cells, especially in hepatocytes. Such as fat degeneration and necrosis can reduce the ability of cell regeneration, causing permanent damage to cell death.[3] One of the drugs classified as a drug that can induce liver injury is acetaminophen. Acetaminophen belongs to a class of analgesic and antipyretic drugs. Acetaminophen is safe and effective, but excessive use can cause liver damage. Liver damage due to excessive use of acetaminophen is caused by the metabolism

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of acetaminophen in the form of N-acetyl-p-benzoquinone (NAPQI), which cannot be completely neutralized by hepatic glutathione. NAPQI will bind to liver cell proteins and cause liver damage.[4]

Liver damage can be measured through the activity of transaminase serum. Two well-known transaminase serums are serum glutamate pyruvate transferase (SGPT) and serum glutamate oxaloacetate transferase (SGOT). SGPT can be found in liver cells, heart, muscle, and kidneys. The greatest portion of SGPT can be found in the liver cytoplasm. On the other hand, SGOT can be found inside the heart cells, liver cells, muscles, kidneys, brain, pancreas, lymph, and lungs. The highest level of SGOT can be found inside the heart cells. The change of permeability causes the increase of SGPT and SGOT or damages the cell wall. Thus, SGPT and SGOT can be used as markers of liver cell damage.[5]

Treatment of liver damage can be done with chemical drugs. Alas, the development of liver damage drugs is still not well developed. It has caused several medication-related problems (MRPs), which happen when the usage of therapeutic drugs does not result in any positive effects. However, on the contrary, it results in adverse effect.[6] Based on that fact, the exploration of the hepatoprotective effect of herbal medication and the composition of active ingredients in herbs shall be done. Herbal medicine does not tend to cause MRPs or any side effects, is safe for long-term usage, can be obtained at low prices, and contains a lot of substances with phytoestrogenic effects, antioxidants, and nutrition. [7]

Some herbal plants have been proven to have a hepatoprotective effect; one is bean sprouts. Based on research conducted by Liu et al.,[9] bean sprouts have been proven to have a hepatoprotective effect on the liver, even on the livers that are already damaged in mice.[8] This is due to the flavonoid in bean sprouts. Tumeric also has the potential as a hepatoprotective agent besides bean sprouts. Due to its potent antioxidant effect and anti-inflammation, turmeric can act as a hepatoprotective agent. Another herbal plant that has hepatoprotective potential is dates. Dates (*Phoenix dactylifera*) are a large commodity and an essential crop in hot, arid regions such as Saudi Arabia and Egypt. In these countries, dates are commonly used as medicine and cosmetics and consumed by humans and animals.[10] Apart from these countries, dates are also famous in Indonesia because of their sweet taste, many benefits, and the fact that they can easily be obtained. Based on the result of phytochemical analysis, it was reported that dates contain alkaloids, steroids, flavonoids, vitamins, and tannin. Flavonoids are known to have membrane stabilizing, hepatoprotective, and antioxidant activity.[11] Dates also contain vitamin C and bioactive components such as carotenoid, sterol, tannin, isoflavonoid, flavonoid, and phenolic acid. These components have an inhibitory effect against oxidative damage.[12,13] Based on research by Alothaid, date extract was proven to improve antioxidant activity in the liver by stimulating the production of CAT, GPx, SOD, and GSH.[14]

This research is conducted to observe the potential hepatoprotective effects of dates on transaminase serum in mice. This research is expected to provide an overview of the potential hepatoprotective effect of dates. In addition, the result of this study is also expected to be used as a basis of consideration and a reference for future research on using dates as hepatoprotective agents.

Methods

The research was conducted at the IRatCo Group's eLRosa Laboratory Research Facility. The research was held from June 2022 to July 2022. The research was carried out from June 2022 to July 2022. The research procedure of using mice has previously met the ethical rules from the Health Research Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences IPB University with a certificate of ethical clearance Number. 060/KEH/SKE/XII/2021. Mice that were used were mice with ddY *Strain*, aged eight weeks, all female, and were not pregnant. Mice were grouped into five groups, with each group containing 5 mice. The number of experimental animals used was calculated with the Federer formula (1997). The calculation of the experimental animal is as follows.

Based on the calculation with the Federer formula, it was concluded that each treatment needed at least 5 mice. Acclimatization of experimental animals is a step that must be done before using them in the research, with consideration of possible differences in the environment from the place of origin to the new environment. In this study, acclimatization was carried out for 14 days to make sure that the mice could adapt to their new environment. The treatment given during acclimatization was the administration of anthelmintics (10 mg/kg

body weight) orally once a day on the 1st and 7th days via oral gavage. Experimental animals were given the antibiotic amoxicillin (15 mg/kg body weight) orally twice a day for five days, from day 2 to day 6, via oral gavage. Animals were also given antiprotozoal sulfamethoxazole (30 mg/kg body weight) orally twice a day from day 8 to day 10. Dosage of drug administration refers to the book of veterinary drugs.15 The treatments given during acclimatization were done to make sure that all mice were free from microorganisms and parasites, such as worms and tick.

The room used for rearing the animals used an air conditioner with temperatures ranging from 22-24°C and humidity of 60-80%. The room was well-lit, quiet, and regularly cleaned once every two days. The experimental animal cages were made of plastic containers with wooden shavings as a base, replaced twice a week. The cages were closed with lids made of woven wire, which was strong, anti-rust, and bite-resistant. The experimental animals were fed using special pellet feed for mice, given twice a day with drinking water sourced from mineral water, and given ad libitum.

This study was done to observe the hepatoprotective effect of date extract on liver cell damage induced by acetaminophen. The animals used were male mice with ddY *strain*. The mice used were 8 weeks old. Experimental animals were divided into 5 treatment groups, each consisting of 5 mice. Mice were divided into dose group 0 as a negative control and groups 1, 2, and 3 as doses administered with commercial date extract in viscous liquid form. The dates used were those on the ripening stage, with a 1460 mg/mL concentration. Group 1 was given 30 µL of dates extract with a dose of 1300 mg/kgBW, and group 2 was given 60 µL of dates extract with a dose of 2900 mg/kgBW; group 3 was given 100 µL of dates extract with a dose of 5000 mg/kgBW, and group 4 as a positive control. All extracts were given using a micropipette for 20 days straight, once a day. The volume of the extract was given based on the treatment group. Groups 0 and 4, as negative and positive control, were not given any preparations. After the 20th day, acetaminophen was induced in the mice with the dosage of 300 mg/kgBW via the intraperitoneal route for two days, in groups 1, 2, and 3 as dose groups and group 4 as positive control group. Group 0, as the negative control group, was not induced by acetaminophen and was used as a normal baseline. Blood was collected on the first 24 hours after induction and on the 26th day after mice were terminated. After termination, the livers of the mice were collected.

A blood examination (hematogram/hematology) was done on day 26. Blood collection was done in every treatment group. The collection of blood samples was done via the intraorbital route. Samples were put inside vacuum tubes containing tripotassium ethylenediaminetetraacetic acid (K3EDTA) anticoagulant. A blood sample was analyzed using a hematology analyzer. A blood chemistry test was also done. Blood biochemistry parameters were serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT). Data were collected in table form using the software Microsoft Office Excel 2019. Data analysis was done using the Statistical Product and Service Solutions (SPPS) with the statistical data normality test using the Kolmogorov-Smirnovv method and ANOVA test,

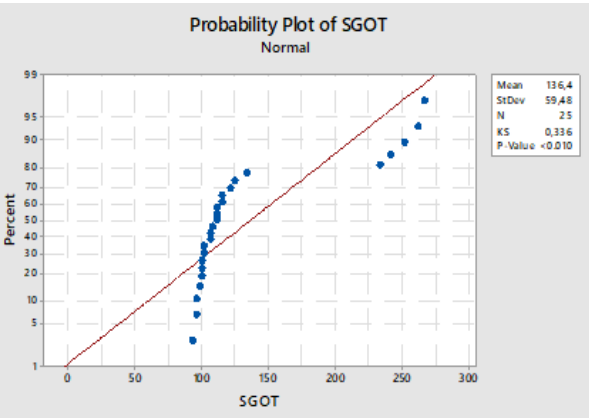

Figure 1 SGOT Data Distribution in A Form of Probability Plot

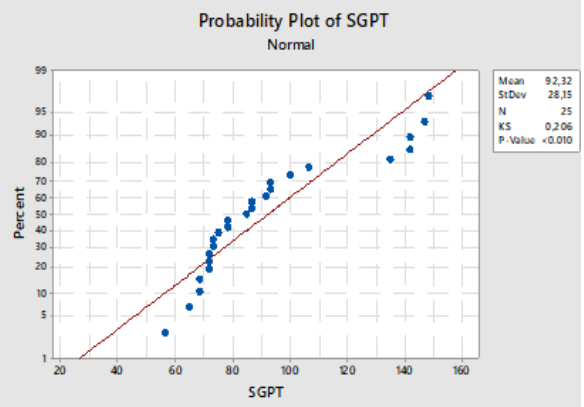

Figure 2 SGPT Data Distribution in A Form of Probability Plot

followed by the Tukey test.

Result

Statistical analysis was carried out to determine the homogeneity and normality of the data. This method was carried out as a reference for the following data analysis stage. This analysis results in the data being normally distributed or not normally distributed. Based on the Kolmogorov test, data that is normally distributed will have a good level of accuracy and interpretation compared to data that is not normally distributed.

The results of the normality analysis of SGPT and SGOT data in this study are presented in Figure 1 and 2. SGPT and SGOT data collected in this study are normally distributed. This refers to the Kolmogorov-Smirnov data's normality coefficient value, which states that the KS value is less than 0.349 for normally distributed data. The KS value for the SGOT data is 0.336, and the KS value for the SGPT data is 0.206. Normally distributed data were suitable for analysis between groups using the ANOVA method and Tukey's follow-up test.

The result done with the ANOVA method showed that mice that were given date extract (K1, K2, K3) had lower serum levels of SGPT and SGOT than mice in the positive group (K4) ($p<0.05$). Figure 3 provides a box plot comparison of each group's average levels of SGOT. The SGOT level in the treatment dose group was shown to be lower than the positive group and was close to the negative group level. The lowest mean SGOT value was found in the negative group, followed by 100 µL treatment dose, 60 µL treatment dose, and 30 µL. The highest value was seen in the positive control. Based on the result, the average level of SGOT decreased along with the increase in the date extract given to the mice. The larger the extract dose, the lower the SGOT level produced.

SGPT levels in mice that were given date extract were lower than in mice in the positive group and closer to the negative group. Graph 4 provides a box plot comparing SGPT levels for each group. The lowest value of SGPT levels was found in the treatment of 100 µL, followed by the negative group, treatment of 30 µL, and treatment of 60 µL. The highest level was seen in the positive group. Based on observations, the average level of SGPT decreased was almost in line with the increase in the dosage of extract

Table 1 The Effect of Date Extract on Levels (µL) of SGPT and SGOT in Mice

Group	SGPT	SGOT
1 (30 µL)	86,0±14,6[a]	114,66 ±13,35[a]
2 (60 µL)	86,3±13,1[a]	111,66±9,35[a]
3 (100 µL)	72,3±12,6[a]	105,32± 7,67[a]
4 (Positive)	142,7±5,2[b]	250,97±13,77[b]
5 (Negative)	74,3 ±4,3[a]	99,39±4,96[a]

*a, b: Different superscript letters on the same line showed significant differences ($p<0.05$)

Figure 3 Effect of the Dosage of Dates Extract on Levels (µL) of SGOT in Mice

given to mice. SGPT level will decrease along with the increase in the dosage.

Table 1 presents data on the levels of SGPT and SGOT in mice that were given the extract, as well as in the positive and negative control groups. The data obtained were tested with a 95% confidence interval and a significance level 0.05 (p=0.05). The test results showed that the levels of SGPT and SGOT were close to the negative group, which was not given any treatment nor induced by acetaminophen. Data analysis of the average value showed a significant difference between the groups that were given the extract and the positive group, which was not given any treatment but was induced by acetaminophen (p<0.05). No significant difference (p>0.05) was seen between the treated mice compared to the negative group. However, a decrease could be seen in the levels of SGPT and SGOT, along with the increase in the dosage.

Discussion

Acetaminophen is a drug with analgesic and antipyretic effects. However, acetaminophen with inappropriate dosage and long-term usage can cause liver damage due to the hepatotoxic property of the drug. Acetaminophen at a toxic dose is biotransformed by cytochrome P450 (CYP2E1 isozyme), producing an unstable, reactive, toxic metabolite, namely N-acetyl-para-benzoquinone imine (NAPQI). Under normal conditions, this metabolite is detoxified by conjugating with glutathione to form mercapturic acid. Administration of acetaminophen in toxic dosage causes many toxic reactive metabolites to form and deplete the glutathione supply

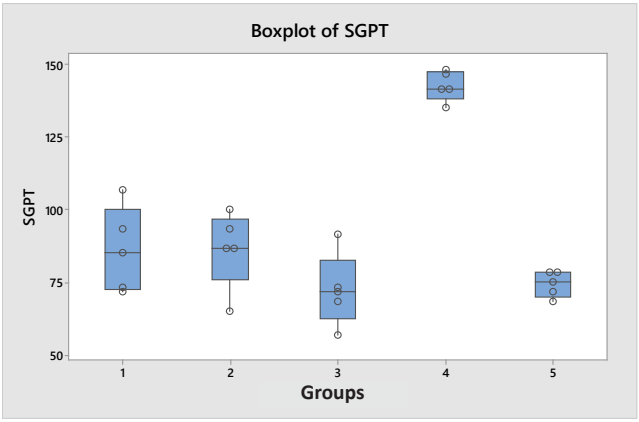

Figure 4 Effect of The Dosage of Dates Extract on Levels (µL) of SGPT in Mice

to conjugate these substances so that the reactive drug metabolites bind to the protein components of liver cells, causing liver damage. Acetaminophen oxidation by cytochrome p450 enzyme also produces free radicals. Suppose free radicals are associated with unsaturated fats such as cell membranes. In that case, there will be lipid peroxidation, which causes damage to the cell membrane structures and functional disorder that will increase serum transaminase levels.[16] Elevation of serum transaminase is one of the strongest signs of cellular leakage and impaired functional integrity of hepatocyte membrane. [17]

Based on the result of the research that has been obtained and statistically analyzed using the ANOVA analysis test, groups that were given the extract show lower SGPT and SGOT levels compared to the positive control or group that was not given any extract ($p<0.05$). The hepatoprotective effect of dates was shown from the level of SGPT and SGOT of the groups that were given the extract. The decrease in the levels of SGOT and SGPT was almost linear with the increase in the extract dosage administered. The decrease in SGOT level followed the increase of the extract dosage, starting from the dosage of 100 µL, followed by the dosage of 60 µL and 30 µL. The SGOT level of mice given the extract showed no significant difference ($p>0.05$) compared to the negative control or mice not given any treatment. On the other hand, the SGOT level of mice that were given the extract showed a significant difference ($p<0.05$) compared to the positive control group. Meanwhile, the decrease in SGPT level was almost in line with the increase in dates extract, with a 100 µL dosage followed by 30 µL and 60 µL. The SGPT level of mice that were given the extract did not show any difference ($p<0.05$) compared to the negative control group.

The results showed that the date extract had a hepatoprotective effect as seen from the comparison between SGPT and SGOT levels in the treatment group with the negative control group, followed by the positive control. This is due to the components contained in the dates. Dates can be consumed as fresh fruit or called the besser stage (short shelf life), or tamer stage (long shelf life). Analysis of phytochemical composition during different ripening stages showed that the highest number of polyphenolic compounds, carotenoids, and anthocyanins were found in the early stages (kimri and besser) and decreased during directed ripening stages showed that the highest number of polyphenolic compounds, carotenoids, and anthocyanins were found in the early stages (kimri and besser) and decreased during direct ripening. The besser stage was found to be rich in flavonoids, tannins, and phenolic acids such as ferulic acid, caffeine, p-coumaric, and protocatechuic acids and catechins, which are rich in antioxidant activity.[18] Antioxidant content is believed to reduce free radicals and reduce oxidative stress levels. This has an impact on the repair of a damaged liver. Other polyphenols found in dates are catechins, quercetin, and luteolin. Based on research conducted by Abdelaziz and Ali,[19] the administration of this polyphenol content in distilled water has been shown to improve liver lesions in the form of vacuolization and fibroblast proliferation in the liver of rats.

Other than having an antioxidant effect, dates also have anti-inflammatory activity through the downregulation of cyclooxygenase-1 and 2 (COX-1 and COX-2) enzymes. This activity is attributed to compounds rich in polyphenols, namely glycosides, and flavonoids.[20] Apart from inhibiting the COX-1 and COX-2 enzymes, dates also can inhibit the CYP2E1 enzyme. The CYP2E1 enzyme is an enzyme that can be found in hepatocytes and functions to metabolize molecules such as ethanol, acetaminophen, and pro-carcinogens. CYP2E1-mediated metabolism produces toxic intermediates and excessive amounts of ROS. High ROS levels due to this enzyme's activity are the main cause of various liver diseases, especially those caused by chronic alcohol consumption.[21]

Based on the GC-MS analysis conducted by Nehdi et al.,[22] dates contain several saturated and unsaturated fats, such as stearic acid, palmitic acid, 9-octadecenoic acid, fumaric acid, and -linolenic acid. 9-octadecenoic acid, another name for oleic acid, is classified as an anti-inflammatory agent because of its inhibitory effect on pro-inflammatory signals. The presence of linoleic acid in dates can reduce oxidative stress caused by injury to liver lesions, liver steatosis, and non-alcoholic liver disease.[23]

Based on the results of the study, the administration of date extract was proven to have a hepatoprotective effect on mice. This is evidenced by the significant decrease in SGPT and SGOT levels compared to the positive control group and the levels of SGPT and SGOT, which were not so different from the negative control group. Further research must be done to utilize dates and their contents as hepatoprotective agents.

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