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HEPATOPROTECTIVE ACTIVITY OF WATER KEFIR

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ABSTRACT

Acute liver failure is a type of liver disease in which the liver has decreased or even lost its function in a relatively short time. Previous research had shown that water kefir had strong antioxidant and anti-inflammatory activity. Therefore, this current study aimed to evaluate the ability of water kefir to prevent and treat acute liver failure. This experiment was conducted using two methods: the prevention and treatment of acute liver failure. In both methods, the inducing agent used was acetaminophen 2 g / kg bw administrated orally. Water kefir was evaluated in 3 doses: 90 mL / kg BW, 180 mL / kg BW, and 270 mL / kg BW; with silymarine as the standard drug. The parameters were AST, ALT, ASP, and total protein level, and Manja Roenigk Histopathology Score. The results showed that in both methods, water kefir in all doses was able to significantly improve AST, ALT, ASP, and total protein level compared to the positive control. The improvement in these levels was also supported by the results of the histological score which also decreased. It means that there was an improvement in the level of liver necrosis. However, water kefir that showed comparable results to silymarine was a dose of 180 mL / kg BW for prevention and 270 mL / kg BW for the treatment of acute liver failure. It could be concluded that water kefir had the ability to prevent and treat acute liver failure caused by acetaminophen.

Keywords: acetaminophen, acute liver failure, hepatoprotective, water kefir

INTRODUCTION

Liver is the largest organ in the human body and plays an important role in various physiological processes, such as the metabolism of carbohydrates, lipids and

amino acids; detoxification; blood clotting process; the body's defence process against infection; and others **[1]**. Acute liver failure is a type of liver disease in which the liver has decreased or even lost its function in a relatively short time. This condition was initially associated with drug-induced liver damage, although it could eventually be applied to other situations. Acute liver failure usually begins with acute liver injury characterized by a two to threefold increase in transaminases in patients without chronic liver disease **[2, 3]**. Acute liver failure can be caused by drug toxicity, viral and autoimmune hepatitis, and hepatic ischaemia **[4]**. Patients with acute liver failure show an increase in oxidative stress which is marked by high levels of SOD and lipid peroxidation **[5]**.

Water kefir is a homemade fermented beverage. Water kefir has a long history and different names in each place, such as Tibi grain, California bees, African bees, Ale nuts, Balm of Gilead, Japanese Beer Seeds, or Sugary kefir grains **[6-9]**. Water kefir contains various microbial species such as *Lactobacillus casei / paracasei, Lactobacillus harbinensis, Lactobacillus hilgardii,Bifidobacterium psychraerophilum/ crudilactis, Saccharomyces cerevisiae,* and *Dekkera bruxellensis.* The main metabolites of fermented water kefir are ethanol and lactic acid; Glycerol, acetic acid, and mannitol are also produced in small quantities. Some aromatic and volatile compounds are also produced (relative to their threshold values) such as ethyl acetate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, and ethyl decanoate **[10]**. Another research showed that kefir water had activity as an antioxidant, antihyperglycemia, antihyperlipidemia, antiobesity, anticancer, and activity in repairing disorders in the digestive tract **[11-15]**. Based on this explanation, this experiment aimed to evaluate the activity of water kefir in preventing and treating acute liver damage.

MATERIALS AND METHODS

Materials

Water kefir grain (purchased from Yogyakarta, Indonesia), sugar, dried fruit, rats aged 2 - 3 months and weighing 200-250 g, silymarin, ethanol, formaldehyde buffer, sugar, hematoxylin-eosin, raisins, acetaminophen, reagent kits for the determination of AST, ALT, ALP and total protein.

Preparation of Water Kefir

The water kefir solution was made by fermentation, starting with the preparation of 100 grams of water kefir seeds, 60 grams of sugar, 2 grams of raisins and 1 L of aqua mineral distillate. The sugar was mixed with warm distilled water in a beaker glass, then put the water kefir seeds and raisins in the sugar solution. The beaker glass was

closed using a thin cloth and the fermentation process was carried out for 48 hours at room temperature ± 25 ° C. The filtrate was used for evaluation, while the kefir grain was reused for the next production **[16]**.

Water Kefir Activity in Liver Damage Prevention

This evaluation aimed to evaluate the ability of water kefir to prevent liver damage due to cytotoxic agent administration, in this case was acetaminophen at a dose of 2 g / kg BW **[17]**. Rats were divided into 6 groups: the negative control group, the positive control group, the 200 mg / kg BW silymarin group, and the water kefir group with doses of 90 mL / kg BW, 180 ml / kg BW, and 270 mL / kg BW. The evaluation was carried out for 14 days, where the water kefir and acetaminophen were administered simultaneously. The parameters measured were AST, ALT, ALP, and total protein on days 0, 7, and 14 of treatment. At the end of the evaluation, the animals were sacrificed and their liver was isolated for histological analysis using the Manja Roenigk Histopathology Scoring **[18]**.

Water Kefir Activity in Liver Damage Treatment

This evaluation aimed to evaluate the ability of water kefir to treat and improve liver damage due to cytotoxic agent administration, in this case was acetaminophen at a dose of 2 g / kg BW **[17]**. In this evaluation, the procedure was divided into 2 stages: the induction stage and the treatment stage. At the induction stage, animals were divided into 2 groups: the group that was not induced (negative control) and the group that was induced. Induction was carried out for 14 days using acetaminophen 2 g / kg BW. The induced rats were divided into 5 groups, that were the positive control group, the silymarin group 200 mg / kg BW, and the water kefir group with the dose of 90 mL / kg, 180 ml / kg, and 270 mL / kg BW. The treatment was carried out for 14 days. The parameters measured were AST, ALT, ALP, and total protein at before treatment, after induction, and after treatment. At the end of the test, the animal was sacrificed and the liver was isolated for histological analysis using the Manja Roenigk Histopathology Scoring **[18]**.

RESULTS AND DISCUSSION

The results of evaluation of water kefir activity in preventing and treating liver damage due to hepatotoxic agent were shown in **Table 1-4**.

Tables 1, 2, 3, and 4 showed that all animals were in a homogeneous state before given treatment. In the prevention method, administration of high doses of paracetamol caused a significant increase in

the AST, ALT, and ALP level of the positive control group when compared to all other groups on days 7 and 14. Significant changes also occurred in total protein level, where the total protein in the positive control group indicate a significantly lower number compared to the other groups. While in the treatment method, acetaminophen administration at a dose of 2 g / kg BW for 7 days caused significant changes in all parameters. AST, ALT, and ALP level were significantly increased in all induced groups compared to the negative control group. Meanwhile, the total protein level decreased significantly. It indicate that the administration of paracetamol at dose of 2 g / kg bw could damage the physiology of the liver.

Acetaminophen is an analgesic and antipyretic drug that is safe to use in therapeutic doses. However, in high doses, it can cause liver damage. From the result above, we could see that paracetamol was able to induce liver damage, characterized by increased levels of AST, ALT, ALP, and decreased levels of total protein in the positive control group in both methods used. Acetaminophen-induced hepatotoxicity begins with the formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), which results in reduced cellular glutathione and the formation of additional proteins in mitochondrial proteins. These events lead to mitochondrial oxidative and nitrosative stress, which is accompanied by activation of c-jun N-terminal kinase (JNK) and translocation to mitochondria. This further amplifies mitochondrial oxidantive stress, leading to translocation of Bax and dynamin related protein 1 (Drp1) to the mitochondria, which induces mitochondrial fission and induces mitochondrial membrane permeability transition (MPT). These induction triggers the release of intermembrane proteins such as apoptosis inducing factor (AIF) and endonuclease G into the cytosol and their translocation to the nucleus, causing fragmentation of nuclear DNA and ultimately activating the regulated necrosis **[19]**.

Water kefir administration at various doses for 14 days resulted in improvements in the level of AST, ALT, ALP, and total protein, both in the prevention and treatment methods, which was marked by significant differences in the parameters level of the treated group and the positive control group. However, the group that showed hepatoprotecvtive activity comparable to silymarine as the standard drug on all parameters was the 270 mL / kg bw water kefir group. It could be interpreted that the administration of silymarine and water kefir was able to prevent and treat damage

caused by the toxic effect of high doses of acetaminophen. This was supported by the result of the histological analysis which showed a significant difference from the liver histology scores calculated using the Manja Roenigk measurement method, as shown in **Table 5**.

Table 1: AST level

Groups	AST level at days- (Prevention Method)			AST level at days- (Treatment Method)		
	0	7	14	0	7	21
Negative control	113.4±11.3	123.2±4.4*	117.8±2.8*	113.4 ± 11.3	121.0 ± 5.7*	117.8 ± 2.8*
Positive control	120.2±6.8	204.2±6.1	219.6±3.4#	115.6 ± 8.6	207.0 ± 4.0	219.0 ± 6.9#
Silymarine 200 mg/kg bw	118.0±15.6	125.0±12.2*	118.4±3.1*	119.4 ± 12.3	205.6 ± 8.3	121.2 ± 3.1*
Water kefir 90 mL/kg bw	121.8±5.5	129.4±5.9*	125.6±2.1*#	122.6 ± 8.1	209.0 ± 8.0	130.0 ± 5.1*#
Water kefir 180 mL/kg bw	117.8±9.8	129.4±7.3*	121.8±2.8*	118.6 ± 4.9	208.8 ± 6.7	128.2 ± 4.1*#
Water kefir 270 mL/kg bw	114.6±8.1	124.6±2.1*	119.4±1.7*	119.2 ± 6.6	206.8 ± 8.6	125.8 ± 5.1*

Note: Data are presented as mean ± SD, * means significantly different compared to the positive control group, # means significantly different compared to the silymarine group, p<0.05, n = 5 mice/group.

Table 2: ALT level

Groups	ALT level at days- (Prevention Method)			ALT level at days- (Treatment Method)		
	0	7	14	0	7	21
Negative control	68.2±1.9	72.6±2.7*	69.8±1.3*	68.2 ± 1.9	72.2 ± 1.9*	69.8 ± 1.3*
Positive control	65.8±4.0	144.6±8.4	151.8±4.2#	66.6± 4.4	188.2 ± 4.4	204.0 ± 5.8#
Silymarine 200 mg/kg bw	64.6±3.4	75.4±3.6*	68.6±2.4*	69.4 ± 5.9	183.0 ± 4.4	70.2 ± 3.7*
Water kefir 90 mL/kg bw	67.6±3.1	76.4±3.8*	73.6±2.3*#	68.6 ± 5.7	183.0 ± 4.3	76.2 ± 1.9*#
Water kefir 180 mL/kg bw	66.6±2.7	74.6±3.1*	70.2±3.0*	64.0 ± 1.6	181.4 ± 6.7	73.6 ± 3.4*
Water kefir 270 mL/kg bw	62.4±2.7	75.2±3.4*	67.2±2.6*	65.8 ± 6.0	180.8 ± 6.6	71.0 ± 2.5*

Note: Data are presented as mean ± SD, * means significantly different compared to the positive control group, # means significantly different compared to the silymarine group, p<0.05, n = 5 mice/group.

Table 3: ALP level

Groups	ALP level at days- (Prevention Method)			ALP level at days- (Treatment Method)		
	0	7	14	0	7	21
Negative control	110.6±8.0	111.8±6.4*	109.4±2.4*	110.6 ± 8.0	111.8 ± 6.4*	109.4 ± 2.4*
Positive control	112.0±7.0	234.4±4.7	235.0±5.4#	113.2 ± 4.3	240.6 ± 2.4	254.8 ± 4.1#
Silymarine 200 mg/kg bw	108.4±6.0	111.0±5.3*	110.0±2.9*	116.2 ± 6.1	241.0 ± 4.7	112.4 ± 3.0*
Water kefir 90 mL/kg bw	114.2±8.1	117.0±6.6*	112.4±4.8*	116.2 ± 4.4	239.2 ± 7.5	120.6 ± 3.3*#
Water kefir 180 mL/kg bw	110.2±5.9	113.6±3.7*	110.6±2.4*	114.0 ± 9.3	238.0 ± 2.5	114.6 ± 6.9*
Water kefir 270 mL/kg bw	101.0±1.0	112.8±2.8*	108.6±2.4*	114.6 ± 7.1	238.0 ± 11.4	113.4 ± 8.3*

Note: Data are presented as mean ± SD, * means significantly different compared to the positive control group, # means significantly different compared to the silymarine group, p<0.05, n = 5 mice/group.

Table 4: Total protein level in liver failure prevention method

Groups	Total protein level at days- (Prevention Method)			Total protein level at days- (Treatment Method)		
	0	7	14	0	7	21
Negative control	6.39±0.29	6.79±0.14*	6.52±0.07*	6.4 ± 0,3	6.8± 0.1*	6.5 ± 0.1*
Positive control	6.65±0.45	5.37±0.28	4.33±0.06#	6.7 ± 0.3	5.0 ± 0.1	4.9 ± 0.04#
Silymarine 200 mg/kg bw	6.60±0.22	6.64±0.09*	6.71±0.05*	6.8 ± 0.4	5.0 ± 0.1	6.8 ± 0.1*
Water kefir 90 mL/kg bw	6.51±0.33	4.92±0.09*	5.61±0.22*#	6.5 ± 0.3	5.1 ± 0.1	6.1 ± 0.1*#
Water kefir 180 mL/kg bw	6.45±0.31	6.07±0.07*	6.21±0.20*#	6.3 ± 0.3	5.0 ± 0.2	6.5 ± 0.4*#
Water kefir 270 mL/kg bw	6.47±0.30	6.59±0.07*	6.60±0.05*	6.7 ± 0.2	5.1 ± 0.1	6.7 ± 0.1*

Note: Data are presented as mean ± SD, * means significantly different compared to the positive control group, # means significantly different compared to the silymarine group, p<0.05, n = 5 mice/group.

Table 5: Manja Roenigk histological score

Groups	Prevention Method	Treatment Method
Negative control	1.15±0.16*	1.15 ± 0.09*
Positive control	3.26±0.24#	2.85 ± 0.06#
Silymarine 200 mg/kg bw	1.38±0.14*	1.21 ± 0.18*
Water kefir 90 mL/kg bw	1.76±0.05*#	1.64 ± 0.07*#
Water kefir 180 mL/kg bw	1.57±0.05*	1.47 ± 0.05*#
Water kefir 270 mL/kg bw	1.34±0.08*	1.18 ±0.03*

Note: Data are presented as mean ± SD, * means significantly different compared to the positive control group, # means significantly different compared to the silymarine group, p<0.05, n = 3 mice/group.

Table 5 showed that the lowest histopathological score was in the negative control group and the highest was in the positive control group. The score close to the number 1 indicates normal conditions, while the score closer to the 4 indicates the elevated number of cell deaths. The score in the negative control group differed significantly from the positive control group. These meant that acetaminophenled to the histological damage inanimalmodel's liver. Meanwhile, the silymarine and water kefir groups showed a histological score closer to 1, although only the silymarine group and the water kefir group at dose of 180 mL / kg bw in the prevention method and 270 mL / kg bw in the treatment method showed values that were not significantly different from the negative control group.

The result showed that water kefir at all doses was able to improve acute liver failure condition either as prevention or treatment, which was characterized by significant improvement of AST, ALT, ALP, and total protein compared to the positive control group. Elevation in the dose indicated an increase in the intensity of the hepatoprotective effect. However, doses of 180 mg / kg bw rats and 270 mg / kg bw rats showed a hepatoprotective effect comparable to silymarineas prevention and treatment, respectively. The ability of water kefir to treat acute liver failure had also been shown by other studies **[20]**.

If we look at the pathophysiology of acute liver failure, free radicals and inflammation play an important role throughout the process. Patients with acute liver failure show an increase in oxidative stress which is characterized by high levels of SOD and lipid peroxidation **[5]**. Therefore, drugs with strong antioxidant and anti-inflammatory activity, as shown by water kefir, have the potential to act as a drug for acute liver failure **[16]**. The liver plays a role in the metabolism of various substances that have the potential to produce free radicals. However, the body is able to produce endogenous antioxidants that are able to maintain a balance of

oxidative level in the liver. When oxidative compounds are more dominant, there will be conditions of oxidative stress which can cause damage such as lipid peroxidation, DNA oxidative damage, and protein damage **[21]**. Increased oxidative stress will also trigger an inflammatory response through the release of cytokines such as transforming growth factor-ß (TGF-ß), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Cytokines play an important role in cytokines mediate hepatic inflammation, apoptosis and necrosis of liver cells, cholestasis and fibrosis. Among the various types of cytokines, TNF-α has been shown to play a key factor in various aspects of liver damage **[22]**. A high systemic inflammatory response can ultimately lead to organ failure and death **[23]**. Several substances that have antioxidant activity have been shown to have the ability to repair liver damage, such as curcumin, coffee, quercetin, silymarine, and others **[24]**.

Liver disease is also frequently associated with the gut microbiome. Our bodies contain approximately 100 trillion microbes consisting of bacteria, viruses, and eukaryotes, each of which interacts with each other, including with us as hosts **[25, 26]**. Most of the microbiome is located in the intestine and plays an important role in immune function and metabolic function which ultimately affects nutrition and body physiology **[26, 27]**. The metabolites produced by the gut microbiome and their interactions with the host affect normal physiology and the body's ability to withstand disease. The disorder in this condition is called dysbiosis and can affect health **[28]**. Genetics, age and external factors such as diet, exposure to toxic substances can affect the gut balance of the microbiome **[22, 28]**.

The condition dysbiosis is also found in patients with liver damage. Qualitative changes (imbalance between harmful and beneficial microbiome) and quantitative (changes in the total microbiome) of the gut microbiome affect liver health **[28]**. These changes can cause disturbances in the composition of the product produced by the microbiome. In addition, dysbiosis can also cause intestinal inflammation, intestinal barrier damage, and translocation of microbial products **[29]**. Consumption of probiotics, especially those containing lactic acid bacteria, can improve the balance of the composition of the gut microbiome **[30]**. Water kefir contains various lactic acid microbial species **[10]**. Consumption of kefir, either milk kefir or water kefir, had also been shown to improve dysbiosis **[31, 32, 33]**. Another role of the gut microbiome is its activity in modulating the metabolism of amino acids

and glutathione in the host **[34]**. Liver damage due to drugs, alcohol, diet, and pollutants, one of which is characterized by glutathione homeostasis disorders **[35]**. Glutathione is a tripeptide compound composed of glutamate, cysteine and glycine. Glutathione functions as a cellular antioxidant and protects cells from oxidative damage. The balance between reactive oxygen species (ROS) and glutathione in hepatocytes is critical in various pathogenesis of liver disease. The decrease in the mitochondrial glutathione levels will induce mitochondrial ROS and will eventually lead to cell death **[36]**.

CONCLUSION

Water kefir had the ability to prevent and treat acute liver failure caused by acetaminophen.

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