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ANTI INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF KEFIR WATER

ALIGITA W*, TARIGAN PN AND SUSILAWATI E

Department of Pharmacology, Faculty of Pharmacy, Bhakti Kencana University, Jl. Soekarno
Hatta 754 Bandung, Indonesia

*Address of Correspondence: E Mail: widhya.aligita@bku.ac.id

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ABSTRACT

Significant increase in non-communicable diseases (NCD) makes people tend to switch to a healthier lifestyle, by consuming functional food. One material that has the potential as a functional food is water kefir. This study aimed to determine the anti-inflammatory and antioxidant activity of water kefir. Antiinflammatory evaluation was carried out in vitro by examine the ability of kefir water red blood cell (RBC) stabilization at various concentrations; and in vivo by evaluate the decrease in edema volume of mouse feet after administration of water kefir at dose of 112.5 mL/kgBW, 225 mL/kgBW, and 337.5 mL/kgBW. Antioxidant evaluation was performed using the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) method. The parameter for antioxidant activity was inhibition concentration (IC₅₀). Results showed that the in vivo anti-inflammatory activity had the effective dose at 45 mL/kgBW. Anti-inflammatory in vitro evaluation showed water kefir's membrane stabilizing activity did not differ significantly compared with diclofenac sodium. Antioxidant evaluation showed that the IC₅₀ water kefir result was 92.65 ppm which could be classified as a powerful antioxidant. The conclusions of this study indicated that water kefir had strong anti-inflammatory and antioxidant activity. The mechanism of action of anti-inflammatory was by stabilizing the cell membrane.

Keywords: Anti inflammatory, antioxidant, DPPH, water kefir

INTRODUCTION

Diseases involving the inflammatory process have a fairly high incidence rate. Most of these diseases are non-communicable diseases, such as

cardiovascular disease, diabetes, arthritis, and cancer. The spread of the disease is universal, regardless of the level of socioeconomic development within and between countries [1, 2]. About 50% of deaths due to NCD are in low and middle income countries, especially those aged 15-65 years. Treatment for NCD in general must be done for life, so that it requires compliance and appropriate treatment to get the optimal therapeutic effect. Consequently, the cost of treatment is relatively high. It is estimated that NCDs and mental health conditions will cost US \$ 30 trillion for the world economy between 2010 and 2030 if preventive measures are not taken [3]. Because of this, the community began to consider a healthy lifestyle to improve their health status both as a preventive and curative measure. Nutrition therapy is one of the solutions that can be used to prevent the emergence of the disease.

Most of the NCD is a disease that is closely related to the inflammatory process and free radicals. Free radicals are destructive, highly reactive and able to react with cell macromolecules, such as proteins, lipids, carbohydrates, or DNA. The reaction between free radicals and these molecules will result in damage to DNA in the cell nucleus, protein damage, or lipid peroxide damage. Oxidative damage or damage

caused by free radicals in the body can be minimized by endogenous antioxidants such as the enzyme catalase, glutathione peroxidase, superoxide dismutase, and glutathione S-transferase. Endogenous antioxidants are naturally produced by the body as a protective mechanism against free radical attacks. However, if there are too many free radicals, endogenous antioxidants are unable to neutralize them, so additional intake of antioxidants is needed. Antioxidants are classified into two types: enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants such as the enzyme superoxide dismutase (SOD), catalase and glutathione peroxidase. Non-enzymatic antioxidants, consisting of: fat-soluble antioxidants, such as tocopherol, carotenoids, flavonoids, quinon, and bilirubin and water-soluble antioxidants, such as ascorbic acid [4, 5, 6]. Inflammation is a vital process in the immune system. Inflammation can occur when the body has to deal with dangerous stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation [7, 8]. In response to injured cells, the body will secrete compounds that will activate the positive chemotaxis ability of leukocytes. The activated leukocytes will then stimulate an inflammatory response [2]. The inflammatory process itself can be considered a pathological process, while

the anti-inflammatory response of endogenous compounds is considered as an attempt by the body to maintain its homeostasis [9]. Metabolic syndrome, chronic and catastrophic diseases are diseases that are closely related to inflammatory processes and free radicals. Inflammatory processes that occur continuously cause tissue destructive [10]. Based on these descriptions, antioxidant and anti-inflammatory activities are vital activities in preventing or treating diseases that involve the inflammatory process. And compounds that have these two activities in theory can be one solution for the prevention and treatment of NCD. One example of food that is potentially used as functional food is water kefir.

Water kefir is a homemade fermented beverage with a solution of sucrose and dried fruit as a substrate. Water kefir contains various microbial species such as *Lactobacillus casei* / *paracasei*, *Lactobacillus harbinensis*, *Lactobacillus hilgardii*, *Bifidobacterium psychraerophilum* / *crudilactis*, *Saccharomyces cerevisiae*, and *Dekkera bruxellensis*. Fermented water kefir has a slightly acidic taste, fizzy, sometimes sweet, and a little alcoholic. The main metabolites of fermented water kefir are ethanol and lactic acid; Glycerol, acetic acid, and mannitol are also produced in

small quantities. In addition, some aromatic and volatile compounds are produced (relative to their threshold values) such as ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate [11]. 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 [12-15]. Water kefir has been empirically proven to have many pharmacological effects, especially in diseases that involve inflammation, such as hypersensitivity and cancer. Previous research showed promising results related to the activity of kefir water as an antioxidant, antihyperglycemia, antihyperlipidemia, antiobesity, anticancer, and activity in repairing disorders in the digestive tract [16-20]. Based on the description above and previous research, this research would evaluate the antioxidant and anti-inflammatory activities of water kefir.

MATERIALS AND METHODS

Materials

Water kefir grain (purchased from Yogyakarta, Indonesia), sugar, dried fruit, 2,2-diphenyl-1-picrylhydrazyl (DPPH), phosphate buffer pH 7.4), ascorbic acid, diclofenac sodium, citric acid.

Preparation of Water Kefir

Preparation of kefir water began with the manufacture of 8% sucrose solution, sucrose was dissolved in aqua distillate at a temperature of 70°C while stirring until homogeneous, filtered. To 1 L sucrose solution was add 100 g kefir grain and dried fruit. The container was covered with cloth, fermented at room temperature for 48 hours. The product of the 48-hour fermented water kefir were obtained by filtering the solution containing the starter. The filtrate was used in the evaluation, while the water kefir grain could be reused to produce the next water kefir.

Antioxidant Activity Evaluation

The filtrate was dried using freeze dry. Freeze-dried kefir water of 25 mg was then dissolved with methanol in a 25 mL volumetric flask, so a 1000 ppm water kefir sample was obtained. Antioxidant activity was tested by DPPH free radical reduction method by spectrophotometry [21]. Ascorbic acid was used as a standard drug. Sample and standard in various concentrations, each 10 mL, were added with 190 mL DPPH stock solution (0.01 mM). The mixture was then incubated in a dark room at room temperature for 30 minutes. Absorbance measurements were carried out at a wavelength of 516 nm [22]. Percentage of inhibition in DPPH absorbance was calculated using the formula:

$$I (\%) = [(A_0 - A_s)/A_0] \times 100\%$$

Where I is the percent decrease in absorbance of DPPH, A₀ is the absorbance of the control solution, and A_s is the absorbance of the sample / drug solution after adding DPPH. The inhibition concentration value of 50 (IC₅₀) was determined from the regression equation obtained by entering a 50% reduction value as an independent variable [23].

Anti-inflammatory Activity Evaluation

These evaluations were divided into 2 methods: the acute anti-inflammatory activity using the in vivo method and the red blood cell stabilization method (in vitro).

Acute Anti-inflammatory Activity

Male Wistar rat 2-3 months old weighing 150-200 g were kept at standard laboratory conditions at 24-26 ° C, humidity 70-75%, and 12 hours light / dark cycle. Animals were fed with standard chow and water ad libitum. The methods in this study were performed in accordance with ethics and guides for animals care and use. Ethical approval was published by the Padjadjaran University Ethics Committee with registration number 0219020261.

Rats were divided into 6 groups, namely the negative control group, positive control group, the standard drug sodium diclofenac 4.5 mg / kgBB, water kefir at a dose of 112.5 mL / kg bw, 225 mL / kg bw, and

337.5 mL / kg bw. Each rat measured its initial leg volume (V0) using a pleitismometer. Then, the rats were treated according to their group. After 30 minutes, a solution of 5% egg yolk as much as 0.3 mL was administered intraplantar as an induction of acute inflammation. Rat's foot volume was measured every 60 minutes for 360 minutes.

Red Blood Cell Membrane Stabilization Method

A rat blood sample of 10 mL was inserted into a centrifuge tube containing 10 mL of a sterile (anticoagulant) Alsever solution. The mixture was then centrifuged at 3000 rpm for 10 minutes at 27°C. The supernatant formed is separated using a sterile pipette. The remaining blood cells were then washed with isosalin solution and re-centrifuged. The process was repeated 4 times until isosalin was saturated. Blood cell volume was measured

and suspended with isosalin so that a red blood cell suspension with a concentration of 10% v/v was obtained. The blood cell suspension was stored at 4°C if not used immediately [24].

Evaluation of anti-inflammatory activity of kefir water *in vitro* was carried out by measuring the stabilization ability of erythrocyte membranes in hypotonic solutions. The 10% v / v blood suspension divided in several groups, namely the negative control group, positive control group, sodium diclofenac (50, 100, 200, 400, 800 ppm), and water kefir (50, 100, 200, 400 , 800 ppm). The composition of the solution for each test group is shown in **Table 1**. Each mixture was incubated at 37°C for 30 minutes and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and measured using UV-Vis spectrophotometer at 560 nm [25, 26].

Table 1: Mixture composition

Component	Group			
	Negative control	Positive control	Water kefir	Sodium diclofenac
Phosphate buffer 0.15 M, pH 7.4	1 mL	1 mL	1 mL	1 mL
Blood suspension	0.5 mL	0.5 mL	0.5 mL	0.5 mL
Hiposaline	0	2 mL	2 mL	2 mL
Isosaline	2 mL	0	0	0
Sodium diclofenac	0	0	0	1 mL
Water kefir	0	0	1 mL	0

RESULTS AND DISCUSSION

Kefir water is a fermented beverage which is the result of a symbiosis of bacteria and fungi that produces the main products, namely: lactic acid, ethanol in small amounts and carbon dioxide [23]. Characterization results showed that kefir water had 1.08% lactic acid content, 0.76% alcohol content, and pH 4. Lactic acid and alcohol are the main metabolites produced by kefir water. The bacteria contained in kefir water are mostly a group of lactic acid bacteria (LAB) [11]. This group of bacteria has the ability to metabolize carbohydrates and produce lactic acid as the end product. The main genus of LAB are *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* [27].

Antioxidant activity of water kefir

The result of antioxidant activity evaluation showed that water kefir had IC_{50} at a concentration of 92.65 ppm, while ascorbic acid as a standard drug had IC_{50} 6.35 ppm. Based on IC_{50} values, water kefir could be categorized as strong antioxidants (51-100 ppm) and very strong antioxidant for ascorbic acid (<50 ppm). The potential of kefir water as a great antioxidant was also shown from the other study [16]. The strong antioxidant activity exhibited by kefir water was caused by the lactic acid bacteria contained in it. The antioxidant activity of lactic acid bacteria had been

extensively studied and shown positive results [28-30]. The antioxidant activity provided by kefir water occurred because kefir water in its fermentation process produced bioactive components in the form of exopolysaccharides that had the ability to increase the body's immune system, inhibit oxidative stress, reduce oxidative damage, reduce the production of Reactive Oxygen Species (ROS). Exopolysaccharide production was influenced by lactic acid bacteria found in water kefir [31].

Antiinflammatory activity of water kefir

Antiinflammatory activity testing was carried out in vivo and in vitro. The results of in vivo anti-inflammatory evaluation were shown in **Table 2**.

The induction process was carried out by administering 0.3 mL of 5% egg yolk. From **Table 2**, it could be seen that the induction process was successfully carried out, shown by the significant differences between the induced groups and the negative control group at 1st hour. A decrease in edema volume occurred at the 4th hour, marked by a significant difference shown by the sodium diclofenac group to the positive control group. Sodium diclofenac has the ability to inhibit the enzyme cyclooxygenase 2 (COX-2) in the process of biosynthesis of prostaglandins, inhibits neutrophil aggregation, inhibits chemotaxis, decreases pro-inflammatory

levels of cytokines [32]. At the same time, a significant decrease in edema volume was also demonstrated by the 3 groups of water kefir. Reduction in the volume of edema water kefir also did not show significant differences with the sodium diclofenac group. This mean that at the 4th hour, water kefir had the same onset and activity as the standard anti-inflammatory drugs. The same results were also shown at the 6th hour of evaluation. The percentage of edema inhibition at the 6th hour for the sodium diclofenac group, water kefir 112.5 mL / kg bw, 225 mL / kg bw, and 337.5 mL / kg bw were 60%, 38%, 47%, and 51%;

respectively. Anti-inflammatory ability of kefir water was in line with the previous research [33].

Anti-inflammatory activity was also evaluated in vitro using the red blood cell membrane stabilization method. **Table 3** showed that water kefir had a membrane stabilizing activity that played a role in its anti-inflammatory ability. From the value of percent stability, water kefir activity was similar to sodium diclofenac's. From these two anti-inflammatory evaluations, it was concluded that water kefir had anti-inflammatory activity with the mechanism of action of stabilizing cell membranes.

Table 2: Edema volume during treatment

Group	Edema Volume at Hour(s)-							
	0	1	2	3	4	5	6	24
Negative control	0.13±0.05	0.13±0.05* #	0.13±0.05* #	0.13±0.05* #	0.13±0.05* #	0.13±0.05* #	0.13±0.05*	0.13±0.05
Positive control	0.13±0.05	0.43±0.05	0.58±0.09	0.73±0.05	0.75±0.10#	0.70 ± 0.09#	0.55 ± 0.05#	0.15±0.57
Diclofenac sodium	0.15±0.05	0.40±0.08	0.47±0.05	0.63±0.13	0.48±0.09*	0.35 ± 0.09*	0.23 ± 0.09*	0.15±0.05
Water kefir 112.5 mL/kg BW	0.13±0.05	0.38±0.05	0.48±0.09	0.73±0.09	0.55±0.12*	0.48±0.05* #	0.35±0.13* #	0.15±0.05
Water kefir 225 mL/kg BW	0.15±0.05	0.45 ± 0.05	0.58 ± 0.09	0.70±0.08	0.52±0.13*	0.45±0.05* #	0.30±0.08*	0.15±0.05
Water kefir 337.5 mL/kg BW	0.13±0.05	0.43 ± 0.05	0.53 ± 0.09	0.58 ± 0.08	0.45±0.09* #	0.35±0.08*	0.28±0.05*	0.13±0.05

Table 3: Percentage of membrane stabilizing activity

Concentration (ppm)	Percentage of Stability (%)	
	Sodium diclofenac	Water kefir
50	29.770 ± 1.630	23.450 ± 0.120
100	32.160 ± 1.620	26.650 ± 0.911
200	33.410 ± 2.480	29.121 ± 0.809
400	36.050 ± 2.060	31.186 ± 0.669
800	40.500 ± 0.900	33.738 ± 0.305

CONCLUSION

The conclusions of this study indicated that water kefir had strong anti-inflammatory and antioxidant activity. The mechanism of action of anti-inflammatory was by stabilizing the cell membrane.

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REFERENCES

- [1] World Health Organization. Non communicable diseases fact sheet 2017. <http://www.who.int/mediacentre/factsheets/fs355/en/>
- [2] Libby P. Inflammatory mechanisms: the molecular basis of inflammation and disease. *Nutr Rev.* 2007; 65:S140-S146.
- [3] Bloom DE, Cafiero-Fonseca E, Candeias V, Adashi E, Bloom L, Gurfein L, et al. Economics of Non-Communicable Diseases in India: The Costs and Returns on Investment of Interventions to Promote Healthy Living and Prevent, Treat, and Manage NCDs. 2014.
- [4] Lamprecht, Manfred. Antioxidants in Sport Nutrition. Austria: CRC Press. 2015.
- [5] Charles, Denys. Antioxidant Properties of Spices Herbs And Other Sources. United State of America: Springer; 2013.
- [6] Alessio, Helaine dan Ann E Hagerman. Oxidative Stress, Exercise and Aging. Miami University USA: Imperial College Press; 2006.
- [7] Chertov O, Yang D, Howard O, Oppenheim JJ. Leukocyte granule proteins mobilize innate host defenses and adaptive immune responses. *Immunol Rev* 2000; 177:68–78.
- [8] Jabbour HN, Sales KJ, Catalano RD, Norman JE. Inflammatory pathways in female reproductive health and disease. *Reprod.* 2009; 138:903–19.
- [9] Stankov SV. Definition of inflammation, causes of inflammation, and possible anti-inflammatory strategy. *The Open Inflammation Journal* 2012; 5:1-9.
- [10] Khanna HD, Karki K, Pande D, Negi R, Khanna RS. Inflammation, Free Radical Damage, Oxidative Stress and Cancer. *Interdiscip J Microinflammation* 2014;1:109.
- [11] Laureys and De Vuyst. Water kefir as a promising low-sugar probiotic fermented beverage. *Archives of Public Health* 2014; 72(Suppl 1):P1.
- [12] Lutz ML. Recherches biologiques sur la constitution du Tibi. *Bulletin de la Societe Mycologique de France*, 1899; 15: 68–72.

- [13] Pidoux M. The microbial flora of sugary kefir grain (the gingerbeer plant): bio- synthesis of the grain from *Lactobacillus hilgardii* producing a polysaccharide gel. MIRCEN Journal, 1989; 5: 223–38.
- [14] Pidoux M, de Ruiter GA, Brooker BE, Colquhoun IJ, Morris VJ. Microscopic and chemical studies of a gelling polysaccharide from *Lactobacillus hilgardii*. Carbohydrate Polymers, 1990; 13:351–62.
- [15] Kebler LF. California bees. Journal of the American Pharmaceutical Association 1921; 10 (12): 939–43.
- [16] Muneer Alsayadi MS, Al Jawfi Y, Belarbi M, Sabri FZ. Antioxidant Potency of Water Kefir. Journal of Microbiology, Biotechnology, and Food Science 2013; 2(6): 2444-7.
- [17] Muneer Alsayadi MS, Al Jawfi Y, Belarbi M, Soualem-Mami Z, Merzouk H, Sari DC, Sabri F, Ghalim M. Evaluation of anti-hyperglycemic and anti-hyperlipidemic activities of water kefir as probiotic on streptozotocin-induced diabetic wistar rats. Journal of Diabetes Mellitus 2014; 4: 85-95.
- [18] Koh WY, Uthumporn U, Rosma A, Effarizah ME, Wan Rosli WI. Assessment of yeast, acetic and lactic acid bacteria isolated from water kefir grains and their application as starter culture in the production of fermented pumpkin-based water kefir beverages in improving gastrointestinal tract digestive tolerance and inhibition against alpha-glucosidase. International Food Research Journal 2019; 26(2): 429-39.
- [19] Bourrie BCT, Cotter PD, Willing BP. Traditional Kefir Reduces Weight Gain and Improves Plasma and Liver Lipid Profiles More Successfully than a Commercial Equivalent in a Mouse Model of Obesity. Journal of Functional Foods 2018; 46: 29-37.
- [20] Hatmal MM, Nuirat A, Zihlif MA, Tahat MO. Exploring the influence of culture conditions on kefir's anticancer properties. Journal of Dairy Science 2018; 101(5): 3771-7.
- [21] Blois, M.S. Antioxidant determination by the use of stable free radicals. Nature 1958; 181: 1199-200.
- [22] Locatelli M, Gindro R, Travaglia F, Colsson D, Rinaldi J, Arlorio M. Study of the DPPH-Scavenging activity: Development of a free software for the correct interpretation

- of data. Food Chemistry, 2009; 114: 889-97.
- [23] Deeseenthum S, Luang-In V, John SM, Chottanom P, Chunchom S. Effects of Kefir Fermentation on Antioxidant Activities (in vitro) and Antioxidative Stress (in vivo) of Three Thai Rice Milk Varieties Prepared by Ultrasonication Technique. Pharmacog J 2018;10(5): 1061-6.
- [24] Kosala K, Widodo MA, Santoso S, dan Karyono S. In vitro and in vivo Anti-inflammatory Activities of *Coptosapelta flavescens* Korth Roots Methanol Extract. Journal of Applied Pharmaceutical Science 2018; 8(9): 42-8.
- [25] Gadamsetty G, Maru S, Sarada NC. Antioxidant and Anti-inflammatory Activities of the Methanolic Leaf Extract of Traditionally Used Medicinal Plant *Mimusops elengi* L. Journal of Pharmaceutical Science & Research 2013; 5:125-30.
- [26] Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red Blood Cell Membrane Stabilizing Potentials of Extracts of *Lantana camara* and its Fractions. International Journal of Plant Physiology and Biochemistry 2010; 2(4): 46-51.
- [27] Khalid K. An overview of lactic acid bacteria. International Journal of Biosciences 2011; 1(3): 1-13.
- [28] Amaretti A, di Nunzio M, Pompei A, Raimondi S, Rossi M, Bordoni A. Antioxidant properties of potential probiotic bacteria: in vivo and in vivo activities. Applied Microbiology and Biotechnology 2013; 90(2): 809-17.
- [29] Lin MY, Yen CY. Antioxidative ability of lactic acid bacteria. Journal of Agricultural and Food Chemistry 1999; 47(4): 1460-6.
- [30] Coda R, Rizzello CG, Pinto D, Gobbetti. Selected lactic acid bacteria synthesize antioxidant peptides during sourdough fermentation of cereal flours. Applied and Environmental Microbiology 2012; 78(4): 1087-96.
- [31] Barboza KRM, Coco LZ, Alves GM, Peters B, Vasquez EC, Pereira TMC, Campagnaro BP. Gastro-protective effect of oral kefir on indomethacin-induced acute gastric lesions in mice: Impact on oxidative stress. Life Sciences 2018; 209: 370-6.
- [32] Chrisholm-Burns M, Terry LS, Well BG, Malone PM, Kolesar JM,

Dipiro JT. (2016): Pharmacotherapy Principle and Practice 4th Ed. United State: Mc. Graw Hill Education.

- [33] Rodrigues KL, Carvalho JC, Schneedorf JM. Anti-inflammatory properties of kefir and its polysaccharide extract. *Inflammo pharmacology* 2005; 13(5-6):485-92.