

Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
 Open Access Macedonian Journal of Medical Sciences. 2020 May 15; 8(A):293-296.
<https://doi.org/10.3889/oamjms.2020.4285>
 eISSN: 1857-9655
Category: A - Basic Sciences
Section: Pathophysiology



The Effect of Giving Dadih on Malondialdehyde Levels and Renal Interstitial Fibrosis at Aging Kidney

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Abstract

BACKGROUND: The increase in the elderly poses problems in the areas of health, since it can cause aging of the physiological function of organs. It also has an impact on kidney function, which will enhance the chronic kidney disease. One of the theory that causes aging process is the free radical theory, which that accumulation of free radicals is caused by depletion of antioxidants. Therefore, exogenous antioxidants are needed and in this study author use dadih. Dadih is fermented buffalo milk, indigenous from West Sumatera-Indonesia. Peptides found in fermented milk contained antioxidant that can stimulate the formation of endogenous antioxidants and diversification of dadih increases its effect as antioxidant.

AIM: This study aims to prove the effect of dadih antioxidants on malondialdehyde (MDA) levels of kidney tissue and renal interstitial fibrosis.

MATERIALS AND METHODS: An experimental study with post-test only control group design conducted to observe effect of dadih to kidney aging of *Rattus norvegicus* in the Laboratory of Andalas University. It used 30 *R. norvegicus* which were divided into three groups. Group K was a positive control (did not get dadih), Group P1 was given dadih 4.5 g once a day, and Group P2 was given dadih 4.5 g twice a day for 42 days. After that, MDA levels of kidney tissue are examined using the thiobarbituric acid reactive substances examination technique and examination of renal interstitial fibrosis which is done by histopathology with Sirius-red staining. Data were analyzed using the normality test with Shapiro-Wilk.

RESULTS: The results showed that dadih can reduce levels of MDA in kidney tissue, where its decrease very significant in Group P1 (given dadih once a day) and Group P2 (given dadih twice a day) compared to the Group K (control group) 0.97 ± 0.06 pg/ml to 0.75 ± 0.03 pg/ml ($p < 0.05$). Group P1 and Group P2 can also reduce the renal interstitial fibrosis which is obtained a decrease in the average fibrosis rank from Group K to Group P1 and subsequently to Group P2 ($p < 0.05$).

CONCLUSION: This study concluded that dadih can reduce MDA levels in kidney tissue and reduce renal interstitial fibrosis rank on aging kidney.

Edited by: Igor Spiroski
Citation: Harun H, Yanwirasti Y, Purwanto B, Rahayuningsih EP. The Effect of Giving Dadih on Malondialdehyde Levels and Renal Interstitial Fibrosis at Aging Kidney. Open Access Maced J Med Sci. 2020 May 15; 8(A):293-296. <https://doi.org/10.3889/oamjms.2020.4285>
Keywords: Dadih; Malondialdehyde; Renal interstitial fibrosis

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Received: 09-Jan-2020

Revised: 17-Apr-2020

Accepted: 20-Apr-2020

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Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

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Introduction

Life expectancy nowadays is increasing both globally and nationally. Increasing old age is encouraging because of an increase in welfare, but on the other hand it creates problems. This is due to the aging process leading to decreased physiological function of the body's organs progressively. As a result, there will be adaptive disturbances and homeostatic mechanisms that lead to vulnerability to environmental or internal stresses with an increase in various diseases [1].

One of the well-known aging theory states that the normal aging process is caused by free radicals that are formed endogenously as a by-product of metabolic processes that use oxygen. Mitochondria play an important role in the aging process because these organelles are the main source of free radicals

production, as a side effect of energy formation. In young mitochondria, this condition can overcome by the presence of antioxidants found in the body. However, in old mitochondria, it is not enough to produce antioxidants needed to neutralize free radicals that are formed so that there is an imbalance between the productions of free radicals with antioxidants [2].

As a result of this imbalance, between the formations of free radicals (reactive oxygen compounds) with the required antioxidants, there will be damage to body tissue called oxidative damage. Reactive oxygen species (ROS) can attack all types of biomolecules, especially unsaturated fatty acids which are important components of the phospholipids making up cell membranes [2], [3].

When free radicals, especially hydroxide radicals react with these unsaturated fatty acids, there will be a chain reaction known as lipid

peroxidation. Decomposition of lipid peroxidation will produce hydroperoxides which will affect membrane function. One of the products of this hydroperoxide is malondialdehyde (MDA) which is a marker of oxidative damage that can be inhibited by giving antioxidants from outside the body [4].

MDA is a lipid peroxidation product. When lipids react with free radicals, there will be a process of peroxidation to form lipid peroxide. These lipid peroxides will induce endothelial damage and inflammatory responses, inhibit vasodilation and activate macrophages [5], [6]. Activated macrophages will express cytokines, including transforming growth factor-beta 1 (TGF- β 1), tumor necrosis factor-alpha 1, interleukin (IL) -1 beta, IL-6, and IL-8. TGF- β 1 is the dominant cytokine involved in the pathogenesis of interstitial fibrosis and glomerulosclerosis in the aging kidney which can then develop into chronic kidney disease (CKD) marked by albuminuria [7].

Kidney fibrosis is known as a common pathological feature of aging kidney leading to CKD even end-stage renal disease. This condition is characterized by excessive deposition of extracellular matrix (ECM). A number of studies show that TGF- β 1 is a key mediator in CKD associated with progressive renal fibrosis [7]. In general, in aging, endogenous antioxidants are not enough to prevent oxidative damage. Therefore, we need another source of antioxidants that can stimulate endogenous antioxidants. Some researchers found that fermented milk contains peptides which act as antioxidants [8].

Fermented milk peptides have functional properties as antioxidants, antithrombotics, antihypertensives, hypocholesterolemia, antimicrobials, and as immunomodulators [9]. Milk and milk derivative products such as fermented milk contain biologically active peptides. Bioactive peptides are food components that have biological functions on health both to cure and prevent disease [9].

One of the fermented milk which is known as the traditional food of the people of West Sumatra is dadih. Dadih (called "dadih" in West Sumatera) is a processed product from buffalo milk made by natural fermentation by lactic acid-producing bacteria in bamboo at room temperature for 2–3 days and has a distinctive sour taste. Dadih contains nutritious nutrients and probiotics [10].

Recent research has given new hope that probiotics have antioxidant abilities. *In vivo* and *in vitro* probiotics are proven to increase the body's antioxidants. Probiotics can increase SOD and GPX activity and reduce MDA in rabbit serum [11], [12], [13].

Based on the above background, the researchers wanted to prove the effect of giving dadih on MDA and interstitial fibrosis of kidney tissue on the aging kidney.

Materials and Methods

This was an experimental study with post-test only control group design conducted to observe effect of dadih to kidney aging of *Rattus norvegicus* in the Pharmacy Laboratory at Andalas University, Biomedical and Anatomical Pathology Laboratory at Medical Faculty of Andalas University. This study was conducted from January until June 2019.

Samples

Thirty male white rats (*R. norvegicus*) aged 24 months and weighing 450 g were divided into three groups. Each group consisted of 10 animals divided into Group K (control group that was only given aquadest as a dadih solvent), Group P1 (the treatment group was given dadih as much 4.5 g once a day dissolved in aquadest), and Group P2 (group the treatment is given dadih as much as 4.5 g twice a day which is dissolved in aquadest). Giving dadih is given for 42 days. Dadih used came from Air Dingin, Alahan Panjang, Solok, West Sumatra.

Examination of MDA levels

MDA level examination conducted by the thiobarbituric acid reactive substances method in Biomedical Laboratory of the Faculty of Medicine, Andalas University, Padang.

Examination of histopathology tissue

Kidney tissue is used for interstitial fibrosis examination by histopathology examination with Sirius Red staining in Anatomical Pathology Laboratory at Medical Faculty of Andalas University, Padang.

Data analysis

The results obtained MDA levels (pg/ml) and kidney interstitial fibrosis in the initial stages were tested for normality with Shapiro–Wilk. If it is not normally distributed, transformation is performed and then the normality test is performed. If still not normally distributed, the analysis will be non-parametric, and if normal the parametric test is carried out. If the value of $p < 0.05$ means, there is significant.

Research ethics

Ethics clearance was approved by the Ethics Committee of Medical Faculty of Andalas University (No: 152/KEP/FK/2019).

Results

Before analyzing the effect of dadih on MDA levels and kidney interstitial fibrosis, a normality test was performed. The normality test results found that MDA levels and kidney interstitial fibrosis were not normally distributed so transformation was performed with Log10. After the transformation of the data and normality test, it turns out that the MDA has been normally distributed ($p > 0.05$) while the data of kidney interstitial fibrosis is not normally distributed so that the test is a non-parametric Kruskal–Wallis test. The effect of dadih on MDA levels in kidney tissue can be seen in Tables 1 and 2.

Table 1: Effects of giving dadih on MDA levels in kidney tissue based on MDA and TBA (pg/mL) reactions

MDA	n	Average	SD	p-value
K	10	0.97	0.06	0.001
P ₁	10	0.75	0.03	
P ₂	10	0.75	0.03	

MDA: Malondialdehyde, TBA: Thiobarbituric acid, SD: Standard deviation.

Based on Table 1, there was a tendency to decrease MDA levels in the treatment group (Group P1 and Group P2) when compared to the control group (Group K), from 0.97 ± 0.06 to 0.75 ± 0.03 . Statistically, this decrease was significant ($p < 0.05$). Then, the *post hoc* Bonferroni test was performed to determine the effect of giving a gift in each group can be seen in Table 2. It turns out that with the Group P1 alone, there has been a significant decrease in MDA levels ($p < 0.05$). However, there is no difference between Group P1 and Group P2.

Table 2: Post hoc tests treated with MDA levels of kidney tissue in each group

Dependent variable	(I) Group	(J) Group	Average difference (I-J)	p-value
MDA	K (Control)	P1	0.22070*	0.001
		P2	0.22070*	0.001
	P1	K	-0.22070*	0.001
		P2	0.00000	10.001
	P2	K	-0.22070*	0.001
		P1	0.00000	10.001

MDA: Malondialdehyde.

From the results of this study, it can be seen that giving dadih can reduce levels of MDA, which statistically, this reduction is very significant both in Group P1 and in Group P2 ($p = 0.001$). This shows that dadih can inhibit the formation of MDA due to oxidative damage caused by an increase in free radicals caused by aging.

Fibrosis data after giving dadih are not normally distributed, so the test is a non-parametric Kruskal–Wallis test (Table 3). The results obtained in table 3 that significant decrease in the average rank of fibrosis from group K (24.5%) then group P1 (16.30%) and group P2 (5.7%). Figure 1 shows the histopathology of renal fibrosis interstitial in each group. Staining of sirius red in the kidney of experimental control animals (group K), single dose treatment (group P1) and double dose treatment (group P2). Collagen deposition is measured by an imageJ program by extracting red colored areas, converting images to grayscale and measuring the

Table 3: Effects of giving Dadih on kidney interstitial fibrosis (Kruskal–Wallis) (%)

Interstitial fibrosis	n	Average rank	p-value
K (control)	10	24.50	
P1	10	16.30	0.001
P2	10	5.70	

percentage of the total area colored per area. Collagen deposition is lower in experimental animals by giving dadih than controls. The lowest collagen deposition is group P2 with double dose dadih.

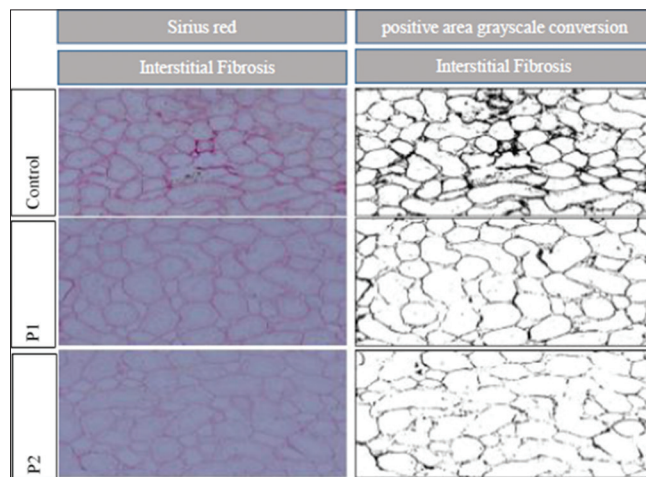


Figure 1. Histopatology of renal fibrosis interstitial in control group, group P1 and group P2

Discussion

From the results of the study, it can be seen that in the treatment Group 1 and treatment Group 2, there was no difference in MDA reduction. This means that giving 4.5 g of dadih once a day is effective enough to inhibit the formation of MDA. Probiotics showed the effect of scavenger activity as an antioxidant by decreasing free radicals formed as oxidative processes [14]. It is suspected that dadih prevents lipid peroxidation through the above process because the dadih components that are functioned as antioxidants are peptides and lactic acid bacteria (LAB), where this LAB can prevent the formation of ROS [14].

Dadhi contains probiotics which are commensal microorganisms [10]. Research on probiotics as antioxidants at this time is very often done and it has been proven that probiotics can modulate antioxidants by correlating metal ions, producing antioxidants themselves, regulating the antioxidant activity of hosts, and reducing the activity of enzymes that produce ROS [11], [13]. This shows that dadih can inhibit the formation of MDA due to oxidative damage caused by an increase in free radicals due to aging.

Kidney fibrosis is known as common pathological features of the aging kidney. This condition is characterized by excessive deposition of the ECM.

Collagen is a major component of ECM in the kidneys and is associated with gradations of disease [7]. The results of this study statistically sign show that giving dadih in the treatment Group 1 and in the treatment Group 2 can reduce fibrosis rate when compared to the Control group. Since the data obtained are not normally distributed, the significant test cannot be carried out. The analysis is only done based on the average rank of fibrosis. Results of staining with Sirius red staining, fibrocollagen matrix deposition is lower in the test animals given dadih.

Research experts show that TGF- β 1 plays a key role in kidney fibrosis. TGF- β 1 also regulates ECM synthesis and increases fibrogenic bFGF₂. TGF- β 1 expression acts as a chemoattractant for fibroblasts in the early phase of fibrosis. TGF- β 1 also induces the transformation of tubular epithelial cells into myofibroblasts and smooth muscle cells through the induction of ECM protein [7]. The results of the study can explained that dadih can reduce renal interstitial fibrosis.

Conclusion

From the results of this study, it can be concluded that dadih is a natural exogenous anti-oxidant, which can reduce oxidative damage in aging kidney by reducing MDA levels in kidney tissue so that it can inhibit renal interstitial fibrosis. Thus, this study can contribute clinically to improve the prognosis and as additional therapy in protection and inhibition of progression of CKD.

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