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RESEARCH ARTICLE

Protective Effect of Goat Kefir on Arsenic-Induced Ovarian Oxidative Stress in Female Rats

Nur Aisyah Laily[1]*, Laras Putri Gamagitta[1], Tri Yudani Mardining Raras[2], I Wayan Agung Indrawan, Bambang Rahardjo[3]

1. *Master of Midwifery Program, Faculty of Medicine, Brawijaya University, Malang, Indonesia.*
2. *Department of Biochemistry and Molecular Biology, Faculty of Medicine, Brawijaya University, Malang, Indonesia.*
3. *Department of Obstetrics and Gynecology, Saiful Anwar Hospital, Malang, Indonesia.*

***Corresponding Author: Nur Aisyah Laily**

Abstract

Objective: To determine the role of goat kefir in ovarian protection from arsenic-induced ovarian oxidative stress. Methods: twenty female wistar rats were randomly divided into five groups; one group was treated as control, one group was treated with arsenic 2 mg/kg/day only, while three groups were treated with different doses (1.25, 2.5, 5 mL/kg/day) of goat kefir and arsenic for 35 days. After 35 days, the rats were terminated, then the ovaries were taken for analysis of MDA levels and SOD activity using spectrophotometry. Results: arsenic exposure significantly increased MDA levels but did not significantly decrease ovarian SOD activity compared to the control group. Kefir administration at doses of 1.25 and 2.5 mL/kg/day could reduce ovarian MDA levels although it was not statistically significant. However, MDA levels were greater in the 5 mL/kg/day group than the arsenic-only group. Kefir increased ovarian SOD activity at all three doses although it was not statistically significant. Conclusion: goat kefir was able to reduce MDA levels and increase ovarian SOD activity in rats that were exposed to arsenic although it was not statistically significant.

Keywords: *Goat kefir, Arsenic, MDA, SOD, Ovarian oxidative stress.*

Introduction

Arsenic is found in water, soil, and air from natural and anthropogenic sources and exists in both inorganic and organic forms. Inorganic arsenic shows greater toxicity than organic arsenic [1]. Humans are exposed to inorganic arsenic through contaminants found in drinking water and food. Arsenic contamination in drinking water mainly occurs in India, Bangladesh, China, as well as several Central and South American countries [2]. Arsenic is naturally contained in high amounts in groundwater in some countries as well as in some food items such as rice, fish, and fruits [3, 4].

The World Health Organization states that arsenic contamination is the biggest threat to global public health. The Occupational Safety and Health Administration (OSHA) suggests that long-term exposure to chemical agents such as heavy metals, pesticides, herbicides, and air pollutants leads to decreased fertility and increased in the incidence of single and recurrent miscarriages [5]. Arsenic accumulates in living systems and can cause severe damage to vital organs, such as the reproductive system, nervous system, gastrointestinal tract, and mucus tissue [6, 7]. Low levels of arsenic contamination (less than 10 µg/L) in drinking water sources might further impair fecundity [8]. Several studies suggest that the main cytotoxicity mediators of arsenic are caused by the formation of Reactive Oxygen Species (ROS) which causes oxidative stress [9, 10, 11].

Arsenic causes oxidative stress through interactions with antioxidants and increases inflammation which then results in the accumulation of free radicals in cells [12]. The ROS generated by arsenic-mediated reactions causes DNA damage, lipid

peroxidation, and protein modification as well as changes in antioxidant defenses [13].Subchronic arsenic exposure in rats can decrease the activity of antioxidant enzymes and increase levels of lipid peroxidation (MDA) in the ovaries [14]. Oxidative stress due to arsenic results in an imbalance of endogenous antioxidants in the body. Several studies have shown that giving external antioxidants can improve or prevent the adverse effects of arsenic toxicity [10, 11].

Kefir is a probiotic product derived from the fermentation of goat's or cow's milk using kefir grains which have a complex microbiological composition, including lactic acid bacteria, yeast, and fungi [15]. Kefiran, which is a potential exopolysaccharide in kefir grains, has strong antioxidant activity.

Apart from being a potent antioxidant, kefir is also used as an antimutagenic, anti-tumor, anti-inflammatory, scavenging radicals, and oxidative stress-reducing agent [16,17]. Kefir at the right dose is expected to prevent damage to the reproductive organs due to arsenic toxicity. Therefore, the authors are interested in determining the role of goat kefir in ovarian protection from arsenic-induced ovarian oxidative stress.

Material and Methods

Goat Kefir

Kefir was purchased from the Natural Probiotic Lab, Faculty of Animal Husbandry, Brawijaya University. Kefir was obtained immediately after production, divided into aliquots, then frozen and stored at -20^{o} until being used.

Animals

Twenty wistar female rats aged 8-10 weeks and weighing between 100-150 g were used in this study. Rats were maintained at the Laboratory of Animal Bioscience, Brawijaya University. All rats were kept at the standard condition with a 12 L: 12 D cycle and room temperature 27-28^{o}C, with free access to food and water.

They were acclimatized for seven days before being given treatment. This research protocol was approved by the Ethics Commission of the Faculty of Medicine, Brawijaya University (approval number: 39/EC/ KEPK-S2/04/2020).

Experimental design

Arsenic used in this study was Arsenic Trioxide (As_2O_3) powder produced by Loba Chemie dissolved in saline. Rats were randomly divided into five groups. Control group was treated with vehicle alone (saline), one group was treated with arsenic 2 mg/kg/day only, while three groups (Treatment 1, Treatment 2, and Treatment 3) were treated with different doses (1.25, 2.5, 5 mL/kg/day, respectively) of goat kefir and arsenic. All groups were treated for 35 days. After the 35th day, the rats were sacrificed by decapitation then the one ovary from each rat was taken for analysis.

Measurement of Ovarian malondialdehyde Content

Malondialdehyde (MDA) levels in ovarian tissue were measured biochemically [18]. Ovarian tissue MDA levels were measured according to the BioAssay System protocol kit.

Ovarian Superoxide Dismutase Activity

The activity of ovarian-superoxide dismutase (SOD) was measured by using spectrophotometry according to the BioAssay System protocol kit.

Statistical analysis

All results were expressed as means ± sem. Statistical analysis was performed using SPSS 23.0 software. Parametric one-way ANOVA and Least Significant Difference (LSD) were performed on MDA levels, while non-parametric tests with Kruskal Wallis were performed on SOD activity. The value p <0.05 was considered significant.

Results

Measurement of ovarian MDA levels showed an increase in the arsenic-only group (83.99 ± 7.41 μM) and was significantly higher than the control group (23.06 ± 2.85 μM). Kefir administration at doses of 1.25 and 2.5 mL/kg/day decreased ovarian MDA levels although not statistically significant (p> 0.05). Meanwhile, ovarian MDA levels at a dose of 5 mL/kg/day of kefir (94.53 ± 6.85 μM) showed higher results compared to the arsenic-only group.

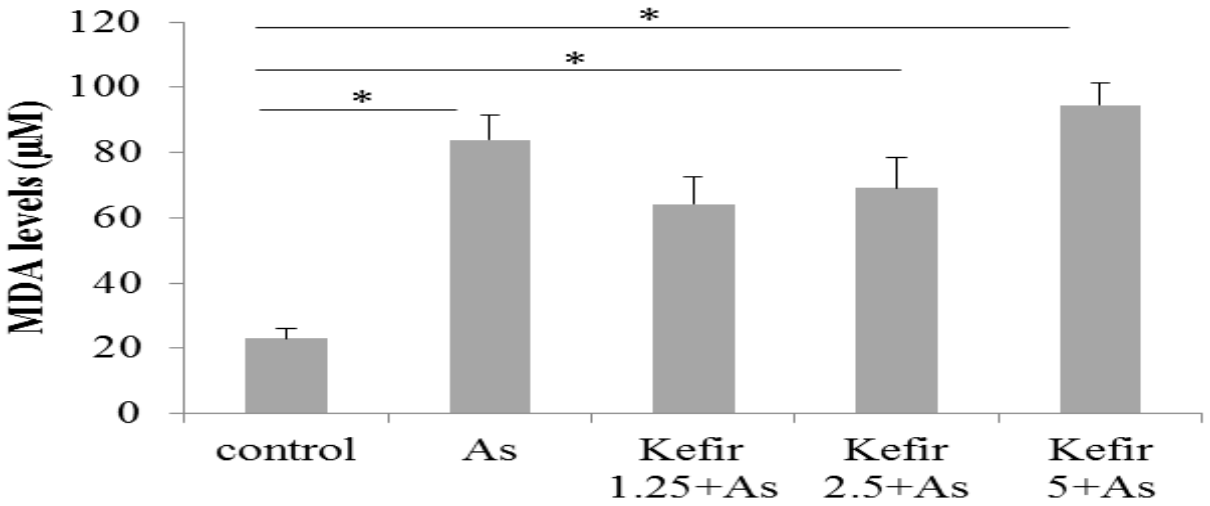

Fig. 1: MDA levels of ovaries in all groups of female rats. Values are expressed as mean ± sem, n=20. Measurement of ovarian MDA levels was observed from one ovary. The differences in means were analyzed by one-way ANOVA (*p*=0.034)

Ovarian SOD activity decreased in the arsenic-only group (110.23 ± 6.51 U/mL) compared with the control group (151.67 ± 6.26 U/mL). SOD activity increased at all three doses of treatments: 1.25 mL/kg (144.38 ± 8.59 U/mL), 2.5 mL/kg (127.44 ± 3.43 U/mL), and 5 mL/kg (152.92 ± 6.81 U/mL) compared with the arsenic-only group. However, the difference between the treatment groups showed that the results were not statistically significant ($p > 0.05$).

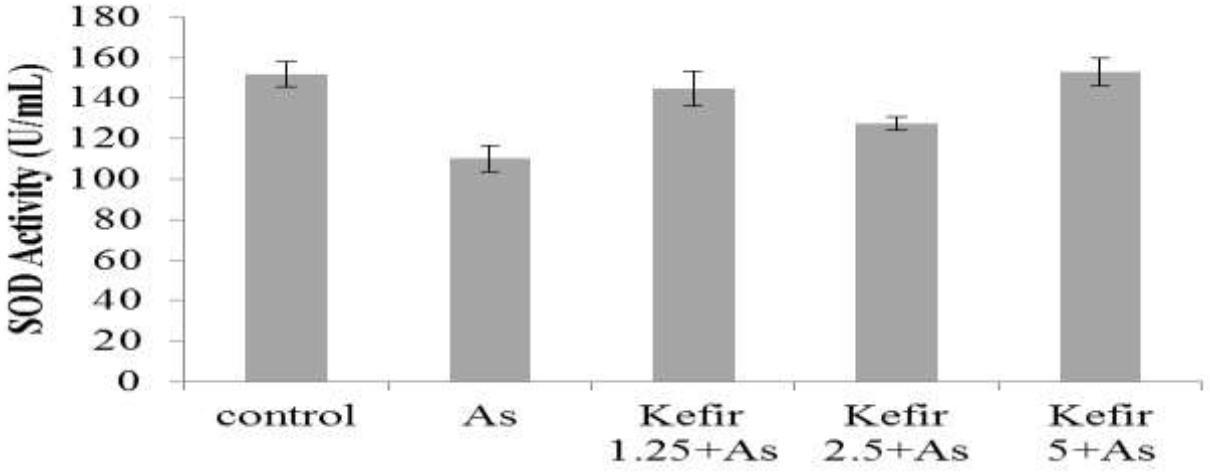

Fig. 2: Ovarian SOD activity in all groups of female rats. Values are expressed as mean ± SEM, n = 20. Measurement of ovarian SOD activity was observed from one ovary. The differences in means were analyzed by Kruskal Wallis (*p*=0.509)

Discussion

Arsenic exposure can increase MDA levels and decrease SOD activity in rat ovaries. This is consistent with previous studies that subchronic exposure to arsenic in rats for 30 and 60 days was able to significantly increase MDA levels and decrease ovarian SOD activity [14].

Oral exposure to an arsenic dose of 3 ppm/day in rats for 30 days also showed a significant increased MDA level and decreased the SOD activity in the ovary [19]. The earliest response to arsenic toxicity is increased levels of ROS in blood and soft tissue [20]. The mechanism of toxicity occurs through the formation of free radicals and inhibition of antioxidant enzymes that cause oxidative stress on target organs including reproductive organs [9]. Excessive levels of ROS are harmful to the body and can result in the accumulation of oxidative damage to cells which can have highly toxic effects on DNA, proteins, and lipids. ROS-mediated damage can ultimately affect physiological function and lead to pathological conditions [21].

Lipid peroxidation is a biomarker of oxidative stress. Malondialdehyde (MDA) is a product

of lipid peroxidation resulting from the reaction of oxygen radicals with polyunsaturated fatty acids (PUFA) in phospholipid membranes and shows damage to cells [22].

Arsenic-induced ROS directly attacks the hydrogen atom of the methylene group adjacent to the unsaturated carbon atom. PUFA are more sensitive to free radical damage and form MDA which can be measured as an indirect indicator of oxidative stress [23]. High MDA concentrations are associated with antioxidant status, when the antioxidant status is high in the body, MDA levels are low [24].

Thus, a high oxidation process in the cell membrane is characterized by high concentrations of MDA in plasma or tissue, indicating an oxidative stress state [25]. Kefir administration was considered ineffective to significantly reduce MDA levels and increase ovarian SOD activity in rats exposed to arsenic, although there were differences in the mean quantitatively. This MDA level reduction and increased SOD activity indicated that kefir had a positive effect on arsenic-induced oxidative stress in rats.

Organic acids produced by lactic acid bacteria in kefir are synergistic by providing H + ions to free radicals, thereby increasing the amount of primary antioxidant activity. This synergistic activity acts as a hydrogen donor to free radicals so that it can regenerate primary antioxidants [26].One of the primary antioxidants is SOD [27]. Synergistic antioxidants also provide an acidic environment that increased the stability of the antioxidants. The increase in the antioxidant activity of kefir also occurs due to the activity of yeast and lactic acid bacteria (LAB) through the formation of phenols and the formation of several other vitamins that can increase the value of kefir antioxidant activity [28].

The exopolysaccharide (EPS) which is formed by kefir shows high antioxidant activity in protecting protein from oxidative damage and becomes a scavenger of superoxide radicals and nitric oxide radicals as well as chelating on Fe metal [29, 30]. The hydroxyl (OH) radical is the most reactive oxygen species and causes damage to adjacent biomolecules.

Some polysaccharides are known to release hydrogen protons to react with hydroxyl radicals, causing a decrease in the attack rate of hydroxyl radicals [31, 32]. This is consistent with previous studies which suggest that milk kefir had significant antimutagenic and antioxidant activity as indicated by inhibition of linoleic acid peroxidation and scavenging activity which was much greater than DPPH (1,1-diphenyl-2-picrylhydrazyl) [33].

So that consumption of kefir can be considered in everyday life to prevent mutagenic and oxidative damage. This study provides an interesting result that the administration of kefir at a dose of 5 mL/kg increase greater ovarian MDA levels compared to the arsenic-only group. The authors suspect that an overly high dose of kefir may increase the risk of an antioxidant-to-prooxidant-switch reaction. Prooxidant or antioxidant activity is highly dependent on the concentration.

The balance between oxidant production and antioxidant protection is believed to be very important in maintaining the health of the biological system. Physiological doses of exogenous antioxidants are required to maintain or rebuild redox homeostasis [34]. However, antioxidants at high doses can act as prooxidants and disrupt the redox balance causing cellular dysfunction [35]. Therefore, in this study, there was an increased ovarian MDA level in the higher kefir dose. This illustrates that exogenous antioxidants, such as kefir, can give the unwanted effect if given lower or higher than the right dose.

Conclusion

Kefir can reduce levels of malondialdehyde (MDA) and increase superoxide dismutase (SOD) activity of arsenic-induced female rats ovaries although not statistically significant.

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