



Impact of Mar'ke Bilar Healthy Drink on Blood Levels of Malondialdehyde, Superoxide Dismutase, and Hemoglobin in Soccer Athletes at PPLP North Sumatra

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Abstract

Background: High-intensity soccer activities due to overtraining can cause the emergence of reactive oxygen species (ROS), resulting in an imbalance of free radicals in the form of malondialdehyde (MDA) and endogenous antioxidants in the form of superoxide dismutase (SOD). As a result, it can interfere with the formation of hemoglobin (Hb) needed by an athlete to take in oxygen (VO_{2max}).

Objectives: This study was designed to determine the effect of giving a healthy drink Mar'ke Bilar on MDA, SOD, and Hb levels in soccer athletes at PPLP North Sumatra, Indonesia.

Methods: This type of research is quasi-experimental with a one-group pre- and post-test design. The population and research samples amounted to 33 soccer athletes at PPLP North Sumatra. The administration of Mar'ke Bilar as much as 200 ml was carried out for 14 days, and then MDA, SOD, and Hb levels were measured before and after. A normality test was conducted with the Kolmogorov-Smirnov test, followed by a different test using a paired *t*-test.

Results: Malondialdehyde levels decreased from 17.5 to 10.9 $\mu\text{mol/L}$. There was an increase in SOD from 45.945 to 53.256 pg/mL . However, the results of Hb also showed an increase from 14.3 to 15.1 gr/dL . There was a significant effect on levels of MDA ($P = 0.001$), SOD ($P = 0.001$), and Hb ($P = 0.002$).

Conclusions: Mar'ke Bilar healthy drink consumption has an effect on reduced MDA levels and increased SOD and Hb.

Keywords: Soccer Athletes, Mar'ke Bilar, MDA, SOD, Hb

1. Background

Soccer in Indonesia has experienced ups and downs in terms of achievement, ranking 152 in the world; however, for the region in Southeast Asia, Indonesia is ranked below Vietnam (96) and Thailand (111) (1). Soccer is the most popular sport in the world that requires strength (power) and endurance that lasts for 90 minutes (2). In general, professional soccer athletes do overtraining when conditions are approaching a match or competition; the long-term consequences can be free radicals in the form of reactive oxygen species (ROS) (3, 4).

The onset of ROS can be characterized by increased levels of malondialdehyde (MDA) in blood serum and decreased levels of endogenous antioxidants, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in cell membranes (5, 6). Malondialdehyde is

one of the results of lipid peroxidation due to free radicals, especially during high-intensity physical exercise. Malondialdehyde can be used as an indicator to determine the amount of free radicals and assess the oxidant capacity in the body (7). Superoxide dismutase is the first barrier against excessive free radicals (8).

The increased levels of MDA and decreased SOD can cause disruption of the formation of hemoglobin (Hb) needed by a soccer athlete in his/her capacity to take oxygen, known as $VO_2 \text{ max}$. Low Hb levels indicate the occurrence of anemia in soccer athletes, called sports anemia (9). This can be caused by a low intake of nutrients, such as iron, protein, folic acid, and vitamin B12. (10). The results of a study conducted by Senturk in 2017 stated that increased oxidative stress can lead to sports anemia, which causes soccer athletes to be vulnerable to injury. The

results of preliminary tests conducted on October 27, 2022, from 8 soccer athletes at PPLP Medan, Indonesia, who were taken randomly, showed 2 individuals who had Hb levels below 13 g/dl (11).

One alternative to minimizing and preventing the occurrence of increased oxidative stress and preventing sports anemia is consuming functional food ingredients (Nutraceuticals), which contain bioactive substances and antioxidants (12). Functional food ingredients obtained from colored fruits and tubers are alternative supplies that must be consumed by an athlete in the form of healthy drinks (13).

Purple passion fruit, persimmon, and purple sweet potato are food ingredients that grow in many places in Indonesia and have bioactive substances (phenols, tannins, anthocyanins, and flavonoids) and antioxidant content (beta-carotene, vitamin C, mineral Zn) which can prevent the increase in free radicals and can prevent the occurrence of sports anemia in soccer athletes (14). The concoction of the three food ingredients is made into a healthy drink called Mar'ke Bilar, which can complement the shortcomings and advantages of each ingredient, both in terms of nutrient content and bioactive substances, such as anthocyanins, tannins, flavonoids, and quercetin (15-17). Purple sweet potatoes have a sweet taste and contain bioactive substances anthocyanins, with levels of 61.85 mg/100g. In addition to being high in anthocyanins, it also contains the mineral zinc 0.27 - 1.89 mg Zn/100 g (18, 19). Purple sweet potato can be used as a sugar substitute, thickener, and as a natural colorant. Purple passion fruit is rich in vitamin C content and contains 88 mg of vitamin C in 100 g (20).

The content of Mar'ke Bilar (passion fruit, persimmon, and purple sweet potato) formulation, was firstly processed through the organoleptic test, the result was 1 which means highly preferred with the composition (passion fruit 30 mL, persimmon and purple sweet potato 50 mL, and water 70 mL). The results of the Mar'ke Bilar content test in 100 ml contain total antioxidants 39.73 mg/mL, anthocyanins 233.53 ppm, phenols 0.57%, beta-carotene 4.49 μ g/g, vitamin C 56.91 mg/g, zinc 9.2 mg, and iron 4.68 mg (21).

2. Objectives

This study aimed to analyze the effect of giving the healthy drink Mar'ke Bilar on MDA, SOD, and Hb levels in soccer athletes at PPLP North Sumatera.

3. Methods

The total population in the study was first screened based on the inclusion criteria: Soccer athletes built by PPLP North Sumatra, not in a state of illness (injury), consuming food provided by PPLP, getting the same supplements from PPLP, and willingness to fill out and sign informed consent. From the inclusion criteria screening, the entire population of 33 subjects was eligible to be sampled. The study was conducted on February 12-28, 2023, and was quasi-experimental with a one-group pre- and post-test design without control. The data were collected in the form of sample characteristics, nutrient intake, MDA, SOD, and Hb. Nutrient intake was collected through the 2 \times 24-hour food recall method before and after giving Mar'ke Bilar.

The Mar'ke Bilar healthy drink was prepared in the Laboratory of Nutrition Department of Poltekkes Kemenkes Medan. Mar'ke Bilar healthy drink was mixed (30 cc purple passion fruit, 50 cc persimmon, 50 cc purple sweet potato, and 70 cc water) and then packed in a 200 ml beverage cup which was first closed using a cup sealer press machine. The drinks were put into a styrofoam box which was first filled with blue ice to avoid damage. The styrofoam box was ready to be taken to the research location for the samples. The Mar'ke Bilar drink was then given to the sample for 14 consecutive days from 17.00 to 18.00 Western Indonesia Time (WIB) after finishing the exercise.

Blood sampling was done on the left upper arm using a 2.5 cc syringe by medical laboratory technology analysts. Then, the blood was put into a tube containing ethylenediaminetetraacetic acid (EDTA) solution and examined at the Molecular Laboratory of the Faculty of Medicine, Brawijaya University Malang, Indonesia. Malondialdehyde and superoxide dismutase examination was carried out using enzyme-linked immunosorbent assay (ELISA) method with a spectrophotometer; nevertheless, Hb examination was performed using the hematology analyzer method with a spectrophotometer.

The data were analyzed univariately and bivariately. The data normality test was conducted using the Kolmogorov-Smirnov test. In this study, the data were normally distributed; therefore, the test performed used the t-dependent test. This study received ethical approval number 01.1437/KEPK POLTEKKES KEMENKES MEDAN 2023.

4. Results

4.1. Characteristics of the Sample

In this study, the characteristics of the sample consisted of age, education, and nutritional status.

Based on age, predominantly 17 years old, there were as many as 14 subjects (42.4%). The entire sample has a high school education equivalent. Based on nutritional status, predominantly normal nutritional status, there were as many as 30 subjects (90.9%). Moreover, there were overweight samples as many as 3 subjects (9.1%).

4.2. Intake of Macronutrients and Micronutrients

The measurement of sample nutrient intake was carried out as a control to avoid bias, and the impact of Mar'ke Bilar healthy drinks can be seen in Table 1. The distribution of average nutrient intake before and after treatment are shown in Table 1. Macronutrient and micronutrient intake in the sample had no difference with $P > 0.05$. Based on the energy needs of soccer athletes of 3 500 kcal, only 61.09% was met. Protein requirements in soccer athletes were 89.6 gr, and the need for vitamin C 200 mg/day was only fulfilled by 40%. However, vitamin A requirements for ages 15 - 19 years were met by 100% of the 2019 RDA (600 - 700 Re).

Table 1. Mean Macronutrient and Micronutrient Intake Before and After Treatment

Nutrition	Mean \pm SE		P-Value
	Before	After	
Energy (Kcal)	2242.66 \pm 22.33	2243.03 \pm 22.32	0.418
Protein (g)	96.59 \pm 0.43	96.61 \pm 0.41	0.906
Fat (g)	80.54 \pm 0.23	80.09 \pm 0.44	0.256
Carbohydrates (g)	274.76 \pm 5.07	270.56 \pm 7.14	0.448
Iron (mg)	9.12 \pm 0.19	9.28 \pm 0.17	0.251
Zinc (mg)	10.19 \pm 0.23	10.29 \pm 0.13	0.636
Vitamin C (mg)	76.75 \pm 3.16	79.58 \pm 1.59	0.352
Vitamin A (Re)	625.90 \pm 12.05	632 \pm 8.15	0.415

4.3. MDA, SOD, and Hb Levels in Soccer Athletes

Mar'ke Bilar healthy drink, containing several antioxidants such as beta-carotene, vitamin C, and zinc, and bioactive substances (anthocyanins and phenols), has a significant effect. The levels of MDA ($P = 0.001$), SOD ($P = 0.001$), and Hb ($P = 0.002$) can be seen in Table 2. However, the distribution of ups and downs and static levels of MDA, SOD, and Hb can be seen in Figure 1. The percentage results for the decrease, increase, and steady state of MDA, SOD, and Hb can be seen in the bar chart (Figure 2).

5. Discussion

5.1. Characteristics of the Sample

In this study, the age range in which the athletes were trained to become professional soccer athletes was 15 -

18 years (high school education). This is because peak performance occurs at the age of 17 - 24 years (22).

The nutritional status of soccer athletes is measured using IMT/U parameters. Based on the obtained results, the athletes with normal nutritional status amounted to 30 subjects (90.9%), and only 3 cases (9.1%) were overweight. These results are in accordance with research conducted by Alfitasari in 2019, which states that most of the soccer athletes in the club he studied (14 subjects, 87.5%) had a normal nutritional status (23). Nutritional status determines the fitness of a soccer athlete to perform optimally because if you are overweight, it will affect movement, and the ability to process the ball will be disrupted (24).

5.2. Effect of Mar'ke Bilar Healthy Drinks on MDA, SOD and Hb Levels

Mar'ke Bilar healthy drink is made from purple passion fruit, persimmon, and purple sweet potato as a thickener and sweetener. Mar'ke Bilar was given to soccer athletes for 14 days at a dose of 200 ml per drink (Figure 3). Mar'ke Bilar was tested for antioxidant content at the Laboratory of Agricultural Product Technology and Faculty of Mathematics and Natural Sciences, Brawijaya University Malang, East Java. Mar'ke Bilar in 100 ml was observed to contain 39.73 ppm total antioxidant, 4.01 g fiber, 4.49 mg beta-carotene, 233.53 ppm anthocyanin, 57% phenol, 9.2 mg zinc, 4.68 mg/dL iron (contribution to Hb), and 56.81 mg vitamin C. The examination of antioxidant content aims to contribute exogenous antioxidant substances that will affect the inhibition of MDA and can stimulate the work of endogenous antioxidants (SOD). The mechanism of the antioxidant content of Mar'ke Bilar and Fe content can be reviewed in the next discussion.

5.2.1. Malondialdehyde

The results showed the effect of Mar'ke Bilar administration on Malondialdehyde (MDA) reduction ($P = 0.001$). While the decrease in MDA was percentaged, it was observed that all samples (100%) experienced a decrease in blood MDA levels. Antioxidant supplementation in the treatment has a positive effect on the synthesis of several antioxidants in reducing MDA levels. In this study, there was also a decrease of 6.7 $\mu\text{mol/L}$. However, when compared to normal MDA concentrations (0 - 1 $\mu\text{mol/L}$), it is still high (25).

The decrease in MDA levels can occur due to the total antioxidant content of antioxidant intake of 39.73 mg/mL consisting of nutrients, such as beta-carotene 4.49 mg, vitamin C 56.81 mg, and zinc 9.2 mg. These antioxidants can reduce and inhibit lipid peroxidation and activate peroxidase, which is an endogenous antioxidant in the

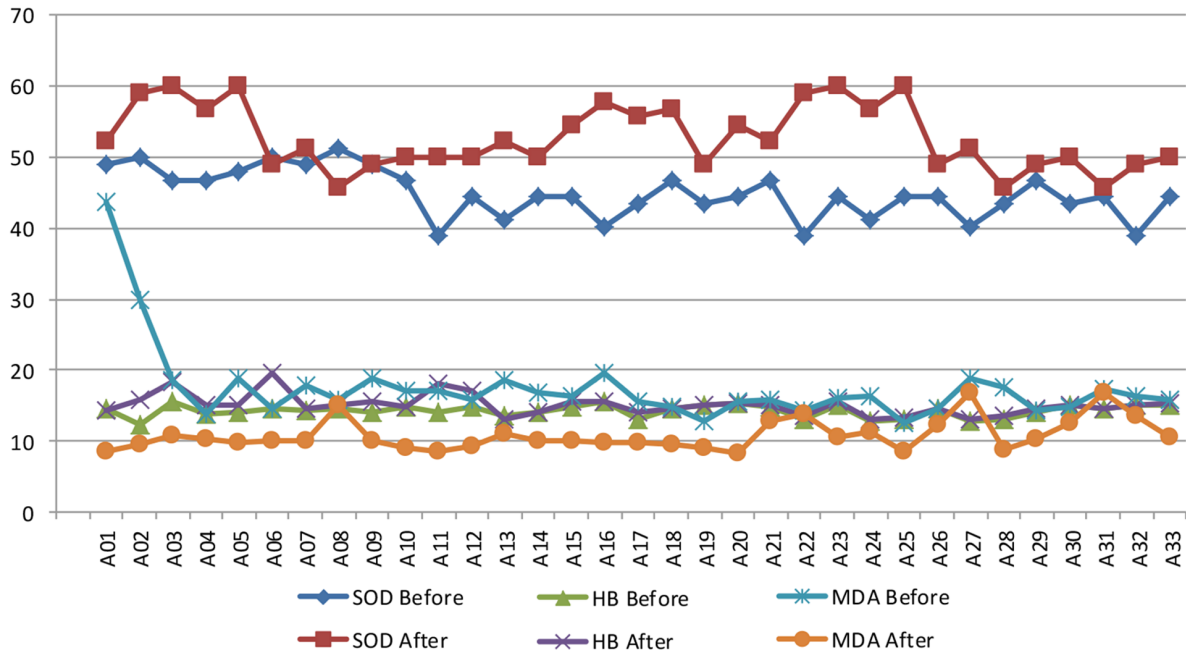


Figure 1. Distribution of increase and decrease of MDA, SOD, and Hb for each sample

Table 2. Average Levels of Malondialdehyde (MDA), Superoxide Dismutase (SOD), and Hemoglobin (Hb) Before and After Treatment of Mar'ke Bilar Healthy Drinks

Variables	Minimum	Maximum	Mean	SE	P-Value
MDA					
Before	12.682	43.6895	17.511	0.96	0.001
After	1.089	16.854	10.564	0.49	
SOD					
Before	39	51.222	45.945	0.41	0.001
After	45.667	60.778	53.256	0.28	
Hb					
Before	12.4	15.5	14.3	0.14	0.002
After	13	19.6	15.1	0.26	

Abbreviations: MDA, malondialdehyde; SOD, superoxide dismutase; Hb, hemoglobin.

body. Antioxidants in the form of vitamin C also help convert free radicals into less reactive ascorbyl radicals. Vitamin C is often referred to as free radical scavenging because it can stop free radical reactions from becoming reactive by providing hydrogen ions (26). Beta-carotene in this drink can also neutralize singlet oxygen, which is also a free radical ROS.

The results of this study are in accordance with research conducted by Prihadi (2019), Pratiwi (2020), Winara (2021), and Dewangga (2022), who used one of the healthy drinks Mar'ke Bilar which also reduced blood MDA

levels (27-30).

5.2.2. Superoxide Dismutase

The results showed the effect of Mar'ke Bilar administration on increasing Superoxide Dismutase (SOD) (P = 0.001). While the increase in SOD was percentaged, it was observed that 91% experienced an increase and only 6% experienced a decrease in blood SOD levels. Mar'ke Bilar, besides containing vitamins as antioxidants, also contains the mineral zinc, which can act as an enzyme co-factor; therefore, it can increase the SOD enzyme

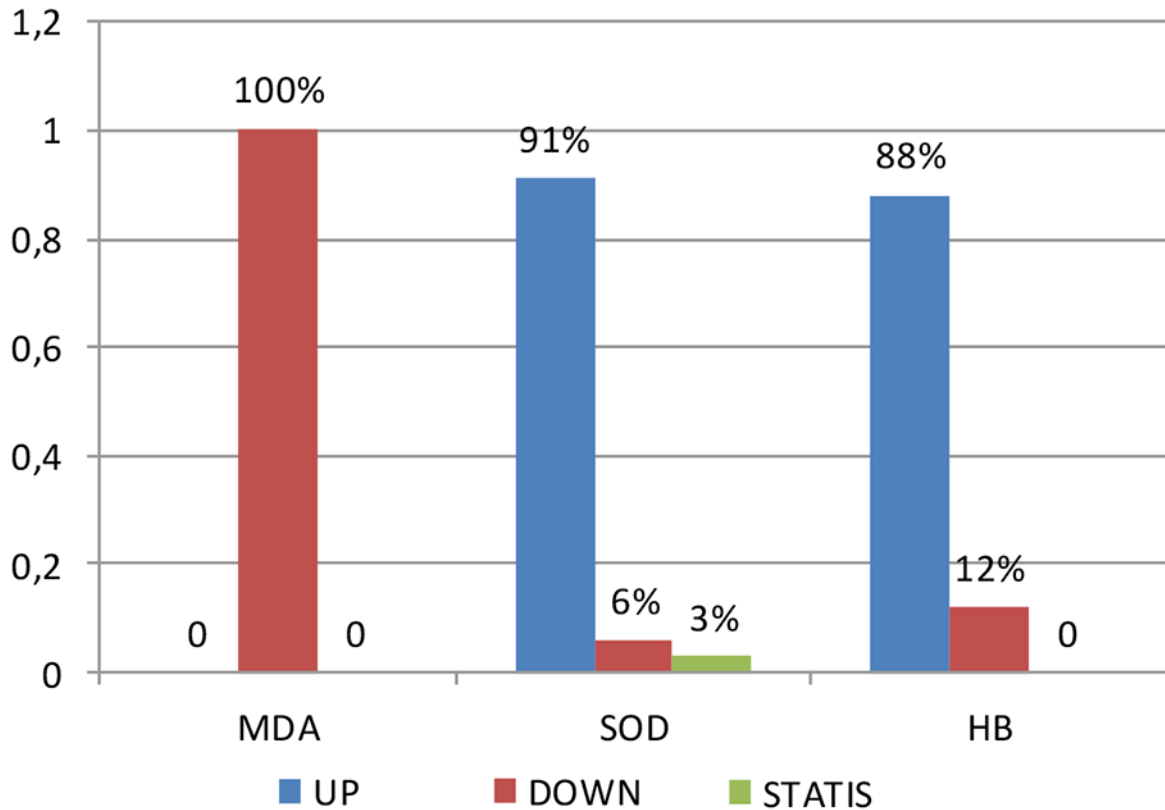


Figure 2. Percentage of Increase and Decrease of Malondialdehyde (MDA), Superoxide Dismutase (SOD), and Hemoglobin (Hb)

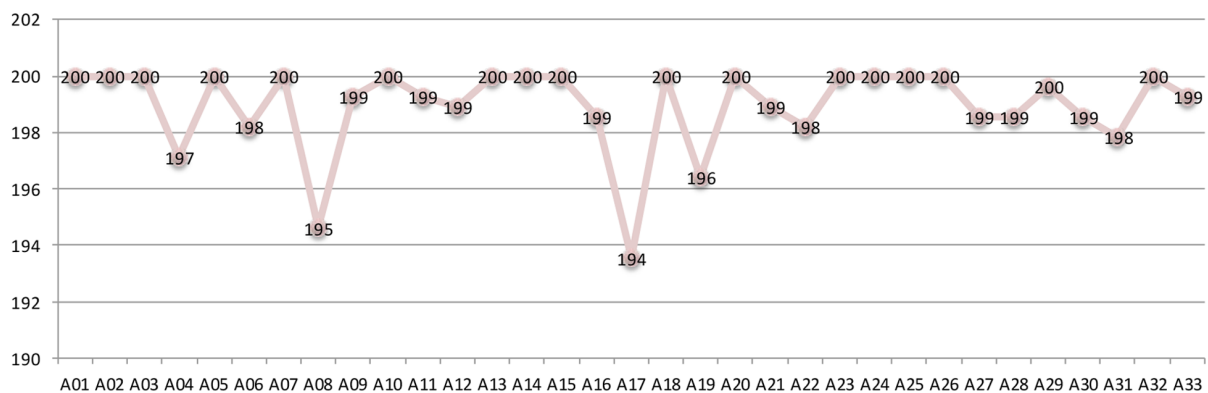


Figure 3. Average consumption of Mar'ke Bilar healthy drinks

in the body (5). The mechanism of antioxidants as anti-free radicals endogenously will stimulate the defense system by activating endogenous enzymes in the form of antioxidants (catalase and SOD). Antioxidants (SOD) protect against free radicals by preventing damage to

deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, and lipids that trigger the formation of new free radicals (31). Anthocyanins, which are one of the ingredients in purple sweet potatoes, can increase the expression of antioxidant enzymes through an increase in

the Nrf2 protein, which is an indirect antioxidant (32).

The results of this study are in accordance with research conducted by Yunarsa (2018) and Susanto (2023), which used one of the ingredients for making Mar'ke Bilar healthy drinks which can increase SOD levels (33,34).

5.2.3. Hemoglobin

The results indicated the effect of the Mar'ke Bilar administration on the increase in Hemoglobin (Hb) ($p = 0.002$). While the increase in Hb was percentaged, it was observed that 88% experienced an increase and only 12% experienced a decrease in blood Hb levels. Mar'ke Bilar contains iron (Fe) of 4.68 mg, which is the main substance forming Hb. Meanwhile, vitamin C and zinc are also contained in Mar'ke Bilar, which can help the absorption of Fe, especially Fe nonheme sources (35). Mar'ke Bilar also contains bioactive substances that can prevent cell damage due to free radicals, including spinal cord and liver cells that produce Hb (10). The results of this study are in accordance with research conducted by Sinaga (2015), Dieny (2016), Tombokan (2021), and Penggalih (2021), who also provided food ingredients containing anthocyanins, iron, vitamin C, and zinc (2, 21, 36, 37).

5.3. Conclusions

The results of this study indicated that the provision of healthy drinks Mar'ke Bilar contributed to a decrease in MDA ($P = 0.001$) and an increase in SOD ($P = 0.001$) and Hb ($P = 0.002$).

5.4. Limitations of the Research

Researchers have limited research to soccer athletes, including a small sample size, because the researcher only took one junior soccer club as the sample. This study did not use a sample control group minimized by looking at nutrient intake before and after the study. The problem of time duration was compared to several previous studies that used the same ingredients, and the time is shorter (≤ 14 days) (Sinaga research in 2017, Mutafiah research in 2019, Rusiani research in 2019, Dewanga in 2022). For hydration status, it was not observed that the research was limited in manpower, and the research time was not too long.

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Footnotes

Authors' Contribution: Ginta Siahaan conceived and designed the evaluation and drafted the manuscript. Eko Widodo participated in designing the evaluation, performed parts of the statistical analysis, and helped to draft the manuscript; re-evaluated the clinical data, performed the statistical analysis, and revised the manuscript. Dini Lestrina collected the clinical data, interpreted them, and revised the manuscript. Riris Oppusunggu re-analyzed the clinical and statistical data and revised the manuscript. All the authors read and approved the final manuscript.

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