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Research Article

Borassus flabellifer Effectively Reduces MDA Levels in High Oxidative Stress Population in Jakarta, Indonesia

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ABSTRACT

Background: Malondialdehyde (MDA) is commonly used to assess oxidative stress levels. **Objective:** This study aimed to investigate the effect of *Borassus flabellifer* fruit consumption on MDA levels in individuals with high oxidative stress, providing insights into the fruit's potential as a natural antioxidant therapy. **Methods:** A prospective interventional study was conducted in Jakarta, recruiting fifty participants aged 30–60 years with baseline MDA levels >10 nmol/L. Participants consumed 150 grams of Borassus flabellifer fruit daily for 30 days. Blood samples were collected at baseline and post-intervention to assess MDA levels. **Results:** The average MDA level decreased significantly from 14.35 ± 2.43 nmol/L to 12.00 ± 1.74 nmol/L after 30 days of fruit consumption ($p < 0.0001$). The Cohen's d value of 2.92 indicated a large effect size, demonstrating the intervention's substantial impact. A strong positive correlation ($r = 0.91$, $p < 0.0001$) between baseline MDA levels and reduction was observed, suggesting that participants with higher baseline oxidative stress benefited more from the intervention. **Conclusion:** *Borassus flabellifer* fruit consumption effectively reduces MDA levels in individuals with high oxidative stress. The results support the inclusion of this fruit in recommendations for managing oxidative stress-related conditions.

Keywords: antioxidant therapy, *borassus flabellifer*, malondialdehyde, oxidative stress

1. Introduction

Oxidative stress is linked to the progression of cardiovascular diseases, diabetes mellitus and neurodegenerative conditions. These conditions occur when the body's defense mechanisms are overwhelmed by Reactive Oxygen Species (ROS) accumulation. In general, antioxidants maintain the balance between ROS production and elimination. However when this particular balance is upset, excessive ROS causes oxidative damage at the cell level, destroying cell structures including lipids, proteins, and even Deoxyribonucleic Acid (DNA) [1].

Malondialdehyde (MDA), a result of lipid peroxidation, is among the most commonly used biomarkers for assessing oxidative stress. Lipid peroxidation: ROS attack polyunsaturated fatty acids in cell membranes and produce MDA as an end product. Increased MDA levels indicate increased oxidative damage and have been associated with severity of disease progression under various conditions. Monitoring MDA is thus mandatory for the evaluation of oxidative stress status in patients with chronic or degenerative diseases [2, 3].

Attempts to change oxidative stress have embraced both pharmacological and non-pharmacological approaches. Dietary antioxidants from natural sources such as fruits phenolic rich in flavonoids and vitamins, seem to have anti-oxidative damage and MDA levels lowering effects. *Borassus flabellifer* (buah lontar) is one of the many tropical fruits. These compounds can counter oxidative stress, and *Borassus flabellifer* is attracting increasing scientific attention for its therapeutic and preventive activities [4]. Previous studies show that dietary antioxidants lower oxidative stress markers such as MDA, but the efficacy varies with intervention type. Some fruits have had some effect such as pomegranate and grapes; However, other antioxidants have not produced similar results in different populations. Additionally, nearly all studies on antioxidant-rich fruits focus on short term effects and very little data exist for populations with chronic illnesses or high oxidative stress [5, 6].

Despite these results, studies on *Borassus flabellifer* are still limited, especially when considering its direct effect on oxidative stress biomarkers. The few studies that exist are focused more on its traditional uses and general nutritional benefits and do not provide insight into how regular consumption affects biochemical markers such as MDA. Furthermore, most prior studies haven't specifically dealt with populations high in oxidative stress, so studies to fill this gap are needed.

Figure 1. *Borassus flabellifer* (lontar fruit)

The aim of the present study is to assess the effect of *Borassus flabellifer* fruit consumption on MDA levels in a population under high oxidative stress. In exploring this relationship, the study aims to empirically demonstrate the fruit's potential as a natural antioxidant therapy. This research fills a gap in the literature by : evaluation of *Borassus flabellifer* against a specific oxidative stress biomarker (MDA), targeting a high-risk population to understand the practical benefits of antioxidant consumption and giving insights into dietary recommendations and complementary therapies for managing oxidative stress-related conditions.

2. Methods

This was a prospective interventional study in Jakarta to assess the effect of *Borassus flabellifer* fruit consumption on Malondialdehyde (MDA) levels in high oxidative stress. Participants were recruited from West Jakarta during May – August 2023. Aged 30-60 years, baseline MDA > 10 nmol/L, indicating high oxidative stress. Exclusion criteria were chronic inflammatory diseases, acute infections or persons receiving antioxidant or anti-inflammatory therapies. In total, 50 participants were enrolled in the study, which ensured statistically sufficient sample size to detect significant effects using preliminary power analysis.

Intervention & Materials

Participants consumed *Borassus flabellifer* fruit at a daily dose of 150 grams for 30 days. Participants consumed 150 grams of *Borassus flabellifer* fruit per day, divided into two equal portions taken in the morning and evening. The fruit was sourced from Koperasi UMKM Indofarma, Bekasi, Indonesia, and no other dietary modifications were imposed to minimize confounding variables.

Blood samples were collected at baseline (pre-intervention) and post-intervention to measure MDA levels. MDA concentrations were assessed using the Malondialdehyde (MDA) Colorimetric Assay Kit (TBA Method) (Catalog Number EEA015, Thermo Fisher Scientific, Vienna, Austria). This kit measures MDA levels using thiobarbituric acid (TBA) to produce a red compound with a maximum absorption peak at 532 nm. The assay was performed using the Malondialdehyde (MDA) Colorimetric Assay Kit in accordance with the manufacturer's instructions (Thermo Fisher, 2023). The core reagents provided in the kit included 3 mL of clarificant, 4 mL of acid reagent, a chromogenic agent in powder form, 5 mL of a 50 µmol/L standard solution, and one microplate accompanied by two plate sealers.

In addition to the kit components, several supplementary materials were required for the procedure. These included distilled or deionized water, normal saline (0.9% NaCl), phosphate-buffered saline (PBS) at 0.01 M and pH 7.4, and glacial acetic acid with a purity of ≥99.5%. The instrumentation used comprised a microplate reader capable of detecting absorbance in the range of 530–540 nm and an incubator maintained at a constant temperature of 37 °C. For safety, all handling was done following the Safety Data Sheets (SDSs), with the use of protective eyewear, gloves, and appropriate clothing.

Sample Preparation and Assay Procedure

Blood samples, either serum or plasma, were analyzed directly for malondialdehyde (MDA) levels. In cases where the samples appeared turbid, they were first centrifuged at 10,000 g for 10 minutes at 4 °C to obtain a clear supernatant suitable for testing. For reagent preparation, the chromogenic agent was dissolved in 14 mL of deionized water that had been preheated to 90–100 °C, followed by the addition of 14 mL of glacial acetic acid. The acid reagent was prepared by diluting it with distilled water in a 1.2:34 ratio.

During the assay procedure, 0.02 mL of the sample, standard, or control was transferred into 1.5 mL microcentrifuge tubes. To each tube, 0.02 mL of clarificant, 0.6 mL of acid reagent, and 0.2 mL of the chromogenic agent were added. For the control group, 50% acetic acid was used in place of the chromogenic agent. The tubes were then sealed and incubated in a water bath at 100 °C for 40 minutes. After incubation, the tubes were cooled to room temperature and centrifuged at 9,569 g for 10 minutes. Subsequently, 0.25 mL of the resulting supernatant was transferred into a microplate well, and the optical density was measured at a wavelength of 532 nm using a microplate reader.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviation (SD) for continuous variables. To evaluate the effect of the intervention, paired t-tests were applied because the data followed a normal distribution. Effect sizes were reported using Cohen's d with 95% confidence intervals (CIs) for precision. Correlations between baseline MDA levels and post-intervention reduction were assessed using Pearson's correlation coefficient (r). The analysis emphasized effect size and precision rather than relying solely on p-values.

3. Results

3.1 Descriptive Statistics

Mean baseline MDA amount was 14.35 2.43 nmol / L, dropping to 12.00 1.74 nmol / L following intervention. This reduction indicates that Borassus flabellifer fruit consumption reduced oxidative stress in the participants.

Table 1. Participant characteristics

Variable	Mean ± SD	Range
Age (years)	44.8 ± 9.1	30 – 60
Baseline MDA (nmol/L)	14.35 ± 2.43	10.5 – 19.0
Post-Intervention MDA (nmol/L)	12.00 ± 1.74	9.2 – 16.1

3.2 Test of Normality

Shapiro-Wilk test showed normal distribution of baseline and post-intervention MDA levels (p-values > 0.05:): Baseline MDA: W: 0.9573, p: 0.0684 ; Post-Intervention MDA: W = 0.9676, p = 0.4444. Since the data had been normally distributed, the paired t test was suitable for analyzing the outcome of the intervention.

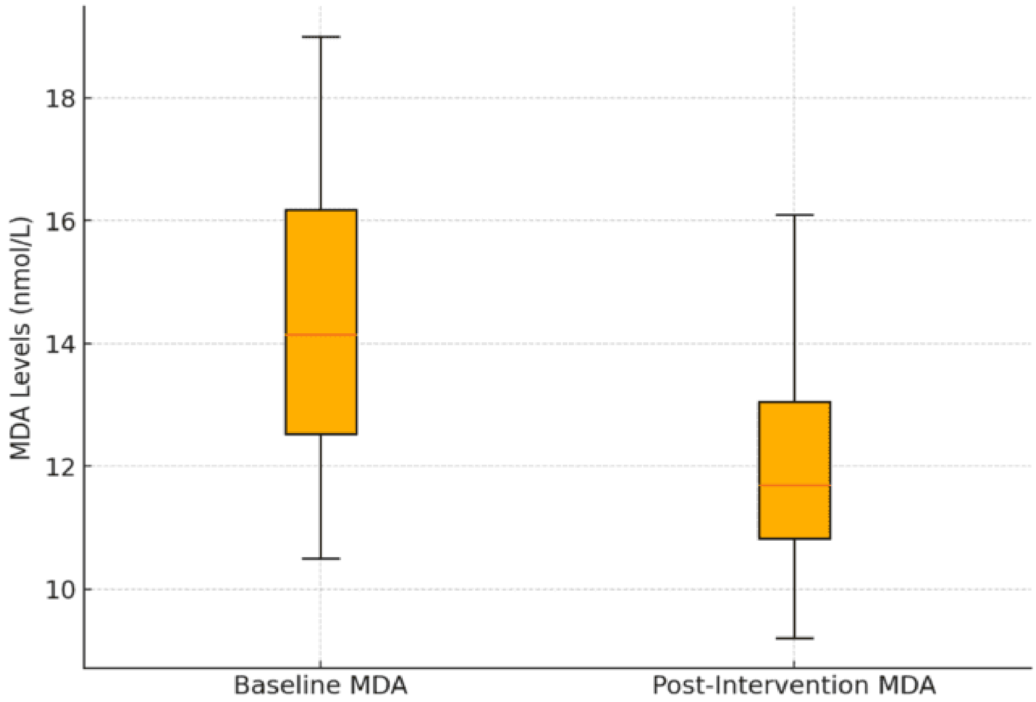

Figure 2. MDA levels (pre- and post-intervention)

3.3 Paired T-Test

The paired t-test showed statistically significant decrease in MDA levels after the intervention: T-Statistic: 20.68; *p*-Value: 0.0000. The test demonstrates significant ($p < 0.05$) variation in MDA levels between post-intervention and baseline.

3.4 Effect Size

Cohen's d value was 2.92, which indicated large effect size. This shows the intervention significantly reduced oxidative stress as shown by the decrease in MDA levels.

3.5 Correlation Analysis

A Pearson's correlation coefficient of r = 0.91 (p= 0.001) confirmed a good relationship between baseline MDA and MDA reduction after intervention. This suggests that participants with higher baseline MDA levels had larger reductions.

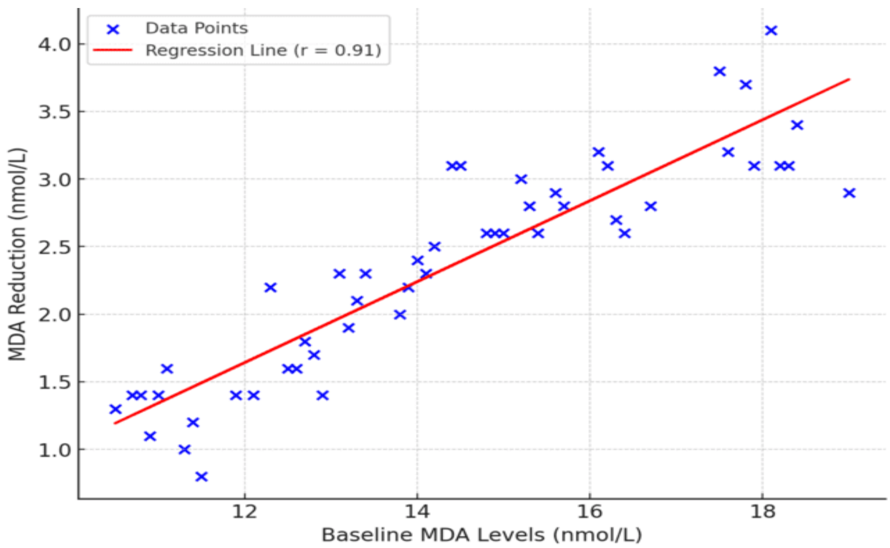

Figure 3. Regression line for correlation analysis (r = 0.91)

4. Discussion

This study shows that fruit consumption of *Borassus flabellifer* fruit reduces oxidative stress in subjects with high baseline levels of Malondialdehyde (MDA) biomarker of lipid peroxidation. MDA levels of participants decreased from 14.35 2.43 nmol / L to 12.00 1.74 nmol / L over 30 days, indicating the fruit's antioxidant capacity. This is in line with the study aim of assessing *Borassus flabellifer* as a dietary intervention in patients with high oxidative stress, highlighting the fruit's potential as a therapeutic fruit.

The intervention resulted in a large effect size (Cohen's d = 2.92) indicating reduced oxidative stress after fruit consumption. Moreover, the positive correlation (r = 0.91) of baseline MDA and MDA reduction suggested that individuals with higher initial oxidative stress gained much more from the treatment. This indicates that the greater the oxidative damage, the greater the potential for antioxidant-rich interventions such as *Borassus flabellifer* to attenuate it.

The results are of particular interest for populations in danger of oxidative stress-related conditions including cardiovascular diseases, type 2 diabetes and neurodegenerative disorders. These diseases are known to take place when the balance between reactive oxygen species (ROS) and the body's antioxidant defences is disturbed leading to cumulative cell harm. This study shows that *Borassus flabellifer* can be a natural and accessible intervention to restore oxidative balance through lowering MDA levels and reducing the risk of oxidative damage.

The findings also address a gap in existing research. Despite its phytochemical richness, several fruits with antioxidant activity (pomegranate and grapes) have been studied to inhibit oxidative markers; however, *Borassus flabellifer* has received limited scientific attention. This study demonstrates the fruit's potential as a general antioxidant and an intervention for high risk populations with high oxidative stress. Consistency of these findings supports the inclusion of *Borassus flabellifer* in dietary recommendations for the management of oxidative stress-related diseases.

Previous research on dietary antioxidants has mixed results, with the efficacy of interventions varying across populations and types of antioxidants used. Some studies report promising effects of fruits such as pomegranate and grape extracts while others report inconsistent results depending on the studied population or targeted biomarkers. This study provides new insights by focusing on a high risk population with high oxidative stress that few prior research has dealt with [7, 9].

In contrast to previous studies which were often limited to short-term effects or employed controlled populations with low oxidative stress, the present study demonstrates that *Borassus flabellifer* exerts a sustained effect over a 30-day period in high oxidative stress [10]. The positive correlation of baseline MDA levels with reduction suggests that individuals under more severe oxidative stress will benefit more, a finding that has not been reported widely in other antioxidant studies.

Additionally, the normal distribution of MDA levels prior to and after the intervention warrants the usage of paired t-tests in statistical analysis. This rigorous methodology bolsters the dependability of the findings and also offers a foundation for more studies.

The high phenolic and flavonoid content of *Borassus flabellifer* explains the significant reduction in MDA levels in this study [4, 10, 11]. These compounds counter oxidative stress by neutralizing free radicals and also stopping lipid peroxidation, lowering MDA production (4,10). The antioxidant properties of the fruit likely mediated a central restoration of oxidative balance in participants, leading to a reduction in oxidative damage.

The large effect size (Cohen's d = 2.92) demonstrates the significant effect of *Borassus flabellifer* on oxidative stress reduction. This indicates that the intervention was statistically significant and clinically meaningful, as is required for dietary guidelines inclusion of the fruit. The strong correlation (r = 0.91) between baseline MDA levels and reduction further indicates that individuals with higher oxidative stress benefited most and the fruit may represent an attractive target for targeted interventions in chronically ill populations.

Practical implications of the findings of the study

Borassus flabellifer is widely available and relatively inexpensive and may represent an accessible dietary intervention for oxidative stress-associated conditions in individuals at high risk of oxidative stress. Its inclusion in daily diets could complement other antioxidant-rich foods and help the body cope with oxidative damage. The antioxidant potential of *Borassus flabellifer* is largely attributed to its high content of polyphenols, flavonoids, and vitamins such as vitamin C and E, all of which are known to neutralize reactive oxygen species (ROS) through multiple biochemical pathways. These bioactive compounds act by donating electrons to unstable free radicals, thereby stabilizing them and preventing further oxidative reactions that can damage cellular lipids, proteins, and DNA [12].

One key mechanism is the inhibition of lipid peroxidation, the very process that produces malondialdehyde (MDA) as a byproduct. Polyphenols and flavonoids in *Borassus flabellifer* scavenge lipid radicals and terminate the chain reaction of lipid peroxidation. As a result, the generation of MDA is significantly reduced, which aligns with the findings of your study where MDA levels decreased post-intervention [12, 13, 14]. Additionally, certain phytochemicals found in *Borassus flabellifer* may also upregulate endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). These enzymes work synergistically to eliminate superoxide anions and hydrogen peroxide, two major contributors to oxidative stress [15, 16].

The fruit may also possess anti-inflammatory properties that reduce oxidative stress indirectly by downregulating pro-inflammatory cytokines and inhibiting pathways like NF-κB, which are often activated in chronic inflammatory and oxidative states [16]. Altogether, the phytochemical composition of *Borassus flabellifer* enables it to act through both direct radical scavenging and indirect modulation of cellular antioxidant defenses, making it a promising natural intervention for oxidative stress-related conditions [12, 18]. This is especially important for chronic conditions individuals who might experience more oxidative stress as a part of their disease progression.

Despite promising results, some limitations must be dealt with in upcoming studies. The sample size of fifty people, although adequate for preliminary analysis, limits generalisability of the findings to larger groups. Future researches need to think about larger sample sizes to validate reproducibility of these results across demographic groups. Another limitation is MDA has been identified as the primary biomarker of oxidative stress. While MDA is a well-established indicator, oxidative stress has multiple pathways and is complex. Future researches may examine *Borassus flabellifer* impact on various other oxidative stress markers as superoxide dismutase (SOD) or glutathione peroxidase (GPx) to further make clear its antioxidant potential. Furthermore, the analysis was conducted over a 30 day period, which limits capacity to evaluate long-term negative effects of the intervention. Longitudinal studies are beneficial to assess if the reduction of oxidative stress can be maintained over longer time frames. Such studies could also investigate the effects of *Borassus flabellifer* in association with other antioxidant-rich foods to enhance the therapeutic effect.

Results reveal a substantial decrease in MDA level from 14.35 2.43 nmol / L to 12.00 1.74 nmol / L following 30 days of fruit use indicating the possibility of fresh fruit as healthy antioxidant treatment. The large effect size (Cohen's d = 2.92) reflects the substantial effect of the intervention. Also the positive correlation ($r = 0.91$) between baseline MDA levels and MDA reduction suggested that people with greater initial oxidative stress got much more benefit from the treatment. The results also pave the way for the development of complementary therapies with *Borassus flabellifer*. For instance, the fruit might be added to functional food or supplements to curb oxidative damage of people with cardiovascular diseases, diabetes or neurodegenerative problems(3,4,10,18–25). The simplicity of the intervention and its demonstrated efficacy make it a good complement to preventive healthcare and disease control plans.

5. Conclusion

Borassus flabellifer fruit consumption reduces oxidative stress as evidenced by a significant decrease in Malondialdehyde (MDA) level in high oxidative stress individuals. These findings confirm the therapeutic use of *Borassus flabellifer* and shed light on its practical applications in managing oxidative stress-related conditions.

6. Data Availability Statement

The datasets generated and analyzed during the current study are not publicly available due to privacy and ethical considerations but are available from the corresponding author upon reasonable request.

7. Ethical Statement

Ethical approval for this study was obtained from the Specialist Medical Centre (SMC) (Approval No.: X/ESMC/IX/2023). The research was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in the study.

8. Author Contributions

Each author has made substantial contributions to this study, including conceptualization, study design, implementation, data collection, analysis, and interpretation. All authors have participated in drafting, revising, and critically reviewing the manuscript. They have provided final approval of the version to be published and have been involved in the decision regarding the journal for submission. Furthermore, all authors agree to take full responsibility for every aspect of the work.

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This study did not receive any specific funding from external sources, organizations, or sponsors. All costs related to the design, data collection, management, analysis, and interpretation of the data were covered by the authors. The suppliers, including Koperasi UMKM Indofarma, which provided the *Borassus flabellifer* fruit, had no involvement or influence in the design of the study, data management, analysis, interpretation, or the preparation and review of this manuscript. The decision to submit the manuscript for publication was made independently by the authors.

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11. Conflict of Interest

The authors declare that there are no conflicts of interest related to this research. The study was conducted independently, and there were no financial or personal relationships that could have influenced the outcomes or interpretations of the findings.

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