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ORIGINAL ARTICLE

Effectiveness of bay leaf extract in reducing malondialdehyde levels

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Abstract

Excessive physical activity can lead to oxidative stress, resulting in elevated levels of free radicals in the human body. Oxidative stress is believed to be a precursor of various degenerative and chronic disorders, including arthritis, autoimmune diseases, cardiovascular diseases, neurodegenerative disorders, inflammatory diseases, and cancer. Malondialdehyde serves as a biomarker for oxidative stress and lipid peroxidation. Hence, the use of antioxidants is imperative to restore the balance in the body. The aim of this study was to investigate the effect of bay leaf administration on malondialdehyde levels in male Wistar rats. A pre-test–post-test design with a control group was employed in this study. Bay leaf extract was prepared using a maceration method with 70% ethanol. The rats were divided into four groups, including one control group and three treatment groups based on the dosage. Technical terms were explained when first used, and concise objective language was employed throughout the report. The study adhered to the conventional structure with standard citations and was free from grammatical, spelling, and punctuation errors. Blood samples were collected from the retro orbital plexus on days 1 (pretest) and 15 (posttest). Blood MDA levels were measured using the thiobarbituric acid reactive substances (TBARs) method, which involves reacting blood plasma with 20% TCA, 1% TBA, and 50% glacial acetic acid. To determine the significance of differences between the test groups, one-way ANOVA was used at a 95% confidence level ($p < 0.05$). Post Hoc Test with LSD technique was conducted for additional analysis or testing. Phytochemical test results indicated the presence of flavonoids, steroids, and tannins as secondary metabolites in the bay leaf extract. Administering bay leaf extract significantly lowered the MDA levels. The group receiving a 5 ml dose demonstrated greater effectiveness in reducing MDA levels in male white Wistar rats induced with excessive physical activity, based on average MDA levels.

Keywords: bay leaf extract, oxidative stress, MDA, wistar rats

Introduction

Physical activity, defined as any movement generated by skeletal muscles requiring energy expenditure, such as walking, cycling, or sports, has significant health benefits.[1] Regular physical activity reduces the risk of non-communicable diseases, including hypertension, coronary heart disease, stroke, type 2 diabetes, several cancers, and mental illnesses.[2–4] Physical activity enhances bone and functional health, maintaining energy balance, and moderate-intensity physical activity improves immune function, but excessive and prolonged high-intensity activity can jeopardize immune function and control body weight.[5] Moderate-intensity physical activity improves immune function, but excessive and prolonged high-intensity activity can jeopardize immune function.[6] Research indicates that excessive physical activity increases oxidative production in muscles, leading to the formation of reactive oxygen species (ROS). Chronic exposure to high ROS levels can be toxic, exhausting enzymatic and non-enzymatic antioxidant systems and causing cellular dysfunction, macromolecular damage, apoptosis, and necrosis. Therefore, excessive physical exercise can be detrimental to both untrained and trained individuals.[1,7,8]

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Free radicals are molecules or molecular fragments with one or more unpaired electrons in atomic or molecular orbitals. Free radicals originating from oxygen are known as reactive oxygen species (ROS).[9] Free radicals are produced during normal cell function and are a part of the natural physiological processes of all living organisms. When excess free radicals cannot be gradually processed, their accumulation in the body leads to oxidative stress, a term commonly used to imply random and indiscriminate damage to various biomolecules.[10] Oxidative stress reflects an imbalance between oxidation and reduction and is understood as the disproportionate relationship between oxidants and/or pro-oxidants and antioxidant molecules.[11] Oxidative stress is generally considered the starting point for the onset of several diseases and plays a crucial role in the development of chronic and degenerative disorders such as arthritis, autoimmune disorders, cardiovascular and neurodegenerative diseases, inflammation, and cancer.[12]

Oxidative stress induces lipid peroxidation, which involves initiation, termination, and propagation of lipid radicals. This process involves oxygen consumption, rearrangement of double bonds in unsaturated lipids, leading to the damage of Polyunsaturated Fatty Acids (PUFAs).[13] Furthermore, the toxic signaling end products are considered as free radical biomarkers that act both as signaling molecules and cytotoxic products causing covalent changes in lipid peroxidation products. Due to the nature of oxidative-induced damage, these compounds are considered as disease mediators in the pathophysiology of many neurodegenerative diseases, including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis (ALS), diabetes, atherosclerosis, chronic inflammation, asthma, and liver injuries, functioning as potential biomarkers in disease signaling mechanisms.[14] Lipid peroxidation in membranes results in the end product, malondialdehyde (MDA). MDA, a three-carbon molecule generated by free radicals, serves as a biomarker for oxidative stress and lipid peroxidation.[15] MDA emerges after the peroxidation of fatty acids containing three or more double bonds and appears in blood and urine. Due to its strong correlation with lipid peroxidation levels, MDA measurement is used as an indicator of lipid peroxidation levels.[16]

The elevation of malondialdehyde (MDA) expression is a consequence of hyperoxidative conditions, representing an imbalance between antioxidants and pro-oxidants.[17] To address this imbalance, and antioxidants are required to address this imbalance. Antioxidants are molecules that are capable of scavenging and safely interacting with free radicals. Specifically, antioxidants can help reduce the negative chain reactions caused by free radicals and protect cell membranes from oxidative damage. Antioxidants can be endogenous (originating from within the body) or exogenous (coming from outside the body, through dietary habits or supplements, for example).[18] Therefore, it can be understood that the production of free radicals can be prevented by optimizing nutrition, especially by increasing the content of antioxidant nutrients in food.[19]

One plant known for its antioxidant properties is the bay leaf (*Syzygium polyanthum*).[20–22] Bay leaves are traditionally used as a food additive and has been scientifically proven to possess medicinal properties. The leaves, fruits, and bark of bay trees are traditionally used for various medicinal and non-medicinal purposes. The roots and fruits are consumed to counteract the effects of alcohol intoxication, while the leaves are traditionally consumed to treat various diseases, such as diabetes mellitus, hypertension, gastric problems, diarrhea, skin diseases, and infections. Another alternative for medicinal use of bay leaves is by making a decoction, usually by boiling several bay leaves in water until it becomes concentrated.[23] Phytochemical screening conducted by Widjajakusuma et al.[24] revealed that bay leaves contain tannins, alkaloids, steroids, triterpenoids, and flavonoids. These bioactive compounds are known for their antioxidant, anti-inflammatory, anticancer, antimicrobial, and antiviral properties.[25] This study aimed to examine the effects of bay leaf administration as an antioxidant in reducing malondialdehyde levels in male Wistar rats (Rattus norvegicus). This research is deemed necessary for further exploration, considering that herbal medicine is believed to have lower side effects and is relatively more cost-effective compared to synthetic drugs.[26]

Method

This study employed a pre-test post-test design with a control group to analyze the effects of bay leaf extract administration in reducing blood malondialdehyde levels in white rat samples. The study samples consisted of male Wistar white rats weighing 160-200 grams and aged 2-3 months. Rats were chosen as research subjects because of their characteristics and physiology that closely resemble humans, making them one of the most commonly used animals in biomedical research. Additionally, rats are larger than mice, and their adaptability to laboratory environments is superior. The selected rats had to meet the inclusion criteria, including male white rats, weighing–160-200 grams, aged 2-3 months, and having good physical health with no anatomical abnormalities. Various tools were used in this study, including rat cages, digital scales, blenders, stirrers, rotary evaporators, porcelain cups, measuring flasks, a tank or bucket for calming the rats, a stopwatch, 3 ml and 5 ml syringes, gloves, masks, blunt-tipped needle syringes, capillary blood pipettes, and a buncher funnel. The materials used included bay leaves, 90% ethanol, NaCl 0.9%, MDA Asay Kit (containing glacial acetic acid, trichloroacetic acid/TCA, and thiobarbituric acid/TBA), Mg, amyl alcohol, distilled water, HCL, male Wistar white rats, rat food, and water.

The process of producing bay leaf extract begins with the preparation of crude drugs (*simplicia*), followed by maceration using 70% ethanol solvent. The obtained solution was then filtered using a Buchner funnel and the filtrate was evaporated using a vacuum rotary evaporator at 70°C, resulting in a concentrated extract. For this study, 20 male Wistar white rats weighing 160-200 grams and aged 2-3 months were used. The rats were divided into four groups, with each group consisting of five rats, and subjected to treatment for 14 days. The control group underwent physical exercise using an Animal Treadmill at a speed of 20 m/min for 50 min/day for aerobic exercise and received distilled water per day per rat for 14 days. Three groups of rats were treated with bay leaf extract at doses of 3, 4, and 5 ml, respectively. After excessive physical activity, rats were given for approximately 1-hour rest. Subsequently, each rat received the bay leaf extract orally through a probe, ensuring careful insertion into the stomach without entering the lungs. Once the probe reached the stomach, the extract was pumped into it. Blood samples were collected via the retro-orbital plexus on the 1st day as a pretest and on the 15th day as a posttest. Blood malondialdehyde (MDA) levels were measured using the TBARs method, which involves the reaction of blood plasma with 20% TCA, 1% TBA, and 50% glacial acetic acid.

To assess the significance between experimental groups, one-way analysis of variance (ANOVA) was conducted at a 95% confidence level ($p < 0.05$). Further analysis or post hoc tests were performed using LSD.

Results

The phytochemical analysis conducted revealed the presence of secondary metabolites in bay leaf extract, including flavonoids, steroids, and tannins. These compounds are expected to contribute to reducing malondialdehyde (MDA) levels in the serum of Wistar white rats induced with excessive physical activity. Pre-test and post-test examinations of MDA levels in the white rat (Rattus norvegicus) serum were performed using the TBARs method, with the results designated as pre-test and post-test data. Based on the observations made in each experimental group, there were variations in the MDA levels in the serum of both the control and treatment groups.

Table 1. Serum Malondialdehyde (MDA) Levels

Group	Replication	*Pre-test* MDA level (nmol/ml)	*Post-test* MDA level (nmol/ml)
Control	1	1.21	7.12
	2	1.14	7.13
	3	1.12	7.16
	4	1.18	7.03
	5	1.09	7.07
	Average	1.14	7.10
Treatment 1	1	1.22	5.01
	2	1.05	5.09
	3	1.13	5.04
	4	1.12	5.02
	5	1.13	5.11
	Average	1.07	5.05
Treatment 2	1	1.15	3.98
	2	1.12	3.87
	3	1.11	3.71
	4	1.07	3.89
	5	1.16	3.96
	Average	1.12	3.88
Treatment 3	1	1.18	2.76
	2	1.12	2.61
	3	1.07	2.69
	4	1.05	2.77
	5	1.11	2.81
	Average	1.10	2.72

In Table 1, it can be observed that Treatment Group III with a dose of 5 ml exhibited lower post-test results compared to the other groups, with an average of 2.72 nmol/ml. The control group, subjected to excessive physical activity and provided with only distilled water (aquades), showed an average post-test MDA level of 7.10 nmol/ml, which is higher than the other groups. Treatment Group I with a dose of 3 ml yielded a post-test result with an average value of 5.05 nmol/ml, and Treatment Group II had an average of 3.88 nmol/ml.

Tabel 2. Post hoc LSD test results

Group		Mean difference	*p*
Control	Instrument 1	2.04800*	.000
	Instrument 2	3.22000*	.000
	Instrument 3	4.37400*	.000
Instrument 1	Control	-2.04800*	.000
	Instrument 2	1.17200*	.000
	Instrument 3	2.32600*	.000
Instrument 2	Control	-3.22000*	.000
	Instrument 1	-1.17200*	.000
	Instrument 3	1.15400*	.000
Instrument 3	Control	-4.37400*	.000
	Instrument 1	-2.32600*	.000
	Instrument 2	2.04800*	.000

Based on the Kolmogorov-Smirnov test, a significance value of 0.200 was obtained for all groups, indicating that the data is normally distributed ($p > 0.05$). After confirming the normal distribution of the data, a homogeneity test using the Levene test was conducted to determine whether the variances of each group in this research population were the same or homogenous. The homogeneity test results showed that the control group, treatment group I, treatment group II, and Treatment Group III originated from populations with the same variances, or these groups were homogenous ($p > 0.05$). The One-Way Anova test results showed a significant difference between the control and treatment groups ($p < 0.05$). Further post hoc LSD analysis was conducted to analyze the differences in the average MDA levels between the groups. With a significance value of 0.000 ($p < 0.05$), it can be concluded that all groups were significantly different from each other.

Discussion

The aim of this study was to examine the effectiveness of bay leaf extract and determine the most effective dosage for reducing the levels of malondialdehyde (MDA) induced by excessive physical activity in male Wistar white rats. Physical activity is defined as any body movement generated by skeletal muscle contraction that increases energy expenditure above the basal level, contributing to overall health.[27] While physical activity and sports are key factors in preventing, treating, and controlling chronic diseases, excessive physical activity can lead to adverse conditions.[28] Intense physical activity triggers changes in energy needs and strong stimulation of muscle tissue, resulting in the production of free radicals.[27] The accumulation of free radicals in the body leads to a phenomenon known as oxidative stress. Oxidative stress induces lipid peroxidation and generates the end product malondialdehyde (MDA). Malondialdehyde, or MDA, is a three-carbon molecule produced by free radicals, serving as a biomarker for oxidative stress and lipid peroxidation.[15] Increased MDA expression is caused by hyper oxidative conditions, indicating an imbalance between antioxidants and pro-oxidants. Therefore, antioxidants are required to address this imbalance[17]

Based on the obtained results, this research demonstrated a decrease in MDA levels in rats induced by excessive physical activity in each experimental group. The difference in the reduction in MDA levels can be observed in the varied average post-test values. The control group, which received aquades only, had an average MDA level of 7.10 nmol/ml. Treatment Group I, which received bay leaf extract at a dosage of 3 ml, had an average value of 5.05 nmol/ml. Treatment Group II, with a dosage of 4 ml, had an average value of 3.88 nmol/ml, and the last group, Treatment Group III, had an average result of 2.72 nmol/ml. Based on this comparison, it can be seen that bay leaf extract at a dose of 5 ml is more effective in reducing MDA levels in male Wistar white rats induced by excessive physical activity.

This effectiveness is attributed to the content of the bay leaf extracts, including flavonoids, tannins, and steroids. These bioactive compounds serve as antioxidants.[29] Their properties help the body by providing additional antioxidants to counteract the free radicals produced by excessive physical activity. In conclusion, bay leaf extract is effective in reducing MDA levels induced by excessive physical activity in male Wistar white rats.

Conclusion

The administration of bay leaf extract significantly reduced MDA levels, and based on the average MDA levels, it can be observed that the group with a dosage of 5 ml was superior compared to the other groups in reducing MDA levels in Wistar male white rats induced with excessive physical activity.

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