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Shiitake Mushroom Extract (*Lentinus Edodes*) Increases Superoxide Dismutase Levels and Collagen Quantity in Male Wistar Strain Rats (*Rattus Norvegicus*) Exposed to Ultraviolet B Light

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ABSTRACT:

Global warming boosts ultraviolet (UV) B rays, which penetrate the epidermis, reach the upper dermis, interact with cellular chromophores, damage DNA, raise oxidative stress, and accelerate photoaging. Shiitake mushroom extract (*Lentinus edodes*) contains protein, dietary fiber, essential amino acids, vitamins (C, D, B1, B2, B12, E), lipid molecules, polysaccharides, sterols, and terpenoids that have antioxidant, antitumor, antiviral, antihypertensive, and immune-modulating properties against UV exposure histology. This study investigated whether shiitake mushroom extract increases superoxide dismutase (SOD) and collagen levels in UVB-exposed male Wistar rats (Rattus norvegicus) skin. This randomized posttest-only control group study separated 36 healthy male Wistar rats, 200-220 grams and 3 months old, into two groups. The control group received UVB and aquabidest, while the treatment group received shiitake mushroom extract. ELISA was used to measure SOD levels in the blood after 28 days of therapy, while Adobe Photoshop and Image J were used to analyze collagen. The control and treatment groups had different collagen and SOD levels (73.917 and 79.806 pixels, respectively). Shiitake mushroom extract significantly increased SOD and collagen levels in rats exposed to UV light, as indicated by Saphiro-Wilk test p-values of 0.000 and Independent t-test p-values of 0.000 ($p < 0.05$). In conclusion, shiitake mushroom extract (*Lentinus edodes*) enhances serum SOD and collagen levels in UVB-exposed male Wistar rats (*Rattus norvegicus*).

Keywords: Superoxide dismutase, collagen, ultraviolet B, shiitake mushroom (*Lentinus edodes*).

Abstract

Purpose: The objective of this research was to examine the impact of shiitake mushroom (*Lentinus edodes*) extract on the levels of superoxide dismutase (SOD) and collagen in the skin of rats subjected to ultraviolet B (UVB) exposure. Additionally, the study aimed to assess the potential of this extract as a natural intervention for mitigating the effects of photoaging.

Patients and methods: Rats were divided into two groups: a control group, which only given distilled water, and a treatment group, which was administered the Shiitake mushroom extract. Both groups were exposed to UVB exposure. SOD levels and collagen amount in the skin tissue were measured and compared between the two groups.

Results: The treatment group showed significantly higher SOD levels and collagen amount compared to the control group.

Conclusion: The results suggest that shiitake mushroom extract has a positive effect on SOD levels and collagen amount and may be a potential natural agent for preventing photoaging due to UVB exposure.

Keywords: shiitake mushroom, superoxide dismutase, collagen, photoaging, ultraviolet B radiation

Introduction

The increase in environmental alterations has led to an elevated level of ultraviolet B (UVB) exposure. This exposure is well acknowledged as a catalyst for photoaging, a form of accelerated aging that occurs due to extended and repetitive contact with sun radiation[1]. The process of photoaging should not be seen solely as a matter of aesthetics, as it carries significant implications for the development of chronic illnesses[2]. Despite the global increase in life expectancy, there is a tendency to underestimate the significance of proactive health maintenance and disease prevention as individuals age[3]. Despite

the extensive efforts of the global scientific community to present more than 300 theories aiming to comprehend the process of aging, the quest for a universally applicable explanation continues to be challenging and unresolved. The free radical theory posits that the process of aging is attributed to the accumulation of molecular damage caused by ROS. This theory provides significant insights, with a particular emphasis on the decline in mitochondrial activity and the role of antioxidants as protective agents against aging [2,4]

The integumentary system, which encompasses the skin, serves as the outermost protective barrier of the human body. This vital organ is susceptible to various forms of environmental harm, particularly the detrimental effects of UVB radiation, which arise as a consequence of the loss of the ozone layer. The aforementioned radiation has the potential to induce photoaging, photo immunosuppression, and photocarcinogenesis. UVB radiation, characterized by its elevated energy levels, can permeate the outermost layer of the skin known as the epidermis. This penetration leads to several molecular alterations, such as the activation of transcription factors and the breakdown of collagen[3,5]. The occurrence of oxidative stress due to exposure to UVB radiation is a result of imbalances between enzymatic activity that promotes oxidation and the presence of oxidants. This imbalance contributes to the development of many pathological conditions, such as cancer and neurological illnesses[4]

Recent studies have highlighted the importance of antioxidants in mitigating the adverse impacts of free radicals[6]. Specifically, molecules such as SOD have been identified as effective agents in preventing oxidative harm[7]. The Shiitake mushroom (*Lentinula edodes*) is a dietary supplement that shows promise in boosting antioxidant capacity and maybe decreasing photoaging.

The shiitake mushroom, which is grown in several areas such as China, Japan, and more recently in certain regions of Europe and North America, serves as both a highly regarded ingredient in gastronomy and a longstanding component of traditional medicinal practices[8,9]. The mushroom possesses vital nutrients and bioactive compounds, such as lipids, polysaccharides, and terpenoids, which are recognized for their antioxidative, antineoplastic, and immunomodulatory characteristics[10]. Moreover, recent studies have revealed the efficacy of Shiitake extract in suppressing deleterious enzymes triggered by ultraviolet (UV) exposure, thereby demonstrating its potential as a constituent in anti-photoaging cosmetics[11].

This study seeks to examine the impact of oral administration of Shiitake mushroom extract on the levels of SOD and collagen amount in skin tissues following exposure to UVB radiation, taking into account the previously described obstacles associated with photoaging and the untapped potential of Shiitake mushroom extract in this context.

The primary objective of this study is to analyze the effect of administering shiitake mushrooms extract as a counteract against the effects of photoaging. Specifically, the research seeks to analyze the administered shiitake mushroom extract on the increase of SOD levels in of healthy male Wistar rats exposed to UVB rays.

Material and methods

The present work employs an experimental research methodology, specifically utilizing a randomized posttest-only control group design. The population under investigation in this study comprised male Wistar rats (Rattus norvegicus) aged between 3 months, which is considered similar to the age of an 18-year-old human[12,13]. The rats selected for the study had a weight range of 200-220 gram[s] [14]. The rats were accommodated, and subjected to daily monitoring, and samples were obtained at the Integrated Biomedical Laboratory Unit of the Udayana University Medical Faculty.. The shiitake mushroom extract was prepared at the Central Food and Nutrition Study Laboratory Unit of Udayana University. Concurrently, the assessment of SOD levels and skin collagen amount was conducted at the Integrated Biomedical Laboratory Unit of Udayana University. The study encompassed a duration of 6 weeks, which can be delineated as follows: During the initial week, the focus was on the preparatory measures, selecting procedures and the subsequent adjustment of the experimental animals. The administration of treatment specific to each group occurred for the duration of week II-V, totaling a period of 28 days. At last, during week VI the emphasis was placed on the assessment of SOD levels and collagen amount.

The primary ingredients utilized in the investigation encompassed desiccated shiitake mushrooms for extraction, aquabidest, Rat Food 594® sourced from PT Charoen Pokphand, mineral water, 96% ethanol, picro Sirius Red dye, and other additional substances. The necessary equipment consisted of plastic rat cages containing husk bedding, equipped with drinking bottles and feeding containers, and topped with wire mesh. In the experiment, a UVB light sourced from the Kernel brand, specifically the KN-4003 series, was employed in conjunction with several other instruments including hair shavers, a Tanita scale capable of measuring weight in grams, a 3 ml syringe, surgical knives, and stomach probes. The equipment utilized for the extraction of shiitake mushrooms encompassed a rotary evaporator, blender, centrifuge, Eppendorf pipette, test tubes, and vortex. In addition, the study employed several tools and equipment, including notebooks and data recording instruments, protective gloves and face masks, a Rat Superoxide Dismutase ELISA Kit for the examination of SOD levels, object glasses, microscopes, cameras, and computers equipped with software for the analysis of microscopic images.

The research design strategy is depicted in Figure 1. The male Wistar strain rats will be partitioned into two distinct groups. The initial group, referred to as the control group, comprises male Wistar rats that were subjected to ultraviolet B exposure and administered a placebo in the form of 2 cc aquabidest. The second group, referred to as the treatment group, consists of male Wistar rats that were subjected to UVB exposure and afterward administered with 2 cc of shiitake mushroom extract.

After collecting the data, the data will be analyzed statistically using the Statistical Package for the Social Sciences (SPSS) version 27 software[15]. The primary objective of a descriptive analysis is to provide a comprehensive presentation of the data through the use of tables and narrative format. The Shapiro-Wilk test is utilized for assessing data normality in this study, as the sample size in each group is smaller than 30. If the outcome of the analysis yields a p-value exceeding 0.05, it indicates that the data adheres to a normal distribution, hence allowing for the continuation of parametric analysis.

Subsequently, the process of Homogeneity Testing is undertaken. The Levene test is employed during this step to assess if the variance of the data is homogenous or heterogeneous. If the analysis yields a p-value exceeding 0.05, it suggests that the variance of the data is homogeneous. Once the normality and homogeneity of the data have been confirmed, a Comparative Analysis is performed to compare the treatment and control groups for each variable. The parametric independent t-test is used when the data is normally distributed and exhibits homogeneous variance. In contrast, when the data does not exhibit a normal distribution, the non-parametric Mann-Whitney test is employed. When the resulting p-value in the analysis is less than 0.05, it suggests that the independent variable exerts a statistically significant impact on the dependent variable.

Results

The research was carried out for a duration of 28 days at the Integrated Biomedical Laboratory of the Faculty of Medicine, Universitas Udayana. During the course of the study, two cohorts of Wistar rats were examined: a control group consisting of 18 rats that were subjected to ultraviolet B exposure and received a 2 cc dose of distilled water, and a treatment group that were exposed to ultraviolet B exposure and administered 400mg/kg/day of shiitake mushroom extract. Subsequently, both samples were sent for analysis to determine the amounts of SOD and collagen amount[14].

As it has been shown in Table 1, the investigation yielded a p-value of 0.000 ($p<0.05$) for the levels of SOD, suggesting a statistically significant impact of the shiitake mushroom extract in elevating SOD levels in rats subjected to UVB exposure. In a similar vein, the statistical analysis revealed a p-value of 0.000 ($p<0.05$) for collagen quantification, indicating a noteworthy impact of the shiitake mushroom extract in augmenting collagen amount in rats subjected to UVB exposure.

According to the depicted Figure 2, there is a notable augmentation in the number of collagen pixels observed inside samples subjected to treatment (T) with shiitake mushroom extract. The observed variations in the field of view between samples C and T. The figure 3 displays a histopathology image of a collagen pixel at a magnification of 400X.

Based on the visual representation provided, it can be observed that the control group samples (C) exhibit a reduced presence of collagen fibers and a lower overall amount in comparison to the treatment group samples (T), which display a greater abundance and amount of collagen fibers. The quantification of collagen amount in skin samples was performed through the application of the picro Sirius Red staining technique, and the samples were thereafter examined under an Olympus CX41 microscope.

Discussion

Effects of Shiitake Mushroom Extract (Lentinus edodes) on Superoxide Dismutase Levels and Collagen Amount in Wistar Male Rats (Rattus norvegicus) Exposed to Ultraviolet Light B

The detrimental consequences associated with sunlight's UV radiation encompass oxidative damage, inflammation, immunosuppression, premature skin aging, and DNA damage. Ultraviolet sunlight encompasses three distinct spectral regions, namely UVA (320-400 nm), UVB (280-320 nm), and UVC (200-280 nm). Merely a small fraction, around 5%, of UVB exposure can penetrate the surface of human skin, yet it is widely acknowledged as possessing significant toxicity. The capacity of UVB exposure to effectively traverse the epidermal layer results in direct alterations to the DNA structure, hence giving rise to various difficulties such as apoptosis. In addition, exposure to UV radiation leads to an overabundance of reactive oxygen species within cells, the production of inflammatory cytokines, and the development of matrix metalloproteinases.

The mitigation of skin damage can be achieved by the utilization of personal care products and cosmetics[16].

Superoxide dismutase is frequently included in these products owing to its capacity to mitigate the harm caused by free radicals.

Metalloenzymes are a collective of enzymes that protect against harm caused by reactive oxygen species (ROS). These enzymes facilitate the dismutation process of superoxide free radicals, converting them into molecular oxygen and hydrogen peroxide. It is worth mentioning that the shiitake mushroom, scientifically known as *Lentinus edodes*, has been seen to enhance the activity of SOD.

Lentinus edodes possess advantageous elements that contribute not only to general well-being but also to the augmentation of SOD levels, the promotion of collagen production, and the prevention of skin aging [17]. Prior research has demonstrated the robust antioxidant efficacy of the subject under investigation, resulting in the mitigation of cellular oxidative stress and elevation of SOD concentrations. *Lentinus edodes* is known to possess bioactive constituents such as carbs, proteins, fibers, lipids, vitamins, folate, niacin, and minerals, hence rendering it a valuable dietary resource characterized by high protein content and low-fat content. Moreover, shiitake mushrooms exhibit a wide array of bioactive compounds, with polysaccharides being the most prominent, renowned for their antioxidative, anti-neoplastic, anti-geriatric, and various other advantageous properties[8,10].

The present investigation involved the use of a sample comprising 18 Wistar rats, with an average weight of 200-220 grams, who were subjected to UVB exposure and administered a daily dosage of 400 mg/kg/day of shiitake extract for a period of 28 days. The results of the research revealed that the experimental group exhibited statistically significant elevations in SOD and collagen amount compared to the control group. The Independent t-test analysis demonstrated a statistically significant elevation in SOD levels within the group that received treatment. This finding suggests that the administration of either 400 mg/kgBB/day of shiitake extract had a beneficial impact on these levels in Wistar rats that were exposed to UVB exposure.

The process by which Shiitake mushroom extract enhances the activity of SOD is attributed to its elemental composition, which includes iron, zinc, and manganese[18]. The aforementioned elements play a pivotal role in facilitating the catalytic degradation of superoxide anions. The metallic cofactors present in mushrooms are involved in the process of catalyzing the single-electron oxidation and reduction reactions of superoxide anions. The presence of ergothioneine in shiitake mushrooms also serves as an antioxidant, leading to an elevation in serum SOD levels and a decrease in malondialdehyde (MDA). The concentration of ergothioneine in *Lentinus edodes* exhibits variability[19], however, it possesses the ability to stimulate intracellular antioxidant pathways, hence modulating the activity of enzymes such as SOD.

Moreover, the examination of collagen reveals a noteworthy impact of shiitake extract on the levels of collagen in the experimental rodents. This finding is consistent with the research conducted by Lee [11], which emphasized the mushroom's ability to regulate ROS following exposure to UV A and B exposure. *Lentinus edodes* is known to possess bioactive substances such as polyphenols and β-glucans, which have been found to exhibit inhibitory effects on matrix metalloproteinases and enhance the expression of collagen type I. The ability of Shiitake mushrooms to enhance collagen amount can be attributed to their constituent components, such as copper, manganese, and iron[20,21]. These compounds play a crucial role in promoting cross-linking between collagen and elastin, which is vital for maintaining skin health and providing protection against UV-induced damage. Copper, for example, plays a crucial role in the activation of the lysyl oxidase enzyme, which is responsible for facilitating collagen maturation and tissue connectivity. This underscores the significance of minerals like copper in maintaining skin health.

Benefits of shiitake Mushroom Extract (Lentinus edodes) as anti-aging

Aging is attributed to both internal and external factors. One of the key external factors is excessive exposure to UV rays, which increases oxidative stress[22]. The fundamental principle behind anti-aging suggests that the aging process can be prevented or slowed if we avoid these causative factors, leading to an improved quality of life [6]. The shiitake mushroom (*Lentinus edodes*) plays a significant role in preventing aging. This is due to its high antioxidant content, notably l-ergothioneine, believed to not only prevent oxidative damage but also enable the repair of DNA in UV-irradiated cells[23].

Lentinus edodes contains compounds like proteins, carbohydrates, fibers, phosphorus, potassium, copper, zinc, thiamine, riboflavin, niacin, magnesium, selenium, pantothenate, vitamin B6, folate, choline, and vitamin D[10]. Some of these components, such as copper, riboflavin, niacin, selenium, and zinc, are essential for the health of the skin, hair, nails, and other tissues and play an active role in preventing premature aging, enhancing skin brightness, treating skin issues, increasing collagen production, rejuvenating the skin, and preventing acne[24]. Previous research has shown that shiitake mushroom extract enhances the levels of SOD and collagen in UVB-exposed skin[7]. Hence, this extract is a promising anti-aging remedy, especially against oxidative stress due to UVB exposure.

Oxidative stress is a primary factor in premature aging caused by UVB exposure. This stress can be reduced by increasing antioxidants. While various compounds such as vitamins C and E, as well as riboflavin independently serves as an antioxidant or as a component of the glutathione redox cycle. Bourgonje et al[25] suggest that riboflavin can protect the body from oxidative stress by boosting SOD levels.

Another vital mineral found in shiitake mushrooms is copper, crucial for all body tissues. Studies have shown that copper actively participates in skin repair processes[26], and has therapeutic applications in anti-aging cosmetics, enhancing the hyaluronic acid levels for improved skin appearance[23,26]. Lentinus edodes also contains vitamin C (L-ascorbic acid), which is water-soluble and vital for skin care. Vitamin C acts as a cofactor for enzymes crucial for collagen stability[16]. Furthermore, a combination of ascorbic acid and vitamin E has been shown to combat UVB-induced skin damage.

Lycopene, in particular, offers protection against UV-induced oxidative damage to tissues. Another compound involved in anti-aging is vitamin D. It acts as a prohormone in humans and is synthesized primarily through UVB-mediated reactions in the skin. As we age, the skin's capacity to produce vitamin D3 diminishes, reducing its protective effects[26]. Based on the literature, shiitake mushrooms have various components that can prevent aging by counteracting oxidative stress from excessive UVB exposure. Hence, the phytochemicals in shiitake mushrooms can potentially elevate superoxide dismutase and collagen levels, delaying skin aging. Further research can elucidate the effectiveness of shiitake mushrooms against UVB-induced oxidative stress and early aging.

Conclusion

Based on the research conducted, it can be concluded that the analysis results show a significant difference between the treatment group (rats given shiitake mushroom extract) and the control group (rats not given shiitake mushroom extract) in terms of SOD levels and the amount of collagen in the skin.

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Disclosure

The author reports no conflicts of interest in this work.

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Table 1 Analysis of the effect of shiitake mushroom extract on superoxide dismutase and collagen levels in serum of test rats exposed to ultraviolet B light.

	N	*Mean*	SD	p-*value*	95% CI
SOD Level					
Control	18	0,2546	0,07446	0,000	0,22-0,28
Treatment	18	0,3965	0,07284		0,36-0,42
Collagen Count					
Control	18	73,917	4,4895	0,000	71,84-75,87
Treatment	18	79,806	4,8222		77,54-81,91

Figure 1 Research Design

Figure 2 Pixel Comparison Chart of Collagen Samples Control and Treatment

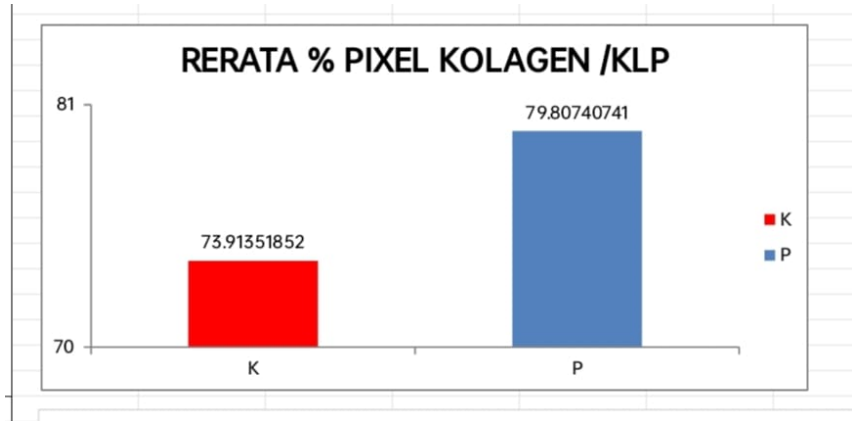

Figure 3 Comparison of 400X magnification collagen pixel histopathology images of samples Control (C) and Treatment (T).