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The Effect of *Bajakah* Wood (Spatholobus Littoralis Hassk.) Extract on Carboxymethyllysine (CML) Glycation Product in Rats (Rattus Norvegicus) and its Review from an Islamic Perspective

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Keywords
Kayu Bajakah, Carboxymethyllysine (CML), Glycation, Skin Aging, Islamic Perspective

Abstract
This study addresses the growing concern of skin aging associated with glycation processes, particularly the accumulation of advanced glycation end products (AGEs) such as Carboxymethyllysine (CML), which contribute to oxidative stress and tissue damage. The research aims to evaluate the effect of *bajakah* wood (Spatholobus littoralis Hassk.) extract on CML levels in D-galactose-induced aging model rats and to examine its relevance from an Islamic perspective. An experimental quantitative design was employed using 36 male Sprague-Dawley rats divided into six groups, including control and treatment groups receiving varying doses of *bajakah* wood extract. CML levels were measured using the ELISA method after treatment. The results showed that although there were variations in mean CML levels across groups, statistical analysis using One-Way ANOVA indicated no significant effect of *bajakah* wood extract on reducing CML levels ($p > 0.05$). The positive control group treated with vitamin C exhibited the lowest CML levels, while treatment groups showed fluctuating results. In conclusion, *bajakah* wood extract demonstrated limited in vivo antiglycation effects under the experimental conditions. However, its bioactive compounds indicate potential for further investigation, particularly with improved dosage, duration, and methodological approaches, while aligning with Islamic principles of halal and beneficial use.

INTRODUCTION

The skin has various physiological functions such as an initial barrier against pathogens, ultraviolet (UV) rays, chemicals or other injuries, maintaining body temperature, preventing water loss from the body, producing pro vitamin D, detecting and fighting infections and providing sensory information (touch, pressure, temperature, and pain nosiception) (Knaggs and Lephart, 2023). Skin aging is a process in which skin quality decreases with age due to the synergistic effects of chronological aging, photo-aging, hormonal deficiencies and environmental factors (Chaudhary et al., 2019).

In 1950, the number of elderly people was found to be nearly 205 million worldwide. This number almost increased 4 times in 2012 to a total of 810 million. The world's population aged 60 and over is expected to increase by 22% by 2050. One in four people will be over 60

years old, it is estimated that more than 8% of the population of the Southeast Asian region is over 60 years old (Chaudhary et al., 2019). Skin aging can be divided into chronological aging (intrinsic aging) and biological aging (external aging). Chronological aging of the skin occurs throughout the body caused by internal factors such as hormone levels, genotype and metabolism that occur naturally and are difficult to change. Biological aging is caused by external factors namely ultraviolet radiation, nutrient levels, and chemical pollution, this aging can be delayed by avoiding these factors (Cao et al., 2020). In some studies, it has been shown that a close relationship between sugar intake and amino acids forms a glycation reaction (Andersen & Winter, 2019; Bangar et al., 2022; Boismal et al., 2020). The mechanism is related to the skin's advanced glycation (AGE) end product (Cao et al., 2020). Glycation is a non-enzymatic reaction that takes place gradually in the form of Amadori compounds followed by the formation of Maillard and AGE's compounds. AGE products affect the formation of oxidative stress and inflammatory factors (Salazar et al., 2021).

Carboxymethyllysine (CML) is one of the main AGE products in the skin that serves as an indicator of collagen glycation. Glycated collagen induces CML expression in the dermis and epidermal compartments and then produces the aging phenotype (Zheng et al., 2022). CML levels increase with age, which can then cause damage to proteins and tissues by forming cross-bonds, which can interfere with normal protein function. These modifications cause cellular dysfunction and contribute to the aging process. CML can accumulate thereby activating the inflammatory pathway (NF-κB) through its interaction with RAGE (Receptor for Advanced Glycation End products). RAGE can also increase the production of reactive oxygen species (ROS) which can cause oxidative stress which contributes to aging (Chaudhuri et al., 2018).

Oxidative stress caused by increased ROS due to RAGE interaction with CML can be inhibited by logwood extract which acts as an antioxidant, anti-inflammatory, and photoprotective, as well as anti-aging (Kursiussamawati et al., 2024; Kusuma et al., 2023; Mahfudh et al., 2024; Nurfitri et al., 2021). *Bajakah* plants (Spatholobus littoralis Hassk) come from the Leguminosae family which contains ethanol extract and has high antioxidant activity. The phytochemical composition of *bajakah* includes flavonoids, saponins, steroids, terpenoids, tannins, and phenol compounds (Denayu Pebrianti et al., 2023; Gu et al., 2020; Hou et al., 2019). Flavonoid compounds inhibit the activity of age-related enzymes such as tyrosinese, elastase, collagenase, and hyaluronidase (Novalia Rahmawati Sianipar et al., 2023). *Bajakah* wood is a material derived from plants so it is categorized as halal in substance (halal li dzatih) because there are no sharia provisions that prohibit its use, so that all types of plants can be used for consumption and medicine (Păcularu-Burada et al., 2024; Rosmarwati et al., 2022; Umbayev et al., 2020). The halalness of a product is determined by four main aspects, namely the halal of the substance, the halal way of obtaining it, the halal processing process, and not harming the user or fulfilling the concept of thayyib (Jumiono et al., 2023). The Qur'an describes that Allah provides various plants as a source of nutrition, medicine, and means of sustaining life as stated in QS. An-Nahl verse 69.

This study used a test animal of male white rats of the Sprague-Dawley strain, this type of rat has many physiological and genetic similarities with humans making it a suitable model for studying human diseases and biological processes such as glycation reactions (Khan et al., 2019). In addition, research in the laboratory allows controlled variables such as the environment to be manipulated to study the specific effects of AGE (Zgutka et al., 2023). This

research is within the framework of al-Maslahah al-Dhahuriyyah, which is an effort to obtain important scientific benefits for human health while still paying attention to the principles that do not cause harm (لا ضرر ولا ضرار) and ensuring that the animals used are treated appropriately so that they are in line with the rules of fiqh where something that was originally mubah can still be used if the purpose of its use brings greater good and is carried out within ethical boundaries that has been determined by the sharia. This study was made to examine the effect of *bajakah* wood extract whose compounds can inhibit the glycation activity of carboxymethyllysine so that it can delay aging in the skin. In addition, the author also provides information and ideas that can be used for the development of future research.

METHOD

This research was a quantitative experimental one conducted at the Integrated Research Laboratory of YARSI University. In this study, male white rats of the Sprague-Dawley strain were injected with D-galactose 500 mg/kgBB and will be tested for CML activity by ELISA after being given ethanol extract of red logwood (Spatholobus Littoralis Hassk.).

Research Design

In this study, male white rats of the Sprague-Dawley strain were injected with D-galactose 500 mg/kgBB and will be tested for CML activity levels after being given ethanol extract of red *bajakah* wood (Spatholobus Littoralis Hassk.). The determination of mice that will be included in the blank group, control and treatment was carried out randomly from the study subjects. From each group of treatment induced by D-galactose, CML activity will be measured by ELISA.

The subjects in this study were test animals in the form of 36 male rats (Rattus norvegicus) of the Sprague-Dawley strain, while the object of the study was the activity of Carboxymethyllysine (CML). The study population included mice of the Sprague-Dawley strain selected based on inclusion and exclusion criteria. Inclusion criteria included male white rats of the Sprague-Dawley strain weighing 170–200 grams, aged 2–3 months, and randomly selected from the IPB laboratory. Meanwhile, the exclusion criteria were mice that had health conditions that did not allow them to be involved in the study. The determination of sample size was carried out using Federer's formula (1963). With six treatment groups, the calculation showed a minimum number of four mice per group, so that a total of at least 24 were obtained. To anticipate the possibility of losing samples during the study, the number of test animals was increased to six per group, bringing the total number of mice used in the study to 36. The independent variable in this study was the administration of red logan wood extract (Spatholobus littoralis Hassk.), the bound variable was CML activity, while the control variables included rat type, weight, age, environmental temperature, feed, and cage.

In the study, 36 male rats of the Sprague-Dawley strain were used with a body weight of 175–200 grams and a age of 2–3 months. During the study, the experimental animals were kept at room temperature with a 12-hour light–dark cycle, given standard feed and drinking water ad libitum. The mice were divided into six groups, each consisting of six tails, and placed in cages measuring 17.5 × 23.75 × 17.5 cm. All animals were tried to be acclimatized for seven days to reduce stress and standardize conditions before treatment began. After the adaptation period, on day 8 all groups except the blank group were induced with D-galactose at a dose of 500 mg/kgBB through subcutaneous injection until day 56. The blank group was given only

0.9% saline solution (w/v) through subcutaneous injection from day 8 to day 56. The negative control group was given D-galactose from day 8 and then on days 29 to day 56 were given 0.9% saline. The positive control group was given D-galactose from day 8 and on days 29 to day 56 was given vitamin C at a dose of 50 mg/kgBB. Treatment groups I, II, and III were given D-galactose from day 8, then on days 29 to 56 they were given subcutaneous red manure wood ethanol extract with doses of 25 mg/kgBB, 50 mg/kgBB, and 100 mg/kgBB respectively. On the 57th day, blood and skin organs were collected from all experimental animals to check the level of CML activity in male Sprague-Dawley rats.

RESULT AND DISCUSSION

Description of research data

This study used blood serum from male Sprague Dawley white rats with an age of 7-8 weeks, with a body weight of 170-200-grams of healthy mice, seen with active movement, wanting to eat, and drinking obtained from the Laboratory of Parasitology & Experimental Animals of the Center of the Health Biology Laboratory, Bogor City, West Java. This study used 6 groups of mice, each group of 6 and the study was conducted for 8 weeks, 7 days before adaptation was carried out and then given treatment, and every week weight was weighed. During the study, all the mice given the treatment did not die.

This study was conducted experimentally with different treatments given to each group of rats consisting of 6 groups, for group 1 was given 0.9% NaCl injection treatment in week 2 to week 8 as a blank group, groups 2, 3, 4, 5, and 6 in week 2 to 8 were given D-galactose injection treatment of 500 mg/kgBB, in week 5 to week 8 plus treatment, for the 2nd group was given 0.9% NaCl orally as a group negative, group 3 was given vitamin C 50 mg/kgBB orally as a positive control, group 4 was given 25 mg/kgBB of logged-in ethanol extract orally as treatment 1, group 5 was given 50 mg/kgBB of logged-in ethanol extract orally as treatment 2, and group 6 was given 100 mg/kgBB of logged-in ethanol extract orally as treatment group 3. After being given treatment, the next day, blood was taken to examine skin aging markers, namely CML using ELISA.

Serum that has been stored in a frozen condition is first thawed at room temperature before ELISA analysis is performed. Of each treatment group, the serum used was the serum with the clearest appearance, did not experience hemolysis, and did not show the presence of deposits. This selection aims to minimize matrix disturbances that can affect antigen–antibody reactions and absorbance readings, so that the CML measurement results obtained are more accurate and reliable. The procedure for quantitative testing of mouse CML by the ELISA method starts from the manufacture of a standard CML solution which is prepared as the basis for the formation of a standard curve used for the calculation of sample concentration. The standard high-concentration stock is first reconstituted using a standard diluent as directed, then slowly homogenized and left for a few minutes to allow the protein to dissolve completely. After that, serial dilution is carried out to produce several standard concentrations that are within the working range of the kit (400 ng/ml, 200 ng/ml, 100 ng/ml. 50 ng/ml, and 25 ng/ml). Each standard concentration is prepared sequentially and precisely, as it determines the quality of the standard curve. The standard curve serves as a quantitative reference that relates the absorbance value to the CML concentration.

The test procedure begins with determining the number of microplate wells (wells) to be used, the total number of wells used is 60. Then the standard solution is fed into the wells and the sample into the test well according to the specified volume. Biotinylated anti-CML antibodies are added to the wells, while standard wells do not require the addition of these antibodies. Next, streptavidin-HRP is added to almost the entire well to form an antigen–antibody–enzyme complex. The microplates are then closed and incubated at 37°C for 60 minutes so that the immunological interaction takes place in a maximum and specific manner.

After incubation, the microplate is washed 5x repeatedly using a wash buffer to remove any reagents that are not specifically binding. The washing stage is carried out to remove antibodies and enzyme conjugates that do not bind specifically to the antigen. Proper washing plays an important role in lowering the reaction background and improving the accuracy and precision of ELISA results. Next, chromogenic substrates (substrates A and B) are added to each well, which will react with the HRP enzyme and produce a discoloration. The color reaction is stopped using a stop solution so that the color becomes stable. The absorbance value was then measured using a microplate reader at a wavelength of 450 nm. Standard absorbance data is used to form a standard curve, while the concentration of CML in a sample is determined through interpolation against the curve.

The results of the measurement of Carboxymethyl Lysine (CML) levels showed that the lowest value was found in group 3, namely the positive control group who were given D-galactose induction at a dose of 500 mg/kgBB and vitamin C at a dose of 50 mg/kgBB orally, with an average CML level of 718.385 ng/mL. This value was lower than group 2, namely the negative control group that was induced by D-galactose at a dose of 500 mg/kgBB and administered NaCl orally, with an average CML level of 791.663 ng/mL. Meanwhile, the highest CML levels were found in group 5, namely the treatment group that was given D-galactose induction at a dose of 500 mg/kgBB and red logan wood ethanol extract at a dose of 50 mg/kgBB. with an average CML level of 859.533 ng/mL. The other treatment groups, namely group 4 (red logwood ethanol extract at a dose of 25 mg/kgBB) and group 6 (red logwood ethanol extract at a dose of 100 mg/kgBB), showed average CML levels of 789.028 ng/mL and 771.576 ng/mL, respectively, which are still numerically within the range of variation between groups.

The results of statistical analysis using One-Way ANOVA showed a value of $p = 0.631$ ($p > 0.05$), which indicates that there was no statistically significant difference in CML levels between all treatment groups. Post-Hoc follow-up test results showed that all treatment group pairs did not have statistically significant differences ($p > 0.05$). Although the lowest p value was found in the comparison between group 3 and group 5, administration of red logwood ethanol extract at doses of 25, 50, and 100 mg/kgBB did not have a significant effect on CML levels compared to the control group, both negative and positive control. Overall, although there were differences in mean CML levels between treatment groups, the differences were not statistically significant, suggesting that data variation within each group was still more dominant than the treatment effect on CML levels.

This study is an in vivo study designed to evaluate the effect of administering ethanol extract of *bajakah* wood (Spatholobus littoralis Hassk) in aging model mice induced with D-galactose at a dose of 500 mg/kgBB on CML levels used as a biomarker of skin aging. High doses of D-galactose induction can cause oxidative stress resulting in damage to various organs

and tissues, thereby accelerating the aging process biochemically and morphologically (Kartika et al., 2023).

Effect of *bajakah* wood extract on CML concentration

Carboxymethyllysine (CML) is one of the main products of advanced glycation end products (AGEs) in the skin that acts as an indicator of collagen glycation. Glycated collagen can induce increased expression of CML in the dermis and epidermal compartments, which further contributes to the emergence of the skin aging phenotype (Zheng et al., 2022). The results of this study showed that the administration of ethanol extract of *bajakah* wood (Spatholobus littoralis Hassk) at doses of 25, 50, and 100 mg/kgBB in D-galactose-induced mice did not have a statistically significant effect on CML levels ($p > 0.05$). The One-Way ANOVA test yielded a value of $p = 0.631$, indicating that there was no significant difference in CML levels between all treatment groups, either compared to the negative or positive control groups. However, descriptively, there was a variation in the average value of CML levels between groups. The positive control group given vitamin C showed the lowest levels of CML, while the treatment group showed values that fluctuated between doses. At a dose of 100 mg/kgBB, there was a difference in the mean value of CML levels compared to negative controls, but the difference did not reach statistical significance, suggesting that the variability of the data in the group was more dominant than the treatment effect.

Bajakah plants (Spatholobus littoralis Hassk) from the Leguminosae family are known to be rich in bioactive compounds such as flavonoids, phenolics, and alkaloids that have the potential to inhibit the formation of AGEs including CML (Novalia Rahmawati Sianipar et al., 2023). The study of Wang et al., 2019) reported that extracts rich in phenolic compounds were able to inhibit the formation of CML through the capture mechanism of reactive dicarbonyl compounds such as glyoxal. However, the study was conducted on an in vitro model with a relatively simple system, so that the interaction between phenolic compounds and CML precursors takes place directly. On the other hand, in this study an in vivo model was used, where the effectiveness of bioactive compounds was greatly influenced by bioavailability factors, metabolism, tissue distribution, and elimination of active compounds in the body. This difference in the study model may explain why the inhibition potential of CML reported in in vitro studies has not been fully reflected in the results of this study.

In addition, the insignificance of the difference in CML levels between groups is also suspected to be influenced by several methodological factors. The relatively limited number of samples in each group can degrade the strength of the statistical test, so that biologically significant differences may not have been detected. The relatively short duration of D-galactose induction, which is six weeks, is estimated to be insufficient to produce optimal accumulation of Carboxymethyllysine (CML) because CML is the end product of the advanced glycation process that is formed chronically and gradually from AGEs. In addition, the dose of D-galactose used in this study may not be high enough to induce the formation of CML to the maximum, so in future studies the use of larger galactose doses, for example above 500 mg, as well as a longer duration of induction may be considered. Nastati and Nugraha, (2022) reported that ethanol extract from logan wood has strong anti-inflammatory activity, but the process of forming CML is a long-term non-enzymatic process, so it requires interventions with a certain duration or dose so that its inhibitory effects can be meaningfully detected. Thus, the results of this study show that the administration of *bajakah* wood ethanol extract at a dose of 25–100

mg/kgBB has not been shown to be significantly effective in reducing CML levels in D-galactose-induced aging model mice, although descriptively there is a tendency for differences that indicate biological potential for further research.

CONCLUSION

The findings of this study indicate that the administration of ethanol extract of *bajakah* wood (*Spatholobus littoralis Hassk*.) at doses of 25, 50, and 100 mg/kgBW in D-galactose-induced rats did not produce a statistically significant effect on *Carboxymethyllysine* (CML) levels ($p > 0.05$). Although descriptive analysis showed variations in mean CML levels across treatment groups, the statistical results suggest that the observed differences were not sufficient to confirm a measurable antiglycation effect under the experimental conditions. These results imply that, while *bajakah* wood contains bioactive compounds such as flavonoids and phenolics with known antioxidant potential, there in vivo efficacy in inhibiting glycation processes may be limited by factors such as bioavailability, metabolic pathways, and experimental duration. Nevertheless, the study still highlights the biological potential of *bajakah* wood as a natural compound with possible anti-aging properties, supported by both scientific rationale and its alignment with Islamic principles of halal and beneficial use.

Future research is recommended to address the limitations identified in this study by employing larger sample sizes to increase statistical power and extending the duration of D-galactose induction to allow for more pronounced accumulation of glycation end products. Additionally, exploring higher or more varied dosage ranges, as well as alternative extraction methods, may help optimize the bioactivity of *bajakah* compounds. Further investigations using different experimental models, including in vitro and clinical studies, are also necessary to better understand the mechanisms of action and therapeutic potential of *bajakah* wood in inhibiting AGE formation. Moreover, integrating molecular analyses, such as oxidative stress markers and gene expression profiling, could provide deeper insights into the pathways involved. Such advancements will contribute to strengthening the evidence base for the development of *bajakah* wood as a natural anti-aging agent and its application in biomedical and pharmaceutical contexts.

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