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The Effects of Olive Oil (Olea Europaea L.) on Cathallase Enzyme Levels in White Rats (Rattus Norvegicus) Wistar Strain of Obese Male

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A B S T R A C T

Obesity is a condition of the body resulting from excessive fat accumulation which leads to health problems. The increase in fat metabolism affects increased production of free radicals in the circulation and in adipocyte cells. Free radicals that increase in the body are not in line with antioxidant enzymes, causing a condition called oxidative stress. The body's defense mechanism against free radicals is the synthesis of antioxidants. When there is a decrease in the activity of endogenous antioxidants such as the catalase enzyme, it provides a strong indication of oxidative stress. The presence of polyphenols and monounsaturated fatty acids in olive oil is useful for preventing chronic damage that causes oxidative stress. The catalase enzyme can be used as a parameter for an uncontrolled increase in ROS (Reactive Oxygen Species) or disruption of the function of the antioxidant defense system. To determine changes in levels of the catalase enzyme in an obese male Wistar strain white rat (Rattus norvegicus) animal model that was given olive oil. This research used a true experimental laboratory with a pre-post test design. The results of the study were significant changes in catalase enzyme levels in the olive oil intervention group (p-value 0.003<0.05) on day 14 and day 28 with (p-value 0.002<0.05 and 0.005<0.05). In the group given olive oil, the effect on the level of catalase enzyme was most significant on day 28 (p-value 0.002<0.05).

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INTRODUCTION

Olive oil is obtained by crushing and pressing olives. The contents of olive oil are monounsaturated fatty acids (MUFAs), unsaponifiable compounds (squalene, sitosterol, triterpenes, etc.), and hydrophilic compounds (tocopherol, phenolic compounds).(Mustikyantoro APJ, 2020). Among antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) are crucial for protecting cells from oxidative damage. Consuming exogenous antioxidants from fruits and vegetables can enhance the antioxidant defense system, reducing oxidative stress and the risk of certain cancers where reactive oxygen species (ROS) accumulate and surpass the body's ability to neutralize them. Olive leaves from Olea europaea contain a variety of polyphenolic compounds that exhibit a wide range of biological activities, including antimicrobial, antiviral, anti-inflammatory, anti-carcinogenic, anti-allergic, anti-thrombotic, cardioprotective, and vasodilatory effects (Pennisi et al., 2023).

The presence of polyphenols and monounsaturated fatty acids is useful for preventing chronic damage caused by oxidative stress. The tocopherol contained in olive oil is important for its nutritional and antioxidant properties, so it can protect fat components from excessive oxidation.(Tan ST et al., 2020)

High antioxidants are able to detoxify lipid peroxides, one of which is the endogenous antioxidant produced, namely the enzyme catalase. The catalase enzyme can capture and decompose free radicals in cells which makes substances less reactive. Other important roles can be. catalyzes hydrogen peroxide to H2O and O2 also prevent the formation of CO2 bubbles in the blood.(Arifah MF et al., 2019). Glutathione peroxidase (GSH-Px) is an enzymatic antioxidant that detoxifies hydrogen peroxide and lipid hydroperoxides by converting or preventing the formation of free radicals into less reactive molecules. Its activity is reduced in diabetic conditions. Alloxan, a derivative of uric acid, induces pancreatic beta cell necrosis by increasing oxidative stress, which leads to decreased insulin secretion and resulting hyperglycemia. Catalase, a specific peroxidase enzyme, decomposes hydrogen peroxide into oxygen and water, serving as a crucial defense mechanism against free radicals in the human body (Okvenda et al., 2023).

Previous research has demonstrated that olive leaf possesses significant antioxidant properties, attributed largely to its phenolic compounds. In insects, the immune system comprises both cellular and humoral defense mechanisms, with circulating hemocytes playing a crucial role in defending against potential pathogens. These hemocytes' immune functions are closely linked to the secretion and release of endogenous enzymes through processes like exocytosis and degranulation. Enzymes such as superoxide dismutase (SOD), peroxidase, and catalase (CAT) are vital antioxidants that help eliminate harmful reactive oxygen species (ROS) while combating infections. Activated hemocytes can undergo an "oxidative burst," releasing ROS and retaining various oxygen ions, free radicals, and peroxides. Due to their unpaired valence electrons, these molecules are highly reactive. ROS are essential for cell signaling and the activation of immune-related genes, and recent studies have highlighted their crucial role in invertebrate immune responses. However, an excessive "oxidative burst" can lead to tissue damage if ROS production overwhelms the cell's antioxidant defenses, causing oxidative stress. Antioxidants are crucial for mitigating this damage by removing excess ROS and protecting the host from oxidative harm (Okvenda et al., 2023).

Obesity is a condition of the body resulting from excessive fat accumulation which leads to health problems such as psychosocial problems, abnormal glucose metabolism, sleep apnea, increased risk of cardiovascular disease, complications of bone problems, gastrointestinal and liver disorders, and even death (Zulfahmidah et al., 2021). The condition of obesity in Indonesia has increased from year to year. According to data (Ministry of Health, 2018) the prevalence of obesity among those aged 18 years and over increased from 14.8% in 2013 to 21.8% in 2018. In obese sufferers, morbidity and mortality rates increase with shorter life expectancy. Increased fat metabolism affects the production of free radicals in the circulation and in adipocyte cells. Free radicals that increase in the body are not in line with antioxidant enzymes, causing a condition called oxidative stress. Oxidative stress that occurs in the body results in damage to cells, organs or tissues. The body's defense mechanism against free radicals is in the form of antioxidant synthesis. When there is a decrease in the activity of endogenous antioxidants such as the enzyme catalase, it provides a strong indication of oxidative stress. Oxidative stress can cause cell damage which can lead to various diseases. The catalase enzyme can be used as a parameter for an uncontrolled increase in ROS (Reactive Oxygen Species) or disruption of the function of the antioxidant defense system.(Salma Rihadatul Aisy N et al., 2021; Silviani D et al., 2022)

In the results of (Jimenez-Lopez C et al., 2020) stated that the phenolic content of olive fruit oil has anti-inflammatory, antimicrobial, antioxidant, antitumor activity and is able to modulate gene expression to protect proteins that play a role in cellular mechanisms involved in inflammatory processes, lipid metabolism and oxidative stress resistance, apart from the results of research by (Mazzocchi A et al., 2019), it is stated that apart from phenolics, another content in olive oil, namely hydroxytyrosol, is able to modulate the gene expression profile of adipocytes.

Considering the background provided and supported by existing studies, the investigation into the impact of olive oil on catalase enzyme levels in obese male mice is crucial due to the lack of prior research in this area. The study was conducted using Wistar strain white rats (Rattus norvegicus) as experimental animals, induced with obesity.

THEORETICAL REVIEW

Olive oil is obtained from olives (Olea europapea L.) which is a plant found in the Mediterranean. Since the time of the prophet, olives have been used for cooking and making medicines. This plant is also distributed in South and East Asia, Macronesia and South and East Africa.(Mustikyantoro APJ, 2020; Rahmasari EN & Puspitorini A, 2020)

METHODOLOGY

This research employed the true experimental laboratory method with a pre-post test design, utilizing one group as the intervention group and a control group to determine the effect of olive oil administration on catalase enzyme levels in obese male Wistar strain white rats (Rattus norvegicus).

The samples were grouped using the cluster random sampling method and met the inclusion and exclusion criteria. The samples were divided into 3 groups. Each experimental group consisted of 9 rats, with a total of 3 treatment groups. Additionally, there were 6 backup samples per treatment group, making a total of 45 rats used in this study. Data analysis was conducted using the SPSS (Statistical Program for Social Science) program.

RESULTS

Enzyme Levels in White Rat Sample Groups

Table 2. Catalase Enzyme Levels in Groups of Obese White Rats without Treatment (K1), Obese White Rats with Orlistat (K2), Obese Rats Treated with Olive Oil (K3)

Group Intervention	Average Sample Enzyme Levels		
	Pre	Post 14 days	Post 28 days
Obese rat without treatment	0.349±0.068	0.354±0.067	0.317±0.084
Obese rat with orsilat	0.0376±0.123	0.551±0.283	0.484±0.131
Obese rat with treatment	0.277±0.047	0.417±0.195	0.514±0.141

In this study, showed that the average enzyme level in G1 before intervention was 0.349 kU/L; post 14 days of intervention was 0.354 kU/L and post 28 days was 0.317 kU/L. In G2 before intervention it was 0.376 kU/L; post 14 days intervention 0.551 kU/L; and post 28 days 0.484 kU/L. and catalase enzyme levels in G3 before intervention were 0.277 kU/L; post 14 days of intervention was 0.417 kU/L; and post 28 days of 0.514 kU/L.

Correlation of Enzyme Levels in White Rats Based on Time

Table 3. Correlation of Catalase Enzyme Levels on Pre-Test, Post-Test Day 14 (Post H14), and Post-Test Day 28 (Post H28)

Group	Time		*P-Value*	
G1	*Pre-test*	*Post*H14	0.496[b]	0.056[a]
		*Post*H28	0.078[b]	
	*Post*H14	*Pre-test*	0.496[b]	
		*Post*H28	0.021[b]	
	*Post*H28	*Pre-test*	0.078[b]	
		*Post*H14	0.021[b]	
G2	*Pre-test*	*Post*H14	0.013[b]	0.038[a]
		*Post*H28	0.529[b]	
	*Post*H14	*Pre-test*	0.013[b]	
		*Post*H28	0.073[b]	

	*Post*H28	*Pre-test*	0.529[b]	
		*Post*H14	0.073[b]	
G3	*Pre-test*	*Post*H14	0.005[b]	0.003[a]
		*Post*H28	0.002[b]	
	*Post*H14	*Pre-test*	0.005[b]	
		*Post*H28	0.598[b]	
	*Post*H28	*Pre-test*	0.002[b]	
		*Post*H14	0.598[b]	

[a]*Oneway Anova Test*
[b]*PostHoc Test*

The study found a significant relationship between catalase enzyme levels and time differences in both the group treated with orsilate (p-value 0.038) and the group of white mice treated (p-value 0.003<0.05). Notable changes in catalase enzyme levels were observed between the Pre-test and Post day 14 (p-value 0.005) and the Pre-test and Post day 28 (p-value 0.002) in the treated group of white mice.

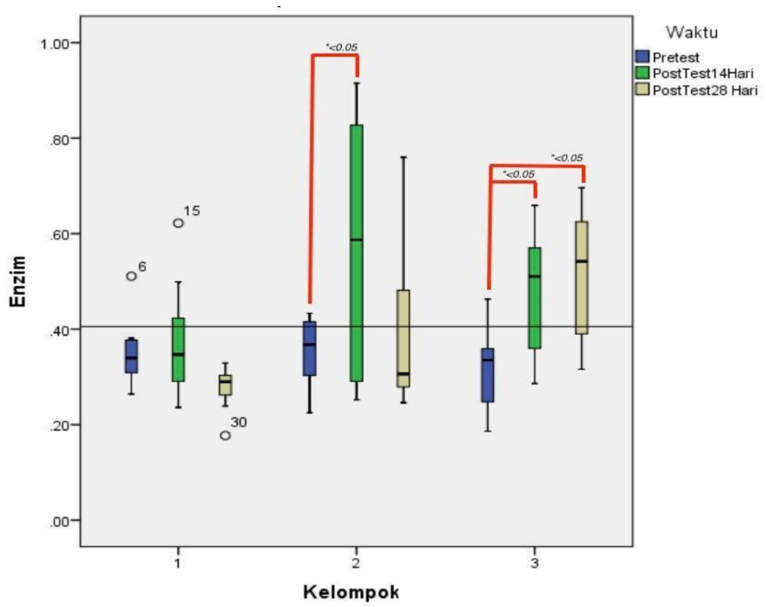

Figure 1. BoxSplot Changes in Catalase Enzyme Levels Over Time

DISCUSSIONS

The results of the research showed that through the Oneway Anova test there was a significant change in catalase enzyme levels in the group of obese white mice given olive oil based on the pre-test and post day 14 (p-value 0.005) and pre-test and post day 28 (p-value 0.002). These results are in line with research from (Milanizadeh S et al., 2017) regarding "The Effects of Olive Leaf Extract on Antioxidant Enzymes Activity and Tumor Growth in Breast Cancer" where analysis of catalase activity data showed a significant increase in the group treated with olive oil at a dose of 150 mg/kg/day ($p < 0.05$). No change in catalase activity was observed with higher doses of olive oil. This research shows that olive oil can have a positive influence on the activity of the catalase enzyme in the body. Catalase is an antioxidant enzyme that plays an important role in fighting oxidative stress by breaking down hydrogen peroxide into water and oxygen. Therefore, olive oil, as an antioxidant, is gradually being replaced in the food field. Natural antioxidants, extracted from natural plants, are considered relatively safe antioxidants, including polyphenols, polysaccharides, enzymes, phytic acid and vitamins. Among them, catalase (CAT) is a good antioxidant that can prevent lipid oxidation. Catalase (CAT) is an oxidoreductase, which has good performance in quenching reactive oxygen species (ROS).(Li X et al., 2022)

The results of this study showed that there was a significant change in enzyme levels in the three groups only on Post Day 28 (p-value 0.006), where the box plot showed that the group of obese white mice given olive oil still experienced an increase in catalase enzyme levels, whereas in the group of obese white mice with orsilate drug intervention and the group of obese white mice without olive oil experienced a decrease in catalase enzyme levels. These results are in line with research by (Luo S et al., 2019) regarding "In Vivo and In Vitro Antioxidant Activities pf Methanol Extracts from Olive Leaves on Caenorhabditis Elegans" which investigated the effect of olive oil on the catalase enzyme, where after administration with olive leaf extract, for 48 hours, the activities of catalase, glutathione peroxidase, and superoxide dismutase were found to increase by 10.81%, 52.23%, and 30.97%, respectively, compared with the control group (significant difference in levels from the analysis results p-value 0.01). Catalase, as the main antioxidant enzyme, plays a role in fighting the effects of free radicals by breaking down hydrogen peroxide. Decreased catalase activity may occur in certain genetic situations, aging, or environmental conditions, causing accumulation of hydrogen peroxide and increasing susceptibility to cell damage. Inflammatory conditions or specific diseases such as diabetes can also affect catalase activity, exacerbating redox imbalances in the body.(Göze I et al., 2017)

The first line of defense against oxidative stress involves various enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which are key factors in the cell's defense strategy against oxidation processes, along with antioxidants such as glutathione. Additionally, assessment of oxidation products such as malondialdehyde can help identify the effects of treatment on T. pyriformis cells. Through research by (Essadek S et al., 2023), it was proven that the effect of olive oil products in the form of Virgin Olive Oil (VOO) and Virgin Argan Oil (VAO) significantly ($p<0.05$) increased the catalase enzyme on the specific activity of several antioxidant defense enzymes in T cells. pyriformis stimulated by H2O2. The increase in catalase enzyme levels in white mice given olives on the 14th day could be caused by the body's response to the active compounds contained in olive oil. Olive oil is rich in antioxidants, especially polyphenols, which have been known to have anti-inflammatory properties and protect body tissue from oxidative damage. On day 14, the mice's bodies may have responded to the increase in antioxidants derived from olive oil by increasing catalase production to help overcome oxidative stress and reduce cell damage.(Meilina, 2017)

The decrease in levels of the catalase enzyme on day 28 occurred due to the body's adaptation to excessive exposure to the drug orsilate over a longer period of time. After a period of adaptation, the rat's body may resetting the production of the catalase enzyme based on the new level of need. If the body feels it no longer requires high levels of catalase to deal with oxidative stress, production of this enzyme may decrease. In addition, it is possible that other factors such as metabolism that changes over time or the effects of long-term exposure to the drug orsilate can also influence the regulation of catalase enzyme levels.(Amos DL et al., 2017)

The lack of change in catalase activity with high doses of olive oil could result from several factors. There might be a saturation point where additional olive oil no longer enhances catalase activity, or the body's ability to absorb and utilize polyphenols from high doses could be limited. Additionally, excessive olive oil intake might cause toxicity or adverse effects that counteract potential benefits. The body's regulatory mechanisms could also limit the increase in catalase activity at higher doses (Elhrech et al., 2024). Experimental variations, such as differences in dosing schedules or olive oil quality, might also contribute to these results. Catalase, a crucial enzymatic antioxidant, plays a key role in alleviating oxidative stress, which is often prevalent in diabetic conditions. Our study found that catalase activity decreased in hyperglycemic rats (orsilat group), whereas it increased in rats that received olive oil supplementation. The control group showed the highest levels of catalase activity, highlighting the negative impact of hyperglycemia on catalase function and the protective effect of olive oil's antioxidant properties.

Increasing catalase activity, especially in the context of hyperglycemia, is significant because it helps reduce oxidative stress by converting hydrogen peroxide into water and oxygen. Deficiencies or malfunctions in catalase are linked to the development of various degenerative diseases, including diabetes mellitus. Additionally, the antioxidant properties of phenolic compounds in olive oil not only offer systemic benefits but also exert positive antioxidant effects within the digestive tract. Administering olive oil to hyperglycemic rats notably elevated GPx levels, indicating its effectiveness in strengthening cellular antioxidant defenses. GPx plays a crucial role in reducing lipid peroxides and hydrogen peroxide, thereby preventing oxidative damage, and is essential for mitigating oxidative stress. The increase in GPx activity following olive oil supplementation supports previous research, underscoring olive oil’s potential to enhance antioxidant defenses against oxidative stress induced by hyperglycemia (Elhrech et al., 2024).

This study showed that olive leaf extract (OLE), likely due to its polyphenol content, reduced weight, consistent with previous research. Additionally, we found that treatment with this polyphenol-rich extract enhanced the activity of antioxidant enzymes like CAT and SOD. Since these enzymes protect cells by scavenging oxygen free radicals, an increase in CAT and SOD due to OLE consumption could minimize macromolecular damage and enhance cancer resistance.

CONCLUSIONS

In a study on obese white rats, catalase enzyme levels in the blood were examined before and after interventions. Initial levels were 0.349 kU/L, which slightly increased to 0.354 kU/L after 14 days without olive oil, but notably decreased to 0.317 kU/L after 28 days. Rats treated with orsilate showed higher initial levels (0.376 kU/L), significantly rising to 0.551 kU/L after 14 days and decreasing slightly to 0.484 kU/L after 28 days. Those treated with olive oil had initial levels of 0.277 kU/L, increasing substantially to 0.417 kU/L after 14 days and further to 0.514 kU/L after 28 days. Notably, the olive oil group showed the most significant impact, particularly on day 28 (p-value $0.002<0.05$). Future research should investigate the long-term effects of olive oil and orlistat on catalase levels and oxidative stress, explore different dosages, and examine their mechanisms of action.

This study's limitations include a relatively small sample size of 45 rats, which may affect the generalizability of the findings. The 28-day duration may not be sufficient to fully understand long-term effects and side effects of olive oil and orlistat. Additionally, the study did not account for all potential confounding factors, such as diet or genetic variations, and lacked direct assessment of pulmonary hypertension. Furthermore, the reliance on animal models means the results may not directly translate to human populations, underscoring the need for further human clinical trials.

RECOMMENDATION

Comparing these interventions with other antioxidants, assessing their impact on various oxidative stress biomarkers, and translating findings to human clinical trials are essential. Additionally, studies should include diverse populations and evaluate safety and potential side effects to ensure broad applicability and benefit.

FURTHER STUDY

Given their own limited experience and competence, it is not surprising that the researcher discovered numerous linguistic, writing, and presenting style flaws when composing this essay. The researcher consequently anticipates shrewd criticism and recommendations from a range of sources to ensure the work is flawless.

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