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Malondialdehyde Levels in Wistar Rat Liver after High Fat Diet and Mango Tea Mistletoe Extract Intake

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Abstract

Malondialdehyde (MDA) levels increase due to the accumulation of free radicals that exceed normal limits, leading to oxidative stress. Mango mistletoe and tea mistletoe (MESA-DP) are known to contain secondary metabolites that function as natural antioxidants. The use of antioxidants derived from these mistletoes can serve as an alternative to reduce MDA levels and neutralize free radicals. This study aimed to determine the dose of MESA-DP extract that is more effective in reducing MDA levels. This research employed a Randomized Pre Test–Post Test Control Group design using 35 male Wistar rats (*Rattus norvegicus*), which were divided into five treatment groups with 4 repetition: G1 normotensive as a control without high fat diet (HFD) and without gavage; G2 as an HFD control without gavage; G3 receiving HFD and MESA-DP of 50 mg/KgBW; G4 receiving HFD and MESA-DP of 100 mg/KgBW; and G5 receiving HFD and MESA-DP of 200 mg/KgBW. Data were analyzed using a one-way analysis of variance (ANOVA) to determine differences in hepatic MDA levels among groups. The results showed significant differences in hepatic MDA. Levels among all treatment groups following administration of MESA-DP extracts. The extracts demonstrated an effect in reducing MDA levels and enhance SOD levels thanks to its antioxidant properties. The most effective doses were found in group G3 50 mg/KgBW with an MDA level of 17,604 ± 0,389.

Keywords: high fat diet; malondialdehyde; mango mistletoe; tea mistletoe; wistar rats.

INTRODUCTION

The intake of foods that are rich in fats is a primary factor that can disturb the body's metabolic harmony and initiate oxidative stress. This situation arises when free radical production surpasses the body's ability to defend itself with antioxidants, resulting in harm to cells and tissues. The liver is particularly susceptible to oxidative stress due to its vital functions in metabolizing fats, detoxifying harmful substances, and maintaining bodily balance. Research has demonstrated that feeding wistar rats a high fat diet (HFD) for an extended period leads to fat buildup, lipid oxidation, and damage to liver cells (Puspa et al., 2025).

Free radicals, particularly reactive oxygen species (ROS), are unstable molecules with unpaired electrons and are very reactive. Normally, ROS are generated as part of cellular metabolic activities and play a role in cell signaling. However, when ROS are produced in excess, they can lead to oxidative stress, damaging crucial biomolecules like lipids, proteins, and DNA. A significant outcome of oxidative stress is lipid peroxidation, which involves the oxidative alteration of unsaturated fatty acids in cell membranes, resulting in harmful substances like Malondialdehyde (MDA). Elevated levels of MDA are commonly used as an indicator of oxidative damage and have been found to increase notably in the liver tissues of rats subjected to a high fat diet (Zaric et al., 2023) (Yeskaliyeva et al., 2023).

Both human and animal organisms possess a built-in antioxidant defense system that includes enzymes such as catalase, and glutathione peroxidase, along with non-enzymatic molecules. Nevertheless, this defense mechanism often struggles to neutralize free radicals effectively under severe oxidative stress conditions. Consequently, it becomes necessary to introduce additional antioxidants from outside sources to mitigate the harmful effects of free radicals and avert further tissue injury. These antioxidants can be sourced from natural origins, especially medicinal plants that are high in bioactive compounds (Djati et al., 2025) (Faisal et al., 2025).

Indonesia boasts a remarkable level of biodiversity, presenting extensive opportunities for the cultivation of herbal plants that could serve as sources of natural antioxidants. Various traditional herbs have historically been utilized by local communities as remedies for preventing and treating different ailments, including liver diseases. One notable plant is mistletoe. Despite being

recognized as a parasitic plant that relies on its host, numerous studies have indicated that mistletoe contains beneficial bioactive compounds and has been employed in traditional medicine across several countries (Silvia & Sjakoer, 2024).

Tea mistletoe and mango mistletoe, collectively called MESA-DP, are recognized for containing numerous bioactive substances such as flavonoids, alkaloids, tannins, saponins, steroids, phenolics, and glycosides. These substances possess antioxidant properties, functioning by neutralizing free radicals, preventing lipid peroxidation, and boosting natural antioxidant levels within the body. Various animal studies have demonstrated that tea mistletoe can reduce blood pressure, alleviate oxidative stress, raise nitric oxide (NO) levels, and lower MDA levels in rats with hypertension (Athiroh, 2009) (Athiroh & Sulistyowati, 2013), (Athiroh et al., 2014).

Additionally, mango mistletoe has been noted for its potential anti-cancer effects linked to its flavonoid composition. While a range of other herbal plants like cocoa, moringa seeds, and probiotics have been thoroughly investigated for their antioxidant properties and protection of the liver, studies on the combined effects of tea mistletoe and mango mistletoe remain limited. This suggests the necessity for further research, particularly concerning the prevention of liver damage due to oxidative stress from a high fat diet (Zulkifli et al., 2013).

MDA, a final product of lipid peroxidation, is harmful and can exacerbate damage to liver cells by compromising the integrity of cell membranes and disrupting mitochondrial function. Elevated MDA levels not only reflect the severity of oxidative stress but are also linked to the level of injury in liver tissues. Therefore, a reduction in MDA levels might serve as a sign of the effectiveness of antioxidant treatments in safeguarding liver tissue from oxidative harm (Munawwaroh, 2019).

In light of this, investigating the potential of MESA-DP as a natural antioxidant resource is crucial. This study aims to determine the dose of MESA-DP extract that is more effective in reducing MDA levels. Ultimately, the findings from this study are anticipated to aid the development of safe and effective herbal medicines derived from Indonesian natural resources to help prevent liver damage linked to oxidative stress.

MATERIALS AND METHODS

Plant Materials and Experimental Animals

This study used tea mistletoe purchased from the market in Kepanjen, Malang, and mango mistletoe from an online store. The experimental animals used were wistar rats (*Rattus novergicus*) obtained from a seller of research test animals after receiving a health certificate.

Experimental Animals and Treatment

This study used 35 male wistar rats (*Rattus novergicus*) (aged 8 weeks, weighing 150 g). The rats were acclimated for 10 days before treatment and randomly divide into 5 grups: G1 standard feed. G2 HFD only. G3 HFD + MESA-DP 50 mg/KgBW. G4 HFD + MESA-DP 100 mg/KgBW. G5 HFD + MESA-DP 200 mg/KgBW. The HFD composition was adapted from Murwani et al. (2006) consisting of standard feed, wheat flour, cholesterol, and pork fat, and HFD was administered for 47 days.

Procedures

Extraction

Tea and mango mistletoe are first sorted, dried, ground into powder, and extracted using the maceration method with 90% methanol. Then, a rotary evaporator method was used at a temoerature of 35 ° C to obtain a thick extract.

Measurement of MDA Activity

Measurement of MDA levels was carried out using an Enzyme-Linked Immunosorbent Assay (ELISA) kit. ELISA kit, was a biochemical method used to identify the presence of antibodies or antigens in a sample. ELISA kit was utilized for testing all antigens, haptens, or antibodies (Dita, 2021). Rinse the liver tissue with PBS (Phosphate Buffered Saline) pH 7.4. Chop and homogenize the liver in PBS on an ice glass. The resulting homogenate would be centrifuged for 5 minutes at 5000 xg to obtain the supernatant. After preparing the liver tissue, prepare the reagents (standard solution) from the kit reagents and perform the preparation of 5 standard dilutions and 1 control. Read the OD (optical density) value at a wavelength of 450 nm using a spectrophotometer within ≤ 10 minutes after adding the stop solution.

In detail, the MDA test was carried out as follows. First, prepare the standard solution by taking 120µl of the original standard (12. 8 nmol/mL) and mix it with 120µl of the standard diluent, resulting in a stock solution of 6. 4 nmol/mL to produce five standard solutions and one control with a concentration of 0 nmol/mL. In each dilution step, 120 µL was taken from the previous solution and mixed with 120 µL of the standard diluent. The wash buffer was diluted by mixing 20 mL of the 25× concentrated solution with water to obtain 500 mL of 1× wash buffer. If crystals were present, the solution should be stirred until completely dissolved before use.

After the liver tissue and reagents (standard solutions) are prepared, the MDA test was carried out as follows. In the initial preparation, all materials, including liver tissue, reagents, and standard solutions, was collected and brought to room temperature. The required number of strips was determined and placed into the plate frame to be used, while unused strips was stored at 2–8 °C. A volume of 50 µL of the standard solution was added to

the standard wells. Then, 40 µL of the sample was added to the sample wells, followed by the addition of 10 µL of anti-MDA antibody to the sample wells. Subsequently, 50 µL of streptavidin-HRP was added to both the sample and standard wells, except for the control. The plate was sealed and incubated for 60 minutes at 37 °C. After incubation, the plate was washed five times with wash buffer, soaked for 30–60 seconds, and then dried. Each well was then added with 50 µL of substrate A and B and incubated for 10 minutes at 37 °C until a blue color develops. Next, 50 µL of stop solution was added, causing the blue color to change to yellow. For result reading, the optical density (OD) was measured at a wavelength of 450 nm within 10 minutes after the addition of the stop solution. The MDA levels are determined by reading and analyzing the spectrophotometric measurement results.

Ethical Clearance

The following is the ethical clearance decision letter from RSI Unisma No. 36/KEPK/RSI-U/VI/2025

Data analysis

The data were examined using One-Way ANOVA.

RESULTS AND DISCUSSION

The following presents the average MDA levels in liver tissue and a diagram of the MDA level test results in the form of quantitative data. This data was then statistically analyzed to determine the differences between groups and to identify the most effective dose in reducing MDA levels.

Table 1. Mean hepatic MDA levels in wistar rats.

Group	Treatment	Mean ± SD (nmol/mL)
G1	Normotensive (standard diet)	20,426 ± 2,664
G2	Positive control (HFD)	33,496 ± 2,118
G3	HFD + MESA-DP 50 mg/KgBW	17,604 ± 0,389
G4	HFD + MESA-DP 100 mg/KgBW	11,240 ± 0,229
G5	HFD + MESA-DP 200 mg/KgBW	10,864 ± 0,138

*The average rat liver MDA shows the average MDA level in rat liver after the study.

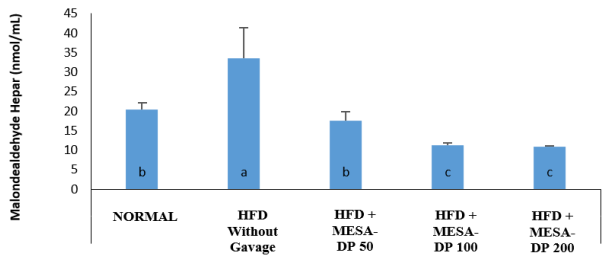

Figure 1. MDA levels in rat liver in all groups. The data were tested using one-way analysis of variance (ANOVA).

Discussion

A diet high in fat, known as HFD, is widely acknowledged as a significant contributor to metabolic disorders and oxidative harm. In the current research, experimental rats were established as a model for HFD related effects, demonstrated by a significant rise in Malondialdehyde (MDA) levels within the liver tissue. An increase in MDA indicates more lipid peroxidation and acts as a trustworthy marker of oxidative stress in the liver.

Prior research has shown variations in the amount of HFD necessary to generate oxidative stress in rodents. Sengupta. (2013) proposed that consuming between 10 to 100 g of HFD per body weight each day could elevate MDA levels, while Curfs et al. (2010) found that approximately 25 - 30 g of food per rat per day is the average requirement. For this study, the HFD was modified from the recipe created by Muwarni et al. (2006). This initial mixture consisted of Confeed PAR-S, wheat flour, cholesterol, cholic acid, lard oil, and water, provided at a rate of 40 g per rat daily for eight weeks.

To enhance the experimental approach, the diet was altered to include 310 g of Confeed PAR-S, 171 g of wheat flour, 11 g of cholesterol, 49 mL of lard oil, and 310 mL of water, which were given to 28 rats for 47 days. The amount of diet is known to greatly affect body weight management and metabolic results in experimental animals. In addition to receiving the HFD, the rats were also given MESA-DP extract, a herbal mixture abundant in bioactive substances, especially antioxidants that help reduce oxidative damage and maintain liver tissue health in Wistar rats (*Rattus norvegicus*).

The protective role of MESA-DP extract against liver oxidative stress was confirmed by statistical analysis through one-way analysis of variance (ANOVA). The rats on HFD alone without any treatment (G2, positive control) showed the highest concentration of hepatic MDA 33. 4955 ± 2,118, signifying severe oxidative damage. Conversely, HFD-fed rats treated with MESA-DP extract (G3, G4, and G5) showed considerably lower MDA levels, recorded at 17,604 ± 0,389, 11,240 ± 0,229, and 10,864 ± 0,138, respectively. Importantly, these measurements were similar to or even lower than those seen in the normal control group (G1, normotensive), which did not experience HFD or treatment 20,426 ± 2,664.

A dose-dependent pattern was noted, where increased dosages of MESA-DP extract led to more significant decreases in hepatic MDA levels. The G3 groups exhibited greater antioxidant effectiveness indicating a positive link between dosage and liver-protective effects. These results suggest that the best therapeutic range of MESA-DP extract may be found in the amounts administered to groups G3. The reduction of MDA levels in the treated groups shows that MESA-DP extract successfully combats oxidative stress caused by HFD.

An excessive consumption of high fat foods triggers the overproduction of reactive oxygen species (ROS), leading to oxidative stress and consequent lipid peroxidation in liver tissues. MDA, which is produced as a result of lipid peroxidation, rises in proportion to the extent of oxidative injury. The introduction of MESA-DP extract greatly curtailed this process, thus diminishing MDA buildup in liver tissue.

Flavonoids serve as effective antioxidants by supplying electrons or hydrogen atoms to unstable free radicals, transforming them into more stable, less reactive molecules. The antioxidant properties of MESA-DP operate through this process, halting the lipid peroxidation chain reaction early on. Consequently, the integrity of cellular membranes is maintained, leading to a significant decrease in MDA production. Crucially, the findings reveal that MESA-DP extract does not cause liver toxicity, as there was no indication of liver damage in the groups that received treatment (Lestari et al., 2023), (Athiroh & Wahyuningsih, 2017).

This research utilized a mix of two herbal sources tea mistletoe and mango mistletoe which might enhance antioxidant activity due to complex interactions among phytochemicals. Combining herbs can lead to various interactions among active ingredients that might either work together or against each other. While counterproductive interactions might lower effectiveness, the results of this study imply that the blended extract shows enhanced antioxidant activity at particular doses (Ma'ruf et al., 2025).

The most significant protective effects on the liver were noted at doses of 50 mg/KgBW. At these levels, antioxidant substances seem to be absorbed and used most efficiently, offering strong protection against oxidative damage to the liver caused by a high fat diet. These findings strongly indicate that MESA-DP extract has the potential to serve as a natural antioxidant that can help reduce liver damage due to a high fat diet.

In summary, the marked decrease in hepatic MDA levels after administering MESA-DP shows its effectiveness in limiting excessive free radical activity and safeguarding liver tissue. The synergistic effects between tea mistletoe and mango mistletoe play a key role in the antioxidant effectiveness of MESA-DP, especially at the dosages of 50 mg/KgBW.

CONCLUSIONS

Based on research conducted on Wistar strain rats (*Rattus norvegicus*) with the administration of a high fat diet model, it was proven that there was an effect on the reduction of MDA levels in liver tissue. The G2 Positive control treatment at 33,496 ± 2,118 increased MDA levels, while treatments G3 17,604 ± 0,389, G4 11,240 ± 0,229, and G5 10,864 ± 0,138 respectively, were effective in reducing these MDA levels, or approaching normal conditions G1 20,426 ± 2,664. This indicates that the tested MESA-DP extract has high antioxidant content, and treatments G3 with a dose of 50 mg/KgBW were effective in lowering MDA levels and preventing damage to liver tissue.

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Competing Interests: The authors declare that there are no competing interest.

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