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In Vivo Test of Combined Leaf Extracts of Tea Mistletoe and Mango Mistletoe on Hepatic TNF-α Levels in Wistar Rats

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Abstract

Tea mistletoe (*Scurrula atropurpurea*) and mango mistletoe (*Dendrophthoe pentandra*) are known to contain bioactive compounds with potential anti-inflammatory activity. Tumor Necrosis Factor Alpha (TNF-α) is a key proinflammatory cytokine whose hepatic expression can be elevated by a high-fat diet. This study aimed to investigate the effect of combined extracts of tea mistletoe and mango mistletoe and determine the most effective dose in reducing hepatic TNF-α levels in Wistar rats induced by a high-fat diet. An in vivo experimental design was employed using 35 male Wistar rats, randomly divided into five groups: negative control, positive control (high-fat diet), and three treatment groups receiving combined extracts at doses of 50, 100, and 200 mg/kg body weight for 37 days. Hepatic TNF-α levels were quantified using ELISA. The results showed that the combination of tea mistletoe and mango mistletoe extracts significantly reduced TNF-α levels in the liver of Wistar rats fed a high-fat diet. Among the tested doses, 200 mg/kg body weight demonstrated the greatest reduction compared to lower doses. In conclusion, the combined extracts exhibit promising anti-inflammatory effects by lowering hepatic TNF-α levels, with 200 mg/kg body weight identified as the most effective dose under the conditions of this study.

Keywords: anti-inflammatory; high-fat diet; mango mistletoe; tea mistletoe; TNF-α.

INTRODUCTION

Indonesia is one of the countries with the highest biodiversity in the world. About 60% of the world's two million plant species can be found here, and people have long used them for food and traditional medicine. Using plants as medicine is a natural way for communities to address health problems. Even though science and technology in the health sector are advancing rapidly, traditional medicine still plays an important role and complements modern treatments (Haba et al., 2022).

Mistletoe is a plant with great potential as a natural medicine. It grows as a semi-parasite, attaching itself to the branches of mature host plants, and is often found in tropical areas. Many people see mistletoe as harmful because it can affect the growth and health of its host. However, researchers have found that mistletoe contains many active compounds that are beneficial for health, such as lowering blood pressure and blood sugar, having anticancer and anti-inflammatory properties, acting as a diuretic, and possibly helping with other health issues (Artanti et al., 2012).

Tea mistletoe (*Scurrula atropurpurea*) and mango mistletoe (*Dendrophthoe pentandra*) are two species from the Loranthaceae family that are rich in secondary metabolites, such as flavonoids, alkaloids, tannins, saponins, and terpenoids. Flavonoid compounds, including flavone, flavonol, catechin, and flavanone, play an important role as antioxidants and anti-inflammatory agents (Nida' et al., 2022).

The presence of these bioactive compounds makes Tea mistletoe and mango mistletoe promising candidates for development as therapeutic agents to reduce inflammation, especially in the liver. Liver inflammation is usually linked to increased oxidative stress and the release of pro-inflammatory cytokines, such as Tumor Necrosis Factor-alpha (TNF-α), which plays a major role in inflammation and liver cell damage (Aini, 2023). TNF-α is often used as a main biomarker in studies of liver inflammation. To better understand these inflammatory mechanisms, Wistar rats are commonly used as test animals because their physiology and immune responses are similar to those of humans, so research results are expected to better reflect human biology (Tiegs & Horst, 2022).

The administration of a combination of tea mistletoe and mango mistletoe extracts is expected to have a synergistic effect in reducing TNF-α expression in liver tissue. This effect comes not only from the antioxidant activity of flavonoids and alkaloids but also from blocking the NF-κB signaling pathway, which is a key factor in the formation of TNF-α and other pro-

inflammatory cytokines (Athiroh et al., 2024). The extract combination may also boost the activity of natural antioxidant enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase, helping to lower oxidative stress and protect liver cells from damage. Therefore, in vivo research is needed to scientifically study the effects of administering combined tea mistletoeand mango mistletoe leaf extracts on TNF-α levels in the liver tissue of Wistar rats, and to determine the most effective dose for significantly lowering TNF-α levels (Ma'ruf et al., 2025)

MATERIALS AND METHODS

Study area

This study obtained ethical approval from the Health Research Ethics Committee of Malang Islamic Hospital (Ethical Clearance) with registration number 36/KEPK/RSI-U/VI/2025. This in vivo experimental study aimed to determine the effect of the combined administration of tea mistletoe and mango mistletoe extracts on hepatic TNF-α levels as an indicator of inflammatory response. The study employed a Randomized Pretest–Posttest Control Group Design involving a total of 35 Wistar rats divided into five treatment groups, each consisting of seven replications with three dosage variations. The variables in this study included control, independent, and dependent variables. The control variables consisted of a negative control in the form of a standard diet (G1) and a positive control in the form of a high-fat diet/HFD (G2). The independent variable was the administration of a combination of tea mistletoe (*Scurrula atropurpurea*) and mango mistletoe (*Dendrophthoe pentandra*) extracts at three doses, namely G3 (HFD + 50 mg/kg BW), G4 (HFD + 100 mg/kg BW), and G5 (HFD + 200 mg/kg BW). Hepatic TNF-α levels were measured using the ELISA method, and the data were statistically analyzed using one-way ANOVA followed by Duncan's test with a significance level of ($p<0.05$) using SPSS.

Tools and Materials

The tools and materials used in this study were categorized according to several stages, including animal maintenance, extraction, dose preparation, surgery, and TNF-α analysis. For animal maintenance, cages (40 × 30 × 25 cm), cage covers, digital scales, gastric sondes, drinking bottles, and feeding containers were used, while the materials included Wistar rats, feed, drinking water, and supporting materials such as husks and tissue paper. The high-fat diet consisted of PARS (57.3%), wheat flour (31%), cholesterol powder (2%), and lard (8.9%). The extraction process utilized equipment such as a blender, oven, freezer, and rotary evaporator, with materials including tea mistletoe and mango mistletoe leaves and 90% methanol. Dose preparation involved measuring cylinders, biolab micropipettes, aquadest, and extract solutions. The surgical procedure involved the use of instruments such as scissors, forceps, and syringes, supported by materials including 75% alcohol, ketamine, xylazine, and 10% Neutral Buffered Formalin (NBF). TNF-α levels were determined using the Enzyme-Linked Immunosorbent Assay (ELISA) method, with tools such as micropipettes, 96-well microplates, an incubator set at 37°C, and an ELISA reader. The assay employed reagents including standards, test samples, biotin-conjugated antibodies, streptavidin-HRP, substrate solutions, sulfuric acid, and a wash buffer.

Procedures

Preparation of Simplicia and Extraction

Tea mistletoe (*Scurrula atropurpurea*) and mango mistletoe (*Dendrophthoe pentandra*) leaves were obtained from Kepanjen, Malang, and online platforms, then processed at the Materia Medica Laboratory in Batu, East Java.

(a) (b)

Figure 1. (a) Tea mistletoe (*Scurrula atropurpurea*) and (b) mango mistletoe (*Dendrophthoe pentandra*) used as plant materials for extraction.

The leaves were dried in an oven at 40–60°C until completely dry, with both fresh and dry weights recorded as parameters. The dried simplicia were ground into powder and extracted using the maceration method at a ratio of 100 g simplicia to 1 L of 90% methanol. The maceration process involved shaking for 60 minutes, followed by soaking for 24 hours to obtain a supernatant. The supernatant was then separated and concentrated using a rotary evaporator at 35°C to produce a viscous extract containing bioactive compounds (Athiroh et al., 2024).

Animal Acclimatization and Maintenance

The experimental animals used in this study were 35 healthy Wistar rats (*Rattus norvegicus*), approximately 8 weeks old and weighing 150–200 g. The animals were maintained in the Animal House of the Faculty of Dentistry, Universitas Brawijaya, under standard feeding and drinking conditions. Prior to treatment, all animals underwent an acclimatization period of approximately 10 days at room temperature (around 24°C) to allow adaptation to the laboratory environment. After the acclimatization period, body weight was measured to determine the appropriate dosage of the combined extracts of *Scurrula atropurpurea* and *Dendrophthoe pentandra*. Body weight was subsequently recorded daily to adjust the administered dose throughout the treatment period (Susanti & Ratnawati, 2012)

Dose Preparation

This study utilized a combination of methanolic extracts of *Scurrula atropurpurea* and *Dendrophthoe pentandra* at doses of 50, 100, and 200 mg/kg body weight (BW). The administered dose was adjusted according to the body weight of each animal. The extracts were prepared in solution form using a ratio of 3:1 (tea mistletoe to mango mistletoe), dissolved in distilled water to a specified volume, and administered according to the required dosage for each animal.

Administration of Extracts

The combined extracts were administered daily for 37 days. During this period, body weight was measured regularly to ensure accurate dose adjustment. The treatment groups consisted of G3 (50 mg/kg BW), G4 (100 mg/kg BW), and G5 (200 mg/kg BW), with each group comprising seven rats as replicates. The extracts were administered orally using a gastric gavage (oral sondation) method with a feeding needle, according to the predetermined dose and volume.

Surgical Procedure and Sample Collection

After 47 days of maintenance and treatment, surgical procedures and blood sample collection were performed on male Wistar rats according to the treatment sequence. The animals were first anesthetized using a combination of ketamine and xylazine until full unconsciousness was achieved. A surgical incision was then made from the abdominal to the thoracic region using surgical scissors. The primary parameter observed in this study was liver tissue. The liver was excised, weighed, and visually examined, then preserved in 10% neutral buffered formalin (NBF) for histopathological analysis. A portion of the liver tissue was wrapped in aluminum foil and stored for subsequent TNF-α analysis (Sulistyowati et al., 2020; Ugwu et al., 2026)

Analysis of Hepatic TNF-α Levels

TNF-α levels were measured using the Enzyme-Linked Immunosorbent Assay (ELISA) method. In this procedure, reagents, standards, and samples were added into microplate wells, followed by the addition of specific antibodies and streptavidin-horseradish peroxidase (HRP). The plate was incubated for 60 minutes at 37°C. Subsequently, the wells were washed five times to remove residual reagents. Substrate solutions A and B were then added, and the plate was incubated in the dark for 10 minutes, resulting in a blue color development. The reaction was stopped using a stop solution, which changed the color to yellow. TNF-α levels were measured using a microplate reader at a wavelength of 450 nm. The obtained optical density (OD) values were compared with a standard curve to determine TNF-α concentrations in the samples (Neun et al., 2020).

Data analysis

The collected data were analyzed statistically using SPSS (Statistical Product and Service Solutions) software. Results were expressed as mean ± standard deviation (SD). Differences among groups were evaluated using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to assess differences between control and treatment groups. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Result

Examination of Hepatic TNF-α Levels

The results showed that the administration of a High Fat Diet (HFD) to Wistar rats increased TNF-α levels in liver tissue compared to the negative control group. This increase indicates that HFD induction successfully triggered an inflammatory condition, as evidenced by the elevated expression of the pro-inflammatory cytokine TNF-α in response to oxidative stress and lipid accumulation in hepatocytes. The mean TNF-α levels in each treatment group are presented in Table 1.

Table 1. Mean TNF-α Levels in the Liver of Wistar Rats (*Rattus norvegicus*) after 37 Days of MESA-DP Extract Combination Administration.

Group	Treatment	Mean ± SD (ng/mL)
G1	Negative Control (standard diet)	148,373 ± 27,992
G2	Positive Control (HFD)	302,310 ± 64,314
G3	HFD + MESA-DP 50 mg/kg BW	300,343 ± 39,474
G4	HFD + MESA-DP 100 mg/kg BW	245,431 ± 21,293
G5	HFD + MESA-DP 200 mg/kg BW	208,066 ± 21,843

* Values are presented as mean ± standard deviation (SD) of hepatic TNF-α levels in Wistar rats after 37 days of experimental treatment.

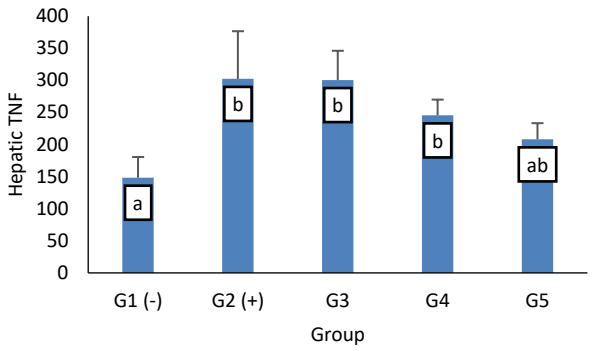

Figure 2. Mean hepatic TNF-α levels in Wistar rats after 37 days of combined MESA-DP extract administration, with different letters indicating significant differences between groups ($p < 0.05$).

Statistical analysis using a one-way ANOVA test showed a significant difference among treatment groups ($p < 0.05$). This indicates that the administration of the combined extract of tea mistletoe and mango mistletoe had a significant effect on reducing TNF-α levels in the liver of Wistar rats. These differences suggest that variations in dosage resulted in different effects on the level of inflammation.

Discussion

The ANOVA test followed by Duncan's multiple range test demonstrated significant differences among the treatment groups ($p < 0.05$), as indicated by the different letter notations assigned to each group. Groups sharing the same letter were not significantly different, whereas groups with different letters showed statistically significant differences. In this study, G1, labeled with the letter a, differed significantly from G2, G3, and G4, which were labeled with the letter b, but did not differ significantly from G5, which was assigned the notation ab. Meanwhile, G2, G3, and G4 did not show significant differences from one another, although all three groups differed significantly from G1. The notation ab in G5 indicates that its value was intermediate between groups a and b; therefore, G5 was not significantly different from either G1 or from G2, G3, and G4. These findings suggest that the treatment administered in G5 began to reduce hepatic TNF-α levels, although the reduction was not sufficient to produce statistically significant differences compared with all other treatment groups.

Based on the measurement results, the negative control group (G1), which received only a standard diet, showed the lowest mean TNF-α levels compared to other groups. This condition indicates that, without exposure to a high-fat diet or additional treatment, liver tissue remained in a normal physiological state with minimal inflammatory response. TNF-α is a pro-inflammatory cytokine produced by immune cells, particularly macrophages and Kupffer cells in the liver, and plays an important role in both systemic and local inflammation. The low TNF-α levels in G1 indicate the absence of significant inflammatory stimuli.

In contrast, the positive control group (G2), which received a High Fat Diet (HFD) without extract treatment, showed a marked increase in TNF-α levels compared to G1. This increase suggests that a high-fat diet can induce metabolic stress and activate inflammatory pathways in the liver. HFD is known to increase lipid accumulation in hepatocytes, trigger lipotoxicity, and activate signaling pathways such as NF-κB, which contribute to the increased production of pro-inflammatory cytokines, including TNF-α. Thus, the data in G2 confirm that the HFD model successfully induced hepatic inflammation.

In the treatment group G3 (50 mg/kg BW), the mean TNF-α level remained relatively high and was close to that of the positive control group. This suggests that a dose of 50 mg/kg BW may not be optimal for suppressing the inflammatory response caused by HFD exposure. However, the variation indicated by the standard deviation suggests the presence of individual responses to the treatment. Bioactive compounds such as flavonoids, alkaloids, and phenolics found in tea mistletoe and mango mistletoe are known to have antioxidant and anti-inflammatory properties, but their effectiveness is highly dependent on dosage and duration of administration. Therefore, a lower dose may not be sufficient to provide maximal protective effects on liver tissue.

The G4 group (100 mg/kg BW) showed a decrease in TNF-α levels compared to G2 and G3. This finding indicates a dose-response relationship, where increasing the extract dose begins to exert a suppressive effect on inflammatory mediators. Biologically, flavonoids can inhibit NF-κB activation, thereby reducing the transcription of pro-inflammatory cytokine genes, including TNF-α. In addition, the antioxidant activity of the extract can reduce oxidative stress, which is a major trigger of hepatic inflammation. This reduction suggests

that the 100 mg/kg BW dose is more effective than the 50 mg/kg BW dose in controlling HFD-induced inflammation.

The most notable result was observed in the G5 group (200 mg/kg BW), where TNF-α levels decreased further and approached those of the negative control group, although they had not fully returned to normal levels. This indicates that the highest dose of the MESA-DP extract combination in this study has a stronger anti-inflammatory potential. This effect may occur through synergistic mechanisms among active compounds that act as free radical scavengers and modulators of inflammatory pathways. The decrease in TNF-α production suggests an improvement in the degree of hepatic inflammation.

Overall, the data pattern shows that HFD exposure significantly increases TNF-α levels, while the administration of the combined extract of tea mistletoe and mango mistletoe gradually reduces these levels in a dose-dependent manner. These findings support the hypothesis that the MESA-DP extract has potential as a natural anti-inflammatory agent that can mitigate the adverse effects of a high-fat diet on the liver. However, TNF-α levels in the treatment groups did not fully return to the levels of the negative control group, suggesting that inflammation may not have been completely resolved or may require a longer treatment duration.

Thus, this study demonstrates that the combination of tea mistletoe and mango mistletoe extracts has the potential to suppress hepatic inflammatory responses, as indicated by reduced TNF-α levels. The highest effectiveness was observed at the 200 mg/kg BW dose, making it the most optimal dose within the range of this study. These findings are consistent with the concept that phytochemical compounds can be utilized as complementary therapies to control inflammation associated with metabolic disorders, particularly those induced by a high-fat diet.

The reduction in TNF-α levels in the treatment groups is likely associated with the presence of bioactive compounds in mistletoe extracts, particularly flavonoids, alkaloids, and saponins. Flavonoids are known for their antioxidant activity, which enables them to scavenge free radicals and reduce oxidative stress, a primary trigger of inflammatory pathway activation. In addition, flavonoids can inhibit the activation of the NF-κB transcription factor, which plays a key role in the production of pro-inflammatory cytokines, including TNF-α.

The more significant decrease in TNF-α levels at the 200 mg/kg BW dose indicates a dose-response relationship, where higher extract doses result in stronger anti-inflammatory effects. This suggests that a higher concentration of active compounds provides a more optimal effect in suppressing inflammatory responses.

Furthermore, the combination of tea mistletoe and mango mistletoe extracts is believed to exert a synergistic effect by enhancing the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase. The increased activity of these enzymes plays an important role in neutralizing free radicals and repairing liver cell damage caused by high-fat diet exposure.

Taken together, the results of this study indicate that the combination of tea mistletoe and mango mistletoe extracts is effective in reducing TNF-α levels in the liver of Wistar rats induced by a high-fat diet. These findings support the potential use of herbal plants as natural anti-inflammatory agents and provide opportunities for the development of alternative therapies based on natural products that are safer and have fewer side effects.

CONCLUSIONS

Based on the results of this in vivo study on the combination of tea mistletoe (*Scurrula atropurpurea*) and mango mistletoe (*Dendrophthoe pentandra*) leaf extracts on TNF-α levels in the liver of Wistar rats, it can be concluded that the administration of the combined extracts significantly reduced TNF-α levels in the liver tissue of Wistar rats induced by a high-fat diet. This indicates the anti-inflammatory activity of the extract combination. The most effective dose in this study was the highest dose (200 mg/kg BW), as it showed the greatest effectiveness in reducing TNF-α levels compared to other doses, thus having the highest potential to inhibit hepatic inflammation.

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Authors' Contributions: Nur Aziza was responsible for manuscript writing, data collection, and data analysis. Nour Athiroh Abdoes Sjakoer contributed to the study design and revised the manuscript for intellectual content. Ari Hayati assisted in data analysis. All authors have read and approved the final version of the manuscript.

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