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Hepatoprotection and Immunomodulation of Natural Killer and CD8 T Cells by Meniran-Turmeric Extract Combination in Mice Injected with 7,12-Dimethylbenz[a] Anthracene

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ABSTRACT

Combination herbal or polyherbal offers advantages by synergizing multiple plants to address health issues more effectively. Meniran and turmeric are traditionally used medicinal plants with notable pharmacological activities due to their bioactive compounds. This study investigated the curative effect of a meniran-turmeric extract combination on immunocompetent cells, particularly NK and CD8 cells, and on liver histopathology in mice administered 7,12-dimethylbenz[a]anthracene (DMBA). Female BALB/c mice (*Mus musculus*) were injected subcutaneously with DMBA at 45 mg/kg body weight (BW) in the mammary gland area and maintained for eight weeks. They were then treated with a 1:1 meniran-turmeric extract combination for two weeks at doses of 100, 300, 900, and 1,800 mg/kg BW, followed by liver and immune cell analyses. The results showed a lower $CD8^+$ T-cell population in extract-treated groups (17.53±1.13%-19.79±1.51%) compared with the DMBA group (20.92±4.74%). Conversely, NK^+ cell populations increased after extract treatment (0.33±0.01%-0.56±0.13%) compared with DMBA alone (0.27±0.02%). Liver histopathology indicated that extract combination administration did not cause more severe damage than that observed in the DMBA group. An extract combination of meniran and turmeric could modulate the immune system, particularly NK and CD8 cells, in mice injected with DMBA. In addition, the study indicated that the combination of meniran and turmeric extract might play a role as an antihepatotoxic agent. This research could serve as a consideration for future studies developing a combination formulation of meniran and turmeric.

1. Introduction

The immune system acts as a defensive mechanism, crucial in regulating and maintaining the homeostasis of the body's biological systems. The immune system functions to regulate immune cells against various infections, including inflammation, cancerous cells, and other diseases, through immunomodulation (Putra and Rifa'i 2019; Km *et al.* 2021; Oo *et al.* 2022; Wiratmini *et al.* 2025). Immunomodulation has three mechanisms of action: activation of the immune response, suppression of the immune response, and restoration of the immune response. Regularly, each type of immune cell has a specific role in its respective posts. Natural killer (NK) and T lymphocyte CD8 (CD8) cells include cytotoxic cells that play roles in defending against infection and eliminating cancerous cells (Vivier *et al.* 2008; Nutt & Huntington 2019).

On the other hand, the liver (hepar) is an organ responsible for detoxifying various compounds

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entering the body. The detoxification and metabolism of these compounds occur in two phases. In Phase I, enzymes such as cytochrome P450 modify compounds to form reactive metabolites. In Phase II, these metabolites undergo conjugation with molecules such as glucuronate or sulfate, facilitating their excretion. These metabolic processes in the liver are essential for the biotransformation and elimination of xenobiotics, ensuring their safe removal from the body through the kidneys or bile. However, exposure to external compounds can lead to structural changes in the liver, such as degeneration, necrosis, inflammation, and even organ dysfunction (Hodges and Minich 2015). Therefore, the toxic effects of these compounds can be identified by examining alterations in liver tissue structure.

7,12-dimethylbenz[a] Anthracene (DMBA) is a form of carcinogenic synthetic polycyclic aromatic hydrocarbons (PAH) that is commonly used to induce cancer in experimental animals. Although the liver is not the primary target of DMBA, once it enters the body, it is metabolized there. Activation metabolism and detoxification of DMBA in the liver lead to the formation of Reactive Oxygen Species (ROS) and DNA adducts, resulting in liver damage (Ashrafi *et al.* 2018). Therefore, it is imperative to devise a plan to address the negative impacts of chemical pollutants, including DMBA.

Herbal medicine, as an alternative therapy, has the potential to influence the body's systems through its bioactive compounds. Herbal medicine is usually used in a single herbal or a combination (polyherbal). The use of polyherbal medicine enables simultaneous modulation of multiple biological pathways, potentially providing a more effective approach to managing complex diseases (Wang *et al.* 2023; Priosoeryanto *et al.* 2025). Previous studies have shown that single herbal medicines can modulate the immune system in DMBA-exposed mice, such as *Sambucus javanica* (Putra and Rifa'i 2019), *Cyperus rotundus* (Rifa *et al.* 2021), and *Morinda citrifolia* (Agustina *et al.* 2020). Additionally, polyherbal products were reported to be immunomodulators for cold and flu (Fathima *et al.* 2022) and COVID-19 (Patankar *et al.* 2021; Chandra *et al.* 2022; Khadke *et al.* 2023). However, research on the effects of polyherbal formulations on the immune system in response to DMBA exposure has not been extensively conducted.

Herbal plants, including Meniran (Phyllanthaceae) and Turmeric (Zingiberaceae), have been used for centuries in various countries, particularly in Indonesia. These herbal medicines exhibit a range of pharmacological effects, including antibacterial, antihypertensive, anti-inflammatory, and anticancer activities (Asyhari *et al.* 2024). Meniran, in particular, has been reported to possess hepatoprotective effects (Harish and Shivanandappa 2006; Ezzat *et al.* 2020; Noviyanti and Yowani 2023; Oktaviona *et al.* 2023). Several studies have been conducted to explore their combination in healthcare applications, such as anticancer (Hermansyah *et al.* 2021, 2023; Puspitarini *et al.* 2022) and immunomodulatory effects in macrophage cells (Puspitarini *et al.* 2024). However, there have been no reports on their hepatoprotective function or on their effects on NK and CD8 cells. Thus, in this study, the main objective was to investigate the curative effect of the meniran and turmeric extract combination on NK and CD8 T cells, and its hepatoprotective impact in mice injected with DMBA.

2. Materials and Methods

2.1. Extraction Process

The materials are dried powders of meniran herb (*Phyllanthus* sp.) and turmeric rhizome (*Curcuma longa*) purchased from UPT. Materia Medika Batu is located in Batu, Indonesia. The extraction process was conducted using the water extraction method (Huang *et al.* 2004; Cao-Ngoc *et al.* 2020; Basak and Annapure 2022). The material was macerated in boiling demineralized water (Hydrobatt) wiat a 1:10 (herb: solvent; w/v) ratio for 4 h. Then, the extract solution was filtered sequentially using a fine cloth, a rough filter, and a paper filter (Whatman). The water from the extract was removed using a freeze dryer and frozen until usage. This study used an extract combination of meniran and turmeric in a 1:1 ratio. In this study, this combination is referred to as the extract combination.

2.2. Ethical Considerations

All animal experiments were performed following the guidelines for the care and use of laboratory animals. The procedures and experimental design were approved by the Brawijaya University Animal Care and Use Committee (Ethic No. 1126-KEP-UB).

2.3. Experimental Design

This study used female BALB/c mice (*Mus musculus*) aged 6 weeks from Maulana Malik Ibrahim State Islamic University of Malang, located in Malang, Indonesia. The mice were allowed to acclimate for one week and kept

at the Animal Anatomy and Physiology Laboratory, Biology Department, Universitas Brawijaya, Malang, Indonesia. Then, the animals were randomly assigned to seven distinct treatment groups for a duration of two weeks. The groups were detailed:

Group 1 (Normal/N): mice injected with corn oil only, without DMBA exposure nor extract treatment.
Group 2 (DMBA): DMBA-induced mice that did not receive any extract intervention.
Group 3 (DMBA+Cisplatin): DMBA-induced mice with administered intraperitoneal cisplatin at 15 mg/kg BW once weekly for two weeks.
Group 4 (DMBA+D1): DMBA-induced mice treated with 100 mg/kg BW of the extract daily.
Group 5 (DMBA+D2): DMBA-induced mice treated with 300 mg/kg BW of the extract daily.
Group 6 (DMBA+D3): DMBA-induced mice treated with 900 mg/kg BW of the extract daily.
Group 7 (DMBA+D4): DMBA-induced mice treated with 1,800 mg/kg BW of the extract daily.

2.4. Antibody Staining and Flow Cytometry Analysis

The spleen from the animal model was isolated and homogenized in Phosphate-Buffered Saline (PBS) (Gibco). The resulting mixture was centrifuged at 2,500 rpm for 5 minutes at 10°C. The pellet was then resuspended in fresh PBS and gently mixed. The 50 µL cell solution was stained with 50 µL extracellular antibodies, i.e., anti-CD8 and anti-NK. PE-conjugated anti-CD8 and FITC-conjugated anti-NK antibodies were among those used (BioLegend). The antibodies were diluted to 0.005 mg/100 µL, as directed by the manufacturer. The cells had been incubated for 30 minutes in dark conditions. Afterward, 400 µL of PBS was added to the sample, which was then transferred to a flow cytometry tube for analysis on a flow cytometer (BD FACSCalibur) to count the target immunocompetent cells. The program BD Cellquest ProTM (BD Biosciences) was used to analyze flow cytometry data. The information was shown as a relative percentage. The cells that were positive for NK or CD8 were written as NK^+ or $CD8^+$.

2.5. Histology Preparation of the Liver

Histology preparations of the liver, including staining with hematoxylin and eosin, were performed in the Pathology Anatomy Laboratory of Kessima Medika, Malang, East Java. The liver was processed for dehydration, cleaning, insertion, and cutting for tissue preparation. Then, the specimen was deparaffinized with xylol and ethanol. The preparation is stained with hematoxylin for 10 minutes and eosin for 1 minute. Lastly, the samples were covered with glass coverslips and mounted in enthelan. Sample later observed under microscope, integrated with Olympus BX51 binoculars, device software Optilab Viewer 3.0, and camera Optilab Advanced Plus.

2.6. Prediction Function Pharmacological as Hepatoprotective

Identification predictions function pharmacological compound active extract as hepatoprotective using Pass Online (Filimonov *et al.* 2014). The compound active to be identified is the one for which LC-HRMS analysis has been previously conducted (Puspitarini *et al.* 2022). Compounds active being analyzed are Quercetin, Ellagic acid, (-)-Epicatechin, 3,4 Dimenthoxycinnamic acid, Caffeic acid, Curcumin, Fereulic acid, and ar -turmerone. SMILE structure of compounds that is used for analysis on PassOnline obtained from PubChem (Kim *et al.* 2023). The predicted activity (Pa) value from PassOnline indicated the accuracy of the predicted functions, meaning that the higher the Pa value, the more accurate the predicted function (Filimonov *et al.* 2014)

2.7. Statistical Analysis

SPSS software was used to analyze flow cytometry data for ANOVA and post hoc Tukey HSD tests. A significant level of $P<0.05$ was deemed appropriate. A mean ± standard deviation (SD) was displayed for each data set.

3. Results

Immunocompetent cells that play an important role in defence against abnormal cells include NK cells and CD8 T cells. The results showed that the relative percentage of $CD8^+$ T cells from the T cell population in the DMBA group had a higher value (20.92±4.74%) than in the N group (12.77±1.2%) ($p<0.05$). Administration of cisplatin to DMBA-induced mice reduced the relative number of $CD8^+$ T cells (20.59±0.96%) compared with the DMBA group, although not significantly. The number of $CD8^+$ T cell populations was lower in the group of mice given the extract combination compared to the DMBA group, namely 17.53±1.13% (DMBA + D1), 19.26±0.79% (DMBA + D2), 18.44±0.23% (DMBA+D3), and 19.79±1.51% (DMBA+D4) (Figure 1).

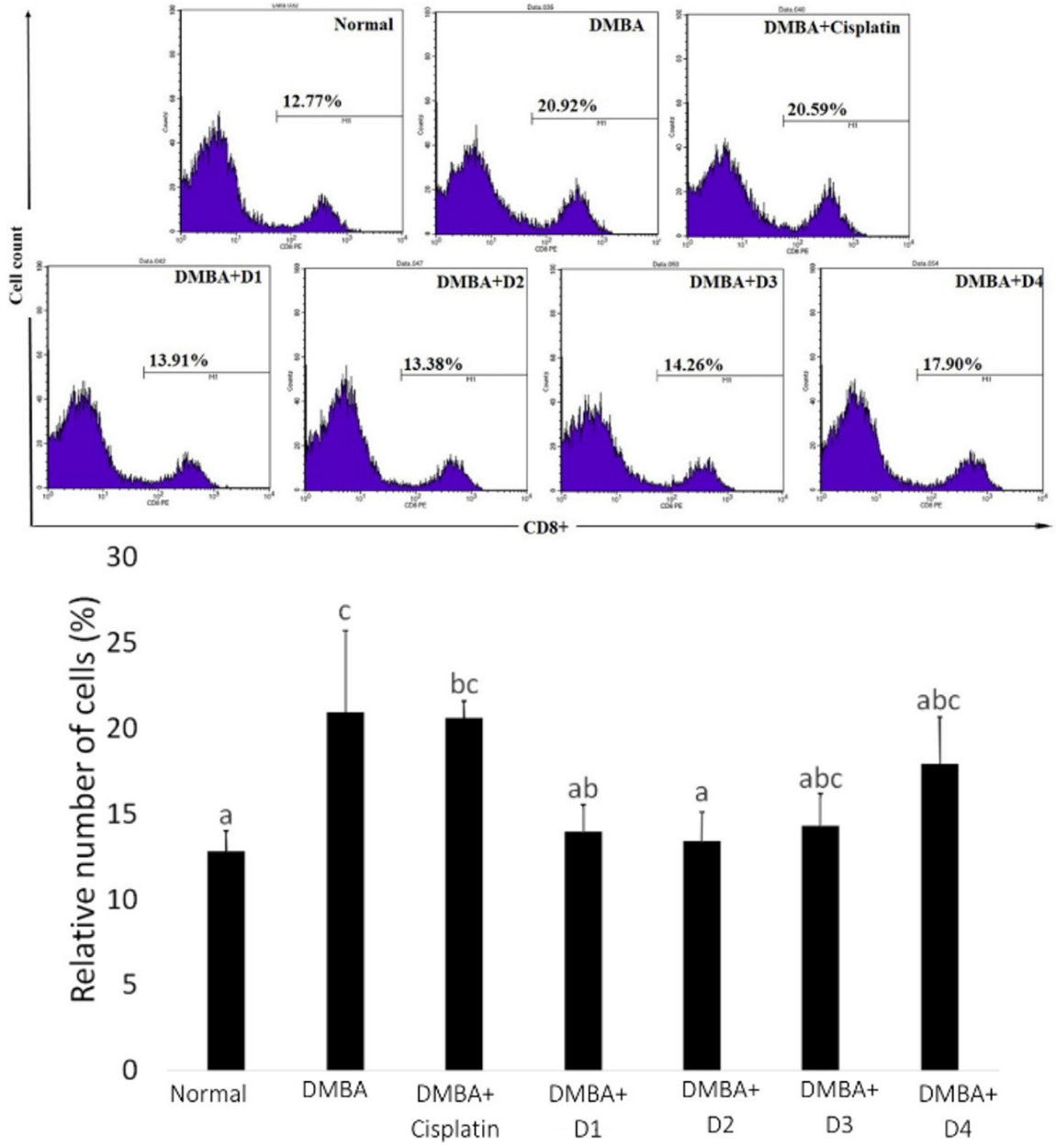

Figure 1. The relative abundance of $CD8^+$ T cells in DMBA-injected mice, with or without the extract combination, was assessed. The extract combination significantly reduced the relative number of $CD8^+$ T cells compared to the DMBA-only group. The left was represented by flow cytometry analysis; the right was represented by data analysis. The bars were represented as mean ± SD, and significantly different symbols were labeled with letters. Normal Normal group; DMBA: DMBA control group (45 mg/kg BW DMBA subcutaneous injection/ DMBA); DMBA-Cisplatin group (DMBA + 15 mg/kg BW Cisplatin via intraperitoneal injection); DMBA-D1 group (DMBA + 100 mg/kg BW extract combination treatment; DMBA-D2 group (DMBA + 300 mg/kg BW extract combination treatment); DMBA-D3 group (DMBA + 900 mg/kg BW extract combination treatment); DMBA-D4 group (DMBA + 1,800 mg/kg BW extract combination treatment)

In contrast to the $CD8^+$ cell population, the percentage of NK+ cells from total lymphocytes in the DMBA group was significantly lower (0.27±0.02%) than in the N group (0.49±0.09%) ($p<0.05$). Giving cisplatin to DMBA-induced mice resulted in a higher percentage of NK^+ cells (0.35±0.03%) compared to the DMBA group. The results showed that the extract combination treatment could increase the number of NK^+ cell populations, namely 0.38±0.07% (DMBA + D1), 0.33±0.01% (DMBA + D2), 0.56±0.13% (DMBA + D3), and 0.35±0.04% (DMBA + D4) compared to the DMBA group (Figure 2).

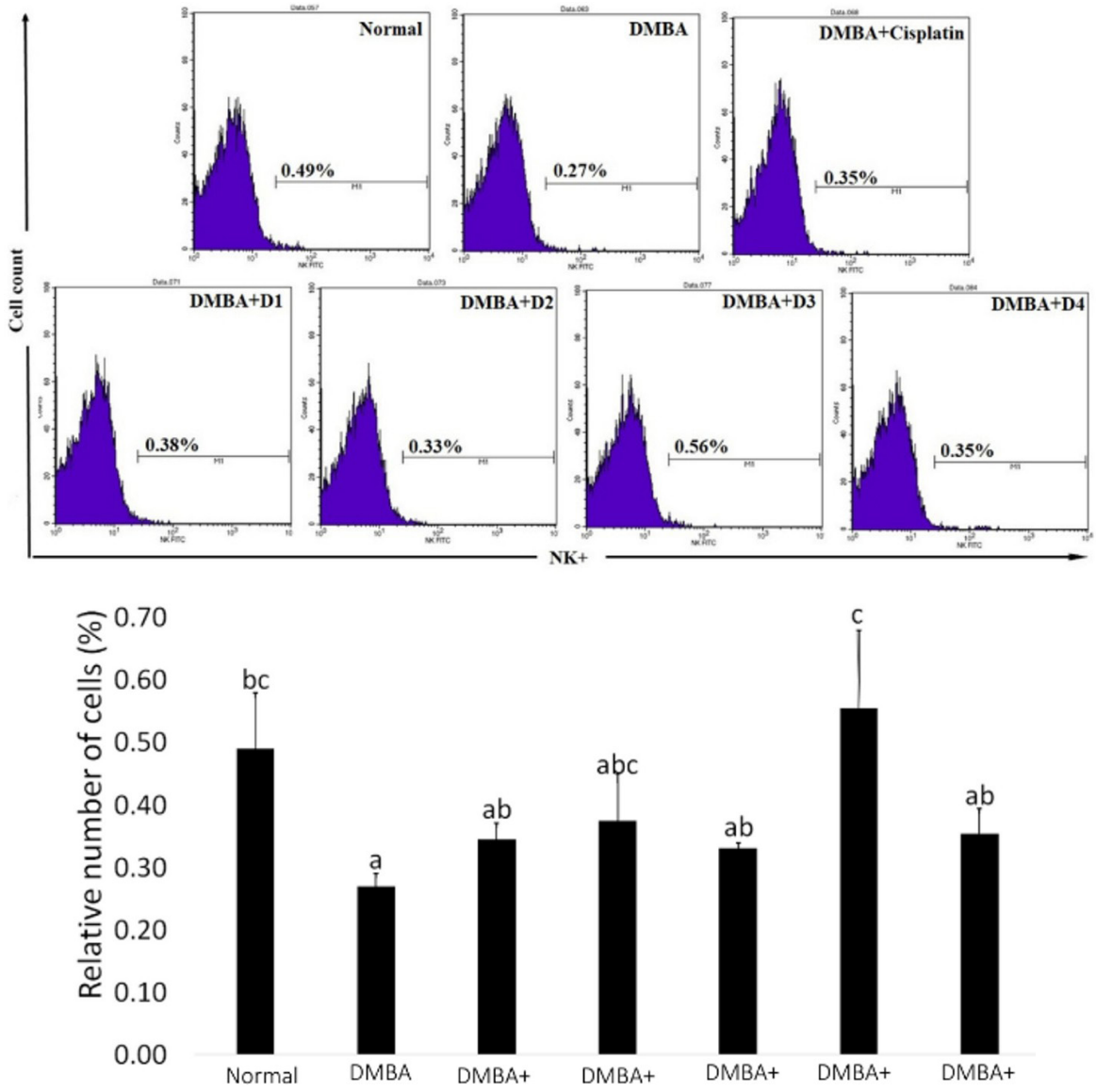

Figure 2. The relative abundance of NK^+ cells in DMBA-injected mice, with or without the extract combination, was assessed. The extract combination led to a decrease in the relative NK^+ cell count compared with the DMBA-treated group. The left was represented by flow cytometry; the right by data analysis. The bars were represented as mean ± SD, and significantly different symbols were labeled with letters. Normal Normal group; DMBA: DMBA control group (45 mg/kg BW DMBA subcutaneous injection/ DMBA); DMBA-Cisplatin group (DMBA + 15 mg/kg BW Cisplatin via intraperitoneal injection); DMBA-D1 group (DMBA + 100 mg/kg BW extract combination treatment; DMBA-D2 group (DMBA + 300 mg/kg BW extract combination treatment); DMBA-D3 group (DMBA + 900 mg/kg BW extract combination treatment); DMBA-D4 group (DMBA-D4 group (DMBA + 1,800 mg/kg BW extract combination treatment)

Furthermore, in liver specimens, the effects of the combination extract on hepatocytes in DMBA-induced experimental animals are shown in Figure 3. The data indicated that, in the DMBA-induced group, inflammatory cells were present in the liver tissue, along with sinusoidal dilation, compared with the normal group. These findings suggest that administering DMBA to experimental animals caused liver tissue damage, as evidenced by infiltration of inflammatory cells. Interestingly, the data showed that in the treatment groups receiving combination extracts, no additional severe liver damage was observed, indicating that no inflammatory cells were detected at any of the administered doses. These results suggest that the combination extract of meniran and turmeric acts as a hepatoprotective agent.

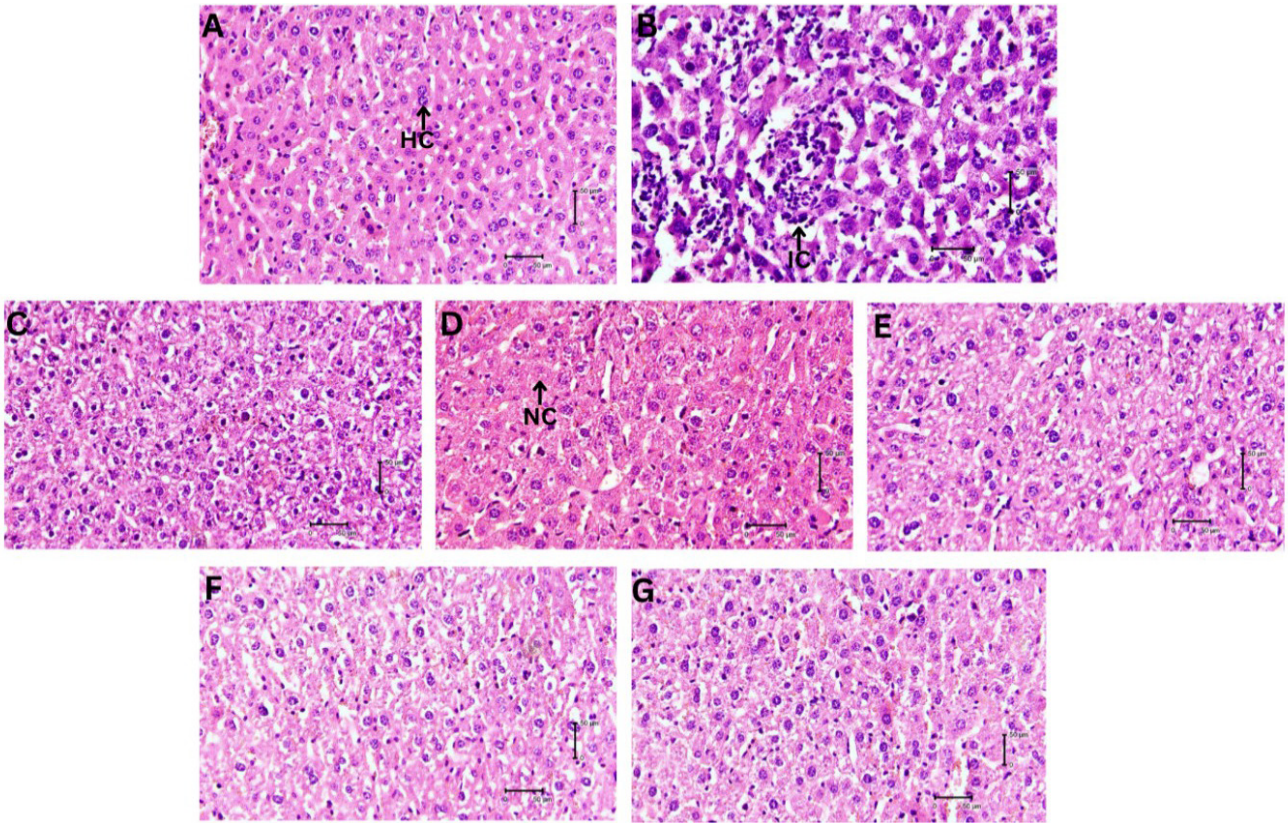

Figure 3. Histology of the liver organ after 8 weeks of DMBA induction (200x magnification). (A) Normal group, (B) DMBA: DMBA control group (45 mg/kg BW DMBA subcutaneous injection/DMBA), (C) DMBA-Cisplatin group (DMBA + 15 mg/kg BW Cisplatin via intraperitoneal injection), (D) DMBA-D1 group (DMBA + 100 mg/kg BW extract combination treatment, (E) DMBA-D2 group (DMBA + 300 mg/kg BW extract combination treatment), (F) DMBA-D3 group (DMBA + 900 mg/kg BW extract combination treatment), (G) DMBA-D4 group (DMBA-D4 group (DMBA + 1,800 mg/kg BW extract combination treatment). Arrows HC: normal hepatocyte cells, IC arrow: inflammation cells, NC arrow: necrosis cells

The potency of the meniran and turmeric extract combination as a hepatoprotective agent will be further analyzed by predicting the active compounds' pharmacological functions. Identification of active compounds with hepatoprotective potential was conducted using PassOnline. Based on the results, the majority of compounds in the combined meniran and turmeric extract exhibit hepatoprotective activity. These compounds function as hepatoprotectants, hepatocyte nuclear factor antagonists, and Hepatocyte Nuclear Factor 4 Alpha (HNF4α) antagonists (Figure 4). The predicted activity (Pa) value indicates the accuracy of the predicted functions, meaning that the higher the Pa value, the more accurate the predicted function (Filimonov *et al.* 2014).

4. Discussion

T $CD8^+$ cells and NK^+ cells are immunocompetent cells that play an essential role in eliminating cancer cells. These cells can promote target cell apoptosis through a combination of perforin/granzyme, the Fas ligand receptor mechanism, and cytokine secretion. T CD8 cells are a group of cells in the immune system that play a role in the adaptive immune system. NK cells are a group of lymphocyte cells that play a role in the innate immune system response (Nutt & Huntington 2019; Sari *et al.* 2023). These cells need to modulate their function to optimize their ability to kill cancer cells. This study showed that the extract combination could modulate $CD8^+$ T cells and NK^+ cells in mice injected with DMBA. Although there was a difference in results between $CD8^+$ cells and NK^+ cells, the DMBA+D3 group showed results close to those of the normal group. This suggests that a dose of 900 mg/kg BW may be the most effective dose compared to the others (Figures 1 and 2). Additionally, this data was supported by a previous study that showed the extract combination could modulate macrophage profiles in mice injected with DMBA (Puspitarini *et al.* 2024).

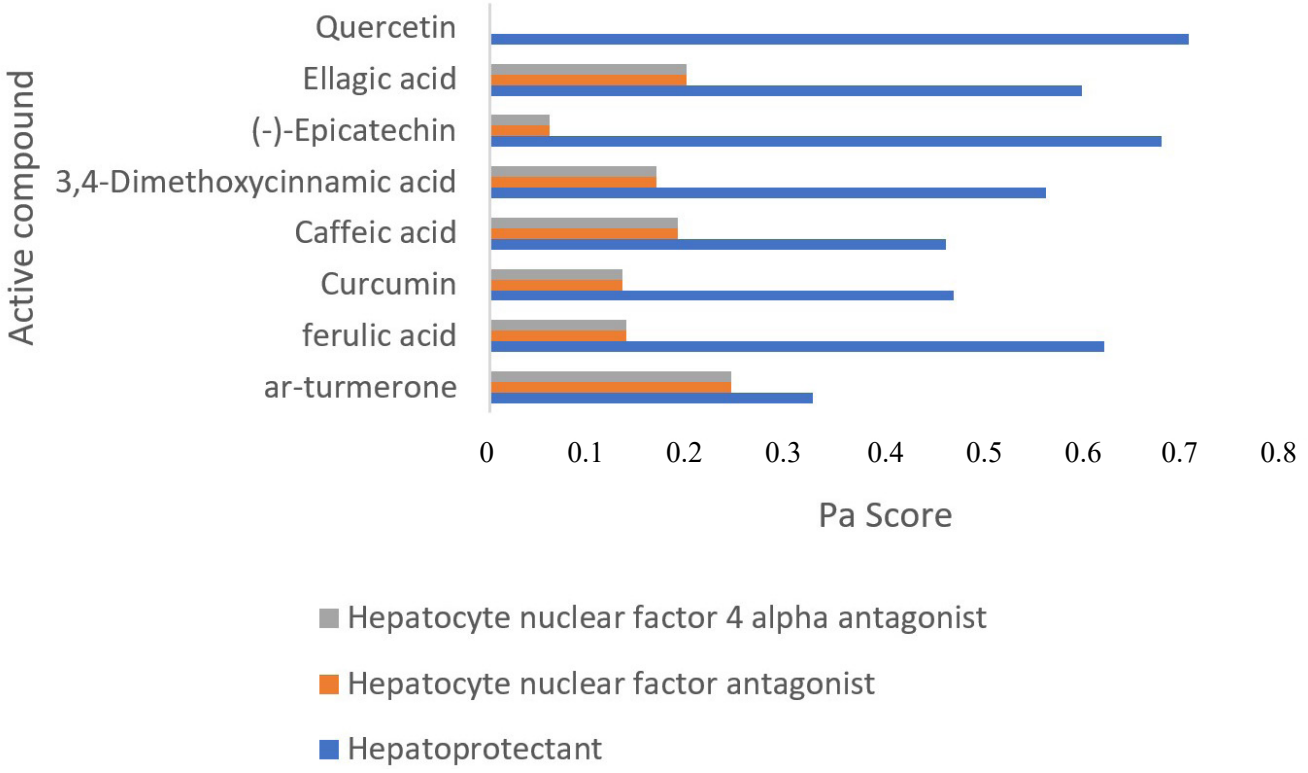

Figure 4. Prediction of pharmacological function as antihepatotoxic for the compounds in the combined extract. Pa score is the mean prediction activity score

Furthermore, the extract combination can also inhibit T47D cancer cell growth (Puspitarini *et al.* 2022).

Modulation of the immune system is essential for maintaining homeostasis and physiological stability in an organism. Immune cells require modulators to optimize their function, including the ability to kill cancer cells. Natural-derived immunomodulators are perceived as safer because they typically exhibit fewer adverse effects (Ortuño-Sahagún *et al.* 2017). Based on these results, the extract combination of meniran and turmeric in this study modulated immunocompetent cells. The extract combination reduced $CD8^+$ cells and increased NK^+ cells in mice induced with DMBA. Their potency might be supported by the bioactive compounds they contain. The active compounds in herbal plants have been shown to modulate the immune system. Polyphenol compounds, in particular, are known to alter the composition of immunoglobulins, inflammation, and immune cell populations (Hosseinzadeh and Younesi 2002).

Previous studies have shown that this extract combination contains several active compounds, such as curcumin, ferulic acid, vanillin, 4-coumaric acid, quercetin, and kaempferol (Puspitarini *et al.* 2022). Curcumin contributes to cancer elimination by decreasing the number of T regulatory cells, supporting the generation of reactive oxygen and nitrogen species by macrophages, and enhancing the cytotoxic activity of NK cells. Moreover, in another study, curcumin was shown to restore the number of $CD4^+$ and $CD8^+$ cells, reversing the shift toward Th1-secreted cytokines (Boroumand *et al.* 2018). Additionally, when quercetin was administered to BALB/c mice following injection with WEHI-3L leukemia cells, NK cell activity was elevated (Yu *et al.* 2010). In general, this study demonstrated that the extract combination of meniran and turmeric could modulate $CD8^+$ T and NK^+ cells in mice injected with DMBA.

The liver is an organ that plays a crucial role in metabolic processes, detoxification, and the body's defense system. As such, it is an important parameter for assessing the toxicity of substances that enter the body, including drugs and other chemicals. In addition to acting as a liver protector, a drug must also be safe for consumption without causing severe liver damage. This study examined liver histology in experimental animals treated with a combination of extracts after DMBA induction. The results of the survey show that administering an extract combination of meniran and turmeric at a dose of 1,800 mg/kg BW in mice did

not cause any liver damage compared to the DMBA group. Herbal treatments are often used because their safety levels are generally higher. Meniran has been reported to have hepatoprotective effects (Harish and Shivanandappa 2006; Ezzat *et al.* 2020; Noviyanti and Yowani 2023; Oktaviona *et al.* 2023). Turmeric has also been reported to play a role in anti-hepatotoxicity through its antioxidants (Karamalakova *et al.* 2019). Compared with previous studies on single herbal treatments, the extract combination is predicted to act synergistically as a hepatoprotective agent. The active compounds in herbal plants act as antioxidants and free radical scavengers, helping repair tissue damage caused by free radicals.

Furthermore, the identification and prediction of active compounds with hepatoprotective activity have been supported and confirmed by *in vivo* studies. Quercetin has been shown to protect the liver in mice exposed to the toxic substance acrylamide (ACR) (Ansar *et al.* 2016). Ellagic acid helps protect the liver from alcohol-induced liver disease (Zhao *et al.* 2021). Curcumin has been reported to act as a hepatoprotective agent against liver toxicity caused by various toxic compounds, such as alcohol, carbon tetrachloride (CCl_4), and diethylnitrosamine (Khan *et al.* 2019). Caffeic acid has been found to reduce hepatic lipid accumulation (Mu *et al.* 2021), while ferulic acid is an effective antihepatotoxic agent with no significant side effects (Rukkumani *et al.* 2004). Based on the results, the study indicates that the combination of meniran and turmeric can serve as a safe anti-hepatotoxic agent.

In conclusion, this study concluded that the extract combination of meniran and turmeric has the potential to modulate the immune response, particularly by affecting NK and CD8 cells, in DMBA-injected mice. Additionally, the findings suggest that this combination may serve as an effective antihepatotoxic agent. This research could serve as a consideration for future studies developing a combination formulation of meniran and turmeric.

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References

Agustina, D., Wahyuningsih, M., Widyarti, S., Soewondo, A., Tsuboi, H., Rifa'i, M., 2020. Noni juice (*Morinda citrifolia*) to prevent cancer progression in mice induced DMBA and cigarette smoke exposure. *Pharmacogn. J.* 12, 946–951. https://doi.org/10.5530/pj.2020.12.134

Ansar, S., Siddiqi, N.J., Zargar, S., Ganaie, M.A., Abudawood, M., 2016. Hepatoprotective effect of quercetin supplementation against acrylamide-induced DNA damage in wistar rats. *BMC Complement. Altern. Med.* 16, 327. https://doi.org/10.1186/s12906-016-1322-7

Ashrafi, M., Karimi, B., Sabahi, M., Shomali, T., 2018. Hepatoprotective effect of simvastatin in mice with DMBA-induced breast cancer: histopathological, biochemical and antioxidant status evaluation. *Biomed. Res. Ther.* 5, 2064–2077. https://doi.org/10.15419/bmrat.v5i3.421

Asyhari, F.N., Zulfatim, H.S., Putri, N.T.M., Dliyauddin, M., Jamil, A.S., Soewondo, A., Natsir, M.H., Ibrahim, M., Rahayu, S., Djati, M.S., Rifa'i, M., 2024. Expression of immunoglobulin M (IgM) and immunoglobulin G (IgG) in normal wistar rat post-cheral® administration. *HAYATI J. Biosci.* 31, 1030–1036. https://doi.org/10.4308/hjb.31.5.1030-1036

Basak, S., Annapure, U.S., 2022. The potential of subcritical water as a "green" method for the extraction and modification of pectin: a critical review. *Food Res. Int.* 161, 111849. https://doi.org/10.1016/j.foodres.2022.111849

Boroumand, N., Samarghandian, S., Hashemy, S.I., 2018. Immunomodulatory, anti-inflammatory, and antioxidant effects of curcumin. *J. Herbmed Pharmacol.* 7, 211–219. https://doi.org/10.15171/jhp.2018.33

Cao-Ngoc, P., Leclercq, L., Rossi, J.C., Hertzog, J., Tixier, A.S., Chemat, F., Nasreddine, R., Al Hamoui Dit Banni, G., Nehmé, R., Schmitt-Kopplin, P., Cottet, H., 2020. Water-based extraction of bioactive principles from blackcurrant leaves and *Chrysanthellum americanum*: a comparative study. *Foods.* 9, 1478. https://doi.org/10.3390/foods9101478

Chandra, K., Das, A.K., Banday, S., Rana, N.A., Arora, M., Jain, S., Islam, F., Agarwal, S., Kashyap, V., Joshi, S., Mueed, A., Dudeja, M., 2022. Efficacy of polyherbal formulations for prevention of COVID-19 infection in high-risk subjects: a randomized open-label controlled clinical trial. *Phytother. Res.* 36, 3632–3643. https://doi.org/10.1002/ptr.7531

Ezzat, M.I., Okba, M.M., Ahmed, S.H., El-Banna, H.A., Prince, A., Mohamed, S.O., Ezzat, S.M., 2020. In-depth hepatoprotective mechanistic study of *Phyllanthus niruri: in vitro* and *in vivo* studies and its chemical characterization. *PLoS ONE.* 15, e0226185. https://doi.org/10.1371/journal.pone.0226185

Fathima, S., Durairaj, S.K., Padigaru, M., Cma, B., 2022. Efficacy of a polyherbal supplement in enhancing immune cells in individuals frequently susceptible to cold and flu. *Curr. Dev. Nutr.* 6, 977. https://doi.org/10.1093/cdn/nzac068.006

Filimonov, D.A., Lagunin, A.A., Gloriozova, T.A., Rudik, A.V., Druzhilovskii, D.S., Pogodin, P.V., Poroikov, V.V., 2014. Prediction of the biological activity spectra of organic compounds using the pass online web resource. *Chem. Heterocycl. Compd.* 50, 444–457. https://doi.org/10.1007/s10593-014-1496-1

Harish, R., Shivanandappa, T., 2006. Antioxidant activity and hepatoprotective potential of *Phyllanthus niruri. Food Chem.* 95, 180–185. https://doi.org/10.1016/j.foodchem.2004.11.049

Hermansyah, D., Paramita, Desiree Anggia, Paramita, Deryne Anggia, Amalina, N.D., 2023. Combination *Curcuma longa* and *Phyllanthus niruri* extract potentiate antiproliferative in triple negative breast cancer MDAMB-231 cells. *Asian Pac. J. Cancer Prev.* 24, 1495–1505. https://doi.org/10.31557/APJCP.2023.24.5.1495

Hermansyah, D., Putra, A., Munir, D., Lelo, A., Amalina, N.D., Alif, I., 2021. Synergistic effect of *Curcuma longa* extract in combination with *Phyllanthus niruri* extract in regulating annexin A2, epidermal growth factor receptor, matrix metalloproteinases, and pyruvate kinase M1/2 signaling pathway on breast cancer stem cell. *Open Access Maced. J. Med. Sci.* 9, 271–285. https://doi.org/10.3889/oamjms.2021.5941

Hodges, R.E., Minich, D.M., 2015. Modulation of metabolic detoxification pathways using foods and food-derived components: a scientific review with clinical application. *J. Nutr. Metab.* 2015, 760689. https://doi.org/10.1155/2015/760689

Hosseinzadeh, H., Younesi, H.M., 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC Pharmacol.* 2, 7. https://doi.org/10.1186/1471-2210-2-7

Huang, S.T., Yang, R.C., Pang, J.H.S., 2004. Aqueous extract of *Phyllanthus urinaria* induces apoptosis in human cancer cells. *Am. J. Chin. Med.* 32, 175–183. https://doi.org/10.1142/S0192415X04001849

Karamalakova, Y.D., Nikolova, G.D., Georgiev, T.K., Gadjeva, V.G., Tolekova, A.N., 2019. Hepatoprotective properties of *Curcuma longa* L. extract in bleomycin-induced chronic hepatotoxicity. *Drug Discov. Ther.* 13, 9–16. https://doi.org/10.5582/ddt.2018.01081

Khadke, S., Gupte, P., Mourya, A., Yadav, A., Mane, S., Joshi, A., Mahajan, M., Mishra, M., Bhalerao, S., 2023. Immunomodulatory effect of a proprietary polyherbal formulation on healthy participants: a single- blind, randomized, placebo- controlled, exploratory clinical study. *Perspect. Clin. Res.* 14, 130–138. https://doi.org/10.4103/picr.picr_100_22

Khan, H., Ullah, H., Nabavi, S.M., 2019. Mechanistic insights of hepatoprotective effects of curcumin: therapeutic updates and future prospects. *Food Chem. Toxicol.* 124, 182–191. https://doi.org/10.1016/j.fct.2018.12.002

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., Bolton, E.E., 2023. PubChem 2023 update. *Nucleic Acids Res.* 51, 1373–1380. https://doi.org/10.1093/nar/gkac956

Km, H., Sy, I., Mu, K., A, I., Ab, U., Mh, Y., A, Y., Nk, A., Yb, G., Hy, M., 2021. Immunostimulatory activity of aqueous extract of polyherbal formulation on Th1/Th2 cytokines secretion and cell mediated immune response in rats. *Saudi J. Med. Pharm. Sci.* 7, 64–70. https://doi.org/10.36348/sjmps.2021.v07i01.013

Mu, H.N., Zhou, Q., Yang, R.Y., Tang, W.Q., Li, H.X., Wang, S.M., Li, J., Chen, W.X., Dong, J., 2021. Caffeic acid prevents non-alcoholic fatty liver disease induced by a high-fat diet through gut microbiota modulation in mice. *Food Res. Int.* 143, 110240. https://doi.org/10.1016/j.foodres.2021.110240

Noviyanti, N.P.R., Yowani, S.C., 2023. Potensi aktivitas hepatoprotektor dari meniran (*Phyllanthus niruri* L.) pada penderita penyakit hati. *Pros. Workshop Dan Semin. Nas. Farm.* 2, 654–667. https://doi.org/10.24843/WSNF.2022.v02.p52

Nutt, S.l., Huntington, N.D., 2019. 17-cytotoxic T lymphocytes and natural killer cells, in: Rich RR, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM (Eds.). *Clinical Immunology, 5th edition.* London: Elsevier. pp. 247-259.e1. https://doi.org/10.1016/B978-0-7020-6896-6.00017-X

Oktaviona, E.E.P., Qomariyah, N., Khaleyla, F., 2023. Aktivitas hepatoprotektif ekstrak daun *Phyllanthus acidus* L. pada mencit diabetes mellitus tipe 2. *LenteraBio Berk. Ilm. Biol.* 12, 381–388. https://doi.org/10.26740/lenterabio.v12n3.p381-388

Oo, A.M., Nor, M.N.M., Lwin, O.M., Simbak, N., 2022. Flavonol quercetin: immunomodulatory and anticancer properties. *Asian J. Med. Biomed.* 6, 17–31. https://doi.org/10.37231/ajmb.2022.6.1.452

Ortuño-Sahagún, D., Zänker, K., Rawat, A.K.S., Kaveri, S.V., Hegde, P., 2017. Natural immunomodulators. *J. Immunol. Res.* 2017, e7529408. https://doi.org/10.1155/2017/7529408

Patankar, S.B., Rangnekar, H., Joshi, K., Suryawanshi, K., Soni, P., Gorde, A., Shah, T., Patankar, S., Jha, D., Raje, R., 2021. Efficacy and safety of polyherbal formulation as an add-on to the standard of care in mild to moderate COVID-19: a randomized, double-blind, placebo-controlled trial. (MedRxiv Preprin) https://doi.org/10.1101/2021.05.14.21256900

Priosoeryanto, B.P., Sudaryatma, P.E., Lanniari, N., Setiyaningsih, S., Harlina, E., Wulansari, R., Ridho, R., Rostantinata, R., Wiradana, P.A., Okabayashi, T., 2025. Indonesian herb extracts inhibit the replication of bovine respiratory syncytial virus: *in vitro* study. *HAYATI J. Biosci.* 32, 727–739. https://doi.org/10.4308/hjb.32.3.727-739

Puspitarini, S., Azzamudin, H., Ulinnuha, M., Puspitoputri, A.H., Khansa, S., Widyarti, S., Widodo, N., Rifa'i, M., 2024. Immunomodulator effect of polyherbal extract turmeric-meniran on macrophages profile of mice cancer model by DMBA injection. *Malays. J. Fundam. Appl. Sci.* 20, 579–587. https://doi.org/10.11113/mjfas.v20n2.3332

Puspitarini, S., Widodo, N., Widyarti, S., Jatmiko, Y.D., Rifa'i, M., 2022. Polyherbal effect between *Phyllanthus urinaria* and *Curcuma longa* as an anticancer and antioxidant. *Res. J. Pharm. Technol.* 15, 671–678.

Putra, W.E., Rifa'i, M., 2019. Immunomodulatory activities of *Sambucus javanica* extracts in DMBA-exposed BALB/c mouse. *Adv. Pharm. Bull.* 9, 619–623. https://doi.org/10.15171/apb.2019.071

Rifa, M., Ramadhani, A., Nafisah, W., Isnanto, H., Sholeha, T., Jatmiko, Y.D., Tsuboi, H., Rifa'i, M., 2021. Immunomodulatory effects of *Cyperus rotundus* extract on 7,12-Dimethylbenz[a] anthracene (DMBA) exposed BALB/c mice. *Pharm. Sci.* 27, 46–55. https://doi.org/10.34172/PS.2020.61

Rukkumani, R., Aruna, K., Suresh Varma, P., Padmanabhan Menon, V., 2004. Hepatoprotective role of ferulic acid: a dose-dependent study. *J. Med. Food.* 7, 456–461. https://doi.org/10.1089/jmf.2004.7.456

Sari, F.N., Samoedra, R.S., Pratama, S.K., Rahayu, S., Soewondo, A., Jatmiko, Y.D., Natsir, M.H., Tsuboi, H., Rifa'i, M., 2023. Immunomodulatory effects of unripe sapodilla (*Manilkara zapota*) fruit extract through inflammatory cytokine regulation in type 1 diabetic mice. *Jordan J. Biol. Sci.* 16, 345–352. https://doi.org/10.54319/jjbs/160219

Vivier, E., Tomasello, E., Baratin, M., Walzer, T., Ugolini, S., 2008. Functions of natural killer cells. *Nat. Immunol.* 9, 503–510. https://doi.org/10.1038/ni1582

Wang, H., Chen, Y., Wang, L., Liu, Q., Yang, S., Wang, C., 2023. Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. *Front. Pharmacol.* 14, 1265178. https://doi.org/10.3389/fphar.2023.1265178

Wiratmini, N.I., Pharmawati, M., Sukmaningsih, A.A.S.A., Raharjo, S.J., Widowati, W., 2025. Anti-inflammatory activity of *Phyllanthus niruri* L. leaf extract: *in vitro* study on RAW 264.7 macrophage cells and *in silico* analysis. *HAYATI J. Biosci.* 32, 287–299. https://doi.org/10.4308/hjb.32.2.287-299

Yu, C.S., Lai, K.C., Yang, J.S., Chiang, J.H., Lu, C.C., Wu, C.L., Lin, J.P., Liao, C.L., Tang, N.Y., Wood, W.G., Chung, J.G., 2010. Quercetin inhibited murine leukemia WEHI-3 cells in vivo and promoted immune response. *Phytother. Res*. 24, 163–168. https://doi.org/10.1002/ptr.2841

Zhao, L., Mehmood, A., Soliman, M.M., Iftikhar, A., Iftikhar, M., Aboelenin, S.M., Wang, C., 2021. Protective effects of ellagic acid against alcoholic liver disease in mice. *Front. Nutr.* 8, 744520. https://doi.org/10.3389/fnut.2021.744520