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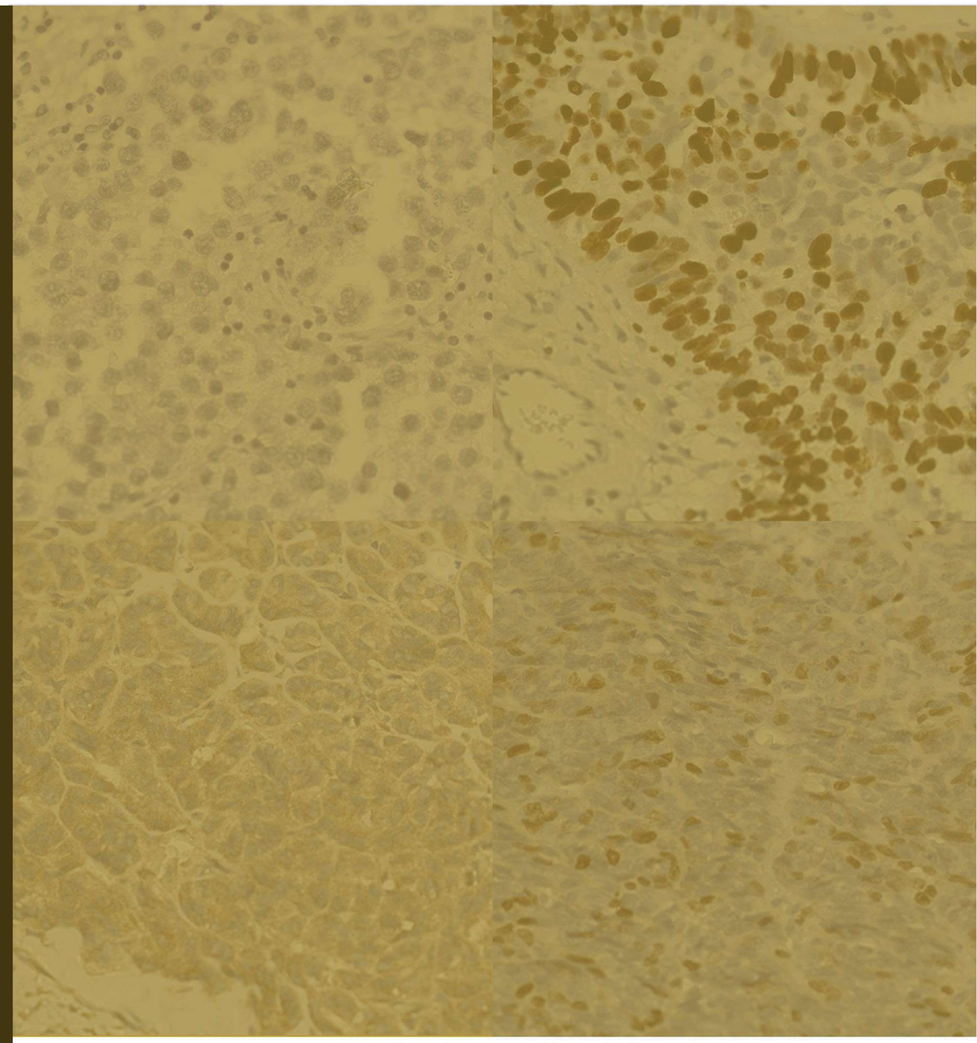

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Campus B Universitas Nahdlatul Ulama Surabaya
Jl. Raya Jemursari No. 51-57, Surabaya, East Java, Indonesia. Zip code 60237
Phone: +62822-3344-123-2
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RESEARCH ARTICLE

Blumea balsamifera and *Sargassum aquifolium* extracts reduce fatty liver damage through lipid metabolism signalling pathways

I Gede Widhiantara [1], Putu Angga Wiradana [1], Anak Agung Ayu Putri Permatasari [1], Ni Kadek Yunita Sari [1], I Wayan Rosiana [1], I Made Gde Sudyadnyana Sandhika [1], Novaria Sari Dewi Panjaitan [2]

[1]Research Group of Biological Health, Study Program of Biology, Faculty of Health and Science, Universitas Dhyana Pura, North Kuta, Indonesia

[2]Center for Biomedical Research, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, Indonesia

Correspondence:
I Gede Widhiantara
Research Group of Biological Health, Study Program of Biology, Faculty of Health and Science, Universitas Dhyana Pura, Jalan Raya Padangluwih, North Kuta, Badung Regency – 80361, Indonesia

Email:
widhiantara@undhirabali.ac.id

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a condition marked by excessive fat accumulation in the liver and poses a significant health challenge. The leaves of *Blumea balsamifera* and *Sargassum aquifolium* have been reported to have anti-atherogenic effects. This study aims to determine the effectiveness of *B. balsamifera* extract (BBLE) and *S. aquifolium* extract (SAE) in preventing and treating liver fat accumulation in Wistar rats induced by a high-cholesterol diet through the expression of the AMP-activated protein kinase (AMPK)/ Sirtuin 1 (SIRT1)/peroxisome proliferator-activated receptor γ (PPARγ) pathway, and the leptin receptor. The experimental design of this study is laboratory-based, involving, 20 Wistar rats were fed a high-cholesterol diet over a period of 21 days. The rats were divided into four groups for the evaluation of BBLE and SAE effect: negative control (P0): induced with a high-cholesterol diet + distilled water, positive control (P1): induced with a high-cholesterol diet + simvastatin, P2: induced with a high-cholesterol diet + 4 mg/kg/bw BBLE, and P3: induced with a high-cholesterol diet + 4 mg/kg/bw BBLE and 4 mg/kg/bw SAE. The treatment duration extended over three months. Immunohistochemical analyses were performed on liver tissues to measure AMPK, SIRT1, PPARγ, and leptin receptor expression. The results indicated that leptin expression was lower in the BBLE+SAE group compared to the simvastatin group, and differences were significant between the BBLE and BBLE+SAE groups. No significant differences were noted in AMPK, SIRT1, and PPARγ expression between the simvastatin and BBLE+SAE groups ($p \geq 0.05$). In conclusion, BBLE and SAE effectively reduce liver lipid accumulation and enhance fat metabolism in hypercholesterolemic rats.

Citation: Widhiantara IG, Wiradana PA, Permatasari AAAP, Sari NKY, Rosiana IW, Sandhika IMGS, Panjaitan NSD. Blumea balsamifera and Sargassum aquifolium extracts reduce fatty liver damage through lipid metabolism signalling pathways. Indones J Med Lab Sci Technol. 2024;6(2):131–142. https://doi.org/10.33086/ijmlst.v6i2.5697

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represent the predominant cause of chronic liver disease on a global scale and affects about 25% of the adult population (1). One in every patient with NAFLD progresses to non-alcoholic steatohepatitis (NASH) (2), which serves as a significant precursor to cirrhosis and hepatocellular carcinoma (3–5). The global incidence of NAFLD has almost doubled influenced by unhealthy lifestyle choices, and it increases along with the rising levels of type-2 diabetes (T2D), obesity, and other metabolic syndromes. Despite the testing of multiple pharmacological agents for NAFLD, none have demonstrated conclusive efficacy (6). Therefore, it is essential to gain a comprehensive understanding of the disease's pathogenesis in order to develop effective therapeutic strategies (7).

High-cholesterol diets are associated with a decrease in energy expenditure, leading to obesity, adipocyte hypertrophy, and inflammation (8). Elevated lipid levels lead to liver steatosis, peripheral insulin resistance, and diabetes, resulting in increased Low-Density Lipoprotein (LDL) and decreased High-Density Lipoprotein (HDL) levels (9). Furthermore, high-cholesterol diets heighten the expression of several pro-inflammatory cytokines while also damaging liver mitochondria, thereby increasing the risk of developing fatty liver and metabolic syndrome (10).

The accumulation of free cholesterol can result in stress on the endoplasmic reticulum (ER), mitochondrial dysfunction, cholesterol crystallization in lipid droplets, and the formation of toxic cholesterol metabolites (oxysterols) that can cause hepatocyte apoptosis, necrosis, or proptosis (11). Previous research has identified a link between harmful cholesterol metabolites with the onset and progression of liver fat accumulation (12). Additionally, the internalization of plasma membrane cholesterol, mediated by the Aster protein, activates inflammatory gene expression and facilitates the transition from fatty liver to fibrosis (13). While understanding the signalling pathways involved in liver cholesterol metabolism due to high-cholesterol diet in rat models is crucial, but comprehensive reports are still very limited.

Sirtuins (SIRT) are a family of proteins identified in various animal species, with SIRT1 playing a pivotal role in numerous physiological functions, including liver glucose uptake, liver gluconeogenesis, fatty acid metabolism, and insulin secretion (14). AMP-activated protein kinase (AMPK) plays a crucial role in energy metabolism functioning as a sensor of cellular energy levels (15,16). An increase in intracellular AMP/ATP ratio activates AMPK, which can inhibit lipid synthesis by blocking fatty acid synthase or promote fatty acid oxidation to enhance energy availability. Studies have shown that the extent of hepatosteatosis is directly linked to a reduction in AMPK levels in liver tissue in rats subjected to a high-fat diet (17).

In addition to AMPK and SIRT1, Peroxisome Proliferator-Activated Receptors (PPARs) serve as ligand-activated transcription factors within the nuclear receptor superfamily. PPARs are integral to the regulation of lipid metabolism, glucose homeostasis, energy balance, inflammation, and atherosclerosis. There are three principal isoforms of PPARs–alpha (α), beta (β), delta (δ), and gamma (γ)–which are expressed variably across different tissues (18). PPARγ is predominantly found in adipose tissue and is responsible for regulating adipocyte differentiation and energy storage. It is also found in the liver, kidneys, lungs, and rectum, where its overexpression lead to increased fat accumulation (19). At the molecularl level, AMPK and SIRT1 collaborate to reduce PPARγ expression, resulting in diminished lipid synthesis (20).

Given the complex aetiology of NAFLD, there are currently limited therapies that have been proven effective. Traditional medicine frequently plays a role in the treatment of both infectious and non-infectious diseases across various Asian countries, including Indonesia. One noteworthy herbal remedy is Sembung leaf (*Blumea balsamifera*), Balinese herbal plant, which is commonly prepared as a traditional beverage known as "Loloh". It has been proven to reduce oxidative stress-induced damage in the testes of rats induced by a high-cholesterol diet (21,22). Additionally, extracts from *B. balsamifera* leaf (BBLE) has been reported to effectively reduce MDA and total cholesterol levels while enhancing the levels of Superoxide Dismutase (SOD), Luteinizing Hormone (LH), and testosterone levels in male rats on a high-cholesterol diet for 21 days (23). Furthermore, research on the effectiveness of BBLE has also been reported to effectively increase the expression of the StAR gene related to the maintenance of testosterone hormone steroidogenesis in hypercholesterolemic male rats (24).

In recent decades, research has increasingly focused on fisheries resources, particularly macroalgae, which are utilized in the pharmaceutical and food industries (25,26). Studies indicate that macroalgae can enhance the immune function and overall physical fitness. Previous research on the brown macroalgae *Sargassum aquifolium* have revealed its capability to produce alginate and other biopolymers (27,28), as well as its potential in maintaining liver health in cases of fatty liver, as evidenced by various animal and human review studies (29). However, the regulatory effects Sargassum aquifolium in conjunction with *B. balsamifera* extract on lipid

metabolism, along with their effectiveness in addressing fatty liver, remain to be validated through in vivo experiments, and the underlying mechanisms of action have not been clarified.

A comparison study has been conducted to analyse the effects of BBLE on lipid accumulation in the liver, both independently and in combination with *S. aquifolium* extract (SAE) in hypercholesterolemic rats. It is anticipated that SAE will enhance therapeutic efficacy in hypercholesterolemia model mice while providing beneficial compounds from BBLE, which is recognised for its high flavonoids content. This approach is further supported by evidence indicating that SAE contains alginate, a biopolymer previously investigated for its health benefit. Therefore, this study aims to evaluate the effects of *B. balsamifera* leaf extract and *S. aquifolium* on fatty liver induced by high-cholesterol diet, focusing on enhancing liver lipid metabolism through the activation of the AMPK/SIRT1/PPARγ signalling pathway and Leptin.

2. MATERIALS AND METHODS

2.1. Ethical Clearance

All experiments were conducted in accordance with the guidelines set forth by the Research Ethics Committee of the Sekolah Tinggi Ilmu Kesehatan Bina Usada Bali (Bina Usada Bali Health Sciences College/STIKES), which holds Ethical Clearance Number: 135/EA/KEPK-BUB-2023.

2.2. Materials and Reagent

The BBLE was obtained from the leaves of *B. balsamifera*, which were sourced from plantations in Luwus Village, Tabanan Regency, Bali Province, Indonesia. The leaf samples underwent identification at the Eka Karya Bali Botanical Gardens affiliated with the National Research and Innovation Agency (BRIN), under Access Number B.206/IPH.7/AP/VIII/2020. SAE was extracted from the brown macroalgae, *Sargassum aquifolium*, collected from Mertasari Beach, Denpasar City, Bali Province, Indonesia, at the lowest tidal conditions in the intertidal zone. The species and morphology of *S. aquifolium* sample were confirmed at the National Research and Innovation Agency (BRIN) in Jakarta, with registration number: 25498.

Male Wistar rats weighing 200 – 300 grams were procured from the Pharmacology Laboratory at the Faculty of Medicine, Universitas Udayana. The commercial feed used in this study was obtained from PT Charoen Pokphand. The high-cholesterol diet formulation comprised 10% (100 grams) of pork oil, equivalent to 108.7 mL, 5% (50 grams) of duck egg yolk, and commercial feed to reach a total of 1,000 grams. A 0.25% solution of Carmoxymethyl cellulose (CMC) was acquired from Sigma-Aldrich with catalog number: 9004-32-4.

For the immunohistochemical analysis, the following antibody kits were utilised: antibodies against AMPK (Ambcam, UK; Catalog number: ab131512), PPARγ (Abcam, UK; Catalog number: ab45036), and HRP (horseradish peroxidase)-conjugated anti-(rabbit IgG) antibody (Abcam, UK, Catalog number: ab97051) and SIRT1 (Abcam, UK; Catalog number: 131611AP). Anti-Leptin Receptor antibody (Abcam, UK; Catalog number: ab216690) was sourced from rabbit polyclonal antibody production.

2.3. *Blumea balsamifera* Leaf Extract (BBLE) Preparation

Fresh sembung leaves (*Blumea balsamifera*) were collected from Luwus Village, Tabanan Regency, Bali Province. These leaves underwent a thorough rinsing with clean water to eliminate any foreign pollutants or organic materials. Following this, the samples were air-dried at room temperature for a period of 24 hours before being further dried in an oven set to 50°C. The dried leaves were then ground using a blender and sieved through a 20-mesh screen to achieve a fine powder. A total of 250 grams of sembung leaf powder was weighed and subsequently soaked in 70% ethanol (Sigma-Aldrich, USA; Catalog number: 64-17-5) for 24 hours in a sealed container. During the extraction process, the mixture was stirred to enhance the effective extraction of metabolites from the leaves. The macerate was then separated using three filtration methods with sterile flannel cloth. To obtain a concentrated extract, the resulting macerate was evaporated using a vacuum rotary evaporator at 40°C and 100 rpm. The preparation standard for the thick sembung leaf extract was aligned with the Indonesian Herbal Pharmacopoeia 2017 (30).

2.4. *S. aquifolium* Extract (SAE) Preparation

Samples of *S. aquifolium* were procured from Mertasari Beach, Denpasar, as identified by the study of Permatasari *et al.* (27). The collection occurred during low tide within the intertidal zone. The macroalgae were carefully placed into sterile plastic bags (30×10 cm) and transported in a cooling box containing ice gel. Following collection, the samples were cleaned with running clean water and dried for three days. The extraction method

for *S. aquifolium* followed the protocol established by Isnansetyo *et al.* (31) with minor modifications. The extraction utilised distilled water (Sigma-Aldrich, USA; Catalog number: 7732-18-5) at ratio of 25 ml of brown algae to 75 ml of solvent (32). The mixture was subjected to stirring for 10 minutes, followed by boiling for 15 minutes, and then centrifugation completed the extraction process. The supernatant was collected after centrifugation, and the extract was concentrated using a vacuum rotary evaporator at 50°C before being tested in the research environment to develop a thick extract preparation. The dosage for both extracts was subsequently determined and combined, with the SAE.

2.5. BBLE and SAE Combination

The dosage for both extract preparations (BBLE and SAE) were established. The dosage for BBLE was set at 4 mg/ml/day, based on prior research findings (24), while the SAE dosage was similarly set at 4 mg/ml/day, as adjusted according to the study by Safhi *et al.* (33). The mixture of both extracts was then homogenized with a 0.25% Carboxymethyl cellulose (CMC) solution (Sigma-Aldrich, USA).

2.6. Animals and Experimental Treatments

The study utilised male Wistar rats (*Rattus norvegicus*), each with an average weight ranging from 150 to 200 grams as experimental subjects. Prior to the initiation of treatment, the Wistar rats were housed in HDPE plastic containers, dimensioned at 40 cm × 35 cm × 20 cm, equipped with wire lids, and the bottom of the containers was covered with husk to absorb waste. Each cage was designated for five rats, accommodating a total of 20 Wistar rats across of four cages.

A 7-day acclimatization phase was implemented, during which the rats were provided with commercial feed sourced from PT. Charoen Pokphand, along with access to sterile drinking water. In instances of illness, affected rats were promptly removed from their housing and referred to a veterinarian for further evaluation and treatment. The high-cholesterol diet used in this study comprised 10% lard (100 g of oil equating to 108.7 ml), 5% duck egg yolk (50 g), and 1,000 g of commercial feed. The dosage schedule for the high-cholesterol diet was established at 20 gr/day, administered *ad libitum* (23) in adherence to protocols outlined by Omagari *et al.* (34).

The investigation was conducted as experimental laboratory research with a completely randomized design. The rat subjects, which had been given a high cholesterol diet for 50 days, were systematically divided into four groups: (1) Negative control group (P0): hypercholesterolemic rat receiving sterile distilled water for 3 months; (2) Positive control group (P1): hypercholesterolemic rat treated with simvastatin at a dose of 20 mg/day/kg BW for 3 months; (3) BBLE treatment group (P2): hypercholesterolemic rat administered BBLE at a dose of 4 mg/ml/day orally for 3 months; (4) BBLE+SAE treatment group (P3): hypercholesterolemic rat receiving BBLE extract at a dose of 4 mg/ml/day + SAE extract at a dose of 4 mg/ml/day orally for 3 months.

The high-cholesterol diet was maintained throughout the 3-month experimental period. Following this period, liver samples from each group were collected for analyses of AMPK/SIRT1/PPARγ protein expression and Leptin receptors using the immunohistochemistry method.

2.7. Sampling of Liver Tissue

A total of 20 liver samples were extracted, with five repetitions from each treatment group. These samples were analysed to determine the protein expression of the AMPK/SIRT1/PPARγ pathway and leptin receptor via immunohistochemistry. The antibodies used in the study included AMPK (ab131512), PPARγ (ab45036), and HRP (horseradish peroxidase)-conjugated anti-rabbit IgG antibody (ab97051) were obtained from Abcam (Cambridge, UK), and SIRT1 (131611AP) sourced from Proteintech (Rosemont, USA). The Anti-Leptin Receptor antibody (ab216690) was produced using rabbit polyclonal antibodies.

2.8. Immunohistochemical Analysis

The liver tissues that were obtained were embedded in paraffin wax; and sections measuring 4 µm were prepared for the evaluation of target protein expression. Immunohistochemistry staining of the liver tissues from each group was performed in accordance with the specific instructions provided with each protein kit as described in subsection 2.7. Colour development was executed using a DAB colour kit, while counterstaining was done with hematoxylin. Following this, the prepared samples underwent differentiation with hydrochloric alcohol. Samples exhibiting a yellow-brown particle coloration were classified as positive for the target proteins (35).

An optical microscope was utilised to assess the expression levels of each protein within the liver tissues. The Remmele scale index, also known as the Immuno Reactive Score (IRS) is determined by multiplying the immunoreactive cell percentage score by the color intensity score of those cells. The data from each sample

represent the average IRS values observed across five fields of view (FOV) at 400× magnification. The focus of this analysis included the expression of AMPK/SIRT1/PPARγ and Leptin receptors, which are responsive to the antigen-antibody interaction in rat liver tissues.

2.9. Statistical Analysis

The average data reflecting the expression of AMPK/SIRT1/PPARγ proteins, as well as leptin receptor, were tabulated using Microsoft Excel (Microsoft, USA). Each group was subjected to five repetitions, with the final results presented as mean ± standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics software Version 23.0 (SPSS Inc., Chicago, IL, USA). The significance of the results was assessed using One-Way ANOVA, followed by Duncan's test, with a significance level established at 95% (P≤0.05). subsequently presented in graphical and pictorial formats, with further processing conducted using GraphPad PRISM software Version 8.0 (GraphPad Software, Inc).

3. RESULTS AND DISCUSSION

The immunohistochemical analysis was conducted to assess the expression of Leptin receptor, AMPK, SIRT-1, and PPARγ proteins within the hepatocyte cells of male Wistar rats. This study aimed to elucidate the molecular mechanism through BBLE and SAE in hypercholesterolemic conditions. Observations were made using the Remmele scale index or Immuno Reactive Score (IRS), which is the result of multiplying the score of the percentage of immunoreactive cells by the color intensity score of immunoreactive cells. The data for each sample reflect the average IRS values that can be observed in 5 fields of view at 400× magnification.

Data analysis indicated that the administration of both extracts had a significant effect on the expression of the Leptin receptor, AMPK, SIRT-1, and PPARγ. The average expression levels of each target protein are illustrated in Figure 1.

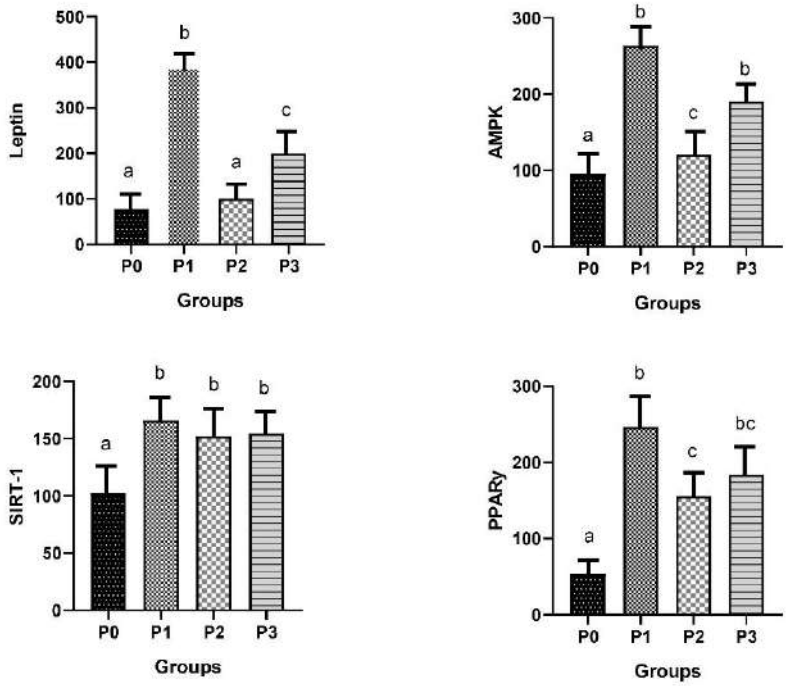

Figure 1. Average expression diagram of Leptin, AMPK, SIRT-1, and PPARγ in Hepatocytes of Wistar Rats. P0 = negative control (High cholesterol diet); P1 = positive control (High cholesterol diet + Simvastatin); P2 = 4 mg/kg BW BBLE + high cholesterol diet; P3 = 4 mg/kg BW BBLE + 4 mg/kg BW SAE and high cholesterol diet. Different letter notations indicate significant differences (p≤0.05) based on DUNCAN's test.

3.1. Expression of Leptin receptors

The results presented in Figure 1 reveal that the average expression of Leptin in group P0 (distilled water) was significantly different (p≤0.05) from groups P1 (simvastatin), P2 (BBLE), and P3 (BBLE+SAE). Specifically, the average expression of Leptin in group P1 (simvastatin) was significantly higher (p≤0.05) compared to groups P2

(BBLE), and P3 (BBLE+SAE). Furthermore, the combination of BBLE and SAE exhibited a significantly increased expression ($p \leq 0.05$) compared to the individual administration of BBLE and distilled water. Figure 2 displays the expression of Leptin protein in the liver of hypercholesterolemic Wistar rats.

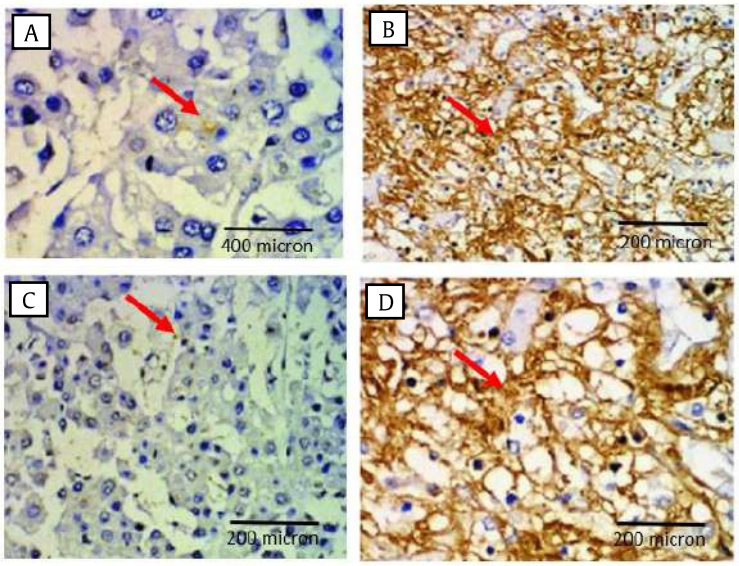

Figure 2. Leptin Expression in Hepatocytes of Wistar Rats. (A) P0 = negative control (Distilled water + High cholesterol diet). (B) P1 = positive control (High cholesterol diet + Simvastatin). (C) P2 = 4 mg/kg BW BBLE + high cholesterol diet. (D) P3 = 4 mg/kg BW BBLE + 4 mg/kg BW SAE and high cholesterol diet. Immunohistochemistry staining Ab. Anti-Leptin at 400x magnification; Nikon H600L microscope.

It is important to note that the physiology of rats consuming a high-cholesterol diet can lead to disruption in energy balance, resulting in glucose intolerance, insulin resistance, leptin resistance, and obesity (36,37). This phenomenon is particularly evident in the P0 group (distilled water + high cholesterol diet), which was administered continuously for 3 months and 21 days, culminating in reduced Leptin expression. Previous study has indicated that bioactive compounds can mitigate oxidative stress and inflammation association with a high-cholesterol diet and Leptin resistance.

For instance, Bergamot leaf extract (*Citrus bergamia*) (BLE) given at a dosage of 50 mg/kg, has been shown to reduce caloric intake, diminish insulin resistance, enhance adipose tissue function and elevate leptin levels in obese rats subjected to a high-fat sugar diet over 20 weeks (38). Additionally, a 30% ethanol extract of *Citrus unshiu* peel has demonstrated efficacy in lowering LDL levels, increasing leptin levels, suppressing fat accumulation, preventing adipogenesis, and inhibiting the production of pro-inflammatory cytokines such as IL-1β, IL-13, and TNF-α (39). Furthermore, Caulerpin, a compound isolated from *Caulerpa racemosa*, functions by activating the AMPK energy sensor, which is dependent on calcium/calmodulin-dependent protein kinase 2 (CaMKK2), in response to elevated AMP/ATP ratio (40). The concurrent administration of BBLE and SAE has proven effective in maintaining leptin levels in hypercholesterolemic rats. Secondary metabolites found in BBLE—including flavonoids, saponins, phenols, tannins, and steroids—are believed to play a significant role in modulating the leptin receptor, thus influencing lipids synthesis in the liver of hypercholesterolemic rats (23). Moreover, brown algae, rich in phlorotannin, act as anti-obesity agents by regulating the leptin signalling pathway in vivo (41).

3.2. AMP-Activated Protein Kinase (AMPK)

AMP-activated protein kinase (AMPK) serves as a critical cellular energy sensor and regulator of metabolic homeostasis, playing a significant role in controlling autophagy, mitochondrial homeostasis, and the metabolism of proteins, lipids, and glucose (42). The activation of AMPK is essential for managing lipid metabolism, which involves inhibiting the synthesis of cholesterol, triglycerides, and de novo fatty acids, while simultaneously enhancing the absorption and oxidation of fatty acids (43). This study demonstrated that the P3 group (4 mg/kg BW BBLE + 4 mg/kg BW SAE) significantly increased AMPK expression in Wistar rats subjected to a high0cholesterol diet. Interestingly, the increase in AMPK expression for this group did not differ significantly ($p \geq 0.05$) when compared to the P1 group (simvastatin). However, it was significantly higher ($p \leq 0.05$) compared to the P2 (BBLE) group and the P0 (negative control) group (Figure 3).

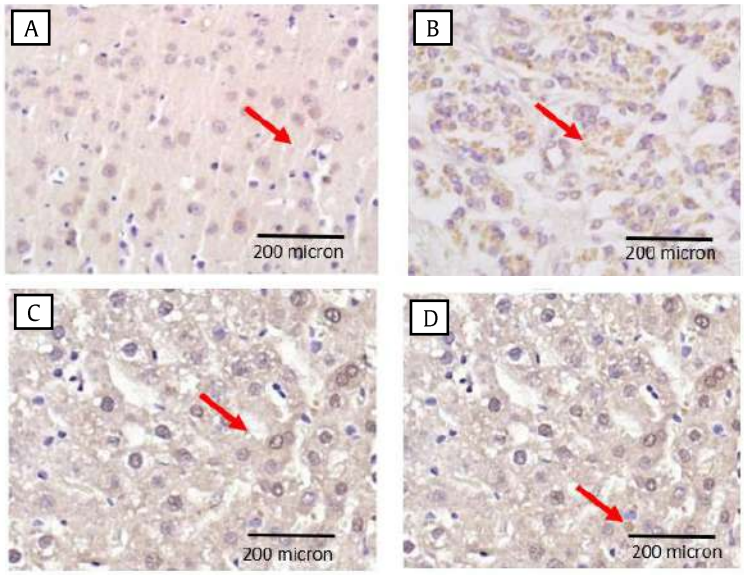

Figure 3. AMPK Expression in Hepatocytes of Wistar Rats. (A) P0 = negative control (Distilled water + High cholesterol diet). (B) P1 = positive control (High cholesterol diet + Simvastatin). (C) P2 = 4 mg/kg BW BBLE + high cholesterol diet. (D) P3 = 4 mg/kg BW BBLE + 4 mg/kg BW SAE and high cholesterol diet. Immunohistochemistry staining Ab. Anti-AMPK at 400x magnification; Nikon H600L microscope.

Pior research has established that Sangyod rice extract (SRE) has capacity to enhance AMPK activation, which plays a critical role in inhibiting adipogenesis, thereby reducing insulin resistance and obesity (44). The active compound quercetin, present in BBLE, is believed to enhance AMPK phosphorylation in adipocytes, contributing to the reduction of adipogenesis (45). Furthermore, other signalling pathways, such as Wnt/β-catenin and Hedgehog (Hh), have been noted to influence adipocyte differentiation and adipogenesis independently of the AMPK pathway (43). Additionally, the extract derived from the brown macroalgae Ecklonia cava, which contains the polyphenol phlorotannin, has demonstrated inhibitory effects on adipogenesis. The role of compounds from this extract in mediating their effects appears to be associated with AMPK activation (46). Similarly, compounds found in brown seaweed, particularly fucoxanthin, have shown protective effects against endothelial cell damage caused by oxidized low-density lipoprotein (oxLDL) while also increasing the expression of AMPK in hypercholesterolemic rats (47).

3.3. SIRT-1

The evaluation of SIRT-1 expression revealed that the P0 group (distilled water + high cholesterol diet) were significantly lower levels ($p \leq 0.05$) at 102.41 in comparison to the other three groups: P1 (simvastatin + high cholesterol diet) at 166.0, P2 (4 mg/kg BW BBLE + high cholesterol diet) at 151.95, and P3 (4 mg/kg BW BBLE + 4 mg/kg BW SAE + high cholesterol diet) at 154.58. These findings suggest that the administration of BBLE and its combination with SAE can enhance fat synthesis function through SIRT-1 signalling pathway in hypercholesterolemic rats (Figure 4). The mechanism through which BBLE and SAE operate to align with those of simvastatin in regulating SIRT-1 expression.

SIRT-1 acts in signalling pathways involved in lipid synthesis and degradation in the liver, and contributes to the pathogenesis of NAFLD (48). The findings of this study indicate that regulation of SIRT-1 signalling may play a role in the therapeutic effects introduced by BBLE and SAE. Puerarin has shown potential in treating NAFLD by enhancing mitochondrial function and activating the AKT-1/SIRT-1 signalling pathway, thereby reducing steatosis, and metabolic disruptions associated with high glucose and fat diets (49). Tetrahydroxy flavone (DHK), a flavonoid, has demonstrated the ability to induce SIRT-1 expression in a model of Acetaminophen (APAP)-induced liver damage, promoting autophagy, reducing oxidative stress, and inhibiting inflammatory responses (50).

3.4. PPARγ

The expression of PPARγ in this study showed a significant increase ($p \leq 0.05$) in the P1 group (simvastatin + high cholesterol diet) compared to the P2 group (4 mg/kg BW BBLE + high cholesterol diet) and P0 (distilled water + high cholesterol diet). However, the average increase observed in the P1 group was not significantly different

(p≥0.05) when compared to the P3 group (4 mg/kg BW BBLE + 4 mg/kg BW SAE and high cholesterol diet), as illustrated in Figure 5.

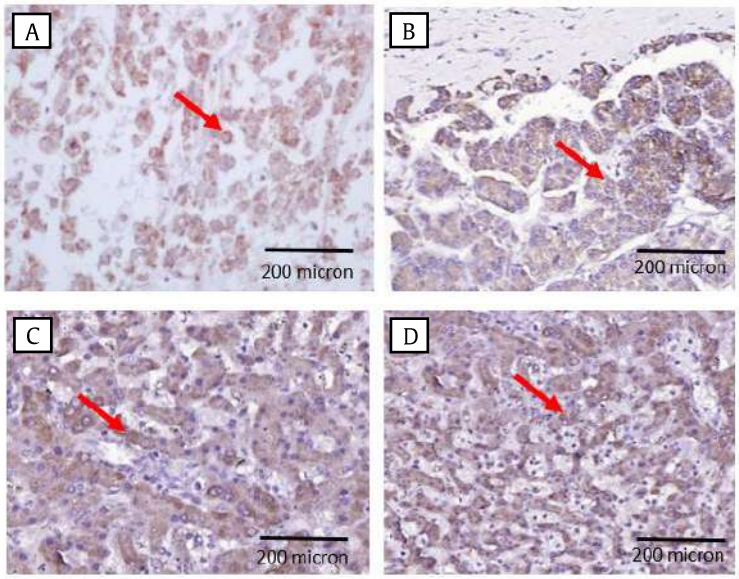

Figure 4. SIRT-1 Expression in Hepatocytes of Wistar Rats. (A) P0 = negative control (Distilled water + High cholesterol diet). (B) P1 = positive control (High cholesterol diet + Simvastatin). (C) P2 = 4 mg/kg BW BBLE + high cholesterol diet. (D) P3 = 4 mg/kg BW BBLE + 4 mg/kg BW SAE and high cholesterol diet. Immunohistochemistry staining Ab. Anti-SIRT1 at 400x magnification; Nikon H600L microscope.

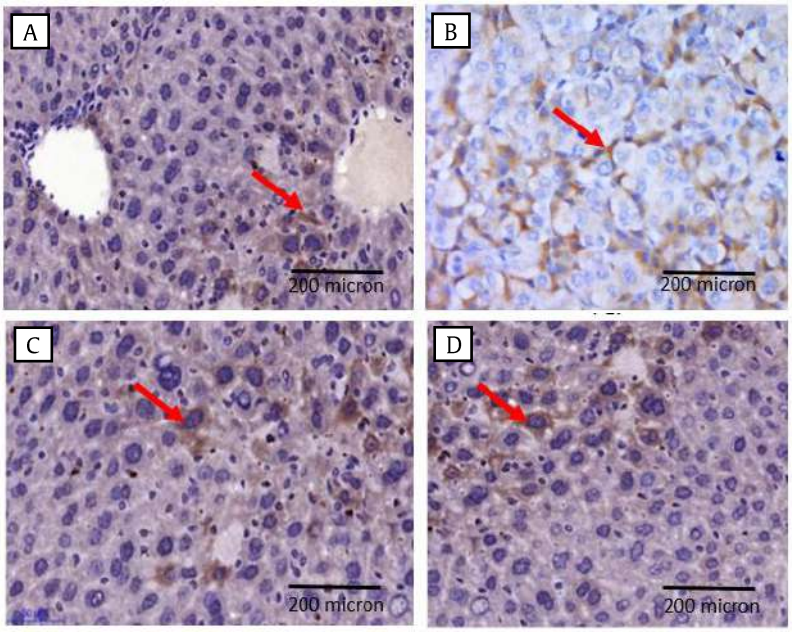

Figure 5. PPARγ Expression in Hepatocytes of Wistar Rats. (A) P0 = negative control (Distilled water + High cholesterol diet). (B) P1 = positive control (High cholesterol diet + Simvastatin). (C) P2 = 4 mg/kg BW BBLE + high cholesterol diet. (D) P3 = 4 mg/kg BW BBLE + 4 mg/kg BW SAE and high cholesterol diet. Immunohistochemistry staining Ab. Anti-PPARγ at 400x magnification; Nikon H600L microscope.

Numerous studies indicate that PPARγ is responsible for the transcriptional regulation of various genes. Among its functions, this receptor significantly influences glucose metabolism by facilitating glucose uptake from the bloodstream via insulin action. In the context of lipid metabolism, PPARγ promotes adipocyte differentiation, enhances adiponectin production, offers neuroprotective effects, and modulates the NF-κB pathway involved in inflammatory process (51,52). Research has also shown that natural compounds with phytoconstituents exhibiting anti-diabetic potential can maintain PPARγ expression in obese rats subjected to a high-fat diet, thereby reducing lipid and fat accumulation in liver and adipose tissue (53). It is hypothesized that the compounds present

in BBLE and SAE may regulate the expression levels of key adipogenesis markers, including PPARγ. Future studies may further explore this hypothesis by measuring additional adipogenesis-related expressions, such as adipogenic and lipogenic markers (54). This study has confirms that the active compounds from both extracts can effectively inhibit lipid accumulation in the liver and adipocytes, subsequently reducing the risk of fatty liver development in Wistar rats fed a high-cholesterol diet.

4. CONCLUSIONS

Overall, BBLE and SAE have a significant potential as anti-hypercholesterolemia agents, particularly in preventing fatty liver. The combination of these two extracts plays a vital role in maintaining lipid metabolism in the liver through the increased expression of Leptin receptor, AMPK, SIRT-1, and PPARγ. The outcomes of this study may pave the way for further investigations, especially concerning patients with lipid metabolism disorders, thereby enhancing the understanding of the underlying molecular mechanisms.

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Ethics statement: All experiments were conducted in accordance with the guidelines of the Ethical Research Committee of Sekolah Tinggi Ilmu Kesehatan Bina Usada Bali (Bina Usada Bali Health Sciences College/STIKES) with Ethical Clearance Number: 135/EA/KEPK-BUB-2023.

Conflict of interest: The author declares that there is no conflict of interest.

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