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Original Article

Exploring the potential of *Holothuria atra* extract in modulating fasting triglyceride index and obesity: In silico, in vitro and in vivo studies

Assyu'ara Al'asyi[1], Irena Ujianti[2*], Rara Nadika[1], Zahirah Zahirah[1], Bimo J. Faizin[1], Devana A. Afifah[1], Randhiva Farhan[1], Salsabila P. Rahmadina[1], Raisya ZN. Lubis[1], Sakinah Ghaisani[1], Kiflan Arkananta[1], Bety S. Lakshmi[3] and Mulyoto Pangestu[4]

[1]Medical Study Program, Faculty of Medicine, Universitas Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia; [2]Department of Physiology, Faculty of Medicine, Universitas Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia; [3]Department of Public Health, Faculty of Medicine, Universitas Muhammadiyah Prof. Dr. Hamka, Jakarta, Indonesia; [4]Department of Obstetrics and Gynecology, Faculty of Medicine, Nursing and Health Science, Monash University, Clayton, Australia

*Corresponding author: irenaujianti@uhamka.ac.id

Abstract

Obesity remains a major global health challenge and is strongly associated with metabolic disorders, particularly cardiovascular disease. This has fueled growing interest in natural interventions that regulate lipid metabolism as potential strategies to mitigate associated risks. Among these, *Holothuria atra* has emerged as a candidate due to its diverse bioactive compounds, though its mechanisms of action and therapeutic efficacy remain inadequately characterized. The aim of this study was to evaluate the combined effects of *H. atra* extract (HAE) and exercise on metabolic regulation, with the goal of determining whether their synergistic use enhances obesity management by targeting multiple metabolic pathways. Specimens of *H. atra* were collected from Tablolong Beach, Indonesia, and ethanol extracts were prepared. An in-silico analysis was performed to assess drug-likeness, quantitative structure-activity relationship (QSAR) properties, and network pharmacology. In vitro test using human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) underwent adipogenic differentiation with or without HAE treatment. This study used male Sprague-Dawley rats that were fed either a control or high-fat diet and further subdivided into groups receiving extract supplementation, swimming exercise, or a combination of both for six weeks. Liquid chromatography-mass spectrometry (LC-MS) analysis identified 6-gingerol and sarcostin as principal bioactive compounds, both of which fulfilled drug-likeness criteria. In silico analyses implicated peroxisome proliferator-activated receptor gamma (PPAR-γ) signaling as a major molecular target. In vitro tests found that HAE attenuated adipogenic differentiation of hUC-MSCs. Both HAE and exercise significantly reduced triglycerides, glucose, and the triglyceride–glucose index in rats fed a high-fat diet. The combination of HAE and exercise produced the greatest improvements, with significant reductions in glucose ($p<0.01$) , triglycerides ($p<0.001$), and the triglyceride–glucose index ($p<0.001$) compared to the high-fat diet control group. Collectively, these findings suggest that HAE, particularly when combined with regular exercise, improves lipid metabolism and may serve as a promising complementary strategy for obesity management.

Keywords: Obesity, triglyceride index, *Holothuria atra,* sea cucumber extract, lipid metabolism

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Introduction

Obesity has become a major global public health concern, with prevalence reaching 18.5% among adult women and 14.0% among adult men in 2022 [1]. It is closely linked to metabolic disorders, including type 2 diabetes, hypertension, and cardiovascular diseases [2]. Sedentary lifestyles and unhealthy dietary patterns significantly contribute to obesity by disrupting lipid metabolism, resulting in elevated fasting triglyceride levels, insulin resistance, and chronic inflammation [3]. These trends highlight the urgent need for natural interventions that can enhance lipid metabolism and reduce obesity-related health risks [3]. Sea cucumber extract has emerged as a promising candidate, given its rich profile of bioactive compounds such as saponins, chondroitin sulfates, glycosaminoglycans, and fatty acids. These constituents exhibit diverse pharmacological properties, including anti-inflammatory, antioxidant, and antidiabetic effects [4]. The extract is biologically relevant for lipid regulation, yet the exploration of its specific mechanisms, particularly those involving triterpene glycosides such as sarcostin and their synergy with other bioactive compounds like gingerol, remains limited [5] . Addressing this gap represents an opportunity to provide novel insights and establish a unique contribution to lipid metabolism research.

Holothuria atra, in particular, has demonstrated the ability to modulate lipid metabolism by inhibiting pancreatic lipase activity, promoting fat oxidation, and reducing fat synthesis [6]. Recent studies have shed light on pathways targeted by its bioactive compounds, particularly those involved in lipid metabolism and fat formation [7,8]. In animal models, *H. atra* supplementation reduced body weight and fat mass while improving insulin sensitivity. In parallel, exercise is widely recognized for its metabolic benefits. Regular physical activity enhances lipid metabolism by increasing fat oxidation, reducing triglyceride levels, improving insulin sensitivity, and preventing hepatic fat accumulation [9]. These adaptations reduce the risk of obesity and metabolic syndrome, making physical activity a cornerstone of obesity prevention and treatment. Given that both *H. atra* and exercise act on complementary pathways in lipid and glucose metabolism, their combination could potentially exert additive or even synergistic effects.

The aim of this study was to investigate the combined metabolic effects of *H. atra* extract and exercise, with the hypothesis that this integrative approach would enhance obesity management by simultaneously targeting multiple metabolic mechanisms. It contributes to innovative findings by demonstrating the ability of sea cucumber extract to improve metabolic health by targeting both plasma lipid levels and the triglyceride index, a promising indicator for assessing metabolic risk. Furthermore, by examining the combination of these bioactives with physical exercise, the study highlights a novel, amplified intervention strategy that has not been previously characterized. These findings offer a foundation for advancing personalized and integrative therapeutic approaches to obesity management.

Methods

Holothuria atra sampling

H. atra was collected by daytime diving in Tablolong Beach, Kupang, East Nusa Tenggara, Indonesia (10°19'07"S 123°27'46") between May 17 and May 30, 2022 (**Figure 1**). Morphological characteristics (elongated, firm body, reddish-black coloration, numerous dorsal papillae, and densely packed ventral tube feet) matched published descriptions and aligned with known features of *H. atra* [10]. Taxonomic classification was as follows: Kingdom Animalia; Phylum Echinodermata; Class Holothuroidea; Order Aspidochirotida; Family Holothuriidae; Genus *Holothuria*; Species *H. atra*. This species is commonly found in Indonesian coastal habitats [11]. DNA barcoding (*COI* gene) analysis from Tablolong Beach, Kupang, confirmed the identification, showing 99–100% similarity to *H. atra* sequences in the National Center for Biotechnology Information (NCBI) GenBank database [12].

Sample extraction

H. atra extract was prepared by removing the body wall, cutting it into small segments approximately 1 cm in size, and drying the material. The dried tissue was then macerated with

ethanol at a 1:5 ratio to break down cellular proteins and extract secondary metabolites. The resulting mixture was concentrated using a rotary evaporator at 40°C for 22 hours.

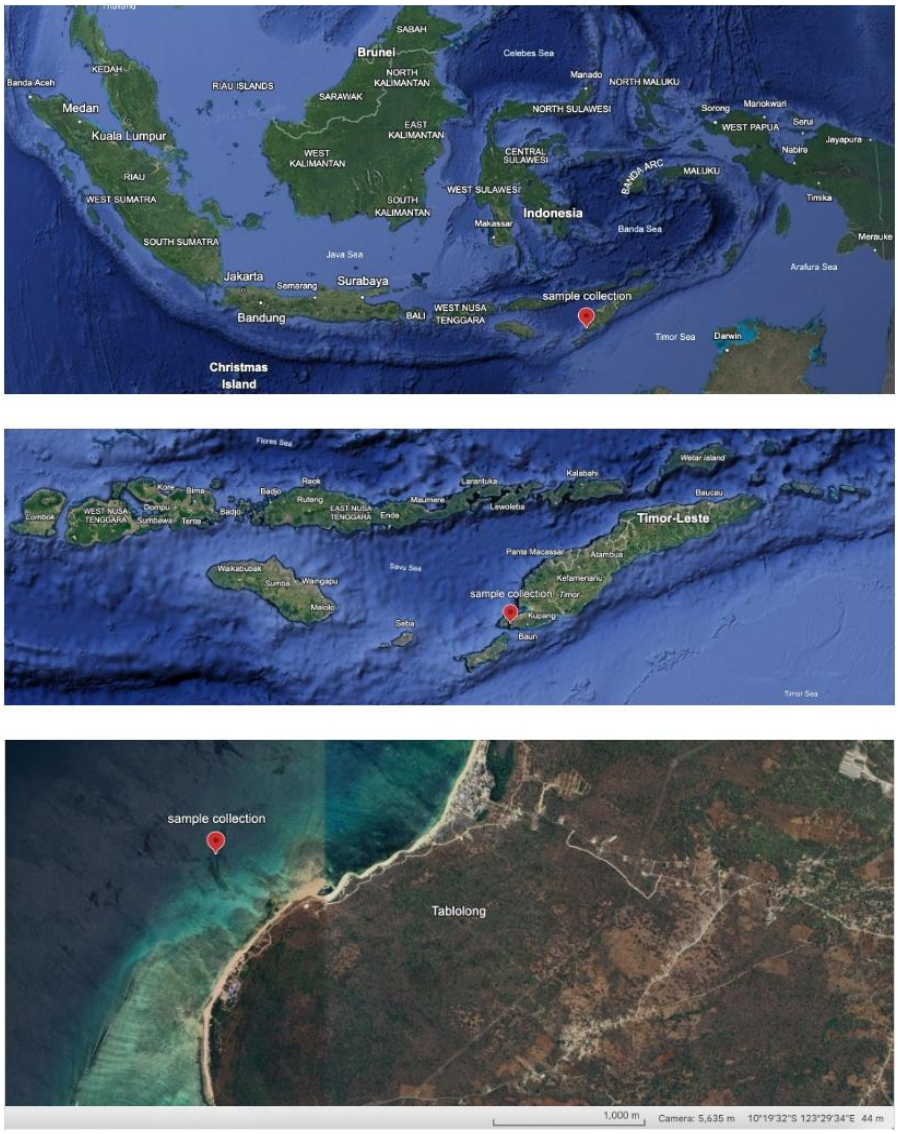


Figure 1. Location of the sample collection (Tablolong Beach, East Nusa Tenggara, Indonesia). The red map pin indicates the exact collection site.

Identification of bioactive compounds and toxicity analysis of *H. atra* extract

H. atra extract was weighed and dissolved in a 10 mL volumetric flask for liquid chromatography-mass spectrometry (LC-MS) or mass spectrometry with quadrupole time-of-flight (MS-QTOF). Standard solutions of biotin and chloramphenicol were prepared to verify instrument performance. Methanol was added, the mixture was sonicated, and the volume was adjusted to the mark with methanol before thorough mixing. The solution was filtered using a GHP/PTFE membrane and injected into the LC-MS/MS-QTOF system. Chromatographic separation was performed on an HSS T3 column using a gradient of 0.1% formic acid in acetonitrile and water at a flow rate of 0.6 mL/min. Mass spectrometric detection was conducted in Tof MS^E mode with electrospray ionization (either positive or negative). Data were processed using UNIFI software (Waters Corporation, Milford, MA, USA) and a mass spectrometry library, with compound identification based on mass error, isotope matching, intensity, and fragment matching.

Bioactive compounds in *H. atra* extract were further evaluated for drug-likeness using Lipinski's Rule of Five. Molecular properties, including hydrogen bond donors and acceptors, molecular weight, and LogP values, were calculated with the ADMETlab 2.0 database to assess their physicochemical characteristics. These parameters were selected because they represent

standard predictors of oral drug-likeness, influencing solubility, permeability, and absorption, and are therefore critical in the drug discovery process.

Quantitative structure-activity relationship (QSAR) analysis

The anti-obesity potential of bioactive compounds in *H. atra* was evaluated using the WAY2DRUG PASS (https://www.way2drug.com/PassOnline/) prediction system. This tool predicts compound activity by comparing their structural features with those of compounds with known properties through structure-activity relationship (SAR) analysis. The reliability of predictions increases with stronger structural similarity to referenced compounds, based on the principle that structurally similar molecules are likely to exhibit similar biological activities. The system generates a probability-to-be-active (Pa) value, ranging from 0 to 1, which indicates the likelihood that a compound will exert a specific effect. Higher Pa values reflect greater confidence in the predicted activity. According to PASS Online criteria, a Pa>0.3 indicates a reasonable likelihood of biological activity.

Prediction of protein targets and network pharmacology analysis

Potential targets of sea cucumber extract were identified using the SuperPred database (https://prediction.charite.de/), with only high-confidence results (≥80% for both accuracy and probability) considered. Target identification was based on the previously determined SMILES notation. Disease-relevant genes and proteins were retrieved from the DisGeNET database (https://disgenet.com/). Protein targets of *H.atra* extract (HAE) were further analyzed using the search tool for the retrieval of interacting genes/proteins (STRING) version 12.0 (https://string-db.org/). The analysis was configured with specific settings: organism set to *Homo sapiens*, network type set to "full STRING network", and medium confidence threshold set at 0.4. The resulting interaction data were exported in TSV format and processed with Cytoscape version 3.10.3 (Cytoscape Consortium, California, United States) to construct and visualize protein-protein interaction networks.

Network analysis was conducted using standard centrality measures to characterize the relationships among target proteins, including stress, degree, betweenness centrality, and closeness centrality. Degree centrality reflects the number of direct interactions a protein has, with higher values indicating potential hub regulators. Betweenness centrality measures the frequency with which a protein appears on the shortest paths connecting other proteins. Closeness centrality reflects the average shortest distance from a given protein to all other proteins in the network.

Mesenchymal cell culture and treatment

Mesenchymal stem cells (MSCs) from human umbilical cord (hUC) were cultured in 12-well plates using Minimum Essential Medium Eagle-Alpha Modification (Alpha-MEM) with nucleosides, supplemented with 10% platelet-rich plasma (PRP). Cells were incubated at 37°C in 5% CO_2. The culture medium was replaced every 2–3 days to maintain optimal growth.

For subculturing, cells were rinsed with phosphate-buffered saline (PBS) and incubated with 1 mL of 0.05% trypsin-EDTA for two minutes. The suspension was transferred to a 15 mL centrifuge tube containing 10 mL of Alpha-MEM supplemented with 10% PRP and centrifuged at 1,200 rpm for 10 minutes at 20°C. The supernatant was discarded, and the cell pellet was resuspended in 1 mL of medium. Cell viability was determined using Trypan Blue assay. Subsequently, the cells were seeded into 12-well plates at a density of 5,000 cells/cm^2 per well and incubated at 37°C in 5% CO_2. Adipogenic differentiation was induced using adipogenesis induction medium, which was refreshed every 2–3 days. Induction was performed concurrently with treatment using sea cucumber extract at concentrations of 9 mg/dL and 19 mg/dL.

In vivo methods

Experimental animals

Ten-week-old healthy male Sprague Dawley rats were obtained from Institut Pertanian Bogor (IPB), Indonesia. Animals were acclimatized for one week in the Central Animal Laboratory, Department of Chemistry, Universitas Indonesia, under controlled conditions (24°C with 50%

humidity). After acclimatization, rats were randomly assigned to five experimental groups (n=5 per group): (1) control diet group, (2) high-fat diet (HFD) group, (3) HFD + extract group, (4) HFD + exercise group, and (5) HFD + extract + exercise group. Rats were housed individually under controlled conditions (18–26°C and 50% humidity), fed twice daily, and provided ad libitum access to water, which was replaced every two days. Body weight, body length, and waist circumference were recorded monthly. At the end of the study, blood samples were collected for analysis of serum lipids, glucose, and insulin levels.

Animal diet and extract supplementation

Laboratory rat feed was obtained from Biorat Laboratory Animal Co., Ltd., consisting of both standard and specialized high-fat, high-salt diets. The standard feed contained 10% fat, 22% protein, 68% carbohydrates, and 0.5% salt. The specialized high-fat diet contained 49% fat, 21% protein, 30% carbohydrates, and 2% salt. Rats in the HFD+extract and HFD+extract+exercise groups were fed the high-fat diet and received daily extract supplementation (300 mg/kg body weight) via gastric administration.

Exercise protocol

Swimming training was conducted in a barrel filled with water maintained at 33–35°C to a depth of 40–50 cm, allowing the rats to swim freely. The initial session lasted 15 minutes, with the duration increased by five minutes per day until reaching 30 minutes. Rats in the HFD+exercise and HFD+exercise+extract groups swam for 30 minutes daily, 5 days per week, for 6 weeks, while rats in the other groups remained sedentary in their cages [13].

Measurement of fasting glucose, fasting triglyceride, and triglyceride index

Each rat's body weight, body length, and abdominal circumference (AC) were measured before and after treatment. AC was measured at the widest region of the abdomen using a non-extensible plastic measuring tape, while body length was defined as the distance from nose to anus. Lee's obesity index was calculated using the formula: Lee's index=cube root of body weight (g)/body length (cm). Following a 12-hour fast, blood samples were collected from the orbital sinus to measure fasting blood glucose (FBG) with a threshold of 90 mg/dL and triglycerides (TG) with a threshold of 120 mg/dL. Measurements were performed before and after treatment using an automatic biochemical analyzer (Hitachi 7600, Hitachi, Tokyo, Japan). The triglyceride-glucose (TyG) index was calculated as a surrogate marker for insulin resistance based on fasting triglyceride and glucose levels. In this study, the following criteria were applied: TyG index<8.3= low likelihood of insulin resistance; TyG index 8.3–8.9= moderate likelihood; and TyG index>8.9= high likelihood [14]. At the end of the experiments, rats were euthanized with an intraperitoneal injection of a lethal dose of ketamine (90 mg/kg) and xylazine (10 mg/kg) diluted in sterile saline. Animals were then placed individually in clean cages and observed until euthanasia was complete [15].

Statistical analysis

For the in vivo study, statistical results are presented as mean ± standard deviation (SD), with experimental groups containing 5–7 animals each. The exact number of animals per group is reported in the corresponding figure legends. Group differences were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A $p<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 20.0 software (IBM Corp., Chicago, USA).

Results

Phytochemical profile and toxicity analysis

LC-MS analysis revealed that 6-gingerol and sarcostin were the principal bioactive compounds in the extract (**Figure 2**). Sarcostin exhibited a peak intensity of 176,739. In the LC-MS/MS component summary, "Intensity" refers to the relative signal strength detected for each compound, indicating its abundance in the sample. The measurement was recorded at a retention time of 18.39 minutes. In comparison, 6-gingerol showed a higher response value of 331,644 at a

retention time of 18.11 minutes, suggesting a substantial contribution to the extract's biological activity.

A total of 15 compounds were identified in the sample using UPLC-QTOF-MS analysis, as presented in **Table 1**. The compounds were annotated based on accurate mass, retention time (RT), fragmentation patterns, and isotope match scores. Retention times ranged from 16.58 to 18.48 minutes. All compounds exhibited mass errors within an acceptable range (−8.7 to 9.6 ppm), supporting the reliability of tentative identifications. Among the detected compounds, 6-gingerol showed the highest response, with a peak intensity of 353,041, indicating its relative abundance in the extract. Other prominent metabolites included sarcostin, ostruthin, and nigalatacine J. In contrast, several compounds, such as cimifoside IV and hederaagenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-α-L-arabinopyranoside, were detected at much lower intensities (responses: 1,119 and 1,059, respectively). The number of fragments observed varied considerably, from 0 (nobilin C) to 143 (cimifoside IV), suggesting differences in fragmentation behavior and reflecting structural complexity. Isotope match scores for both m/z and intensity RMS% further corroborated compound identities, with most exhibiting low RMS values and strong agreement with theoretical isotope distributions. All detected compounds were observed as $[M-H]^-$ adducts, consistent with negative ionization mode typically applied to this class of metabolites. The identified metabolites included diterpenoids, glycosides, and other specialized compounds, highlighting the chemical diversity of the sample. Collectively, these results provide a comprehensive phytochemical profile, supporting further quantitative and bioactivity-guided investigations.

Both 6-gingerol and sarcostin exhibited significant drug-like potential (**Table 2**), fulfilling Lipinski's Rule of Five [16]. 6-gingerol had a molecular weight of 294.39 g/mol, a LogP value of 3.48, two hydrogen bond donors, and four hydrogen bond acceptors. These properties support stability, solubility, membrane permeability, structural flexibility, and moderate lipophilicity. Sarcostin had a molecular weight of 382.5 g/mol, a LogP value of 2.35, six hydrogen bond donors, and six hydrogen bond acceptors, indicating higher hydrophilicity; however, the greater number of hydrogen bond donors and acceptors may affect membrane permeability.

The toxicity plots indicate that 6-gingerol exhibits higher LIPO and FLEX scores compared to sarcostin, while sarcostin shows higher POLAR and SIZE values (**Figure 3**). Both compounds have comparable INSATU and INSOLU scores.

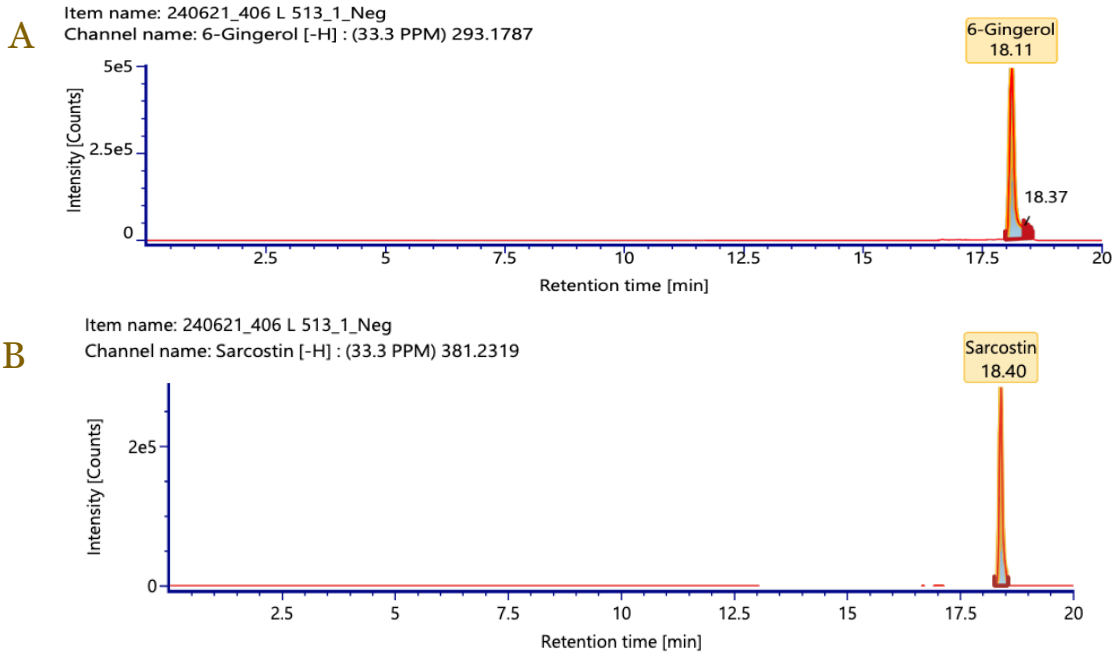


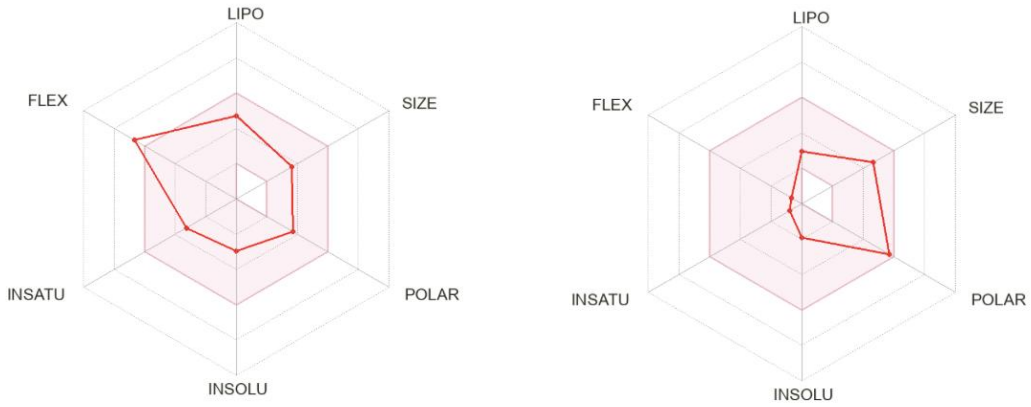
Figure 2. LC-MS analysis of 6-gingerol (A) and sarcostin (B). The mass spectrum shows signal intensity as a function of molecular weight (m/z). Peaks in the graph represent the detected compounds.

Table 1. Identification and analytical parameters of compounds detected by LC-MS analysis

Component name	Observed RT (min)	Fragments found	Match Mz RMS PPM	Intensity RMS percent	Response
3-O-(Z,E) 4,2-Decadienoyl ingenol	16.62	60	4.81	19.15	2,031
6-Gingerol	18.11	13	13.15	16.19	353,041
Andropanideol	17.81	1	8.38	25.11	15,350
Cimifoside IV	16.65	143	21.64	114.91	1,119
Ganoderic acid H	16.58	24	8.22	15.89	2,769
Hederaagenin 3-O-α-L-rhamnopyranosyl-	16.58	76	6.96	81.31	1,059
Isolidylfutoquicinol B	17.51	1	5.98	48.24	1,051
Kukoamine A	18.17	2	6.06	16.18	15,455
Mulberrofururan D	16.64	8	6.00	824.89	3,315
Nigalatacine J	17.88	3	13.39	80.94	74,070
Nobilin C	17.74	0	2.82	15.73	1,125
Ostruthin	18.16	1	12.20	16.55	121,610
Paeonolide G	16.62	68	4.97	44.83	9,365
Sarcostin	18.40	1	12.18	12.96	196,319
β-Cryptoxanthin	18.48	17	4.99	15.50	31,519

Table 2. Toxicity analysis of 6-gingerol and sarcostin identified from *Holothuria atra* extract

Molecule type	Hydrogen bond donors (HBD)	Hydrogen bond acceptors (HBA)	Molecular weight (MW)	Log P
6-Gingerol	2	4	294.4 g/mol	3.48
Sarcostin	6	6	382.5 g/mol	2.35



6-Gingerol Sarcostin

Figure 3. Toxicity profile of key bioactive compounds identified from *Holothuria atra* extract, alongside radar plots depicting the physicochemical property profiles of 6-gingerol (left) and sarcostin (right).

Quantitative structure-activity relationship (QSAR) analysis

Several compounds showed structural similarity to approved drugs, particularly those targeting metabolic disorders. Notably, predicted activities included antidiabetic effects (Pa=0.405), antidyslipidemic activity (Pa=0.328), and anti-obesity potential, suggesting therapeutic relevance for metabolic syndrome prevention (**Figure 4**). In addition to these, the SAR predictions indicated activities related to hyperglycemia control (Pa=0.336), anti-infective properties, and steroid-like effects. The hyperglycemia control prediction (Pa=0.336) is particularly relevant, as even moderate likelihood values (>0.3) in PASS analysis can indicate potential lead compounds for further development in metabolic disease management. Anti-infective activity may contribute to overall health benefits, while steroid-like activity may imply modulation of inflammatory pathways associated with metabolic disorders.

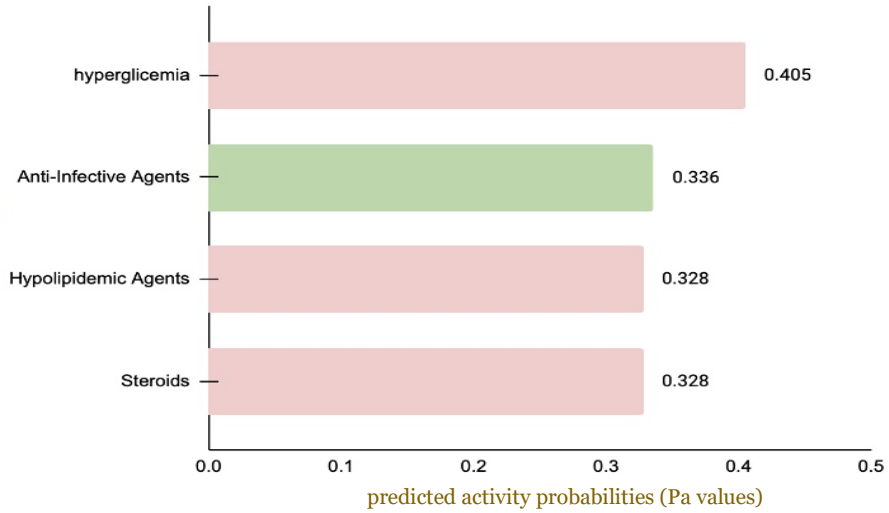


Figure 4. Structure-activity relationship (SAR)-based prediction of bioactive compounds derived from *Holothuria atra* extract for metabolic disease applications obtained using Way2Drug PASS online. Red bars denote metabolism-related activities, while green bars highlight other relevant pharmacological potentials.

Network pharmacology analysis

Key protein targets associated with the biological activity of *H. atra* extract are presented in **Figure 5**. Network analysis identified PPAR-γ as a potential key target due to its high centrality scores and strong biological relevance. Although INS shows the highest values for degree, betweenness, and closeness centrality in the network (**Table 3**), PPAR-γ also functions as a major hub, demonstrating substantial network connectivity with 23 direct interactions. Its established role in glucose and lipid metabolism, along with its involvement in dyslipidemia and insulin resistance, suggests that *H. atra* may exert anti-obesity effects primarily through modulation of the PPAR-γ pathway.

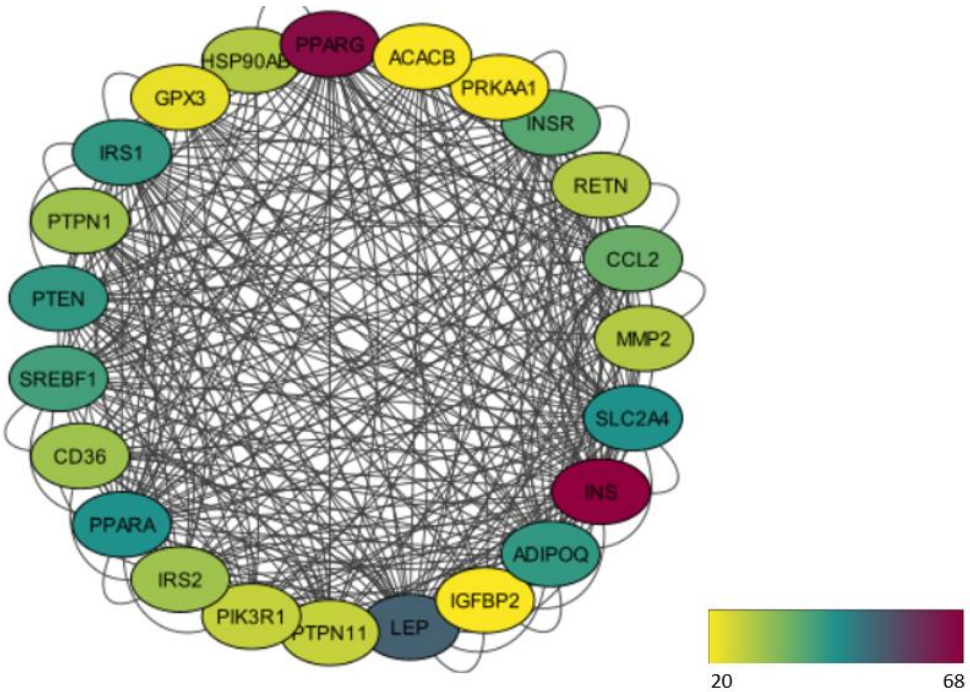


Figure 5. Network analysis of *H. atra* targets. Nodes represent protein targets and edges represent interactions. Node colors are mapped to degree values, where yellow represents low degree in 20 connections and dark purple represents high degree in 68 connections.

Table 3. Network pharmacology results

Protein target	Degree	Betweenness centrality	Closeness centrality
INS	68	0.17	0.85
PPARG	66	0.15	0.82
LEP	52	0.05	0.73
PPARA	44	0.06	0.68
SLC2A4	44	0.02	0.68
ADIPOQ	42	0.02	0.64
IRS1	42	0.03	0.67
PTEN	42	0.08	0.67
SREBF1	40	0.03	0.65
INSR	38	0.03	0.65

In vitro differentiation

At baseline, HU-MSCs exhibited the typical fibroblast-like morphology, characterized by small cell bodies and elongated processes across all treatment groups. By the 5th passage, at the start of the treatment, control group cells differentiated into mature adipocytes, adopting a spherical morphology with cytoplasm filled with large lipid droplets, ultimately forming a single dominant droplet. Control group cells without differentiation retained the fibroblast-like morphology typical of MSCs at the start of the treatment. In contrast, cells treated with *H. atra* extract maintained a fibroblast-like morphology but displayed cytoplasm containing fewer and smaller lipid vacuoles. Interestingly, cells exposed to 9 ug/ml extract developed larger vacuoles compared to the group treated with 19 ug/ml, as presented in **Figure 6**.

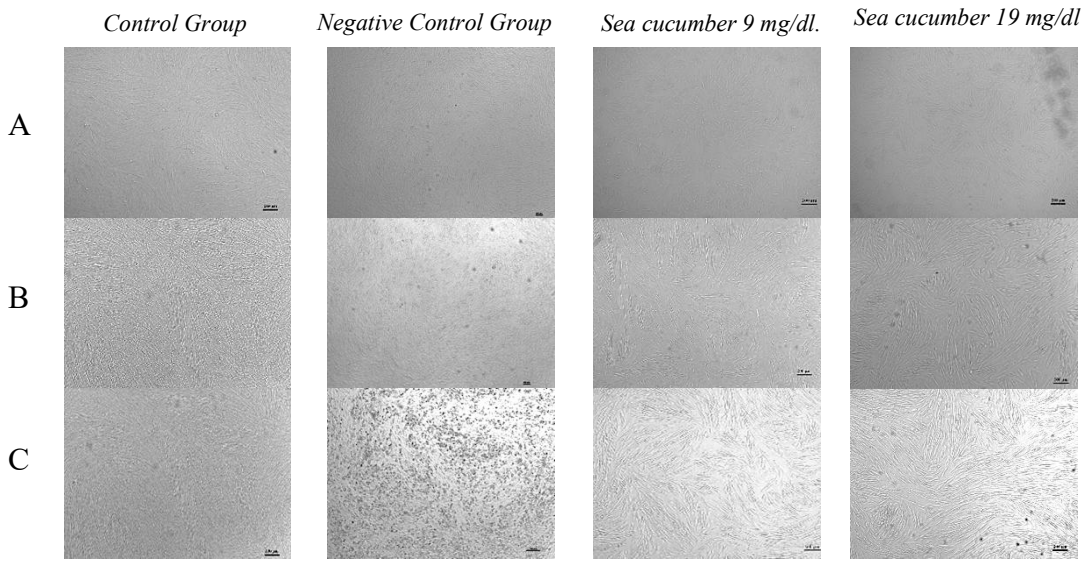


Figure 6. Representative images of MSCs from positive control group, negative control group, and treatment groups (9 mg/dL and 19 mg/dL *H. atra* extract). Differentiation of cells at passage 5 is depicted after 7 days (A), 9 days (B), and 12 days (C).

In vivo biochemical results

Administration of *H. atra* extract and exercise—either individually or combined—significantly improved the disrupted biochemical parameters caused by a high-fat diet, based on the data in **Table 4**. In the HFD group, serum triglycerides were 143.2±7.72 mg/dL, glucose was 100.8±1.68 mg/dL, and the triglyceride–glucose (TyG) index was 8.90±0.05. HFD + extract reduced triglycerides to 100.2±10.2 mg/dL (p<0.01), glucose to 89.0±3.30 mg/dL, and TyG index to 8.40±0.09 (p<0.01). HFD + exercise yielded similar improvements, with triglycerides at 97.0±5.50 mg/dL (p<0.01), glucose at 90.8±2.08 mg/dL, and TyG index at 8.40±0.05 (p<0.01). The greatest effect was observed in the HFD + extract + exercise group, which showed triglycerides of 66.0±0.77 mg/dL (p<0.001), glucose of 84.21±0.29 mg/dL (p<0.01), and TyG index of 8.00±0.05 (p<0.001).

Table 4. Effects of *H. atra* extract on biochemical parameters in high-fat-diet (HFD) rats

Parameter (pasma serum)	Normal diet	High-fat-diet (HFD)	HFD+extract	HFD+exercise	HFD+extract +exercise
Triglyceride (mg/dL)	110.2±10.6	143.2±7.72	100.2±10.2**	97.0±5.50**	66.0±0.77***
Glucose (mg/dL)	75.6±4.41	100.8±1.68	89.0±3.30	90.8±2.08	84.21±0.29**
Triglyceride-glucose (TyG) index	8.30±0.11	8.9±0.05	8.4±0.09**	8.4±0.05**	8.0±0.05***

The effect of sea cucumber extract on animal and biochemical parameters. The data is presented as mean±SD (n=5 per group). The statistical differences were revealed by one-way ANOVA and Tukey's post hoc test. The mean differences were significant compared with the high-fat-diet group.
*Statistically significant at $p< 0.05$
**Statistically significant at $p<0.01$
***Statistically significant at $p<0.001$

In the body weight analysis (**Figure 7**), all groups displayed comparable baseline weights before intervention. After the 8-week treatment, the HFD group showed a significant increase in body weight compared with the normal diet group ($p<0.001$), whereas the groups receiving *H. atra* extract (HFD+HAE), exercise (HFD+Exercise), or their combination (HFD+HAE+Exercise) demonstrated significantly lower post-intervention body weights than the untreated HFD group ($p<0.001$). Notably, the combined *H. atra* extract plus exercise group exhibited the greatest reduction, approaching values similar to the normal diet group, indicating a possible synergistic effect between the extract and exercise. These differences are statistically significant and highlight that the observed weight-lowering effect is not incidental but associated with the specific interventions applied.

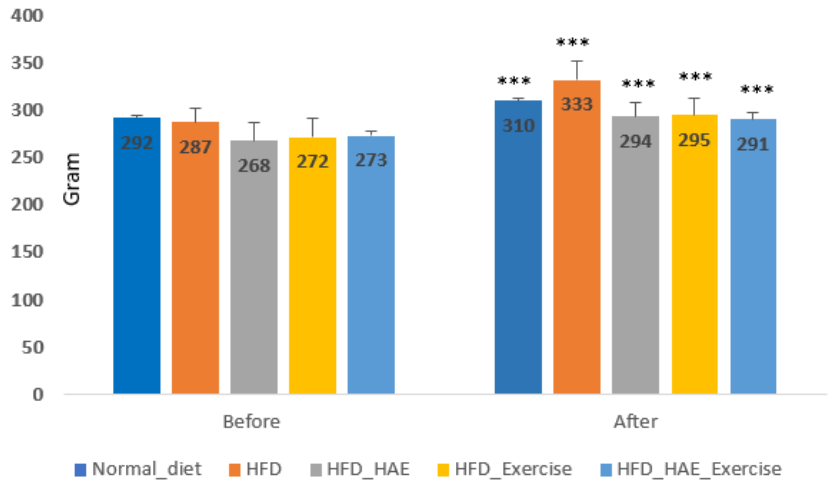


Figure 7. Body weight (g) of experimental groups before and after intervention. Rats were divided into five groups: Normal diet, high-fat diet (HFD), HFD with *H. atra* extract (HFD+HAE), HFD with exercise (HFD+Exercise), and HFD with *H. atra* extract plus exercise (HFD+HAE+Exercise). Data are presented as mean ± SD. Significant differences compared with the HFD group after intervention are indicated (***$p<0.001$).

Discussion

Bioactive compounds from sea cucumbers show significant potential as natural therapeutics for obesity and related metabolic disorders. In silico analyses of this study revealed that these compounds modulate metabolic and inflammatory pathways by increasing adiponectin (ADIPOQ) levels, enhancing insulin sensitivity, and suppressing pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) [17]. They also activate PPAR-γ, restore SLC2A4/GLUT4 activity, improve glucose uptake, and reduce insulin resistance—mechanisms that collectively enhance insulin receptor function and optimize glucose metabolism [18–20]. In vitro evidence supports these findings, showing that sea cucumber extracts lower NF-κB levels, thereby directly suppressing adipogenesis [8,21]. Previous study reported that NF-κB regulates PPAR-γ expression—a critical driver of adipocyte differentiation—highlighting the extract's role

in limiting fat accumulation [22]. These extracts also inhibit lipid storage regulators, including fatty acid synthase (FAS) and the NF-κB/IKK pathway [17], consistent with Parida *et al.*'s observations of reduced lipid accumulation markers such as TNF-α and NF-κB [23]. Together, these anti-adipogenic and anti-inflammatory actions underscore the therapeutic potential of sea cucumber extracts in obesity management.

In high-fat diet (HFD) models, sea cucumber extract significantly reduced body weight compared to controls [24,25]. Key metabolites, such as 6-gingerol, promote lipid metabolism by stimulating lipolysis and thermogenesis while inhibiting lipogenesis. They also exert anti-inflammatory and antioxidant activities [26,27]. Sarcostin may have complementary effects, modulating lipid metabolism and reducing intestinal fat absorption [28]. These mechanisms collectively improve insulin sensitivity, lower triglyceride levels, and reduce metabolic risk factors. Physical activity offers synergistic benefits by further increasing adiponectin, PPAR-γ, and GLUT4 expression, thereby enhancing both glucose and lipid metabolism [29]. Exercise also reduces leptin levels, improves appetite regulation, activates PPAR-α to enhance lipid profiles [13], and suppresses inflammatory mediators. When combined with sea cucumber extract supplementation, these interventions produce additive effects—improving insulin signaling, promoting lipid oxidation, and strengthening resistance to oxidative stress. Supporting this, Chen *et al.* found that HFD-fed C57/BL6 mice receiving sea cucumber saponins or phospholipids enriched with eicosapentaenoic acid showed improved glucose tolerance, reduced adiposity, and lower cholesterol levels, with liposomal formulations exhibiting particularly strong anti-obesity effects [25]. These findings align with broader evidence on the role of marine bioactives in regulating obesity-related molecular pathways [6,30-32].

H. atra extract exerts its anti-obesity effects through multiple complementary mechanisms. It improves insulin sensitivity by increasing adiponectin levels, which enhance insulin utilization and promote fatty acid oxidation [19]. The extract partially activates PPAR-γ, facilitating GLUT4 translocation and glucose uptake without triggering excessive adipocyte proliferation [29]. Simultaneously, it inhibits NF-κB signaling, reducing chronic low-grade inflammation linked to obesity [23]. Bioactive constituents such as 6-gingerol and sarcostin further modulate lipid metabolism by promoting lipolysis and thermogenesis while suppressing lipogenesis. When combined with regular exercise, *H. atra* independently enhances adiponectin levels, PPAR-γ activity, and lipid oxidation; these effects are further amplified, leading to greater fat loss and improved glycemic control [25]. The anti-obesity activity of sea cucumber extract appears to be mediated primarily by PPAR-γ modulation, with terpenoids such as sarcostin demonstrating high target specificity, adenosine monophosphate-activated protein kinase (AMPK) activation, and potent antioxidant activity [33,34]. These pathways collectively suppress adipogenesis, improve glucose metabolism, and reduce systemic inflammation, positioning sea cucumber extract as a promising candidate for integrative obesity therapy. Future research should include rigorously designed human clinical trials [35], as well as studies on bioavailability optimization, active compound isolation, mechanistic elucidation, and evaluation across diverse populations to enhance translational applicability.

However, this study is limited by its reliance on computational models, cell-based assays, and animal experiments, which may not fully recapitulate human physiology. Future investigations should prioritize well-controlled clinical trials to confirm the efficacy, safety, and therapeutic potential of *H. atra* extract in obesity management.

Conclusion

This study demonstrates that *H. atra* extract exerts significant anti-obesity and lipid-modulating effects by inhibiting adipogenesis, reducing systemic inflammation, and improving metabolic parameters, particularly when combined with exercise. These findings support its potential as a natural multi-target intervention for obesity and related disorders. However, further investigations are required to isolate individual active compounds, elucidate molecular mechanisms, determine pharmacokinetic properties, assess long-term safety, and confirm efficacy through rigorous clinical trials.

Ethics approval

The research protocol was approved by the ethics committee of Universitas Muhammadiyah Prof. Dr. HAMKA under permit number KEPKK/FK/058/05/2024.

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Competing interests

All authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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