https://doi.org/10.31925/farmacia.2025.1.17 **ORIGINAL ARTICLE**

ASIAN SWAMP EEL (*MONOPTERUS ALBUS*) OIL AND VITAMIN D3 EMULSION AS NOVEL APPROACH TO IMPROVE LIPID PROFILES AND GLYCEMIC CONTROL IN RAT MODEL

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Abstract

Asian swamp eel (*Monopterus albus*) oil and vitamin D3 have demonstrated promising antihyperlipidemic and antihyperglycemic properties. This study aimed to formulate an emulsion combining swamp eel oil and vitamin D3 (EVD) and evaluate its effects on cholesterol levels and blood glucose in rats treated with a high-fat diet (HFD). Eel powder was extracted, and the oil was emulsified with vitamin D3 and three surfactant agents to form a self-emulsifying drug delivery system (SEDDS). Twenty-five male rats were assigned into five groups: standard diet (SD), HFD, HFD with EVD (1 mL or 2 mL), and HFD with simvastatin (SVT). Treatments were administered daily for 30 days, followed by total cholesterol (TC), blood glucose (BG), and alanine aminotransferase (ALT) level measurements. The resulting EVD emulsion was clear and exhibited high stability. In an animal model, EVD significantly reduced TC, improved glycemic control, and stabilised ALT levels in HFD-fed rats compared to the untreated HFD group ($p < 0.05$). These findings indicate that EVD may offer therapeutic benefits for managing hyperlipidemia and glycemic dysregulation associated with high-fat diets.

Rezumat

Uleiul de anghilă asiatică de mlaștină (*Monopterus albus*) și vitamina D3 au demonstrat proprietăți antihiperlipidemice și antihiperglicemice. Acest studiu a avut ca scop formularea unei emulsii care combină uleiul de anghilă asiatică de mlaștină și vitamina D3 (EVD), precum și evaluarea efectelor acesteia asupra nivelului de colesterol și glucoză din sânge la șobolani hrăniți cu o dietă bogată în grăsimi (HFD). Uleiul a fost emulsionat cu vitamina D3 utilizând trei agenți surfactanți pentru a forma un sistem de cedare autoemulsifiant al medicamentului (SEDDS). Douăzeci și cinci de șobolani masculi au fost împărțiți în cinci grupuri: dietă standard (SD), HFD, HFD cu EVD (1 mL sau 2 mL) și HFD cu simvastatină (SVT). Tratamentele au fost administrate zilnic timp de 30 de zile și s-au determinat colesterolul total (TC), glicemia (BG) și nivelul de alanin aminotransferază (ALT). Emulsia EVD rezultată a fost clară și a prezentat o stabilitate ridicată. În modelul animal, EVD a redus semnificativ TC, a îmbunătățit controlul glicemic și a stabilizat nivelurile de ALT la șobolanii hrăniți cu HFD, comparativ cu grupul HFD netratat ($p < 0.05$). Aceste rezultate indică faptul că EVD poate oferi beneficii terapeutice pentru gestionarea hiperlipidemiei și disfuncțiilor glicemice asociate cu dietele bogate în grăsimi.

Keywords: asian swamp eel, vitamin d3, dyslipidemia, glycemic control

Introduction

Dyslipidemia, particularly hypercholesterolemia, is a major risk factor for cardiovascular diseases (CVD), which continues to be the primary cause of global mortality [1]. Elevated cholesterol levels and lipid imbalances are key factors in the development of coronary heart disease (CHD) [2], which contributes to a global mortality rate of approximately 108.8 *per* 100,000 individuals [3]. In many regions, poor dietary habits, coupled with elevated low-density lipoprotein cholesterol (LDL-C) levels, are among the most significant modifiable risk factors contributing to premature cardiovascular mortality [4]. Research has shown that managing these risk factors through dietary changes, alongside pharmacological treatments, can substantially reduce the incidence of premature cardiovascular deaths [5, 6]. As such, targeting high cholesterol levels and improving diet are vital strategies for reducing the global burden of CVD, particularly in high-risk populations.

Asian swamp eel (*Monopterus albus*), a species abundant in Southeast Asia, is a promising yet underutilised natural resource with significant therapeutic and nutritional values [7]. Recent preclinical studies have demonstrated that the oil extracted from Asian swamp eel has potent antihyperlipidemic effects, effectively reducing cholesterol and triglyceride levels in high-fat diet-induced animal models. [8]. Furthermore, the lipid-lowering efficacy of swamp eel oil surpasses that of other widely studied fish oils, such as cod, trout, and sardine oils [8]. Despite

these promising findings, the potential of swamp eel oil as a functional food supplement has yet to be fully explored.

Vitamin D3, a lipid-soluble vitamin naturally found in fish oil and other animal-based products, has demonstrated potential in regulating lipid metabolism [9, 10]. Meta-analyses have shown that vitamin D3 supplementation can lower serum total cholesterol, LDL cholesterol, and triglycerides, though its effects on HDL cholesterol are less consistent [11]. Additionally, vitamin D3 plays a crucial role in glycaemic control [12], further enhancing its potential as a therapeutic agent for metabolic disorders. Given these properties, vitamin D3 is a strong candidate for combination therapies to address lipid imbalances and glycaemic control.

Therefore, this study aimed to develop a formulation combining swamp eel oil and vitamin D3 and to assess its therapeutic potential in a metabolic disturbance rat model induced by a high-fat diet. By leveraging the lipid-lowering and glycemic control properties of both swamp eel oil and vitamin D3, the results of this study could contribute to the advancement of functional foods and supplements aimed at mitigating cardiovascular and metabolic diseases related to dyslipidaemia.

Materials and Methods

Materials

Vitamin D3 soft gels containing 1000 IU of cholecalciferol (Blackmore, Australia) were purchased from a local pharmacy in Makassar, Indonesia. Simvastatin tablets containing 10 mg of the active compound (Kimia Farma, Indonesia) were obtained from a local pharmacy in Makassar and served as the reference drug. Asian Swamp Eel (*Monopterus albus*) were procured from a local market in Makassar, Indonesia. The species identification was verified at the Zoology Laboratory, Department of Biology, Mathematics and Natural Science Faculty, Hasanuddin University, and the identification number 047/ZOO/BIO/2024 was assigned. According to the FishBase database, 100 grams of *Monopterus albus* provides 1.07 mg of iron, 260 mg of calcium, 18.4% protein, 0.0997 g of omega-3 fatty acids, 30.9 mcg of selenium, 13.2 mcg of vitamin A and 1.44 mg of zinc [13].

Swamp eel preparation and extraction

Swamp eels were processed by removing the internal organs and cutting them into small pieces. The pieces were thoroughly washed with tap water to remove impurities. They were then dried in an oven at 70°C for 15 hours to ensure proper dehydration. The dried eel was ground using a blender to obtain a fine powder, which was sieved to achieve a consistent particle size. The powdered sample was carefully weighed and kept in a dark vessel at -4°C to prevent oxidation and preserve its quality.

A 10 g portion of the powdered eel was mixed with 75 mL of 96% ethanol in a 500 mL Erlenmeyer flask. The mixture was shaken for 1 minute to ensure thorough homogenization. The extraction was then carried out using a microwave-assisted extraction (MAE) system (Panasonic NN-ST34HM, Japan) with an extraction power of 800 W at 60°C. Extraction times were varied at 1, 2, 3, 4 and 5 minutes to determine the optimal time for obtaining the highest extract yield. To prevent the evaporation of the solvent during extraction, the process was performed in a closed vessel [14]. After extraction, the mixture was filtered to separate the solid residues, and the resulting liquid extract was evaporated using a water bath set at 60°C to remove excess ethanol. The concentrated extract was then obtained. The yield percentage of the eel extract was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{weight of concentrated extract}}{\text{weight of dry powder}} \times 100\%$$

Formulation of swamp eel oil and vitamin D3 emulsion

The emulsion system used in this study was designed using a combination of three surfactants to create a self-emulsifying drug delivery system (Table I) [15, 16]. This formulation was adapted from a previous study that identified the most stable emulsion for cod fish oil [17], with modifications to accommodate the specific properties of swamp eel oil. The preparation process involved incorporating the surfactants into the oil phase and stirring for 10 minutes. Once a homogeneous mixture was achieved, water was gently added while stirring continuously for 10 minutes to ensure proper emulsification. The stability of the emulsion was monitored throughout the process to confirm its consistency.

Table I

Composition of eel fish oil and vitamin D3 emulsion

No.	Materials	Concentration (%)
1	**Eel fish oil (oil phase)**	4
2	**Vitamin D3 (oil phase)**	1
3	**Cremophor RH-40 (surfactant)**	35
4	**Glycerine (Co-surfactant)**	35
5	**PEG 400 (Co-surfactant)**	10
6	**Water (up to)**	100

Evaluation of eel fish oil and vitamin D3 emulsion

Organoleptic Properties. The organoleptic characteristics of the emulsion, including its colour, odour, taste, and pH, were qualitatively assessed. These observations provide preliminary insights into the acceptability and potential stability of the emulsion formulation.

Microscopic Analysis of Droplet Distribution and Size. The emulsion's droplet distribution and size were analysed using an optical microscope (Olympus CX23, Japan) with a digital imaging system at 400× magnification. Image analysis was employed to determine droplet size and uniformity [18].

Stability Testing. The physical stability of the emulsion was evaluated through centrifugation testing. A 10 g emulsion sample was placed in a sealed tube and centrifuged at 3000 rpm for 30 minutes. The stability was assessed based on the absence of phase separation post-centrifugation, which indicates a stable emulsion system [19].

Preparation of Diets

Standard and high-fat diets were formulated using local ingredients sourced from a specialised laboratory rat pellet manufacturer (Pa'commo, Makassar, Indonesia). The standard diet contained 62% carbohydrates, 14% protein, 10% sugar, 4% fat, and 5% vitamins and minerals. The high-fat diet (HFD) consisted of 42% carbohydrates, 24% protein, 16.5% fat, 5% fructose, and 5% vitamins and minerals.

Animals and Housing

Twenty-five male Wistar rats weighing 180 - 220 g (8 - 10 weeks old) were purchased from a certified local breeder in Makassar. Upon arrival, the rats were acclimatised for 14 days in a controlled animal laboratory environment with constant temperature (22 - 25°C), relative humidity (50 - 60%), and a 12-hour light-dark cycle system. During the acclimation period, the rats had access to water and were fed a standard diet *ad libitum*. All experimental protocols adhered to the ethical standards of the Institutional Animal Care and Use Committee at Hasanuddin University, following international guidelines for animal experimentation (ethical clearance number: 301/UN4.6.4.5.31/PP36/2023).

Experimental Design

The rats were randomly assigned to five groups (n = 5 *per* group) as follows: the Control Group (SD), which received a standard diet; the HFD Group, which was fed a high-fat diet along with a placebo (water); the HFD + EVD (1 mL) Group, which received a high-fat diet supplemented with 1 mL of the emulsion; the HFD + EVD (2 mL) Group, which was provided with a high-fat diet and 2 mL of the emulsion; and the HFD + SVT Group, which was fed a high-fat diet supplemented with simvastatin at a dose of 2 mg/kg body weight.

The eel oil and vitamin D emulsion (EVD) was formulated to contain 4% swamp eel oil and 1% vitamin D3 (equivalent to 1000 IU/mL). Rats received the EVD at a 1 - 2 mL dose *per* 200 g of body weight, corresponding to 20 - 40 mg/kg of fish oil and 50 - 100 IU/kg of vitamin D3. When extrapolated to human equivalents, this dosage corresponds to approximately 194 - 388 mg/day of fish oil and 483 - 966 IU/day of vitamin D3. Simvastatin was administered to rats at a dose of 2 mg/kg body weight, derived from a human dose of 20 mg/day using the body surface area (BSA) conversion method as described by Nair and Jacob (2016) [20]. All treatments were administered daily by oral gavage for 30 days. Rats were fasted overnight before blood sample withdrawal. Blood collection was *per*formed *via* cardiac puncture under anaesthesia.

Analysis of Body Weight

Rats were weighed daily throughout the 30-day experiment. Body weight gain was calculated from the difference between the body weights before and after initiation of treatments. The percentage of body weight gain was computed using the following formula:

$$\% \text{ weight gain} = \frac{\text{pre-treatment weight} - \text{post treatment weight}}{\text{pre-treatment weight}} \times 100\%$$

Analysis of Serum Biomarkers

The serum from blood samples was collected after centrifugation at 2500 rpm for 10 minutes. Total cholesterol (TC), fasting blood glucose (FBG), and alanine aminotransferase (ALT) levels were analysed with a chemical analyser (Humalyzer 3500, Human Diagnostic Worldwide, Germany) following the manufacturer's protocols provided with the assay kits.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). The data was assessed using the Kolmogorov-Smirnov test before employing a one-way analysis of variance (ANOVA) to determine significant differences among groups. This was followed by a post-hoc analysis with Tukey's Honest Significant Difference (HSD) to identify specific group differences. $p < 0.05$ was considered statistically significant.

Results and Discussion

The Yield of Eel Extract

From an initial 4.5 kg of fresh eel samples, 520.8 g of dried eel powder was produced. The microwave-assisted extraction (MAE) method extracted fish powder with 96% ethanol as the solvent. The volume of oil obtained varied depending on the extraction duration, as shown in Table II. The results demonstrated that a four-minute extraction time yielded the highest percentage of fish oil at 6.1%. Extending the extraction time to five minutes did not lead to a further increase in yield, suggesting that the extraction efficiency plateaued after four minutes.

Table II

The percentage yield of eel extract with different extraction times using microwave-assisted extraction

Time (mins)	Eel extract (g)	Yield (%)
1	0.47	4.7
2	0.50	5.0
3	0.52	5.2
4	0.61	6.1
5	0.61	6.1

Droplet Size and Characteristics of the Swamp Eel Oil and Vitamin D3 Emulsion

The developed emulsion displayed a stable and clear appearance (Figure 1). The organoleptic assessment revealed that the emulsion had a yellowish-clear visual appearance, a mildly sweet taste, and a characteristic fish oil odour. The pH of the emulsion was 6.4, consistent with the expected pH range for oil-based formulations at room temperatures (25 - 30°C).

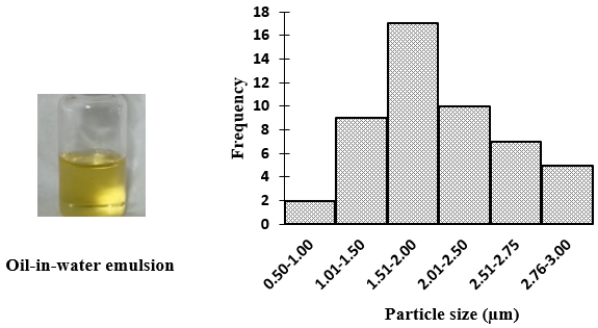

Figure 1.

Frequency distribution of particle size of the swamp eel oil and vitamin D3 emulsion

Particle size analysis showed a mean droplet diameter of 1.96 ± 0.58 µm, with a median droplet size of 1.95 µm. The particle size distribution showed a standard curve, as illustrated in Figure 1, suggesting uniform droplet formation under the applied emulsification conditions.

Body Weight Gain

Table III summarises the percentage of body weight gain in rats following 30 days of treatment with a standard diet, a high-fat diet (HFD), or HFD supplemented with eel fish oil and vitamin D3 (EVD). All groups showed an increase in body weight over the experimental period. Rats fed an HFD without treatment exhibited the highest weight gain percentage (30.1 ± 3.4%), significantly exceeding that of other groups (14.1 ± 5.4% to 21.1 ± 5.8%). In contrast, EVD treatment at a 1 mL/kg dose led to a markedly lower weight gain than the untreated HFD group ($p < 0.05$).

Table III

The percentage of weight gain after 30 days of receiving a standard or high-fat diet with eel fish oil plus vitamin D3 emulsion

Groups	Pre-treatment (g)	Post-treatment (g)	Weight gain (g)	Percentage of weight gain (%)
Control	128.7 ± 20.1	152.7 ± 19.6	24.0 ± 1.0$^{\#}$	19.0 ± 3.3$^{\#}$
HFD	108.8 ± 4.7	144.5 ± 2.0	32.8 ± 3.9*	30.1 ± 3.4*
HFD + EVD 1	160.7 ± 15.1	179.8 ± 21.7	22.8 ± 9.1$^{\#}$	14.1 ± 5.4$^{\#}$
HFD + EVD 2	139.3 ± 10.0	156.5 ± 17.8	26.0 ± 9.1	17.1 ± 5.6
HFD + SVT	128.4 ± 4.2	155.6 ± 3.5	27.3 ± 6.7	21.1 ± 5.8

HFD: High-fat diet; HFD + EVD 1: High-fat diet plus eel fish oil and vitamin D3 emulsion 1 mL; HFD + EVD 2: High-fat diet plus eel fish oil and vitamin D3 emulsion 2 mL; HFD + SVT: High-fat diet plus simvastatin. *$p < 0.05$ compared to the control group. $^{\#}p < 0.05$ compared to the HFD group

Serum Lipid and Glucose Profiles

The serum TC and FBG levels after 30 days of treatment are depicted in Figure 2. Rats on standard diet maintained stable TC levels throughout the experiment. Conversely, rats in the untreated HFD group experienced a significant increase in TC and FBG levels, reaching 150.0 ± 31.9 mg/dL and 143.3 ± 23.2 mg/dL, respectively. Supplementation with EVD significantly reduced serum TC and glucose levels in HFD-fed rats compared to the untreated HFD group ($p < 0.05$). While EVD's lipid-lowering effect was comparable to that of simvastatin, the administration of EVD at 2 mL/kg demonstrated superior efficacy in reducing serum glucose levels compared to simvastatin. These findings highlight the potential therapeutic impact of EVD in managing metabolic disruptions induced by an HFD.

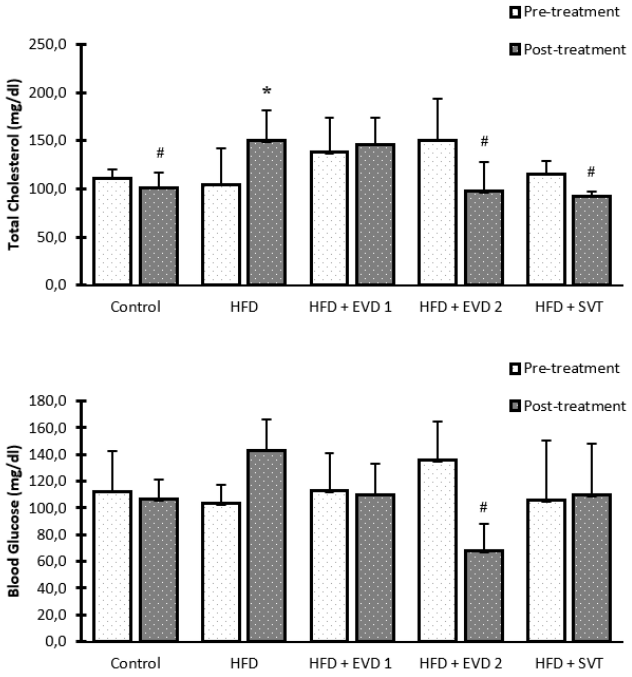

Figure 2.

The levels of total cholesterol and blood glucose in rats receiving a standard or high-fat diet with eel fish oil and vitamin D3 emulsion. HFD: High-fat diet; HFD + EVD 1: High-fat diet plus eel fish oil and vitamin D3 emulsion 1 mL; HFD + EVD 2: High-fat diet plus eel fish oil and vitamin D3 emulsion 2 mL; HFD + SVT: High-fat diet plus simvastatin. *$p < 0.05$ compared to the control group; $^{\#}p < 0.05$ compared to the HFD group

Alanine Aminotransferase (ALT) Analysis

Alanine aminotransferase (ALT) is an important biomarker of the integrity and function of liver cells. ALT may also elevate in dyslipidaemia-induced fatty liver disease. It was found that after 30 days of treatment, the high-fat diet (HFD) group exhibited a mean ALT level of 61.0 ± 4.79 U/L (Figure 3), nearly double that of the control group (38.2 ± 4.79 U/L). However, this elevation did not achieve statistical significance. Meanwhile, the group receiving HFD supplemented with 1 mL of the eel fish oil and vitamin D3 emulsion (HFD + EVD 1) displayed ALT levels comparable to those of the control group, indicating a trend toward normalisation. However, the higher emulsion dose (HFD + EVD 2) did not effectively mitigate ALT elevation, showing levels similar to the untreated HFD group. Unlike the HFD-treated group, rats treated with simvastatin showed a marked increase in ALT levels compared to both the control and HFD + EVD 1 group ($p < 0.05$), suggesting potential hepatotoxic effects associated with simvastatin at the administered dose.

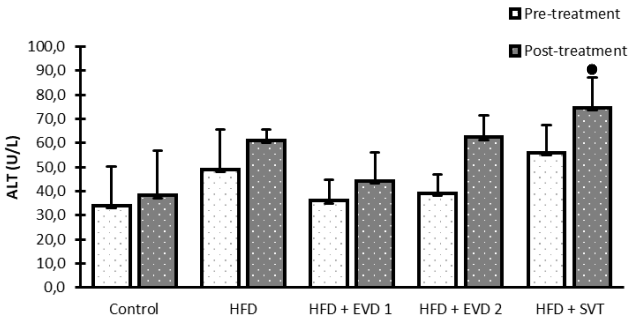

Figure 3.

Mean ALT levels in rats subjected to standard or high-fat diets, with and without supplementation of eel fish oil and vitamin D3 emulsion or simvastatin. HFD: High-fat diet; HFD + EVD 1: High-fat diet plus eel fish oil and vitamin D3 emulsion 1 mL; HFD + EVD 2: High-fat diet plus eel fish oil and vitamin D3 emulsion 2 mL; HFD + SVT: High-fat diet plus simvastatin. ●$p < 0.05$ compared to the control and HFD + EVD 1 group

This study demonstrates the potential of combining Asian swamp eel oil with vitamin D3 in an emulsion as an effective approach for improving lipid metabolism and glycaemic control in a high-fat diet-induced rat model. The results show that the eel fish oil and vitamin D3 emulsion significantly reduced total cholesterol and blood glucose levels, providing evidence for the therapeutic benefits of this combination in managing dyslipidaemia and metabolic disturbances.

In this study, we formulated an oil-in-water (O/W) emulsion by combining eel fish oil with vitamin D3, resulting in a clear yellowish emulsion with particle sizes ranging from 0.79 to 2.89 µm. The emulsion was stable at room temperature, maintaining a pH of 6.4 throughout the experimental period. This stability is important for ensuring the consistency of the formulation over time, which could help improve its practical application as a supplement. Usually, to mask the unpleasant taste and odour of fish oil, it is typical to encapsulate fish oil in soft gels or capsules [21]. However, an O/W emulsion offers a viable alternative, as it can also serve this purpose while potentially improving stability, absorption, and bioavailability [22, 23].

When administered to rats on an HFD for 30 days, the eel fish oil and vitamin D3 emulsion significantly reduced total cholesterol and blood glucose levels while maintaining the rats' body weight. This suggests EVD's potential as a lipid-lowering agent and a tool for improving metabolic health. EVD dose of 1 - 2 mL/day in rats was equivalent to 194 - 388 mg/day of fish oil and 483 - 966 IU/day of vitamin D3 for humans, which falls within the recommended daily intake range of 250 - 500 mg of omega-3 fatty acids and 800 - 1000 IU of vitamin D3 [24, 25]. Fish oil, especially the omega-3 fatty acids it contains, has long been recognised for its beneficial effects on lipid metabolism [26]. Omega-3s have been demonstrated to lower triglycerides, reduce inflammatory response, and improve overall lipid profiles, thus reducing the risk of cardiovascular diseases [27]. Additionally, vitamin D3, a fat-soluble vitamin, has been shown to play a crucial role in regulating blood glucose levels. Several reports have indicated that vitamin D deficiency is related to impaired insulin sensitivity, and supplementation may improve glycaemic control in individuals with metabolic disturbance, such as type 2 diabetes mellitus and obesity [11, 28, 29].

Interestingly, while simvastatin, a widely used statin, effectively reduced total cholesterol levels and maintained body weight, it did not have any benefits on blood glucose levels in HFD-treated rats. In contrast, the eel fish oil and vitamin D3 emulsion lowered total cholesterol and improved glycaemic control. The combination of eel fish oil and vitamin D3 may offer a more favourable metabolic profile. Previously, a combination of omega-3 fatty acids and vitamin D3 has been reported to provide synergistic benefits in improving insulin sensitivity and reducing systemic inflammation [30]. This is especially important given that statins are linked with a possible risk of new-onset type 2 diabetes mellitus in some patients [31]. Therefore, the eel fish oil and vitamin D3 emulsion might offer a safer, alternative therapeutic approach for managing dyslipidaemia and metabolic disturbances, with fewer concerns regarding glycaemic control.

Another key finding in this study was the effect of simvastatin on ALT levels, a common biomarker for liver function. Rats treated with simvastatin showed a significant elevation in ALT levels, suggesting potential hepatotoxic effects, consistent with known side effects of statins [32]. Although effective in reducing cholesterol levels, statins have been associated with adverse metabolic effects, such as impaired glucose metabolism and insulin resistance, particularly at moderate to high doses [33]. Moreover, long-term statin use has been associated with the potential development of non-alcoholic fatty liver disease (NAFLD) and other liver dysfunctions in diabetic and non-diabetic patients [34]. In contrast, the eel fish oil and vitamin D3 emulsion did not result in significant ALT elevation, supporting its potential for preserving liver health and managing lipid levels. This aligns with previous studies that have shown the beneficial effects of fish oil and vitamin D supplementation on liver biomarkers, particularly in conditions such as NAFLD, where liver inflammation and enzyme levels are often elevated [30].

Conclusions

In conclusion, the combination of eel fish oil and vitamin D3 emulsion showed promising lipid-lowering effects, glycaemic control, and preserved liver function in rats fed a high-fat diet. This emulsion may offer a beneficial alternative to statins, effectively lowering total cholesterol without negatively impacting glycaemic control or liver health. The results suggest that the combination of omega-3-rich eel fish oil and vitamin D3 holds potential as a therapeutic strategy for managing dyslipidaemia and preventing liver damage.

However, to validate the clinical applicability of these findings, further research is necessary. The current study primarily focused on basic lipid and liver biomarkers; future investigations should incorporate a more comprehensive analysis of additional biomarkers related to lipid metabolism, inflammatory pathways, and NAFLD progression. Such studies are essential to confirm the long-term effectiveness of eel fish oil and vitamin D3 in lipid management and liver protection, as well as to optimise dosages and therapeutic regimens for human populations. Furthermore, clinical trials are necessary to further determine the safety and

efficacy of this combination in diverse patient groups, including those with varying degrees of dyslipidaemia and metabolic dysfunction.

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Conflict of interest

The authors declare no conflict of interest.

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