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Research Article

Combination of *Sargassum duplicatum* Alginate, Mangosteen Pericarp, and Okra Fruit Extracts Improved Lipid Profiles and Ameliorates Liver Damage in Hyperlipidemic Rats

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Abstract | Hyperlipidemia caused by high fat diet leads to oxidative stress, which impairs lipid profiles, increasing the risk of atherosclerosis and other cardiovascular diseases, in addition to damage to liver. This study aims to determine combination effect alginate derived from *Sargassum duplicatum* for reducing lipid absorption in the digestive tract with mangosteen (*Garcinia mangostana*) pericarp and okra (*Abelmoschus esculentus*) extract acting as antioxidants. High-fat diet (HFD) containing cow fat was given to male Wistar rats for 11 weeks to induce hyperlipidemia, followed by 2 weeks of oral administration of alginate and extract combinations while HFD feeding was continued, representing a post-induction therapeutic intervention aimed at improving hyperlipidemia while limiting further progression of early atherosclerotic changes. Combinations examined were alginate and mangosteen peel extract (SG); alginate and okra extract (AG); and alginate, mangosteen peel, and okra extract (SAG) in total dose of 0,035 g/kg bw. The SG group was effective in increasing HDL levels, lowering LDL levels, and reducing atherosclerosis risk. On the other hand, the SA group was more effective in improving ALT and AST levels, as well as liver histopathology. The superior vascular effect of SG may be related to the combined cholesterol-lowering action of alginate and the anti-oxidized LDL activity of mangosteen xanthones, whereas liver improvement in SA/SAG may reflect the hepatoprotective antioxidant activity of okra flavonoids and polysaccharides. Combination of *Sargassum* alginate, mangosteen peel and okra extract were able to improve lipid profiles and ameliorate liver damage in hyperlipidemic rats.

Keywords | Anti-hyperlipidemia, antioxidants, histopathology, oxidative stress, rats.

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INTRODUCTION

Modern lifestyles characterized by high consumpiton of fat-rich diets. It can increase the risk of hyperlipidemia, which is marked by elevated LDL and reduced HDL levels (Zeng *et al*., 2022). Increased non-HDL cholesterol is strongly associated with cardiovascular disease risk (Cao *et al*., 2019), and LDL cholesterol has become a primary marker for atherosclerotic risk assessment (Carr *et al*., 2019). Oxidized LDL triggers endothelial dysfunction and inflammation, contributing to the development of atherosclerotic plaque in the wall of arteries (Summerhill *et al*., 2019).

The liver plays a central role in regulating and circulating lipoproteins through LDL receptors on its surface (Alabi *et al*., 2021). Excess lipid accumulation in the liver induces oxidative stress and hepatic inflammation, contributing to the development of non-alcoholic fatty liver disease (NAFLD) (Mueller *et al*., 2021). Hepatocellular damage is commonly reflected by elevated serum ALT and AST levels (Ghosh *et al*., 2021), NAFLD has become a major global health concern due to its high prevalence and potential progression to more severe liver conditions (Martín-Fernández *et al*., 2022).

The role of the liver in lipid metabolism is also demonstrated by the synthesis of bile acid from cholesterol by hepatocytes (Thapa *et al*., 2023). The bile acid synthesized in the liver are secreted into the duodenum, where they emulsify dietary lipids, making them available for absorption into the body, and subsequently returned to the liver (Deng and Bae, 2020). The use of a combination substances that play a role in reducing lipid absorption in the digestive tract and antioxidant to prevent tissue damage caused by oxidative stress is a promising alternative solution for reducing the risk of complications due to hyperlipidemia. In this study, we used a combination of alginate from *Sargassum duplicatum*, brown algae abundantly found in the coastal waters of Indonesia, extract of mangosteen peel (*Garcinia mangostana*), and okra fruit extract (*Abelmoschus esculentus*). Both mangosteen peel and okra are widely recognized for their high level of antioxidants (Ilmi *et al*., 2020; Wulandari *et al*., 2021). Mangosteen and okra are abundant in Indonesia and are significant export commodities (Sugiharti *et al*., 2020; EFSA *et al*., 2021).

Given the involvement of lipid absorption and oxidative stress in hyperlipidemia pathogenesis, a combination strategy targeting both mechanism may provide therapeutic benefit. Alginate derived from *Sargassum duplicatum* is a soluble dietary fiber known to bind bile acids and reduce cholesterol absorption (Yan *et al*., 2021). In addition, mangosteen peel (*Garcinia mangostana*) and okra fruit (*Abelmoschus esculentus*) are rich in antioxidants that can neutralize reactive oxygen species and protect tissues from oxidative damage (Wulandari *et al*., 2021; Akram *et al*., 2023; Husen *et al*., 2020). The rationale for including the triple combination (SAG) was based on the hypothesis that alginate, mangosteen peel, and okra extract may provide complementary multi-target actions against hyperlipidemia by simultaneously reducing intestinal lipid absorption, suppressing oxidative modification of circulating lipoproteins, and protecting hepatic tissue from oxidative injury. Such complementary mechanism was expected to provide broader protection than dual combination, although the magnitude of benefit in each target organ might depend on the dominant activity and effective dose of each component. Despite of each natural materials contain chemically distinct bioactive constituents, the administered doses in this study were selected based on previously reported biologically effective doses rather than equivalence purified active compounds. Therefore, the present study was designed as an initial comparative evaluation of biological responses under equal administered weight-based dosing.

This study aims to determine combination effect alginate derived from *Sargassum duplicatum* for reducing lipid absorption in the digestive tract with mangosteen (*Garcinia mangostana*) pericarp and okra *(Abelmoschus esculentus*) extract acting as antioxidants in Wistar rats. Although previous studies have reported the lipid-lowering effects of alginate as well as the antioxidant and hepatoprotective activities of mangosteen peel and okra extracts individually, investigations into the combined effects of these three agents on cardiovascular and hepatic complications associated with hyperlipidemia remain very limited. To date, no study has comprehensively evaluated the complementary action of marine-derived alginate (*Sargassum duplicatum*) combined with antioxidant-rich plant extracts in a hyperlipidemic rat model, particularly with simultaneous assessment of lipid profile, atherosclerosis risk, and liver histopathological alterations. Therefore, this study offers novelty through a new integrative multi-target combination strategy, namely by combining a lipid absorption–inhibiting agent with natural antioxidants to simultaneously suppress multiple pathophysiological pathways of hyperlipidemia.

MATERIALS AND METHODS

Ethical approval

All experimental procedures were approved by the Research Ethics Commission of the Faculty of Dental Medicine, Universitas Airlangga (035/HRECC.FODM/I/2023).

Study area and experimental settings

This experimental study was conducted at the Animal Laboratory, Department of Biology, Faculty of Science and

technology, Universitas Airlangga, Surabaya, Indonesia. All animal handling, treatment administration, and sample collection procedures were carried out under controlled laboratory conditions. This study was performed from August-December 2022. Environmental conditions were maintained at a temperature of 26-28 °C with relative humidity of 65-70% and a 12-hour light/dark cycle.

High fat diet feed

The high-fat diet (HFD) feed was made with composition of 40% cow fat, 30% fish meal, 29% rice flour, and 1% salt (Marques *et al*., 2015). Feed was made into standard size pellet, at 0.8 mm diameter.

Formulation of *Sargassum* alginate, mangosteen peel and okra extracts

The alginate of *Sargassum duplicatum* was formulated based on previous study, as with the extraction of mangosteen pericarp and okra fruit (Ilmi *et al*., 2020; Wulandari *et al*., 2021). Alginate and extracts were used as both standalone and combination treatments. This design compares biological responses under equal administrated mass, not equivalent active compound potency.

Experimental animals

This research used 40 male Wistar strain rats aged 10 weeks, weighing 110-120 g, all of which were in a healthy condition without any signs of injury or illness. The rats were obtained from Experimental Animal Laboratory in Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia. Rat models were acclimated for 14 days, with access to water and feed ad libitum. The temperature and humidity were maintained within the range of 22-24°C and 65-70%, respectively.

Administration of alginate and mangosteen peel and okra extract

Rat models were separated into 10 groups, which were KN (normal group), K- (negative control, HFD only), K+ (positive control, Simvastatin 10 mg/kg BW), KS (*Sargassum* alginate group), KA (okra extract group), KG (mangosteen peel extract group), SA (combination of *Sargassum* alginate and okra extract), SG (combination of *Sargassum* alginate and mangosteen peel extract), AG (combination of okra extract and mangosteen peel extract), and SAG (combination of *Sargassum* alginate, okra extract, and mangosteen peel extract). Each group had 4 rats. All groups except KN were fed HFD feed for 11 weeks ad libitum (Marques et al., 2015). Alginate and extracts were orally administered daily in the last 2 weeks of HFD treatment. The dosage for single groups (KS, KA, KG) was 0.035 g/kg bw each, for two-combination groups (SA, SG, AG) was 0.0175g/kg bw each, and for three-combination group (SAG) was 0.0115g/kg bw each. The total administered dose was maintained at 0.035 g/kg bw across all treatment groups. Therefore, in combination groups, the dose of each component was proportionally adjusted according to the number of combined materials to ensure equal total treatment exposure.

Sample collection

Rats were anesthetized and dissected at the end of treatment. Blood samples were carefully taken intracardially, then transferred into vacutainer vacuum tubes gel clot activator. Subsequently, the blood samples were centrifuged at 3000 rpm for 30 minutes at a temperature of 4°C. The serum was separated and stored at -40°C for further analysis in different vials. The aorta and liver tissues were collected and fixed in a 10% neutral buffered formalin solution for histological preparations.

Lipid profile measurement

The lipid profiles measured in this study included serum HDL and LDL levels. Serum HDL were measured by enzymatic method using rat HDL and LDL cholesterol assay kit (Crystal Chem). Optical density was measured at a wavelength of 600 nm.

Measurement of ALT and AST levels

Serum enzymes ALT and AST were measured using IFCC method based on guideline from ALT and AST kit (Glory Diagnostics). Optical density was measured at a wavelength of 340 nm.

Histopathological evaluation

Fixed aorta and liver were processed into histological sections using paraffin embedding method. Briefly, tissues were fixed in 10% neutral buffered formalin, dehydrated through a graded ethanol series (70%, 80%, 90%, 96%, and absolute ethanol), cleared in xylene, and infiltrated with molten paraffin wax. The tissues were then embedded in paraffin blocks and sectioned at 5 µm thickness using rotary microtome). Sections were mounted on glass slides, deparaffinized, rehydrated, and stained using hematoxylin-eosin according to standart histological procedures (Suvarna *et al*., 2019). Aortic wall thickness was evaluated based on average thickness measured from four different angles (09:00, 12:00, 15:00, and 18:00) using Optilab Viewer 4 software. Histopathological evaluation of aorta was based on criteria from previous study (Bennani-Kabchi *et al*., 2000). A score of 0 was assigned for a normal aorta with orderly arranged muscle cells and centrally located nuclei. Enlarged muscle cells, foam cells, fibrosis, irregularly arranged elastin fibers, fat infiltration, or plaque in the aortic wall was given a score of 1 respectively. The sum of scores was then converted into severity categories of atherosclerosis risk; score 0 indicated normal aorta, 1-2 low/mild atherosclerosis, 3-4 moderate atherosclerosis, and

5-6 severe atherosclerosis. Histopathological analysis of the liver was conducted using light microscope and assisted by Optilab Viewer 4 software to determine the percentage of normal cells, necrosis, and edema, as well as percentage of inflammatory area. Histopathological evaluation of liver sections was performed using light microscopy based on Suvarna et al. (2019) and Kumar et al., (2020). Normal hepatocytes exhibited a polygonal morphology with distinct cell borders, granular eosinophilic cytoplasm, and centrally located round nuclei containing finely dispersed chromatin and prominent nucleoli. Necrotic hepatocyte was characterized by increased cytoplasmic eosinophilia, nuclear changes included pyknosis, karyorrhexis, and karyolysis. Edematous hepatocytes were appeared pale with enlarged size of cells or vacuolated cytoplasm and indistinct cellular boundaries. Inflammatory areas were identified by infiltrates of inflammatory cells within the hepatic parenchyma and/or portal tracts. The area of inflammation was assessed based on the density and distribution of infiltrating cells (Kleiner et al., 2005; Burt et al., 2018).

Statistical analysis

Data were statistically analyzed using SPSS software version 21.0. Serum HDL and LDL levels, aorta histopathological score, percentage of normal, swollen, and necrotic hepatocytes, and ALT levels were tested using Kruskal-Wallis test, followed by post-hoc Mann-Whitney test. Percentage of the inflammatory area was tested using one-way ANOVA followed by Duncan test. Unpaired t-tests were performed for aortic wall thickness and AST levels. A p-value < 0.05 was considered statistically significant (Motulsky, 2014). A formal priori power analysis was not performed before the experiment. The sample size of four animals per group was determined based on previous comparable in vivo studies using similar high-fat diet rat models and natural product interventions, consistent with exploratory preclinical study design.

RESULTS

Clinical observations

During the experimental period, all rats survived until the end of the treatment, and no mortality was observed in any group. Rats fed a high-fat diet (HFD) in the negative control group (K-) exhibited mild clinical signs, including greater body weight gain compared to the normal control group, reduced physical activity, and slightly dull fur appearance. However, no severe clinical symptoms such as diarrhea, severe allergic reactions, behavioral disturbances, or other signs of acute toxicity were observed. Meanwhile, rats receiving combination treatments showed better general conditions compared to the negative control group, with relatively normal activity levels and proper grooming behavior. No visible adverse effects or clinical signs of toxicity were observed in any of the treatment groups throughout the study period.

Serum lipid profiles

Serum HDL and LDL level after treatment is presented on Figure 1. Based on measurement results, HFD induced significant decrease of serum HDL in K- compared to KN. K+, KS, KG, and KA group were not significantly different compared to K-. On the other hand, all combination groups (SA, SG, AG, SAG) were found to be significantly higher to K-. Serum HDL of SA and SG were higher compared to KN ($p<0.05$). The elevation of HDL above normal control levels in SA and SG groups may indicate enhanced reverse cholesterol transport rather than dysregulation, particularly because it was accompanied by reduced LDL levels and improved vascular or hepatic outcomes. Antioxidant compounds from mangosteen and okra may also preserve HDL functionality by reducing oxidative modification, thereby contributing to a beneficial increase in circulating HDL. Serum LDL levels from all groups were lower significantly compared to K-, however they tended to be at similar level compared to KN. In K+, KG, KA, SA, and SAG, serum LDL were significantly reduced compared to KN ($p<0.05$).

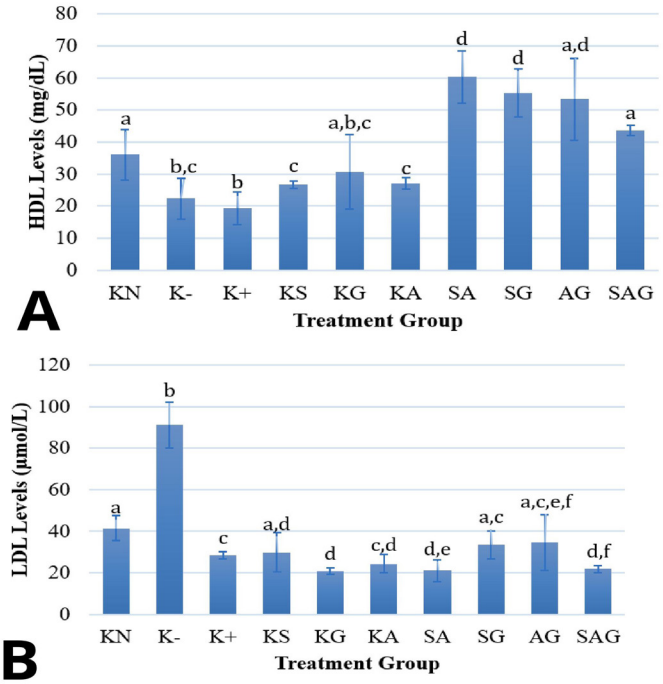

Figure 1: Serum lipid profile in all treatment groups. A: HDL levels; B: LDL levels; KN: Normal control group; K-: Negative control; K+: Positive control (simvastatin); KS: Alginate from *Sargassum duplicatum*; KG: Extract of mangosteen peel; KA: Extract of okra fruit; SG: Combination of alginate and mangosteen peel extract; SA: Combination of alginate and okra extract; AG: Combination of extract of mangosteen peel and okra; SAG: Combination of alginate, okra, and mangosteen peel. Different letters above the diagram indicate significant differences ($p<0.05$).

Histopathological analysis of aorta

Sections of aorta were analyzed for thickness and developed atherosclerosis level. The thickness of the aortic wall did not show significant differences statistically ($p>0.05$) to normal and negative controls (KN and K-) (Figure 2), but noticeable increase of atherosclerosis level was observed in untreated/negative control group K-. Compared to all other treatments, SG combination which was not statistically different to KN and K+. SG group was observed to have the most improvement, with only minimal damage, no foam cells found, and only a small portion of elastin fibers showing irregularities. The histopathological observations of the KG group showed results that were not markedly different from those of the alginate group. There was widening of the elastic fibers accompanied by fibrotic tissue changes. Foam cells were observed in 2 out of 3 samples; however, no lipid infiltration was detected. The KG group was classified as moderate. (Figure 3).

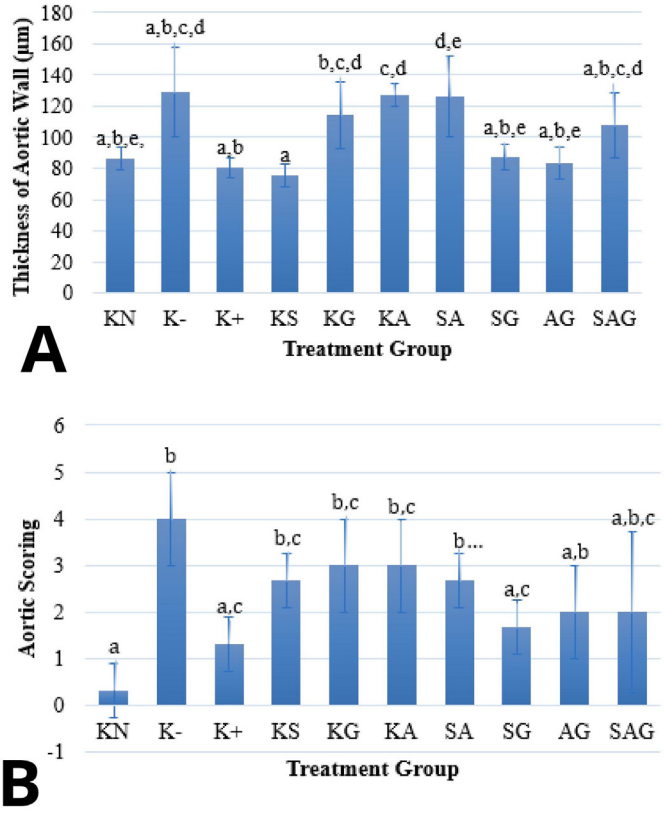

Figure 2: Histopathological analysis of aortic wall in all treatment groups. A: Thickness of aortic wall; B: Atherosclerosis score; KN: Normal control group; K-negative control; K+: Positive control (simvastatin); KS: Alginate from *Sargassum duplicatum*; KG: Extract of mangosteen peel; KA: Extract of okra fruit; SG: Combination of alginate and mangosteen peel extract; SA: Combination of alginate and okra extract; AG: Combination of extract of mangosteen peel and okra; SAG: Combination of alginate, okra, and mangosteen peel. Different letters above the diagram indicate statistically significant differences at $p<0.05$.

Serum ALT and AST levels

Serum ALT and AST levels after treatment is presented on Figure 4. Based on results, ALT level of KA was not different significantly compared to K-, while all other groups were significantly different ($p<0.05$). Meanwhile, comparing to negative control (K-), there were no significant changes in serum AST levels in all groups (Figure 4) ($p>0.05$).

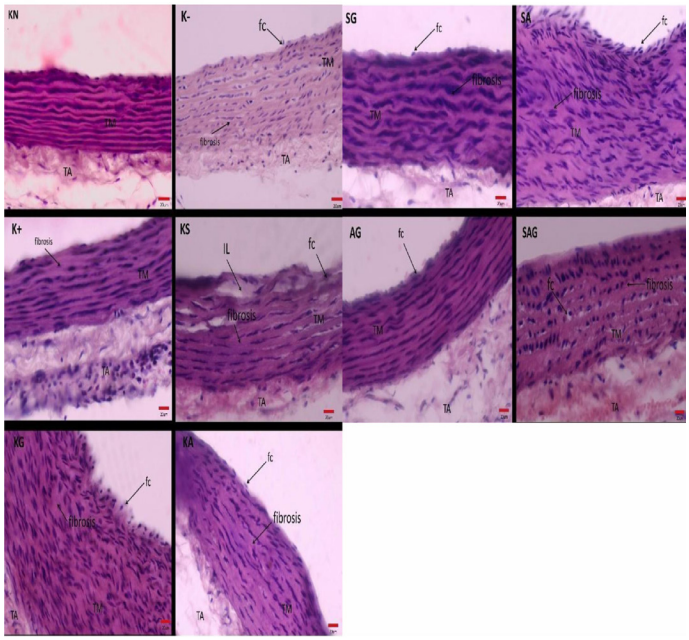

Figure 3: Cross section of aorta in all group of treatments. KN: Normal control group; K-: Negative control; K+: Positive control (simvastatin); KS: Alginate from *Sargassum duplicatum*; KG: Extract of mangosteen peel; KA: Extract of okra fruit; SG: Combination of alginate and mangosteen peel extract; SA: Combination of alginate and okra extract; AG: Combination of extract of mangosteen peel and okra; SAG: Combination of alginate, okra, and mangosteen peel. fc: Foam cell, TM: Tunica media, TA: Tunica adventitia. Scale bar: 20 µm.

Histopathological analysis of liver

Sections of liver from all groups is presented in Figure 5, while evaluation of normal hepatocytes, edema, necrotic, and inflammatory area is presented in Figure 6. It can be seen that K- had significantly increased number of necrotic and edema cells, as well as inflammation area, in addition to lower number of normal cells compared to other groups. No significant decrease in the percentage of normal cells was found in all groups except K- and KA. Increase of edema cells was found in K-, KA, and AG groups, while edema cells in K+, KS, KG, and SG were not significantly different from KN. On the other hand, KS, KG, KA, SG, and SAG were not statistically different compared to K+. Higher number of necrotic cells and larger inflammatory area was also found in K-, while in other groups, both were significantly reduced. Decrease of necrotic cell number was similar in single treatment groups compared to treatment combination groups, except for KA; while inflammatory area was reduced rather similarly in all treatment groups, given single or combined treatments.

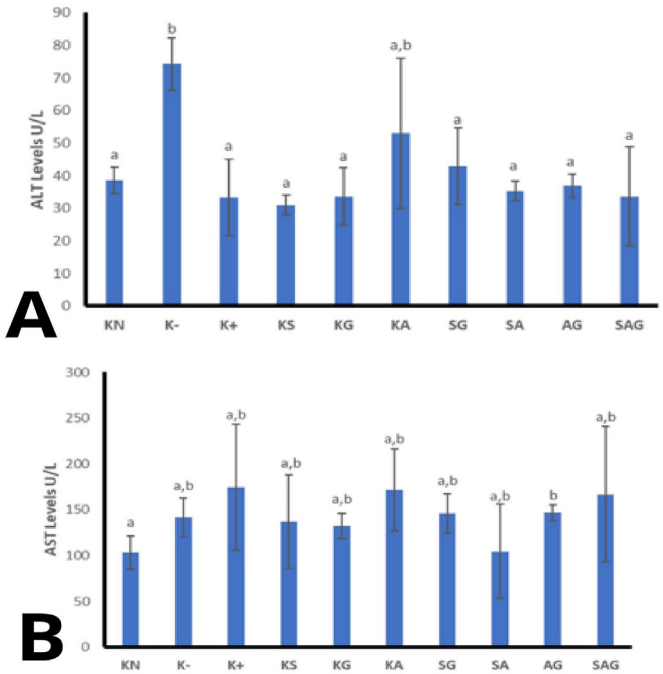

Figure 4: Serum ALT and AST levels from all treatment groups. A: ALT levels; B: AST levels; KN: Normal control group; K-negative control; K+: Positive control (simvastatin); KS: Alginate from *Sargassum duplicatum*; KG: Extract of mangosteen peel; KA: Extract of okra fruit; SG: Combination of alginate and mangosteen peel extract; SA: Combination of alginate and okra extract; AG: Combination of extract of mangosteen peel and okra; SAG: Combination of alginate, okra, and mangosteen peel. Different letters above the diagram indicate statistically significant differences at $p<0.05$.

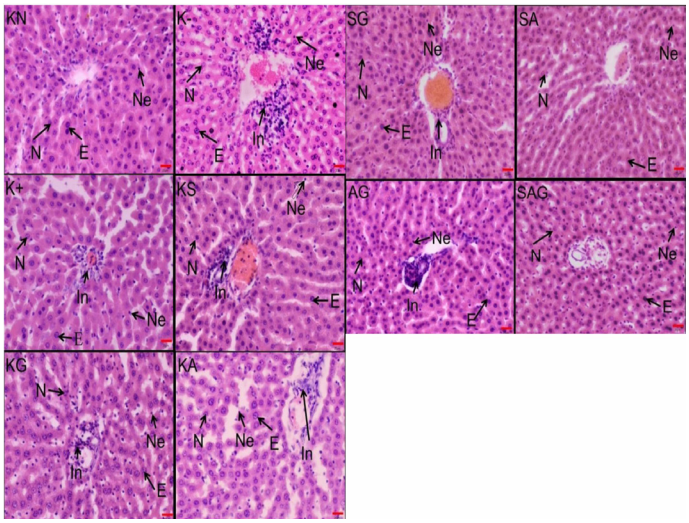

Figure 5: Histology of the liver in all group of treatments. KN: Normal control group; K-negative control; K+: Positive control (simvastatin); KS: Alginate from *Sargassum duplicatum*; KG: Extract of mangosteen peel; KA: Extract of okra fruit; SG: Combination of alginate and mangosteen peel extract; SA: Combination of alginate and okra extract; AG: Combination of extract of mangosteen peel and okra; SAG: Combination of alginate, okra, and mangosteen peel. N: Normal cells; E: Edema cells; I: Inflammation; Ne: Necrosis cells. Scale bar: 20 µm.

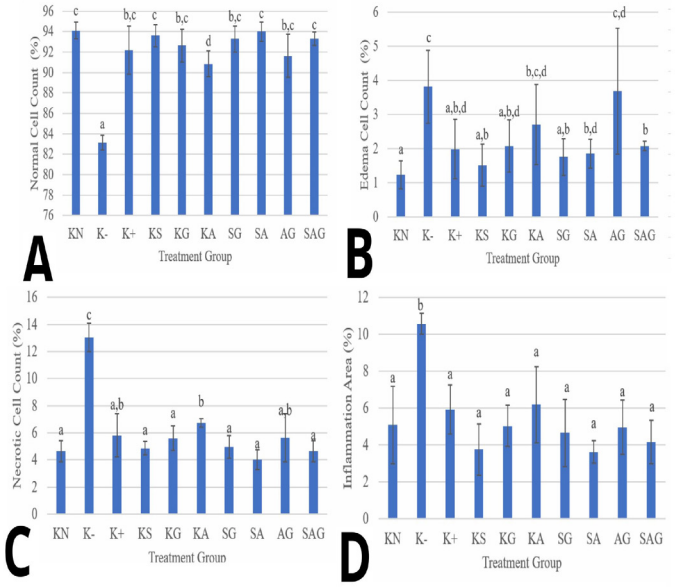

Figure 6: Percentage of hepatocytes in all treatment groups. A: Normal cells; B: Edema cells; C: Necrotic cells; D: Inflammation area of liver; KN: Normal control group; K-negative control; K+: Positive control (simvastatin); KS: Alginate from *Sargassum duplicatum*; KG: Extract of mangosteen peel; KA: Extract of okra fruit; SG: Combination of alginate and mangosteen peel extract; SA: Combination of alginate and okra extract; AG: Combination of extract of mangosteen peel and okra; SAG: Combination of alginate, okra, and mangosteen peel. Different letters above the diagram indicate statistically significant differences at $p<0.05$.

DISCUSSION

Administration of HFD for 11 weeks resulted in significant alterations in lipid profiles, particularly increased LDL and decreased HDL levels, consistent with previous findings (Liang *et al*., 2021). Elevated LDL promotes lipid accumulation in arterial walls and contributes to foam cell formation and early atherosclerotic changes (Meneses *et al*., 2019; Zhu *et al*., 2020). In this study, the negative control group (K-) showed increased atherosclerosis scores, although aortic wall thickness did not differ significantly. It indicated early structural alteration rather than advanced plaque formation.

Excess lipid accumulation als affects hepatic metabolism. Increased free fatty acid influx into the liver promotes oxidative stress and reactive oxygen species (ROS) production, leading to hepatocyte necrosis and inflammation (Hliwa *et al*., 2021; Ma *et al*., 2021). High-fat diet elevated blood ALT and AST levels in mice (Tang *et al*., 2022). ALT is an enzyme primarily found in hepatocytes, while AST is found not only in hepatocytes but also in other organs, excluding bones. Increased ALT in the serum is associated with increased necrosis of

liver cells or increased hepatocyte permeability, whereas increased AST is more nonspecific as indicator of liver damage (Pilling *et al*., 2021). In this study, HFD caused significant increase of ALT while AST did not show a significant increase, indicated that HFD administration resulted in significant liver damage, as evidenced by the significant increase in ALT in the K- group, but not in tissues other than the liver.

Simvastatin as the positive control in this study reduced the ALT levels and improved aortic structure. In the present study, simvastatin also significantly reduced LDL levels compared with the negative control and improved liver histopathological appearance, indicating effective correction of lipid-related tissue injury under the current experimental conditions. It's lipid-lowering and anti-inflammatory effects are associated with inhibition of HMG-CoA reductase and reduction of atherogenic lipoproteins (Gesto *et al*., 2020; Yanai *et al*., 2022). Statins have also been reported to modulate oxidative stress and inflammatory pathways (Koushki *et al*., 2021; Pereira *et al*., 2022), although high doses may induce adverse hepatic effects (Nassir, 2022; Saha and Garg, 2021). Rats administered Simvastatin show a significant increase in linoleic acid levels compared to the HFD group. Linoleic acid (LA) is an essential fatty acid (EFA) that plays a role in regulating lipid metabolism by reducing total cholesterol and LDL levels (Zhang *et al*., 2020). Although statins have been associated with hepatotoxicity under certain conditions, they remain widely used as positive controls in hyperlipidemia studies because their lipid-lowering effect reduces hepatic lipid overload, which may secondarily improve liver structure when administered at controlled doses and limited duration.

Alginate contained soluble fiber or dietary fiber components which can reduce digestion and intestinal lipid absorption (Zhao *et al*., 2022; Kalas *et al*., 2021), disrupt cholesterol and bile acid reabsorption in intestine (Zonouz *et al*., 2023), and decreasing cholesterol levels transported to liver via chylomicrons. This process is accompanied by upregulation of LDL receptors and reduction of lipoprotein secretion to maintain homeostasis in the liver (Liu *et al*., 2021). Fucoidan lipopolysaccharides in alginate can also stimulate inflammatory signaling pathway via the release of obstructive pro-inflammatory cytokines and adhesion molecules (Jayasinghe *et al*., 2023). Lowered lipid supply to the liver also prevents NAFLD occurrence, reducing levels of ALT and AST, as well as ameliorating hepatocyte damage (Zhao *et al*., 2022). Alginates also have been found to inhibit α-glucosidase and pancreatic lipase enzymes, which play a crucial role in controlling glucose and lipid absorption in the body (Magwaza and Islam, 2023).

Administration of high-dose mangosteen extract at 200 mg/kg body weight for 5 weeks during HFD/streptozotocin treatment can improve lipid profile, modulating antioxidant enzyme activity, enhancing liver function, and lowering oxidative stress of rats compared to HFD group (Zonouz *et al*., 2023). Xanthones from the phenolic compound class found in mangosteen pericarp exhibit strong antioxidative activity by directly binding to ROS in the liver (Wulandari *et al*., 2021), resulting in reduced hepatocyte damage, such as necrosis and inflammation (Magwaza and Islam, 2023). Consistent with previous study, mangosteen pericarp extract was able to lower ALT and AST levels, reduce inflammation, modulate key signaling pathways, improve tissue histology, and inhibit acetylcholinesterase activity (Husen *et al*., 2020).

Okra contains an array of antioxidants, such as flavonoids and polyphenols that can act to scavenge free radicals. Flavonoids have hepatoprotective properties and inhibit lipid peroxidation (Sahlan *et al*., 2021). This aligns with previous study which reported that okra extract decreased ALT and AST levels, as well as hepatocyte damage (Wahyuningsih *et al*., 2021). The interaction between antioxidants, paraoxonase-1 (PON1) and HDL also plays important role to prevent progression of atherosclerosis. Antioxidants modify HDL composition, reducing ROS, increase HDL and PON1 concentrations and activities, thus enhance the ability of HDL to promote cholesterol efflux from cells (Otocka-Kmiecik, 2022). Previous study also revealed that administration of okra extract to HFD-induced rats for eight weeks resulted in significant decrease of cholesterol, LDL, and TG levels. Okra extract might be able to reduce the expression of both lipogenic (SREBP-1c, FAS, acetyl-CoA carboxylase), and adipogenic proteins (PPAR-C/EBP-α, aP2/FABP4) (Liao *et al*., 2019). Thus, okra can mitigate HFD-induced obesity through reduction of adipogenesis and lipogenesis.

In summary, complementary action of alginate with antioxidants contained in mangosteen pericarp and okra resulting in reduced lipid accumulation and ROS-induced damage in the (Sahlan *et al*., 2021), resulting in the prevention of NAFLD formation. In the current study, HDL level of SAG did not significantly different from KN, while LDL was lower compared to KN. The number of normal hepatocytes, necrotic cells, and inflammation area of SAG did not significantly different to KN, while edema cells in SAG was slightly higher than KN but significantly lower compared to K-. Previous study was in line with our findings, for example intraperitoneal administration of *Sargassum vulgare*, along with *Bacillus oceanisediminis*, chrysanthemum, and alginate at a dose of 200 mg/kg for one week showcased a notable hepatoprotective effect (Nabil-Adam *et al*., 2023). The complementary action of antioxidant and anti-inflammatory properties to

counteract the hepatotoxicity induced by acetaminophen highlights the potential of marine-derived extracts to ameliorate liver damage.

Although the triple combination was initially expected to produce stronger effects through multi-target synergy, its superiority was not consistently observed across all parameters. This may be explained by the lower individual dose of each component in the SAG formulation compared with dual combinations, potentially reducing the dominant bioactivity of certain compounds. In addition, the results suggest that specific organ responses may depend more strongly on particular bioactive compounds, such as mangosteen-derived xanthones for vascular protection and okra-derived flavonoids for hepatoprotection. Beside of that, the lack of consistent superiority of triple combination may partly reflect dose dilution, since each component in the SAG group was administered at a lower individual dose than in dual combinations. Reduced per-component in SAG likely limited the effect of dominant compounds.

This study has the limitations that should be acknowledged. The molecular mechanism underlying the observed lipid-lowering and hepatoprotective effects were not directly investigated, as oxidative stress markers, inflammatory cytokines, and gene expressions related to lipid metabolism were not measured. Despite of this limitation, the present findings provide important preliminary evidence supporting the complementary action of combining marine-derived alginate with antioxidant-rich plant extracts. Beside of that, this study also has limitation of the extracts were administered at equal weight-based doses without phytochemical standardization of their dominant active compounds. Therefore, direct potency equivalence among extracts cannot be assumed. Another limitation is the relatively small sample size per group, which may limit statistical power and increase the possibility of Type II error, particularly for variables that showed non-significant trends. Beside of that, histopathological scoring was not conducted under blinded conditions, which may introduce observer bias despite the use of predefined scoring criteria. Future studies are recommended to explore the molecular signalling pathways involved in lipid metabolism and oxidative stress, and larger studies are recommended to confirm the reproducibility of these findings.

CONCLUSIONS

This study demonstrates that the combination of alginate derived from *Sargassum duplicatum* with mangosteen peel extract (*Garcinia mangostana*) and okra fruit (*Abelmoschus esculentus*) exerts protective effects in a high-fat diet–induced hyperlipidemic rat model. Among all tested formulations, the combination of alginate and mangosteen peel extract (SG) showed the most pronounced improvement in lipid profile, particularly through increased HDL levels and decreased LDL levels, which correlated with a reduction in atherosclerosis risk scores. Meanwhile, combinations containing okra extract demonstrated more evident improvements in liver function parameters, especially through decreased ALT levels and improved liver histopathological features, including reduced necrosis and inflammatory areas. These findings suggest that the combined administration of alginate, mangosteen peel extract, and okra extract may provide complementary protection against hyperlipidemia-related vascular and hepatic alterations, although formal synergistic interaction was not specifically evaluated in the present study. The triple combination (SAG) was able to maintain lipid parameters and liver structure close to normal conditions, suggesting its potential to be developed as a preventive strategy against hyperlipidemia-related complications, particularly non-alcoholic fatty liver disease. Further studies are needed to elucidate the underlying molecular mechanisms and to evaluate long-term efficacy and safety.

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NOVELTY STSTMENT

This study provides novel evidence regarding the complementary protective effects of a combination of alginate derived from Sargassum duplicatum, mangosteen peel extract (*Garcinia mangostana*), and okra fruit extract (*Abelmoschus esculentus*) against hyperlipidemia-induced vascular and hepatic alterations in Wistar rats. Unlike previous studies that evaluated these natural compounds individually, the present study comprehensively investigated their combined multi-target activity through simultaneous assessment of lipid profile, atherosclerosis risk, liver enzyme levels, and liver histopathological changes. The findings demonstrate the potential of combining marine-derived alginate with antioxidant-rich plant extracts as a natural preventive strategy for hyperlipidemia and non-alcoholic fatty liver disease (NAFLD).

AUTHOR'S CONTRIBUTION

Conception, design, and material: DW, HS, WDM, FRP,

RJK; supervision and resources: DW, HS, WDM, FRP; data collection and literature search: DW, DA, SKR, GFI, DH, FK, CBF, AL; analysis and interpretation: DW, PNM, HBN; writing manuscript: DW, AL, CBF, RJK, EAS, WDM, FRP; critical review and formatting: DW, EAS, RJK, MAH, HAM.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Data availability

All datasets analysed during the current study are available for public.

Abbreviations

AG: Combination of alginate and okra extract; ALT: Alanine Aminotransferase; Ap2/FABP4: Adipocyte Protein 2; AST: Aspartate Aminotransferase; C/EBP-α: CCAAT/Enhancer-Binding Protein Alpha; FAS: Fatty Acid Synthase; HDL: High Density Lipoprotein; HFD: High Fat Diet; LDL: Low Density Lipoprotein; NAFLD: Non-alcoholic Fatty Liver Disease; NASH: Non-alcoholic Steatohepatitis; PON1: Paraoxonase-1; PPAR-γ: Peroxisome Proliferator-Activated Receptor Gamma; ROS: Reactive Oxygen Species; SG: Combination of alginate and mangosteen peel extract; SAG: Combination of alginate, mangosteen peel, and okra extract; SREBP-1c: Sterol Regulatory Element-Binding Protein-1c.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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