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RESEARCH ARTICLE

REVISED Sea grapes extract improves blood glucose, total cholesterol, and PGC-1α in rats fed on cholesterol- and fat-enriched diet [version 2; peer review: 1 approved, 2 approved with reservations]

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Abstract

Background: Sea grapes or Caulerpa racemosa have a lot of phytochemical content, especially unsaturated fatty acids that are beneficial for health. This study aims to evaluate the effects of sea grapes extract on blood glucose levels, total cholesterol-, and Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α in male Wistar rats, which were given per-oral (p.o.) cholesterol- and carbohydrates fat-enriched diets (CFED). **Methods:** Forty male Wistar albino rats weighing between 200 – 250 g were used for this study. Animals were randomly distributed into four groups of ten animals each. Group A served as control (received standard dry pellet diet). Rats in group B were fed on CFED for 4 weeks. Groups C and D were fed on CFED and were administered 150 and 450 mg/kg of sea grapes extract (p.o.), respectively. **Results:** Group C rats indicated a blood glucose reduction and an increase in PGC-1α serum, in

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Reviewer Status ✓ ? ?

	Invited Reviewers		
	1	2	3
version 2 (revision) 13 Sep 2021	✓ report	? report	? report
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1. **Carla F. Kairupan**, Universitas Sam Ratulangi, Manado, Indonesia

comparison to group D (p<0.05). There were no significant differences between group C and D in blood cholesterol reduction (high dose of the extract did not have significant effects) (p=0.222), and both groups had the same effect in lowering total cholesterol in rats. **Conclusion:** Sea grapes extract is proven to improve blood glucose, total cholesterol, and PGC-1α levels in rats fed with CFED.

Keywords
Caulerpa racemosa extract, blood glucose, total cholesterol, PGC-1α, functional food

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Any reports and responses or comments on the article can be found at the end of the article.

> **REVISED** **Amendments from Version 1**
>
> We have made minor revisions as stated by the reviewer:
> 1. The Laboratory of Pharmacology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University where the study was conducted is not in Makassar, it should be a typo and we have changed it to Manado.
> 2. The terminology and composition of diets are inconsistent and unclear. We've made it clear.
> 3. The area where the blood sample was collected is inappropriate. We've made that clear, in the tail of the rats.
> 4. The doses of Sea grapes extract were 150 mg/kg BW and 450 mg/kg BW. How did you decide on these doses? Are there any safety issues? In existing studies, most use 100 mg and 200 mg, therefore the choice of this dose is higher and different from previous studies and 450 is the maximum capacity for a safe rats stomach.
>
> **Any further responses from the reviewers can be found at the end of the article**

Introduction

Reactive Oxygen Species (ROS) are the amounts of reactive molecules and free radicals derived from oxygen in a molecule (*i.e.*, superoxide, peroxide, hydrogen peroxide, hydroxyl radical, *etc.*) (Sies & Jones, 2020). Oxygen-based radicals are produced as a byproduct in the mitochondrial electron transport at the aerobic respiration performed by oxidoreductase enzymes and metal-catalyzed oxidation. A recent study has shown that ROS has a role in cell apoptosis that leads to organ dysfunction (Pizzino et al., 2017).

Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α is a transcription coactivator that regulates the genes involved in energy metabolism. It is the main regulator of mitochondrial biogenesis (Liang &Ward, 2006). PGC-1α stimulates mitochondrial biogenesis and encourages the remodeling of muscle tissue to a fiber-type composition that is metabolically more oxidative and less glycolytic in nature, and it participates in the regulation of both carbohydrate and lipid metabolism (Puigserver & Spiegelman, 2003; S. Yang et al., 2020).

The ability of cell defense against ROS has been associated with aging and contributes to the increased oxidative stress state. This condition can disturb the enzyme activity, especially through the reversible oxidative reaction at the thiol functional group at the side chain of the enzyme structures (Birben et al., 2012). This can lead to the alteration of biomolecule structure and integrity, and enzyme dysfunction (Freitas et al., 2016). As a result, insulin resistance and Type 2 Diabetes can development (Facchini et al., 2001; Meigs et al., 2003). Additionally, the effect of aging on changes in liver mass can increase serum Low-Density Lipoprotein (LDL)-cholesterol level, due to the hepatocytes cell death caused by oxidative stress (Anantharaju et al., 2002; Miller, 1984). Hence, effective control of ROS levels is essential. The aging population tends to have a higher prevalence of chronic disease, thus there is a demand for health-improving foods (Park, 2013). The consumption and production of high-antioxidant as functional foods in recent years are popular due to their capability of reducing Reactive Oxygen Species (ROS), as well as having an impact on several aging and chronic related diseases (Park, 2013; Park et al., 2004). However, there are some challenges that are associated with the utilization of functional food. For example, specific functional foods need to be consumed in high concentrations in order to be biologically effective, therefore, this would require the nutritional facts such as the daily dose of the bioactive compound in each serving size to be determined (Kang et al., 2011). Preliminary studies are needed to determine which bioactive compound is the most beneficial, and what is the quantitative-activity relationship between the bioactive compounds contained in functional foods.

Sea grapes (*Caulerpa racemosa*) or lawi-lawi (Indonesia-local terminology) is a species of editable green alga, seaweed in the *Caulerpaceae* family found in waters surrounding Sulawesi (Pakki et al., 2020). Sea grapes are harvested intensively as they are an important source of macronutrients and micronutrients, especially in East and South-East Asia (grown commercially in ponds and consumed in the Philippines, Indonesia and Vietnam) as a major part of the traditional diet (Chen et al., 2019). Some studies showed that sea grapes contains several bioactive components, such as protein, polysaccharides, polyphenol, flavonoids, and antioxidants (P. Yang et al., 2015; Yep et al., 2019; Taslim & Fahrul, 2021). Moreover, sea grapes have a high antioxidant level, and they have the potential to act as functional food or nutraceuticals (Tanna et al., 2020; Yep et al., 2019; Nurkolis et al., 2021). The extract of sea grapes can reduce glucose level, aspartate aminotransferase (AST), alanine aminotransferase (ATL). Moreover, it appears to have a hepatoprotective activity in diabetic rats (Qudus B Aroyehun et al., 2020). Therefore, this study aims to evaluate the effects of Sea grapes extract on blood glucose levels, total cholesterol, and PGC-1α in male Wistar rats on cholesterol- and fat-enriched diets (CFED).

Methods

This *in vivo* study was conducted at the pharmacological laboratory, faculty of mathematics and natural sciences, Sam Ratulangi University.

Collection and preparation of plant material

Fresh sea grapes (*Caulerpa racemosa)* was collected from the shallow section (5-10 meters from the sea surface) of the Mantehage seawater, North Sulawesi, Indonesia. The botanical identification and authentication were confirmed at the department of pharmacology, faculty of mathematics and natural sciences, Sam Ratulangi University, Indonesia. The specimens were collected for feature references. The sea grapes were rinsed thoroughly with water, air-dried at room temperature and in an 40°C oven, then powdered by an electric mill.

Preparation of sea grapes extracts

Crude powder (one kg) was macerated in 96% ethanol for 72 hours with each extraction performed in triplicates, which resulted in 34% yield. The crude extracts were filtered by Whatman 41 filter paper. The total filtrate was concentrated and evaporated at 40°C with a rotary evaporator RV 8 IKA under reduced pressure (100 millibar) for 90 minutes, and evaporated in an 40°C oven to produce a thick extract. The extract was stored in a refrigerator at 10°C until used in the study.

Animal handling and ethical approval

All experimental rats were kept on standardized free access of feed and *ad libitum* of water. The study was conducted in the Laboratory of Pharmacology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, Indonesia. Forty male Wistar albino rats (*Rattus norvegicus*) (4-5 weeks) weighing between 200 – 250 g were obtained from the Laboratory Animals Farming Makassar, Indonesia, and transported to the research site. The animals were grouped and housed in cages and maintained under standard laboratory conditions (temperature: 27 ± 2^oC), with light and dark cycles (12/12 hours). The rats were acclimatized to laboratory conditions for 10 days before the commencement of the experiment. The research protocol (use of experimental animals) refers to the Declaration of Helsinki. The Council for International Organizations of Medical Sciences (CIOMS) has approved the application of ethical health research protocols online (http://sim-epk.keppkn.kemkes.go.id) RSUP Prof. Dr. RD. Kandou, Manado with No. 086/EC/KEPK-KANDOU/VI/2021. Additionally, all experimental procedures were carried out according to the Institutional Animal Care and Usage Committee (ARRIVE guidelines) (Nurkolis et al., 2021).

In vivo studies of sea grapes extracts to evaluate blood glucose, total cholesterol, and PGC-1α levels

CFED production

Carbohydrates fat-enriched diets (CFED) is a standard mouse food that comes with 1% colic acid, 2% pure cholesterol powder, 20% fat (animal source/pork oil), and 2% corn oil. Additional components are subtly added to the standard CFED and homogenized into a dough with the addition of 1000mL of aqua dest. Small pellets are cut and left to dry at room temperature in sterile conditions. CFED is prepared weekly and stored at 4°C until used to reduce oxidation. CFED consists of carbohydrate (43.57%), coarse protein (12.38%), coarse fiber (4.73%), coarse fat (3.17%), cholesterol (2%), colic acid (1%), animal fat (20%), corn oil (2%), total ash (4.3%), and moisture (6.85%). Compared to a normal diet (standard dry pellet) that contains 58.1% carbohydrates, 16.51% coarse protein, and 0% animal fat, all the other components such as corn oil, cholesterol, and folic acid, were not significantly changed. CFED production guideline was carried out as previously described (Harb et al., 2018).

Sea grapes extract administration scheme

Wistar albino rats were randomly distributed into four groups of ten animals each. Group A served as control (received standard dry pellet diet). Rats in group B were fed on CFED for 4 weeks. Rats in groups C and D were fed on CFED and were given 150 and 450 mg/kg Body Weight (BW) of sea grapes extract, respectively, for 4 weeks. CFED and extract of sea grapes were administered by oral gavage.

Sample collection

Throughout the experiment, all the efforts were made to minimize the pain and distress of the experimental animals. For this purpose after four weeks of extract treatment, rats were kept fasted overnight and given euthanasia under ketamine anesthesia. 2.5 mL of blood samples were collected from the tail and kept in dry and clean tubes without addition of anticoagulants (Tiger-top tube), to allow clotting at room temperature. The samples were then centrifuged for 20 minutes at 3000 rpm. Finally, the sera were collected for the blood glucose, total cholesterol, and PGC-1α analysis.

Biomedical analysis of blood sample

Blood glucose and cholesterol levels were assayed using COBAS Integra® 400 plus analyzer (Roche) (See underlying data) (Nurkolis, 2021). Samples were washed with Phosphate Buffered Saline (PBS, pH 7.4) 1% until the liquid runs clear. The samples were centrifuged at 3000 rpm for 20 minutes to obtain pellets and supernatant. The supernatant is taken

for the PGC-1α examination (See underlying data) (Nurkolis, 2021). The concentration of PGC-1α was measured by using mouse PGC-1α ELISA Kit (Sunlong Biotech Co., Ltd, # MBS288117).

Data management and analysis

The data were statistically analyzed with the use of the MANOVA/Multivariate ANOVA test. The Levene's test was used to determine which posthoc tests should be conducted. In cases where the Levene's test p-value was <0.05 Games-Howell test (equal variances not assumed), and for p-value >0.05 Bonferroni test (equal variances assumed) was used. Statistical analyses were performed by using SPPS 26.0 for the Windows version.

Results

The Levene's Homogeneity test shows that the p-value for glucose and PGC-1α are <0.05, therefore equal variance cannot be assumed, while equal variances can be assumed for cholesterol as the p-value is >0.05.

The results indicate that blood glucose significantly increased in group B, compared to group A (p < 0.05) (Table 2). Blood glucose significantly decreased in both groups C and D (p < 0.05). The effect of sea grapes administration as much

Table 1. Statistical interpretations based on homogeneity test.

		F	df1	df2	P-value
Glucose	Mean	10.495	3	36	.000*
	Median	7.105	3	36	.001*
	Median (adjusted df)	7.105	3	19.296	.002*
	Trimmed mean	10.205	3	36	.000*
Cholesterol	Mean	1.957	3	36	.138
	Median	1.741	3	36	.176
	Median (adjusted df)	1.741	3	23.800	.186
	Trimmed mean	1.853	3	36	.155
PGC-1α	Mean	9.042	3	36	.000*
	Median	6.290	3	36	.002*
	Median (adjusted df)	6.290	3	29.106	.002*
	Based on trimmed mean	8.938	3	36	.000*

F, Levene's Test; DF, Degrees of Freedom.
*Represents p-value <0.05, CI:95%.

Table 2. The low dose of sea grapes is more effective in significantly reducing blood glucose.

	Diet	Mean	P-value
Group A	CFED	−15.3880	.000*
	CFED + Sea grapes 150 mg/kgBW	5.9500	.000*
	CFED + Sea grapes 450 mg/kgBW	2.8900	.001*
Group B	Control	15.3880	.000*
	CFED + Sea grapes 150 mg/kgBW	21.3380	.000*
	CFED + Sea grapes 450 mg/kgBW	18.2780	.000*
Group C	Control	−5.9500	.000*
	CFED	−21.3380	.000*
	CFED + Sea grapes 450 mg/kgBW	−3.0600	.003*
Group D	Control	−2.8900	.001*
	CFED	−18.2780	.000*
	CFED + Sea grapes 150 mg/kgBW	3.0600	.003*

*Represents p-value <0.05, CI: 95%.

as 150 mg/kg BW is more effective than the sea grapes 450 mg/kg BW, in significantly decreasing blood glucose in rats ($p < 0.05$).

As expected the rats in group B had significantly increased blood cholesterol levels compared to group A ($p < 0.05$). In both groups A and B ($p < 0.05$), blood cholesterol significantly decreased in rats given CFED + sea grapes extract 150 mg/kg BW, and CFED treatment + sea grapes extract 450 mg/kg BW. There was no significant difference between the CFED treatment group + 150 mg/kg BW sea grapes extract, and the CFED treatment group + 450 mg/kg BW sea grapes extract, in reducing blood cholesterol (high dose of the extract did not result in significant effects ($p > 0.05$)).

Group B had a significantly decreased PGC-1α serum concentration. PGC-1α serum concentrations significantly increased in group C, as well in group D, compared to groups A and B. The effect of sea grapes administration as much as 150 mg/kg BW is more effective than that of sea grapes 450 mg/kg BW, in the significant increase of PGC-1α serum in rats.

Discussion

This study showed that the supplementation of sea grapes extract managed to lower blood glucose and serum cholesterol significantly in rats that were given cholesterol- and fat-enriched diets (Figure 1). Although compared to the control group, rats that were given cholesterol- and fat-enriched diets with sea grapes extract had lower levels of blood cholesterol and blood glucose.

The Results of this study indicate that sea grapes have the capability of reducing blood glucose levels (Table 2). Similarly, Aroyehun et al., have shown that sea grapes have antidiabetic activity (Qudus B Aroyehun et al., 2020). The plasma analysis in this study has also indicated that the sea grapes treated group had a significant decrease ($p < 0.05$) in their blood glucose levels compared to the untreated diabetic group (Qudus B Aroyehun et al., 2020). Sea grapes extract-treated group demonstrated similar efficacy in lowering blood glucose as Metformin (Qudus B Aroyehun et al., 2020), hence, sea grapes may have an hypoglycaemic effect. A hyperglycaemic state may induce oxidative stress that could be detrimental to insulin-sensitive tissues such as the liver, which may cause damage to the organ (Bugianesi 2005; Manna 2010; Palsamy 2010).

This study showed that sea grapes reduce hyperlipidemia in rats, however this is not in line with the findings by Aroyehun et al., (Qudus B Aroyehun et al., 2020), which states that sea grapes extract has little to no effect on the cholesterol level of

The effect of giving sea grapes (Caulerpa racemosa) on Blood Glucose and PGC-1α Levels

Blood Glucose

The effect of giving sea grapes extract as much as 150 mg/kgBW is more effective than a dose of 450 mg/kgBW in reducing blood glucose in the rats fed on cholesterol- and fat-enriched diet.

Sea grapes Extract 150 mg/kgBW

Rats CFED

PGC-1 α

The effect of giving sea grapes extract as much as 150 mg/kgBW is more effective than a dose of 450 mg/kgBW in increase PGC-1 α in the rats fed on cholesterol- and fat-enriched diet. This suggests that mitochondrial biogenesis, which is one of the main roles of PGC-1 α, is enhanced by sea grapes extract supplementation.

The effect of giving sea grapes (Caulerpa racemosa) extract on Total Cholesterol

Dose of 150 mg/kgBW

Dose of 450 mg/kgBW

Sea grapes Extract

Rats CFED

Cholesterol

There was no significant difference between the CFED + sea grapes extract 150 mg/kgBW treatment group and the CFED + sea grapes extract 450 mg/kgBW treatment group on rats blood cholesterol (more extract doses did not have a significant effect), both of them had the same effect in lowering total cholesterol in subject rats.

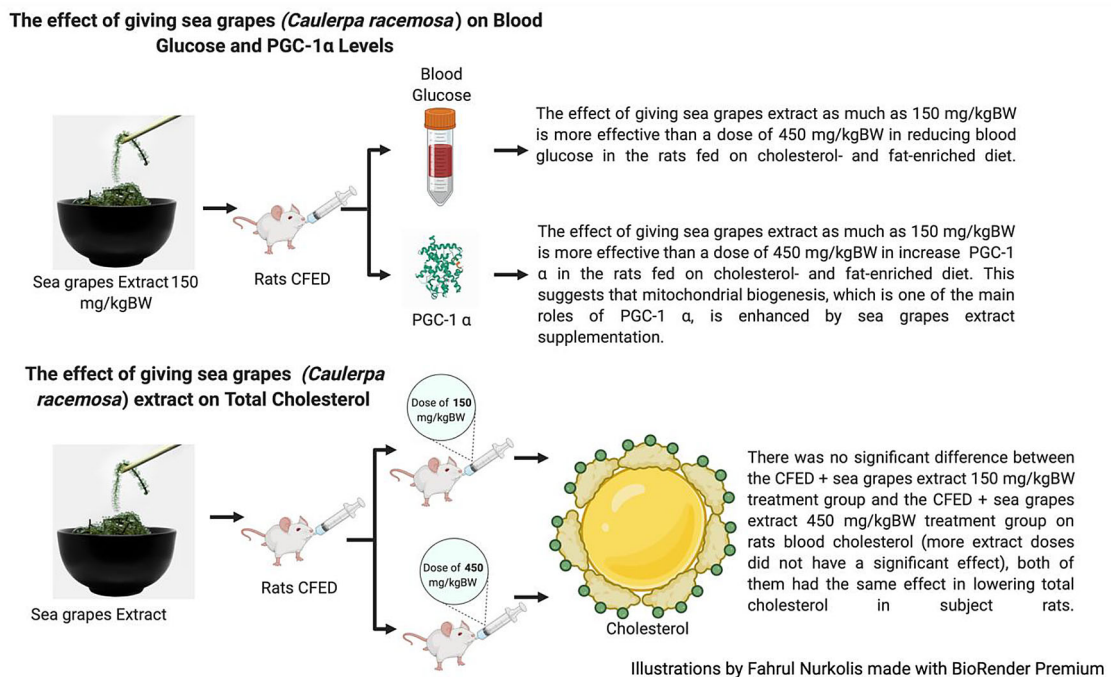Illustrations by Fahrul Nurkolis made with BioRender Premium

Figure 1. Effects of sea grapes extract on subject rats.

induced diabetic rats. In addition, the effect of lower doses of the extract (150 mg/kg BW) was better in lowering blood cholesterol than higher doses of sea grapes extract (450 mg/kg BW) (Figure 1, Table 3). This can be due to the saturated fatty acids content, especially palmitic acid, which dominates the composition of fatty acids, comprising 80% of the total fat in sea grapes (Qudus B Aroyehun et al., 2020). Studies have shown that palmitate acid may raise total cholesterol levels, specifically LDL-cholesterol levels (Clandinin et al. 2000; Mensink, 2013).

Levels of PGC-1α in rats significantly decreased after being given a CFED diet compared to the control group (Figure 1, Table 4). However, PGC-1α levels increased significantly in rats given sea grapes extract, even when compared to the control group. This suggests that PGC-1α, which is one of the major elements in mitochondrial biogenesis, is enhanced by the sea grapes extract. Perhaps the content of flavonoids as well as phenols in sea grapes extract can cause this effect. One study has shown that flavonoid supplementation increases the performance in endurance activities via an increase in expression of PGC-1α as the "master regulator" of biogenesis and skeletal muscle angiogenesis (Khani et al. 2017). In addition, other studies have also shown that antioxidant compounds can upregulate PGC-1α target genes, which not only play a role in preventing oxidative damage, but also reduce mitochondrial ROS levels, ensure mitochondrial integrity

Table 3. **Both doses of sea grapes extract significantly reduce blood cholesterol.**

	Diet	Mean	P-value
Group A	CFED	−22.1100	.000*
	CFED + Sea grapes 150 mg/kgBW	12.0600	.000*
	CFED + Sea grapes 450 mg/kgBW	8.0200	.001*
Group B	Control	22.1100	.000*
	CFED + Sea grapes 150 mg/kgBW	34.1700	.000*
	CFED + Sea grapes 450 mg/kgBW	30.1300	.000*
Group C	Control	−12.0600	.000*
	CFED	−34.1700	.000*
	CFED + Sea grapes 450 mg/kgBW	−4.0400	.222
Group D	Control	−8.0200	.001*
	CFED	−30.1300	.000*
	CFED + Sea grapes 150 mg/kgBW	3.0600	.003*

*Represents p-value <0.05, CI: 95%.

Table 4. **The low dose of sea grapes is more effective in significantly increasing PGC-1α.**

	Diet	Mean	P-value
Group A	CFED	20.9200	.000*
	CFED + Sea grapes 150 mg/kgBW	−19.3500	.000*
	CFED + Sea grapes 450 mg/kgBW	−14.4200	.000*
Group B	Control	−20.9200	.000*
	CFED + Sea grapes 150 mg/kgBW	−40.2700	.000*
	CFED + Sea grapes 450 mg/kgBW	−35.3400	.000*
Group C	control	19.3500	.000*
	CFED	40.2700	.000*
	CFED + Sea grapes 450 mg/kgBW	4.9300	.000*
Group D	control	14.4200	.000*
	CFED	35.3400	.000*
	CFED + Sea grapes 150 mg/kgBW	−4.9300	.000*

*Represents p-value <0.05, CI: 95%.

during cell differentiation (Beldelli et al. 2014), as well as avoiding the cytotoxic effects of ROS accumulation (St-Pierre et al. 2006).

Conclusion

Sea grapes extract is proven to improve blood glucose levels, total cholesterol, and PGC-1α in rats fed with cholesterol- and fat-enriched diets. The results of this study can be used as a reference for clinical trials to further research the beneficial effects of sea grapes for human consumption. However, it is necessary to do the same research with parameters other than blood sugar, cholesterol and PGC-1a, to expand its metabolic scope.

Data availability
Underlying data
Harvard dataverse: Sea grapes extract effect on blood glucose level (BGL), total cholesterol (TC), and serum PGC-1α concentrations.

DOI: https://doi.org/10.7910/DVN/8IKREA (Nurkolis, 2021).

The project contains the following underlying data:

- Raw data for the sea grapes extract effect on blood glucose level (BGL), total cholesterol (TC), and serum PGC-1α concentrations.

Reporting guidelines
Harvard Dataverse: Arrive checklist for Sea grapes extract with blood glucose, total cholesterol, and PGC-1α in rats fed on cholesterol- and fat-enriched diet.

https://doi.org/10.7910/DVN/NXF0IW (Nurkolis et al., 2021).

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Author contributions
M.K and F.N. collated study ideas, designed and experiment, analyzed data, and compiled manuscripts. N.A.T, N. R, N. S, H.K.P, D.S.W and N. M analyzed and interpreted the data and critically revised the manuscript. F. N and F.M.I conducted experiments, analyzed biochemistry, and critically revised the manuscript. N. M, M.R.B, R. R, P.S.A and K.E.K.M, implemented experimental protocols, assisted in statistical analysis, interpreted data, and critically revised manuscripts. All writers read and approve the final manuscript.

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Open Peer Review

Current Peer Review Status: ✔ ❓ ❓

❓ **Achuthan Raghavamenon**

Department of Biochemistry, Amala Cancer Research Centre, Thrissur, Kerala, India

In this study, the authors aim to see the effect of sea grape extracts as a function food on H fat cholesterol fed rats and observed that it improves blood sugar, cholesterol, and PGC-1α. The experimental plan is good and the results collected and analysed look fine. The conclusion derived seems overwhelming, as they collected only very few information. The following demerits have to be addressed:

1. The introduction describes much about oxidative stress, antioxidants, and flavonoid contents of Sea grape, however, no correlating information has been collected in the study.

2. The parameters studied in this manuscript are blood cholesterol, glucose and PGC1-alpha. As per the manuscript title, this article describes the improvement in the above mentioned parameters in rats fed a high cholesterol diet and then treated with sea grape extract. Why did the authors study only these three parameters? How are glucose level, cholesterol and PGC-1 alpha inter-connected? The introduction should discuss all this, so as to reflect a clear logic of the study.

3. Authors have given references for the anti-diabetic and cholesterol lowering effect of Sea grapes. Authors have not attempted to collect any information on triglycerides, HDL cholesterol, LDL cholesterol, etc. Also, what additional advantage blood PGC-1α provides is not clear.

4. The basis of the doses used in this study has not been adequately explained. Has any toxicity study been conducted before to arrive to these doses?

5. Authors have not done any histology of the liver to make sure that there is lower lipid deposition. There is the chance that lowering of blood lipids may be due to the accumulation of lipids in the liver tissue.

6. Tables 2, 3, and 4 have ABC & D groupings, and in each group, there is Control, CFED,CFED +150 mg extract AND cfed+ 450 extract. This is confusing. I suggest to make these tables

direct values.

7. Spelling and grammar errors have to be addressed, for example, the use of present tense in the results section at some places, and use of past tenses at some other places. Such minor corrections can be checked and corrected by the authors while revising the manuscript.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biochemistry , oxidized lipids and degenerative diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 08 November 2021

https://doi.org/10.5256/f1000research.77279.r94240

?   **Chinedum Ogbonnaya Eleazu**

Department of Chemistry, Biochemistry and Molecular Biology, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Nigeria

The article by Kuswari *et al.* has some interesting points. However, the manuscript will require revision by the authors for it to be worthy of indexing.

General comments:
- The authors did not satisfactorily address the comments of the reviewers on their choice of dose of Sea grape extract they used for this study. Was an acute toxicity done to show the safety of the doses of this plant as used in this study? Again, there are several errors in the usage of English Language in this manuscript.

Specific comments:

Abstract:
- L14, what do the authors mean by PGC-1α serum? Or do they mean in the serum? which is a better expression.

Introduction:
- Paragraph 3, suggest to correct 'Type 2 Diabetes development' to 'type 2 diabetes can develop or occur'. The continued sentence, 'additionally, the effect of ageing changes in the liver mass can increase serum low density lipoproteins........' is useless to the manuscript as ageing population was not a factor of interest in the manuscript.

- Paragraph 4, L8 of the introduction, if Sea grapes have been scientifically proven to reduce glucose level, why investigate it again in this study?

- This introduction needs to be re-written with the focus on what the authors determined, and their rationale for determining them.

Collection and identification of plant material:
- The identification number of the plant is missing and should be reported.

Animal handling and ethical approval:
- The statement, 'the study was conducted in the laboratory........' is a repetition.

- The carrier for the administration of the Sea grape extract should also be indicated.

- Why did the authors use up to 10 animals per group? Did they subject their sample size to power testing before the study? The study appears overpowered.

Results:
- Table 1 is not cited in the text and the data on it needs to be explained. The title for table 2 should be changed to blood glucose concentrations of rats. Then, the effect of sea grape extract on blood glucose concentrations of the rats can be written as part of the results.

- The unit of glucose is missing in the data presented in Table 2. Same is applicable for the units of cholesterol and PGC-1α in Tables 3 and 4. Why are some results in negative values? Authors need to revisit these data.

- Finally, in their blood sampling, authors indicated they worked with the sera of the animals, yet in different parts of the manuscript and in the results section, they reported their results on glucose and cholesterol as blood glucose and blood cholesterol. This should be revisited as well.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Diabetes, Antioxidants, Nutraceuticals, Metabolic syndrome

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 30 September 2021

https://doi.org/10.5256/f1000research.77279.r94138

✔️ **Carla F. Kairupan**
Bagian Patologi Anatomi Fakultas Kedokteran, Universitas Sam Ratulangi, Manado, Indonesia

The authors have addressed the comments that I made. I therefore think the revisions are appropriate.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pathology, Molecular Genetics, Herbal Medicine

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 20 August 2021

https://doi.org/10.5256/f1000research.58483.r90945

? **Carla F. Kairupan**

Bagian Patologi Anatomi Fakultas Kedokteran, Universitas Sam Ratulangi, Manado, Indonesia

This is an interesting research, in which the treatment with Sea grapes extract clearly improved blood glucose levels, total cholesterol, and PGC-1α in rats. However, it is not the first study that is done on the beneficial effects of Sea grapes extract.

The paper is well written and the experimental design is set up well, however, the statistical analysis should be better described.

The following points should also be addressed appropriately in the revision.
1. The Laboratory of Pharmacology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University where the study was conducted is not in Makassar.

2. The terminology and composition of diets are inconsistent and unclear.

3. The area where the blood sample was collected is inappropriate.

4. The doses of Sea grapes extract were 150 mg/kg BW and 450 mg/kg BW. How did you decide on these doses? Are there any safety issues?

The article may also benefit from an English language edit.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pathology, Moleculer Genetics, Herbal Medicine

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 31 Aug 2021
Fahrul Nurkolis, State Islamic University of Sunan Kalijaga (UIN Sunan Kalijaga Yogyakarta), Yogyakarta, Indonesia

Dear Dr. Carla F Kairupan, PhD.,

Thank you for the appreciation and constructive review comments to improve this paper.

We have made minor improvements as you mentioned in the review, as follows:
1. The Laboratory of Pharmacology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University where the study was conducted is not in Makassar, **it should be a typo and we have changed it to Manado.**

2. The terminology and composition of diets are inconsistent and unclear. **We've made it clear.**

3. The area where the blood sample was collected is inappropriate.
We've made that clear, in the tail of the rats.

 4. The doses of Sea grapes extract were 150 mg/kg BW and 450 mg/kg BW. How did you decide on these doses? Are there any safety issues? **In existing studies, most use 100 mg and 200 mg, therefore the choice of this dose is higher and different from previous studies and 450 is the maximum capacity for a safe rats stomach.**

Thank you,
Greetings, hope you are well!

Competing Interests: No competing interests were disclosed.

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