



Original Article

The Effect of Add-on *Garcinia mangostana L.* Extract on Endothelial Dysfunction in Type 2 Diabetes Mellitus Subjects with High Risk Framingham Score: A Cohort Study

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ABSTRACT

Background : *Garcinia mangostana L.* has been extensively used for years as antioxidant and anti-inflammation. However, its role in the context of endothelial disease was lacking.

Objectives : To assess the effect of add-on *G. mangostana L.* extracts on endothelial dysfunction in type 2 diabetes subjects with high-risk Framingham score, compared to placebo.

Methods : This was a prospective cohort study conducted in type 2 diabetes subjects with high-risk Framingham score. Subjects were randomized into two groups. The first group received 2,520 mg/day of *G. mangostana L.* extract. The second group was given a placebo for 90 days. The outcome measure of our study was the levels of endothelial progenitor cell (EPC), circulating endothelial cell (CEC), nitric oxide (NO), tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-6, hemoglobin A1c (HbA1c), high-sensitivity C-reactive protein (hs-CRP), malondialdehyde (MDA), Superoxide dismutase (SOD) and fasting blood glucose. Multiple linear regression was used to determine the correlation and effect estimate.

Results: A total of 49 patients were included in our study. Of those, elevated levels of EPC and SOD were observed in treatment group compared to placebo. On the other hand, the level of CEC, IL-1, IL-6, NO, MDA, TNF- α , fasting blood glucose and HbA1c was found significantly lower than placebo.

Conclusion: *Garcinia mangostana L.* extract is associated with an increased levels of EPC and SOD, and it is also correlated with a decreased levels of CEC, IL-1, IL-6, NO, MDA, TNF- α , fasting blood glucose and HbA1c.

1. Introduction

Endothelial dysfunction has been widely known to contribute in the development of atherosclerosis and its complication. Briefly, the endothelium place an important role as a mechanical and biological barrier between the blood and the vascular wall. This process requires a balance between pro and anti inflammatory mediators. In the theory of atherosclerosis, it was known that NO, IL1, and IL6 were involved in the development of endothelial dysfunction. Moreover, recent studies proposed that the development of endothelial dysfunction was also triggered by EPC and CEC.^{1,2} EPCs are immature cells that are capable of differentiating into mature endothelial cells. While CECs are mature cells that separated from the intimal layer as a response to endothelial injury.^{2,4} Therefore, a new potential therapy having a target site at those components might provide a promising outcome.

Garcinia mangostana L. is one of the medical plants found in Asia, particularly in South East Asia. The role of *Garcinia mangostana L.* as anti-inflammatory agent has been widely reported. Subsequently, it was also reported that *Garcinia mangostana L.* plays a beneficial role in type 2 diabetes patients with stable coronary artery disease.^{5,6} However, the evidence of *G. mangostana* effect on type 2 diabetes mellitus subjects with high-risk Framingham scores was limited.

Our present study, therefore, aimed to assess the effect of *Garcinia mangostana L.* extract on the levels of EPC, CEC, NO, TNF- α , IL-1, IL-6, HbA1c, hs-CRP, MDA, SOD and fasting blood glucose. Our current findings were expected to provide the primary data of the role of *Garcinia mangostana L.* extract in the development of endothelial dysfunction.

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2. Methods

2.1. Research Design

A prospective cohort study was conducted in dr. Saiful Anwar Hospital, Malang, Indonesia, during the period November 2018 and January 2019. To guide the protocols in our study, we used Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist.⁷

2.2. Ethical approval

This study has been approved by the Institutional Review Board, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia (No: 64/EC/KEPK/03/2018). All participants included in our present study were explained about the aim, purpose, benefit, and the risk of this study. All participants had given the written informed consent prior to be involved in our study. All participants in our study were voluntary, and no incentive was given.

2.3. Participants & eligibility criteria

Participants were recruited using a random sampling procedure. The inclusion criteria were (1) Adult patients age 50 to 80 years with Framingham score >20 (high-risk) based on clinical, biochemical, anthropometry, and electrocardiographic criteria; (2) clinically stable and recovered; (3) on standard medication of moderate-high intensity statin, anti-diabetes therapy such as metformin, sulfonylurea, acarbose or insulin, ACE inhibitor or angiotensin receptor blocker (ARB) or calcium channel blocker, and/or beta-blocker. Patients with the following criteria: (1) heart failure NYHA class III-IV patients; (2) elevated levels of AST and ALT three times normal upper limit; (3) eGFR <30 ml/min/1.73 m² (by MDRD glomerular filtration rate equation)⁸; (4) creatinine level >1.5 mg/dl; (5) the value of APTT and INR twice of normal limit; (6) ongoing infection or inflammatory conditions (leukocytes >10,500; laboratory cut-off); (7) history of neoplasms or other malignancy were excluded from the study.

2.4. Outcome measure

We collected demographic data including gender, age, body weight and height, body mass index (BMI), blood pressure, heart rate, lipid profile (i.e., triglycerides, total cholesterol, LDL and HDL cholesterol), fasting plasma glucose and HbA1c. The high-risk Framingham score was retrieved from the demographic data and clinical history.⁹

The outcome measure of our study was the levels of EPC, CEC, NO, TNF- α , IL-1, IL-6, HbA1c, hs-CRP, MDA, SOD, and fasting blood glucose after 90 days of treatment.

2.5. Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences software (SPSS) for Windows, version 16, Chicago, USA. Data were presented as mean \pm standard deviation (SD). The correlation and effect estimate in our present study were determined using multiple linear regression. The p-value of less than 0.05 was considered having significant association.

3. Results

3.1. Patients selection

A total of 90 patients were identified during study period. Of those, 13 patients were excluded because of low-intensity statin used (n=11), creatinine level >1.5 mg/dl (n=1), and estimated GFR <30 ml/min/1.73 m² (n=1). Additionally, a total of 28 patients were excluded because of having low risk of Framingham score. Totally, we

included 49 patients in our study. Of them, a total of 23 patients receive *G. mangostana L.* extract. While a total of 26 patients received placebo. A flowchart describing the eligibility process in our present study is provided in Figure 1.

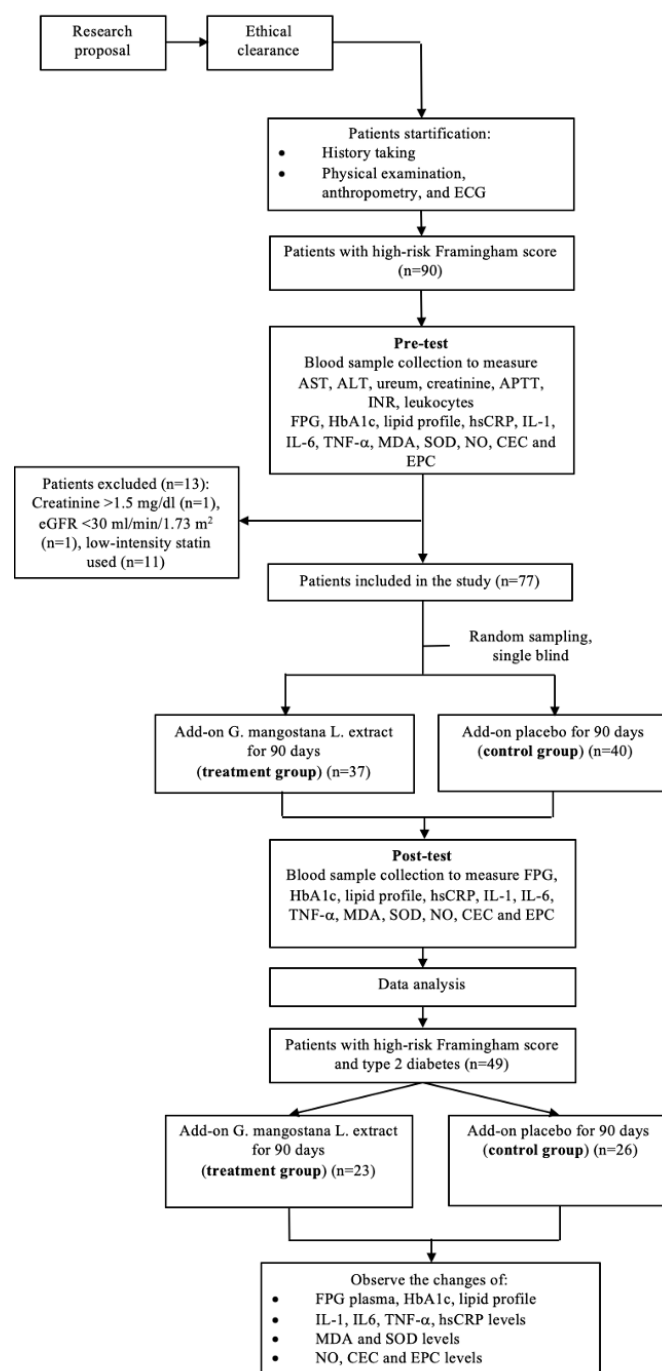


Figure 1. A flowchart of patients selection in this study

3.2. Baseline characteristics

The majority of patients were female (77.55%, n=38), with the mean age was 64.16 years, and the mean BMI was 25.92 kg/m². Other baseline characteristics were provided in Table 1. Our statistical analysis confirmed that the baseline characteristics between treatment and control groups were not significantly different (p>0.05), suggesting that the data between the treatment and control groups were distributed homogenously.

Table 1. Baseline characteristics of patients included in our study

Characteristics	All n=49	Add-on Placebo n=26 (53,1%)	Add-on <i>G.</i> <i>mangostana</i> L. extract n=23 (46.9%)	p-value
Sex				0.911
Male (no. of patients)	11	6	5	
Female (no. of patients)	38	20	18	
Age (yr, mean)	64.16	64.08	64.08	0.910
Weight (kg)	61.12	63.31	58.65	0.152
Height (cm)	153.73	153.31	154.22	0.680
BMI (kg/m ²)	25.92	26.95	24.75	0.117
Systolic BP (mmHg)	149.22	146.62	152.17	0.406
Diastolic BP (mmHg)	87.53	88.35	86.61	0.648
Heart rate (bpm)	76.22	73.23	79.61	0.069
Triglycerides (mg/dl)	154.12	160.38	147.04	0.608
Total cholesterol (mg/dl)	189.94	189.12	190.87	0.882
HDL cholesterol (mg/dl)	47.38	46.79	48.04	0.716
LDL cholesterol (mg/dl)	107.07	108.77	105.15	0.685
Fasting blood glucose (mg/dl)	154.55	163.31	144.65	0.290
HbA1c (%)	8.12	8.45	7.75	0.017

Note; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; bpm, beats per minute.

3.3. Main findings

The levels of lipid profile between treatment and control group were not significantly different. Moreover, the level of fasting blood glucose was not significantly different between treatment and control groups. However, a decreased level of HbA1c was observed in treatment group compared to control group (Table 2, Figure 2).

Concerning antioxidant effect, the level of MDA was observed lower in treatment group compared to control group. Subsequently, the levels of CEC, IL-1, IL-6, NO, MDA, and TNF- α were found significantly lower in treatment group compared to control group (Table 3, Figure 3 and 4). Furthermore, the levels of EPC and SOD were significantly higher in treatment group compared to control group (Table 3, Figure 5).

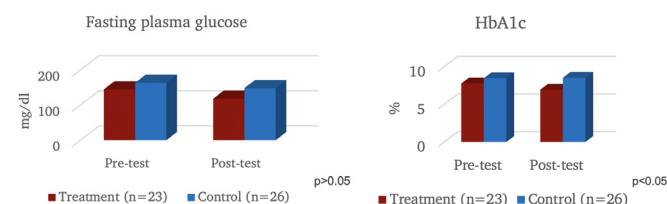


Figure 2. Add-on *G. mangostana* L. extract could improve glycemic profile in comparison to placebo

Table 3. The effect of *G. mangostana* L. extract as antioxidant, anti-inflammation, and improvement of endothelial dysfunction after 90 days of treatment

	<i>G. mangostana</i> extract (n=26)	Placebo (n=23)	P-value
Anti-inflammation			
Δ IL-1	-12.4816 \pm 20.3	11.9 \pm 32.8	0.003
Δ IL-6	-64.330 \pm 121.5	48.6135 \pm 121.5	0.002
Δ TNF- α	-178.8 \pm 108.8	-56.6 \pm 86.6	0.000
hs-CRP	-116.4 \pm 107.6	-49.6 \pm 78.5	0.000
Anti-oxidant			
Δ Malondialdehyde (MDA)	-6.9 \pm 9.2	3.09 \pm 6.3	0.000
Δ Superoxide dismutase (SOD)	0.099 \pm 0.76	-0.40 \pm 0.71	0.023
Parameters of endothelial dysfunction			
Δ Nitric oxide (NO)	-6.87 \pm 12.6	-1.01 \pm 3.22	0.027
Δ EPC	17.27 \pm 23.20	-20.55 \pm 29.6	0.000
Δ CEC	-65.3 \pm 78.07	-6.1374 \pm 39.49	0.002

Note; IL-1, interleukin-1; TNF- α , tumor necrosis factor- α ; EPC, endothelial progenitor cell; CEC, circulating endothelial cell

Table 2. The effect of adding *G. mangostana* L. extract on lipid and glycemic profiles after 90 days of treatment

	Add-on <i>G. mangostana</i> L. extract (n=23)	Add-on Placebo (n=26)	P-value
Lipid profile			
Total cholesterol	-1.0435	3.3846	0.650
HDL	2.2174	-1.0231	0.140
LDL	-9.0652	0.6500	0.116
Triglycerides	-7.6522	-10.0000	0.901
Glycemic profile			
HbA1c	-0.81 \pm 1.01	0.06 \pm 0.80	0.002
Fasting blood glucose	-26.4 \pm 4.7	-15.11 \pm 43.9	0.336

Note; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HbA1c, hemoglobin A1c

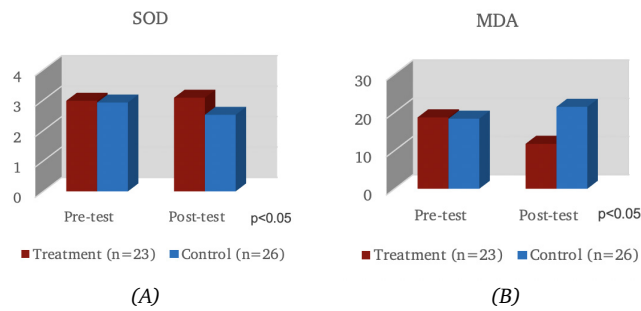


Figure 3. Increase of SOD level (A) and decrease of MDA (B) after adding *G. mangostana L.* extract on top of treatment, compared to placebo

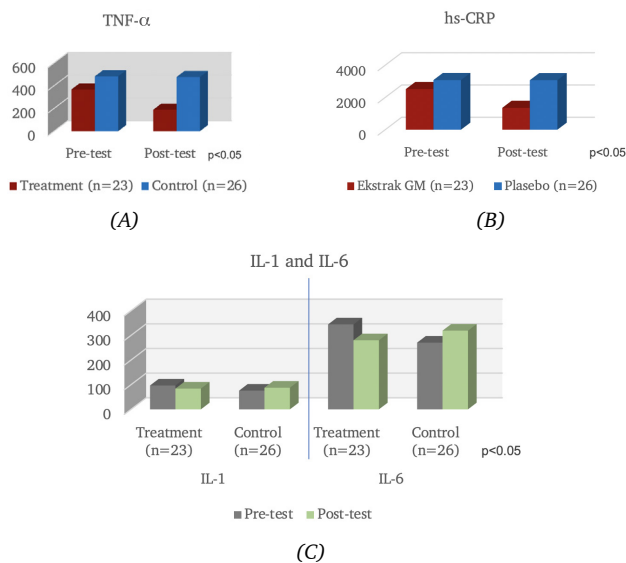


Figure 4. The effect of add-on *G. mangostana L.* extract to reduce the level of TNF-α (A), hs-CRP (B), IL-1 and IL-6 (C) in comparison to placebo

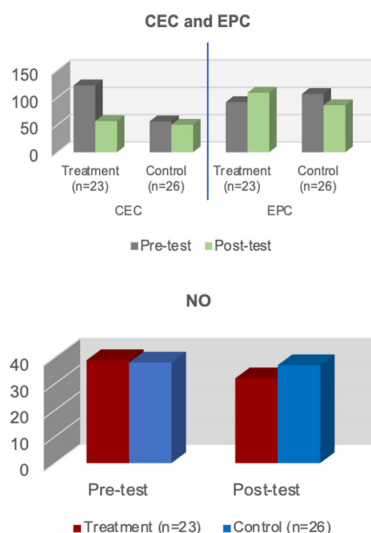


Figure 5. The effect of add-on *G. mangostana extract L.* in improving endothelial dysfunction after 90 days, compared to placebo

4. Discussion

Our current findings confirmed that the administration of *G. mangostana L.* extract was associated with elevated level of EPC and,

SOD and decreased level of CEC, NO, and MDA. Our findings suggested that *G. mangostana L.* extract might play an important role as an antioxidant in the development of endothelial dysfunction. Briefly, in the process of oxidative stress, reactive oxygen species (ROS) accumulates through the protein subunit p66. Antioxidant protection occurs through catalase, SOD, and glutathione peroxidase (GPx), which eliminate the excessive ROS. The alteration to protein p66 and SOD function causes endothelial dysfunction.^{10,11} Atherosclerosis leads to narrowing of blood vessels and is more common in diabetes mellitus. Hyperglycemia and hypercholesterolemia in type 2 DM predispose to the development of atherosclerotic plaque, which can be stable or unstable. Mechanisms such as increased inflammation, oxidative stress, foam cell deposition, endothelial dysfunction, and angiogenesis will facilitate plaque rupture.¹² Furthermore, hyperglycemia has a detrimental effect as in the formation of advanced glycated end products (AGE) and increases the production of reactive oxygen species (ROS) through NADPH oxidase activation; both are the main causes of endothelial progenitor cells (EPCs) apoptosis. AGE itself, in turn, further increases ROS production, and also promotes NF-κB transcription. NF-κB is crucially involved in inflammation via IL-1 and TNF-α.¹³ Xanthone and other bioactive have a role as antioxidants in preventing AGE formation and reducing ROS. Therefore, the extract of *G. mangostana L.* can be used as a supplemental antioxidant therapy.^{10,11}

The evidence concerning the role of *G. mangostana L.* extract in the process of atherosclerosis was limited. However, several studies have reported. Abdallah et al.¹⁴ suggested that *G. mangostana L.* extract could able to inhibit the formation of AGE in the cytoplasm. This anti-AGE property resulted from an inhibition of free radicals and glycation reaction to monosaccharide. Therefore, it prevented the development of diabetic complications, whereas Ibrahim et al.¹⁵ showed that the anti-diabetic property of *G. mangostana L.* extract was found in its bioactive xanthone. However, many clinical trials, both in vivo or in vitro, are required to evaluate further.¹⁶

Moreover, we also found that the levels of IL-1, IL-6, TNF-α, and hs-CRP were significantly lower in treatment group compared to placebo. Our result suggested that the administration of *G. mangostana L.* extract was associated with anti-inflammation effect. Briefly, both inflammatory and oxidative stress lead to endothelial dysfunction, and they were worsened by hyperglycemia in type 2 DM. Besides, the accumulation of ROS and NO bioavailability are imbalanced. Alexandru et al.¹⁰ showed that EPC dysfunction in type 2 DM was related to oxidative stress and ROS formation. SOD activity as an antioxidant enzyme was also found reduced. Mobilization of EPC from bone marrow and homing to the site of injury requires enough NO. Therefore, EPC level decreases in endothelial dysfunction. Unlike EPC, the level of CEC increases during injury as CECs detach from the injured endothelial layer.^{4,13,11, 17-20} On the other hand, a study by Jindarat,¹⁷ suggested that xanthone could increase the activation of MAPK, c-Jun and EIK-1, and NF-κB pathways activation. Enhancing the activation of the NF-κB pathway associates with the increase of cytokines production, including IL-1, IL-6, and TNF-α. Those inflammatory markers reduced when the pathways were inhibited.¹⁷

In our current study, several important limitations were found. First, several factors that might govern anti-inflammation and anti-oxidation effects in endothelial diseases such as psychological stress, drug adherence, and lifestyle status were not observed. Second, the result of our current study should be interpreted with caution due to the relatively small sample size. Third, the design of our present study was prospective cohort. Further studies using randomized control trial might be required to obtain a higher level of evidence. Forth, the sample frame of our present study was limited to a single center. Further studies with multicenter sampling method might be required.

5. Conclusion

Administration of *Garcinia mangostana L.* extract is associated with the improvements of endothelial dysfunction by regulating increased levels of in EPC and SOD and decreased levels of CEC, IL-1, IL-6, NO, MDA, TNF-α, fasting blood glucose and HbA1c. Our findings may provide that *Garcinia mangostana L.* extract has the potential as promising adjuvant therapy in coronary artery disease treatment and the development of diabetic vascular complications.

6. Declarations

6.1. Ethics Approval and Consent to participate

This study was approved by local Institutional Review Board, and all participants have provided written informed consent prior to involve in the study.

6.2. Consent for publication

Not applicable.

6.3. Availability of data and materials

Data used in our study were presented in the main text.

6.4. Competing interests

Not applicable.

6.5. Funding source

Not applicable.

6.6. Authors contributions

Idea/concept: OH. Design: OH. Control/supervision: DS. Data collection/processing: OH. Extraction/Analysis/interpretation: OH, DS, MSR. Literature review: DS, MSR. Writing the article: OH. Critical review: DS, MSR, BS, CTT, DH. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

6.7. Acknowledgements

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