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Article

Supplementation with an Analog Rice in Combination with Metformin Prevent Myocardial Cell Death in Type 2 Diabetic Rats

Andi Muhammad[1] , Jauhar Firdaus[2*], Nindya Shinta Rumastika[3], Hairrudin[4] , Elly Nurus Sakinah[5]

[1] Faculty of Medicine, University of Jember, Jember, Indonesia; andimuhsr@gmail.com
[2] Department of Physiology, Faculty of Medicine, University of Jember, Jember, Indonesia; jauhar_firdaus.fk@unej.ac.id
[3] Department of Anatomical Pathology, Faculty of Medicine, University of Jember, Jember, Indonesia; shintariadi@gmail.com
[4] Department of Biochemistry, Faculty of Medicine, University of Jember, Jember, Indonesia; hairrudin.fk@unej.ac.id
[5] Department of Pharmacology, Faculty of Medicine, University of Jember, Jember, Indonesia; ellyns.fk@unej.ac.id

* Correspondence: jauhar_firdaus.fk@unej.ac.id

Abstract: Type 2 diabetes (T2D) is an urgent health issue that leads to one of the most significant cardiovascular complications worldwide, diabetic cardiomyopathy (DCM), which is associated with high morbidity and mortality. Chronic hyperglycemia and oxidative stress are major contributor for DCM. Analog rice based of MOCAF and corn starch is a functional food recommended as nutritional supplementation to replace staple foods in T2D patients. Analog rice has a high fiber content, high resistant starch and low glycemic index, which can help lower blood sugar levels and prevent DM complications. MOCAF in analog rice is also rich in phenolic compounds, which has potential effect of antioxidant. Metformin is the first line oral therapy for T2D. The present study investigates the potential effects of an analog rice diet in combination with metformin, for the development of DCM. Type 2 diabetic rats were induced by HFD/STZ method and was treated by analog rice and metformin for 4 weeks. Myocardial cell death measured by pyknotic nuclei percentage was analysed. The study result demonstrate that analog rice and metformin prevent the myocardial cell damage observed in type 2 diabetic animals, highlighting analog rice potential as adjuvant therapy, for treating DCM has no clinical significance than metformin therapy alone.

Keywords: diabetes; heart; analog rice; myocardial cell death; metformin; MOCAF

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1. Introduction

Type 2 diabetes (T2D) represents a major global health concern due to its high prevalence and associated complications [1]. Cardiovascular associated complications, including diabetic cardiomyopathy (DCM), are the leading cause of mortality and morbidity in these patients [2]. Numerous glucose-lowering medications are available for the therapy of T2D patients and some of them may lower cardiovascular mortality [3]. However, the risk of death from diabetic cardiovascular complications remains significantly high in this population and requiring a new preventive and therapeutic approaches for diabetic cardiomyopathy treatment [4].

Diabetic cardiomyopathy (DCM) manifests as a clinical condition associated with cardiac structural and functional changes provoked by diabetes [5]. Persistent hyperglycemia, hyperlipidemia and insulin resistance in type 2 diabetes provoke excessive production of superoxide and inflammation which are vital to the pathogenesis of DCM [6]. Overproduction of reactive oxygen species (ROS) and subsequent oxidative stress may lead to cardiac fibrosis, DNA damage and myocardial cell death [7]. In view of this, agents with antidiabetic and antioxidant potential are considered as promising options to slow down the development of DCM.

In the last years, Functional foods are widely used as diabetes mellitus management and prevention due to their antioxidant, anti-inflammatory, and insulin-sensitizing properties [8]. Analog rice is one of functional food in dietary management that have potential to solve T2D problems [9]. Analog rice is formulated from different type of materials which contain low glycemic index (GI), high resistant starch and high fiber ingredients. According to previous researches, analog rice based of modified cassava flour (MOCAF), corn starch and rice flour significantly effective in lowering blood glucose levels in diabetic rats by enhancing GLP-1 and insulin serum levels [10], [11]. MOCAF is also rich in phenolic compounds such as gallic acid, kaempferol, catechin gallate, gallocatechin, and caffeic acid which play a key role in antioxidant activity by binding to negative oxygen ions and free hydroxyl radicals. [12]

Metformin is one of the first line anti-hyperglycemic agent for the treatment of T2D and used by millions of patients worldwide daily, including those with cardiovascular complications [13]. A number of studies have reported metformin may stimulate multiple cardioprotective pathways, including the AMP-activated protein kinase (AMPK) pathway which induce clearance of ROS by increasing antioxidant enzymes, enchance mitochondrial function and reduce endoplasmic reticulum stress [14]. The aim of this study was to investigate the potential additive effect of the analog rice supplementation in combination with metformin for preventing myocardial cell death in an animal model of T2D. The results provide evidence for the beneficial impact of the analog rice diet in combination with metformin, against the development of cardiac structural changes, measured by myocardial cell death in diabetic animals.

2. Materials and Methods

The study was a true experimental research and used posttest only group design with random sampling. The study was conducted in Biomedical Laboratory, Faculty of Dentistry, University of Jember. The study used 3 months old male rats (*Rattus norvegicus*) Wistar strain weighing 200-250 g. The rats were treated in accordance with the Declaration of Helsinki. An ethical approval was obtained from the Ethical Committee of the Faculty of Medicine, University of Jember (No. 5224 UN/25.1.10.2/KE/2024). Rats were placed in cages individually within a well-ventilated room maintained at a temperature of 18-24°C and a humidity level of 50-70%. Lighting conditions were controlled with a 12-hour light-dark cycle, and the cages were cleaned daily.

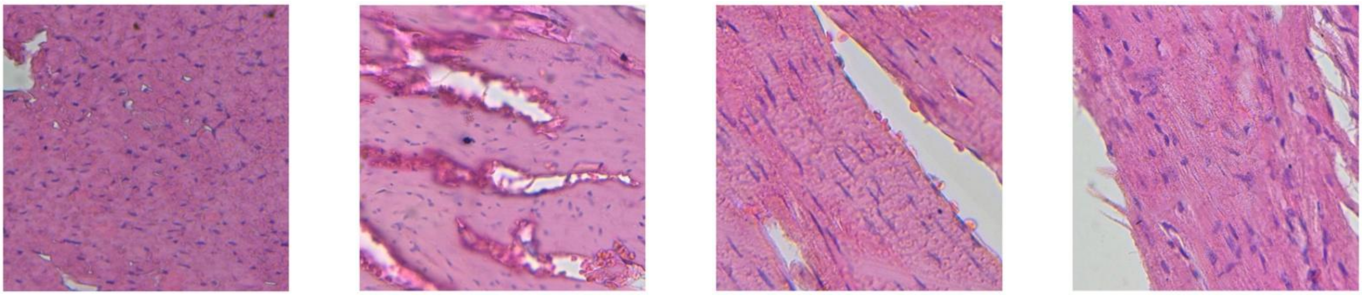
After a week of acclimatization, a total of 24 rats were randomly divided into normal control group (n=6) and experimental group (n=18). The normal control group received with standard feed formula for 7 weeks, while the experimental group was fed with high-fat diet (HFD) containing 23% fat for 3 week. On the 21st day, the experimental group received an intraperitoneal injection of streptozotocin (STZ) (BioWORLD) at a dose of 35 mg/kgBW [15], [16]. After 3 day of STZ injection, fasting blood glucose levels were measured through the tip of the rats tail. The experimental group, with fasting blood glucose (FBG) levels exceeding 200 mg/dl and considered as T2D animal model, and the experimental group randomly divided into three equal groups (n=6), namely P0, P1 and P2 group. In experimental group (P0), diabetic rats were given 20 g of standard pellet and aquadest, experimental group (P1) diabetic rats were given 20 g of standard pellet and 45 mg/kgBW of metformin, and experimental group (P2) diabetic rats were given 20 g of analog rice and 45 mg/kgBW of metformin. Analog rice pellet was formulated with MOCAF (30%), corn starch (30%) and rice (40%) and subtituted 60% of carbohydrate content of standard pellet. Analog rice was produced by using hot extrusion technique in 70°C with twin screws. The experimental groups were fed at a quantity of 20 g per day ad libitum, simultaneously for 4 weeks. At the end of the 4th

treatment week, the rats were terminated, then the hearts organ were taken for the histopathological measurement.

The hearts were immediately excised, washed, and fixed in 10% neutral buffered formalin for 24 hours. Standard paraffin heart blocks were prepared. Then, 5-μm thick sections were placed on adhesive slides and stained with haematoxylin-eosin. The sections were examined under Olympus light microscope (CX23LED), and digital images were captured by microscope camera (Optilab SIGMA). Myocardial cell death was measured by the percentage pyknotic nuclei per total normal cardiomyocyte cells found in five fields of view of the longitudinal cardiac section, observed at 400x magnification. After the procedures were completed, the data were analyzed using SPSS version 26 software.

3. Results and Discussion

The study investigated structural changes of diabetic rats heart through histopathological observation and the analysis was summarized in Figure 1. Histopathological study of cardiac sections of normal control rats as showed in Figure 1a revealed normal morphological appearance with striated muscle fibers and single centrally located oval nuclei. On the contrary, cardiac sections of non-treated diabetic rat as showed in Figure 1b revealed myocardial degeneration, vacuolation, and widened myocardial gap. Cardiac muscle fibers are showing restored striation and neat row of nuclei in both treated diabetic rats and can be seen as in Figure 1c and Figure 1d.



(**a**) (**b**) (**c**) **(d)**

Figure 1. Histopathological observation in all groups (**a**) cardiac section of normal control rats showed normal morphological appearance with striated muscle fibers and single centrally located oval nuclei; (**b**) cardiac section of non-treated diabetic rats showed myocardial degeneration, vacuolation, and widened myocardial gap; (**c**) cardiac section of metformin-treated diabetic rats showed restored striation and neat row of nuclei; (**d**) cardiac section of analog rice and metformin-treated diabetic rats showed restored striation and neat row of nuclei;

Myocardial cell death percentage in the heart was investigated using pyknotic nuclei percentage parameter on cardiac section. Pyknotic nuclei with chromatin margination were still found on cardiac sections of both normal control and experimental groups. The average of myocardial cell death percentage of all groups is shown in Figure 2. The percentage of normal control group was 1.168 ± 0.448, non treated diabetic rat group was 9.075 ± 1.365, metformin-treated diabetic rat group was 1.200 ± 0.607, analog rice and metformin-treated diabetic rat group was 1.738 ± 0.939. There were significant variations in myocardial cell death percentage between the control group and experimental groups. Based on Figure 2, the average of myocardial cell death percentage was significantly increased ($p < 0.001$) in non-treated diabetic group as compared with the control group, metformin treated diabetic group and metformin-analog rice treated diabetic group. The myocardial cell death percentage of diabetic rats without treatment was increased by 7.907% as compared with normal control rats.

Based on Figure 2, the myocardial cell death percentage was significantly lowered in both treated diabetic rat groups, as compared with non treated diabetic group. This finding was expected because both metformin and analog rice has antidiabetic and antioxidant properties which may prevent structural changes in diabetic heart. However, there was no significant difference ($p > 0.05$) between metformin only-treated diabetic rats group and metformin and analog rice-treated diabetic rats group. Metformin and analog rice-treated diabetic rats group had slightly higher percentage with no significant difference. The percentage difference in total was only 0.538% and could be due to random variation rather than a true effect. This insignificant difference could also due to shorter period of type 2 diabetic rats intervention than many previous researches.

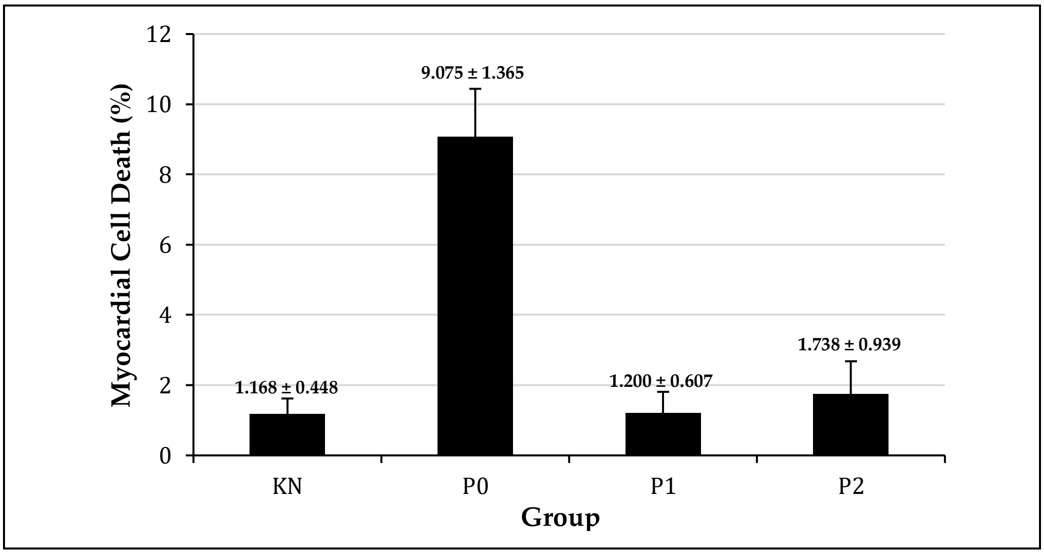


Figure 2. Average myocardial cell death percentage in all groups

Significant structural changes in myocardium was commonly found in diabetic cardiomyopathy [17]. DCM involves complex metabolic pathways involving chronic hyperglycemia-induced changes, alteration in fatty acid metabolism and inflammation [18] DCM causes higher rates of myocardial cell death by both necrosis and apoptosis than in healthy heart [2]. Pyknosis was one of the stage of both apoptosis and necrosis and pyknotic nuclei has been widely used as indicator of cellular damage [19]. Pyknotic nuclei has also been observed on normal and healthy cells due to regulated apoptosis [20].

Analog rice used in this study contained of low GI, high resistant starch (RS) and high fiber material [10]. RS and the fiber contained in analog rice will be fermented by gut microbiota, producing short-chain fatty acids (SCFAs). Subsequently, SCFAs will increase the production and secretion of glucagon-like peptide-1 (GLP-1) in the intestinal epithelium. The increase in GLP-1 induces the proliferation of pancreatic β cells and enhances insulin secretion. As a result, blood glucose levels will decrease and inhibiting hyperglicemia progression [21]. MOCAF contained in analog rice was also rich in phenolic compound, thus it could be antioxidant agent for delaying progression of diabetes complication, including diabetic cardiomyopathy [12].

Many analog rices have demonstrated protective roles against several diabetes pathogenesis due to their antidiabetic and antioxidant activities, and other beneficial functions [9]. However, their effect on cardiovascular complication and with combination with antidiabetic drugs has scarcely been investigated. The present study showed the evidence of potential cardioprotective effect of an analog rice based on MOCAF and corn starch, in combination with metformin, against diabetic cardiomyopathy. In the current study, analog rice and metformin therapy had insignificantly different result as compared with metformin only therapy to prevent myocardial cell death in type 2 diabetic rats. Although analog rice and metformin could still significantly prevent myocardial cell death in diabetic rats, this finding may suggest that analog rice supplementation is not a very promising candidate for adjuvant cardiac therapy, especially for preventing DCM.

4. Conclusions

Based on findings of the present study, analog rice supplementation in combination with metformin prevent myocardial cell death in type 2 diabetic rats. The potential effect of analog rice as adjuvant cardiac therapy is recommended to be investigated in further studies, especially to determine the effect of analog rice alone in preventing DCM progression. Longer duration of type 2 diabetic intervention and higher MOCAF composition could be used in further similar studies.

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