Momordica Charantia Fruit Extract Improvesubcellular Changes in Cardiovascular Tissues of Diabetic Rats

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***Abstract*—Type 1 Diabetes mellitus (T1DM) or insulin deficiencies lead to the development of diabetic cardiovascular complications, due to the imbalance between oxidative stress and anti-oxidants. The present descriptive study focusedon the effect of *Momordica charantia (MC*) fruit extract in the cardiovascular tissue of streptozotocin-induced diabetic rats. A total of 30 adult male Sprague-Dawley rats were used and equally divided into five groups (n=6); Control group (Ctrl), control group treated with *MC* (Ctrl + *MC*), DM untreated group (DM), DM group treated with *MC* (DM + *MC*) and DM group treated with metformin 150g/kg (DM + Met). Diabetes was induced in rats by a single intramuscular injection of streptozotocin (STZ) 60mg/kg of body weight. *MC* fruit extract 1.5g/kg was used for this study. Following four weeks of STZ induction, the treatment was started and continued for 28 days. At the end of the study, the rats were sacrificed; cardiac and aortic tissues were harvested for electron microscopic study. Improved ultrastructural damages of both tissues were observed in DM + *MC* group. In conclusion, oral administration of *MC* fruit extract improved subcellular changes in cardiovascular tissues of diabetic rats.**

***Index Terms*—type 1 diabetes mellitus, *Momordica charantia*, cardiovascular tissue, electron microscope**

I. Introduction

Diabetes mellitus is a chronic endocrine disorder characterized by can be classified as type 1 diabetes mellitus or insulin dependent diabetes mellitus (IDDM), type 2diabetes mellitus (NIDDM) and gestational diabetes mellitus [1]. It was reported that the prevalence of DM among Malaysian increased from 6.3 to 22.9% over the past few decades. Type 1 diabetic patients have 10-fold increase in the risk of developing cardiovascular disease compared to non diabetic individual [2]. In diabetes mellitus, increases in oxidative stress and high rate of fatty acid oxidation increases the reactive oxygen species production. As a result, the increase in oxygen demand and reduces in cardiac efficiency occurs and lead to the development of mitochondrial dysfunction,myocardial apoptosis, fibrosis and vascular complications [3].

Diabetes mellitus influences the myocardium through microangiopathy, metabolic disturbances and cardiac autonomic neuropathy. The untreated diabetic cardiac disease may lead to heart failure and vascular diseases. It was shown that increase blood glucose level enhances the high glucose intake on the endothelial lining of blood vessels resulting in thicker and weaker basement membrane. Marked reduction of cardiac glucose resulted from down regulating myocardial metabolism in chronic insulin deficiency state was documented [4].

Oral hypoglycaemic agents such as metformin, sulphonylureas, alpha-glucosidase inhibitors andthiazolidinediones are the mainstay of treatment for diabetes mellitus. Natural products or herbs also proved to have anti-hyperglycaemic, hypoglycaemic properties and additional anti-oxidant property [5], [6].Therefore, researches have been carried out to study the effect of herbal medicine towards diabetes mellitus and its complications. Recently, a research study documented the positive effect of *MC* leaves extract on diabetic heart tissue with regard to the microscopic changes [7].Admittedly, limited studies were conducted on the effect of *Momordica charantia* (*MC*) fruit extract towards diabetic cardiovascular disease. In the present study, we observed the effect of *MC* fruit extract towards diabetic cardiovascular tissue, with regard to its electron microscopic changes. It is believed that treatment with *MC* fruit extract improve the ultrastructural damages of cardiac and aortic tissues in experimental diabetic rats.

II. Material and Methodology

A. Preparation of Momordica Charantia Aqueous Fruit Extract

The dried fruits of *MC*(5 Kg)were purchased from local market, Malaysia. The whole plant was sent to a

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botanist to identify (UKMB 40067). Dried fruits of *MC*were cut into pieces and underwent aqueous extraction with double distilled water (1:3). The extract was solified and was kept in the -80℃ freezer. It was then freeze dried to obtain a powder form.In this study, the oral dosage of 1.5g/kg body weight was used [8].

B. Experimental Design

A total of thirty (n=30) adult male Sprague-Dawley rats were used. After the acclimatization period, the rats were divided into control and experimental groups. The experimental group of ratsreceived a single intramuscular injection of streptozotocin (STZ)(60mg/kg). The control group received 0.9% normal saline, intramuscularly. Following 72 hours of STZ induction, the fasting blood glucose was measured in all groups of rats. Fasting blood glucose level more than 8mmol/L was labelled as diabetic and remained untreated for 4 weeks [8].At the end of 4 weeks of STZ induction, the rats were further subdivided into control group (Ctrl) and control treated with *MC* extract (Ctrl+*MC*); STZ-induced diabetic groups were further subdivided into untreated diabetic group (DM), diabeticgroup treated with *MC* extract (DM+*MC*) and diabetic group treated with metformin 150mg/kg (DM+Met).The dose of metformin was adopted from the previous study [9].

C. Transmission Electron Microscopic Study

At the end of the study, i.e.following 28 days of treatment, the rats were sacrificed. The cardiac and thoracic part of aortic tissues were collected from each group of rats. Both tissues were cut into 1 mm^3 size at a cross section. Then, they were rinsed with 0.1 M Phosphate buffer saline for about 10 minutes. The tissues were then fixed with 2.5% glutaraldehyde as primary fixation. The tissues were rinsed again with 0.1 M PBS for 10 minutes and en bloc with 3% uranyl acetate as secondary fixation. The tissues were then dehydrated with ethanol series. Infiltration process was taken out with 1:2 resin and propylene oxide. The tissues were then embedded in 100% resin in capsules. The process of polymerization was taken more than 8 hours. After polymerization, the specimen was sectioned with a glass knife and stained with toluidine solution for semi-thin section. In semi-thin section, the required area of tissues was observed and proceeded for ultra-thin section where the blocked was sectioned with a diamond knife. The samples were placed on the copper grid size of 200 networks. All the results were viewed by the two expert observers in double-blinded fashion. Finally, the specimens were viewed under transmission electron microscope Tecnai G2 model in the voltage of 80kV.

III. Result and Discussion

A. Observation of Animal

In the present study, it was noted that all the rats received STZ induction presented with polyuria, polydipsia and diarrhoea compared to control groups of rats. The experimental diabetic groups also presented with polyphagia. Polydipsia, polyuria and polyphagia were observed by the evidence of frequent bottles filling up and changing of bedding. These symptoms may be due to the excessive amount of glucose being excreted through the urine followed by the water. Furthermore, the resistance of the glucose to absorb into the cells leads to polyphagia. However, rats with the *MC* treated group showed no prominent changes in terms of food and water intake. It may be due to the increased glucose utilization in the liver and peripheral tissueswhich lead to minimize the symptoms [7].

B. Cardiac tissue: Transmission Electron Microscopic (TEM) Findings

The left ventricle was used to determine the ultrastructural changes in the cardiac tissue. There were prominent abnormalities noted under TEM for DM group. The changes such as derangement of myofibrils, deformation of mitochondria and invaginations in the nuclear envelope of cardiomyocytes. However, DM+*MC* and DM+Met groups showed less ultrastructural abnormalities. The detailed findings of each abnormality were mentioned below.

Myofibril: The arrangement of myofibrils is defined as regular arrays of sarcomere (between two Z lines). It was revealed that DM group showed irregular and sparse myofibrils with increased in cytoplasmic space as compared to DM+*MC* group. DM+Met group also showed less disorganization of myofibrils. There were no obvious ultrastructural abnormalities found in Ctrl and Ctrl+*MC* groups.

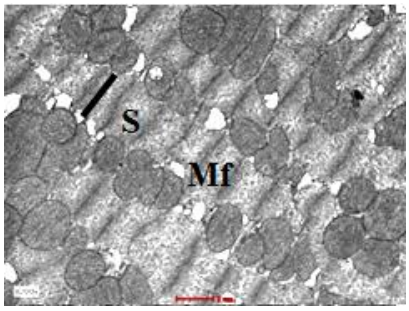
Mitochondria: Ctrl group showed normal mitochondria with regular arrangement. Ctrl+*MC* group also showed no obvious ultrastructural abnormalities compared to Ctrl group. However, DM group showed disarrangement of mitochondria with increased in size. In DM+*MC* group, the architecture of mitochondria was reverted to normal compared to DM group. DM+Met group also showed improve in mitochondria in terms of the size and arrangement (Fig. 1).

Nuclei: Invaginations in nuclear envelope were found to be more prominence in DM group. Invaginations were defined as 0.3 μm in depth and less than 1 μm in width [10]. In the present study, Ctrl group showed only a few invaginations in nuclear envelope, as a normal architecture. Ctrl+*MC* group also showed no obvious changes in cardiomyocytes appearance compared to Ctrl group. However, DM group showed multiple invaginations in nuclear envelope with deformation of nuclei. DM+*MC* group showed less invagination of nuclear envelope compared to DM group. DM+Met group also revealed the improvement in ultrastructure integrity of cardiac tissue in diabetic rats (Fig. 2).

The ultrastructural findings of the present study proved that the organization of cardiac tissue was disturbed in STZ-induced diabetic rats. Previous study had shown myocardial ultrastructure damages in untreated diabetic rats [11]. However, the mechanism by which hyperglycaemia cause diabetic cardiac disease remained obscure. It can be explained that cardiac tissue damage is most probably due to the increase production of superoxide in the mitochondrial electron-transport chain.

Thent et al. 2012 had demonstrated the prominent cardiac tissue cellular injury in diabetic rats under TEM findings following 8 weeks of STZ induction [12]. Lanlua et al. 2012, mentioned that increased levels of cardiac specific transcription factor and myosin calcium ATPase in the diabetes mellitus may be the contributing factors for myofibrils loss.Increased production of ROS and fatty acid level in the state of the diabetes mellitus promotes the deformation of mitochondria via loss of the membrane permeability [13]. Moreover, ROS induce phospholipid cardiolipin in the inner membrane of mitochondria that interacts with ROS to disrupt the cristae. As a result, more cytoplasmic spaces were observed in the DM group due to the deterioration of the membrane of mitochondria.

In the present study, it was demonstrated that the treatment with *MC* fruit extract for 28 days reduced the ultrastructural damages in cardiac tissues of type 1 DM rats. However, the detailed mechanism of *MC* fruit extract in protecting the ultrastructural damage of diabetic cardiac tissue was notexplored, till date. It might be due to the effect of active compound such as charantosides that reduced the ROS and AGEs in the cardiomyocytes. Flavonoid compounds present in *MC* fruit extract reduced the oxidative damage occurred in cardiac tissue.The results were found in accordance with DM+Met group.



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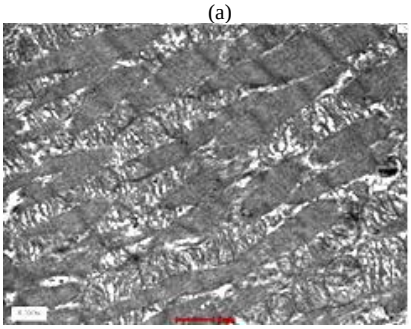

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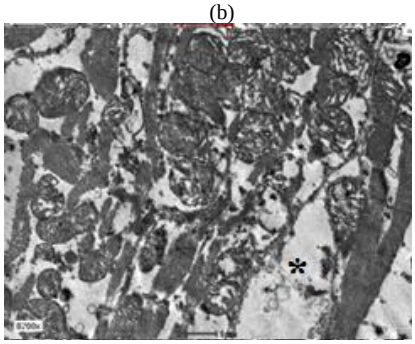

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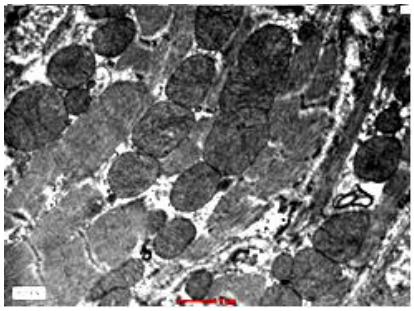

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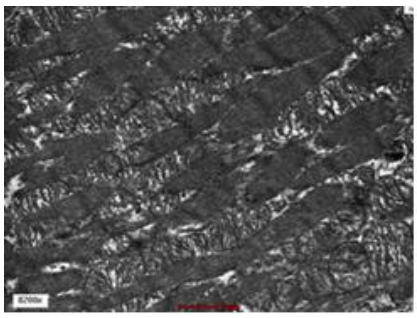

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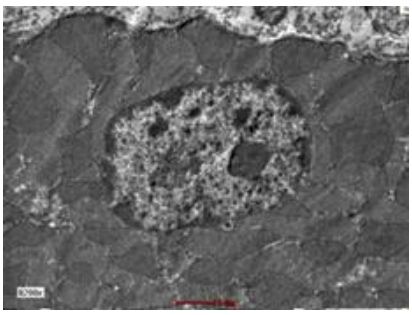
Figure 1. Electronmicrograph showing mitochondria and myofibrils in the cardiac tissue of a(Ctrl), b(Ctrl+*MC*), c(DM), d(DM+*MC*), e(DM+Met) groups. Mf = myofibrils, M = mitochondria,S=sarcomere,* = cytoplasmic space



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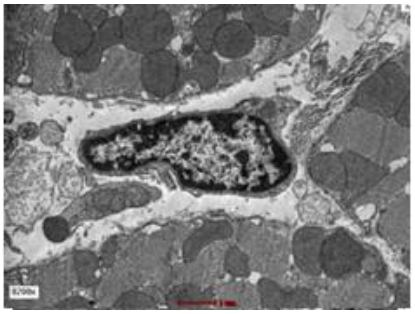

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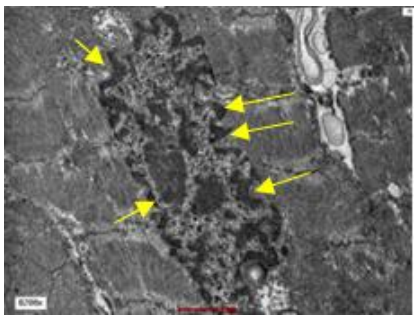

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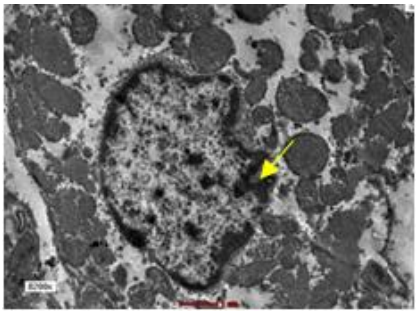

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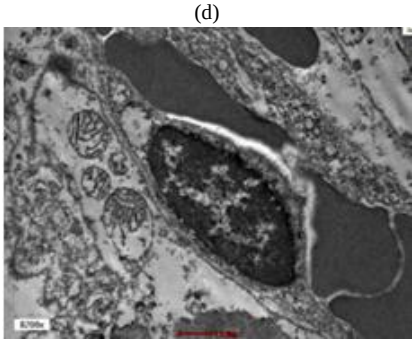

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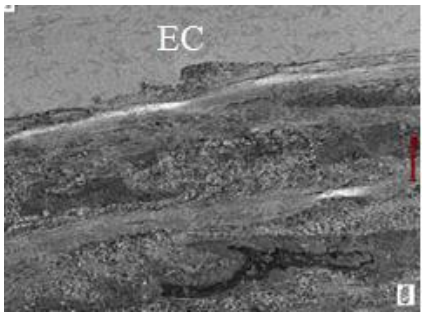
Figure 2. Electronmicrograph showing the nucleus of cardiomyocyte in the cardiac muscle fibres of a(Ctrl), b(Ctrl+*MC*), c(DM), d(DM+*MC*), e(DM+Met) groups. Nuclear invagination = Yellow arrows.

C. Thoracic Aorta: Transmission Electron Microscopic (TEM) Findings

In the present study, TEM findings of thoracic aorta focused on endothelial cells, smooth muscle cells and elastic lamina. In Ctrl and Ctrl+*MC* groups, no definitive changes were observed in the thoracic aorta.

DM group showed severe alterations in the structure of the vascular wall. The endothelial cells showed irregular distributions with atrophic characteristics. The ultrastructural changes observed in endothelial cells indicated the endothelial injury. The subendothelial region was thickened and tunica media showed irregular smooth muscle cells in the elastic lamina with degenerative interstitial matrix. The elastic lamina also appeared to be fragmented and reduplicated. There was a migration of smooth muscle cells observed from the tunica media into the tunica intima. However, DM+*MC* group showed a less irregularity in smooth muscle cell compared to DM group. The elastic lamina was found to be regular, and the intima surface appeared to be intact, with few fragmentations. Furthermore, DM+Met group showed less ultrastructural changes in thoracic aorta (Fig. 3).



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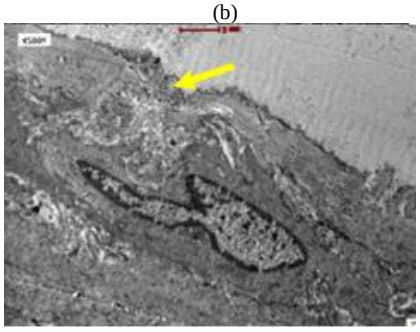

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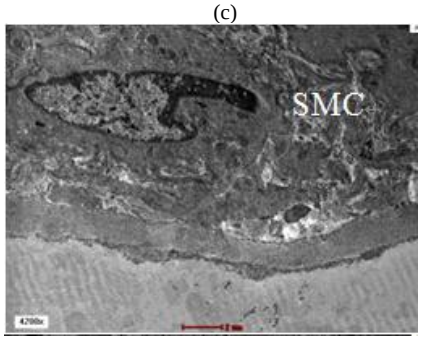

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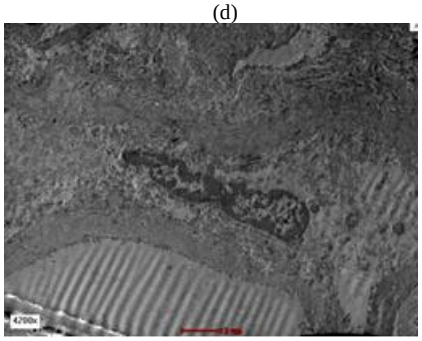

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Figure 3. Electronmicrograph showing ultrastructural organization in thoracic aorta of a(Ctrl), b(Ctrl+*MC*), c(DM), d(DM+*MC*), e(DM+Met) groups. IEL = internal elastic lamina, SMC = smooth muscle cells, EC= endothelial cells, migration of smooth muscle cells =Yellow arrow.

Ultrastructural findings of aorta were reported previously by Balkis-Budin 2009, in which it was mentioned the morphological changes in endothelial cells as an endothelial injury[7]. Infiltration of macrophages into the subendothelial space, migration of smooth muscle cells from tunica media (TM) to tunica intima (TI) and the proliferation of smooth muscle cells in the TM layer were observed in diabetic rats. These findings are considered to be early events in the development of vascular complications in type 1 DM rats.

It wasreported that both endothelium and smooth muscle cells of thoracic aorta were affected in type 1

diabetes mellitus. Hyperglycaemia induces a significant increase in ROS production in the blood vessels via the auto-oxidation of glucose, reduces synthesis of nitric oxide (NO), accelerated inactivation of NO and increases activation of protein kinase C(PKC) [14]. These events, promotethe proliferation of the smooth muscle cells in TM. The overproduction of smooth muscle cells in TM breaks the IEL and migrates toward TI [15]. As a result, the disturbances in the ultrastructural organization of thoracic aorta occurred in DM group. A group of researcher had mentioned the proliferation and migration of smooth muscle cells from TM to TI in type 1 diabetic rats [16]. Irregularity of endothelial cells in DM group was due to an increase in fibronectin. The increased in fibronectin level was found in the endothelium of atherosclerotic subjects [17].

To date, there are limited studies which investigated the effect of fruit extract of *MC* towards the ultrastructural changes on the thoracic aorta. *MC* which is known to have anti-atherosclerotic activity increases the superoxide dismutase (SOD) and total anti-oxidant activity [18]. It is believed by maintaining the blood glucose level and balancing the oxidant – anti-oxidant levels, *MC* could modify the ultrastructure changes against the tissue damages. Therefore, supplementation with *MC* extract improved vascular injury in type 1 diabetic rats. In DM+Met group, the ultrastructural changes of thoracic aorta reverted to the normal cellular integrity.Eventually, it was proved that both *MC* and metformin have similar effects in maintaining the ultrastructural integrity of cardiac and aortic tissues in experimentaldiabetic rats.

IV. Conclusion

From the present findings, it was shown that *MC* aqueous fruit extract improved the subcellular changes of the cardiac and thoracic part of aortic tissues in diabetic animals. The resultswere in accordance withoral hypoglycemic agent, metformin.Hence, we provide the evidence thatoral administration with *Momordica charantia* fruit extract for 28 days in STZ-induced type 1 diabetic rats has positive effectson diabetic cardiovascular complications. Further detailed studies are mandatory to corroborate such facts.

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