

ULTRASTRUCTURAL ANALYSIS OF THE CARDIOMYOCYTES OF BALB/C MICE FED A HIGH-FAT DIET AND TREATED WITH *APIUM GRAVEOLENS* EXTRACT

Abstract

Diet has a significant impact on cardiac function, with lipids being particularly important in pathology prevention and development. The purpose of this study was to look into the preventive impact of *Apium graveolens* heart injury in mice fed a high-fat diet. TEM was used to examine the ultrastructure of cardiomyocytes mitochondria, nuclei, and myofibrils. The mitochondrial ultra structure (M) of the HFD group had morphological abnormalities such as membrane degradation, cristae disorder, which caused them to seem deformed, and cristae loss. Nuclear membrane and nucleolus degeneration may be seen in the nucleus ultra structure. Fewer myofibrils (Mf) show degeneration and sarcomere disruption (Z-line). In TEM micrographs, muscle fiber mass fell considerably, suggesting muscle fiber breakdown. After *Apium graveolens* extract therapy, the normal order of muscle fibers, mitochondria, and nuclei demonstrates that the extract may protect cardiomyocytes from oxidative damage produced by HFD. The findings suggest that *Apium graveolens* may help protect heart muscle against oxidative stress and the illnesses associated with it induced by a high-fat diet.

Keywords: *Apium graveolens*, cardiomyocytes, high-fat diet, mitochondria, nucleus, myofibril.

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I. INTRODUCTION

Coronary heart disease (CHD) and heart failure (HF) are two cardiovascular conditions that are significantly linked to the use of an HFD¹. There has been mounting evidence in recent years linking HF risk to dietary fat consumption¹. Chronic exposure to excess lipids in the circulation can be harmful to the heart due to the accumulation of toxic metabolic by-products like reactive oxygen species (ROS) and ceramides, which can activate specific signalling cascades and lead to myocyte dysfunction and death^{2,3,4,5}. Hypertrophic cardiomyopathy has been linked to high-fat diets, which in turn have been linked to obesity⁶. High-fat diet-induced childhood obesity is a risk factor for cardiovascular disease, one of the world's leading killers. High levels of plasma triglycerides, LDL cholesterol, and fasting glucose, as well as low levels of HDL cholesterol, are all related to being overweight or obese^{7,8}. In addition to its function in the etiology of overweight-related disorders^{9,10}, oxidative stress has been demonstrated to have a significant role in cardiovascular diseases in conjunction with dyslipidemia and hyperglycemia^{11,12}. There is a lack of antioxidant enzymes and a high oxidative capacity in heart tissue, making it vulnerable to oxidative injury¹³. Oxidative damage to mitochondria causes cell dysfunction, makes cells more vulnerable to stress, and can even lead to cell death, which can have devastating, long-lasting pathological effects. Overweight/obesity has been shown to cause cardiac dysfunction by increasing oxidative stress, mitochondrial ROS generation, and cell death^{14,15}.

¹W.C. Stanley, E.R. Dabkowski, R.F. Ribeiro, K.A. O'Connell, "Dietary fat and heart failure: moving from lipotoxicity to lipo protection," *Circ. Res.*, 110:764-776, 2012.

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In addition to pharmacological therapy of cardiovascular risk factors and the use of conventional pharmaceuticals, the role of dietary variables and herbal remedies in the prevention and treatment of CVDs is becoming increasingly well recognized¹⁶. *Apium graveolens* is a herb. It belongs to the Apiaceae family, *Apium* genus, *Apium* species (*graveolens*), and Magnoliophyta division (Plantae). The scientific name for celery is *Apium graveolens*, but it's known by many different names across the world¹⁷. Celery plants may attain a height of 100 centimeters. It has a robust aroma and lasts a long time. Leaves range in size from 5-50 mm in length and have a triangular diamond or spear form with sawtooth or lobate borders¹⁸. Celery has been demonstrated to be effective in the treatment of cardiovascular illnesses. This is because it has been shown to reduce the risk factors for cardiovascular diseases (CVDs), enhance antioxidant enzymes, and lower the expression of cardiovascular disease and inflammatory biomarkers in mice that were fed a high-fat diet¹⁹. On the other hand, very little information is available on celery's potential to protect the heart. Therefore, we set out to see if *Apium graveolens* may improve cardiac ultrastructure in high-fat-diet-induced BALB/c mice.

II. MATERIAL AND METHODOLOGY

- 1. Chemicals:** Sodium cacodylate, HCl, paraformaldehyde, glutaraldehyde, osmium tetroxide, acetone, propylene oxide, Araldite CY2121, dodecyl succinic anhydride (DDSA), 2, 4, 6-tri (dimethylaminomethylphenol), dibutyl phthalate and Uranyl acetate stain.
- 2. Plant Material:** Celery leaves (*Apium graveolens*) were obtained fresh from the market in Iewduh, Shillong, and Meghalaya, India. Following washing in distilled water and drying at 25° C, the leaves were chopped and dried in a 40° C oven for three days. The dried leaves were then powdered using an electric blender and stored at 4° C in an airtight vial.
- 3. Preparation of Extract:** The plant powder was extracted with distilled water at 1:10 (powder/solvent) with occasional shaking for 24 hours. First, the extract was filtered using a muslin cloth, and then, after that, it was filtered through Whatman No. 1 filter paper. The filtrate was lyophilized using a rotary evaporator. Using sterile water, the leftovers were refrigerated at 4° C.
- 4. Experimental Animals:** The Pasteur Institute in Shillong, Meghalaya, India, provided adult Swiss albino mice weighing 20-30g. Five mice were housed in each polyacrylic cage under controlled laboratory settings. They were given distilled water and a regular dry pellet meal (both sourced from Hindustan Lever in Kolkata, India). Mice were

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¹⁹C. Marbaniang and L. Kma, "*Apium graveolens* Aqueous Extract Reduced Cardiovascular Diseases and Inflammatory Biomarkers Expression in High-Fat Diet-Fed BALB/C Mice," *J. Nat. Remedies*, in press.

introduced to the controlled environment of the laboratory 7 days before the experiment began. The procedures involving animals in the experiments met the standards set by the Institutional Animal Ethics Committee (IAEC).

5. **Dosage Selection:** The serum levels of liver enzyme markers were used to determine the effective dose (200 mg/kg b.w.) of aqueous extract of *Apium graveolens* that was used in this investigation. This dose was determined from the previous study²⁰.
6. **Experiment Design:** A total of 20 male BALB/c mice, all of which were Swiss albino and used in the study, were allocated at random into four groups of five mice each, as follows:
 - Group 1- (Normal pellet)
 - Group 2 (200 mg/kg b.w) - Normal pellet + *Apium graveolens* aqueous extract
 - Group 3-High-fat diet (containing 0.15 % cholesterol, 0.5 % sodium cholate, and 21% fat: containing 91% saturated fat, 7% monounsaturated fat, and 2% polyunsaturated fat)²¹.
 - Group 4- (200 mg/kg b.w)- High-fat diet + *Apium graveolens* aqueous extract
 The extract was given to each group orally by gavage for a total of 12 weeks.
7. **Transmission Electron Microscope (TEM) studies:** TEM studies were performed to check for the ultra-morphological effects of control and treated heart tissues using a modified version of the technique described by Massoud²². Small pieces (1 mm) of control and treated tissues were freshly cut and fixed in Karnovsky's fixative and post-fixed in 2% osmium tetroxide in phosphate buffer. After fixation, tissues were dehydrated at increasing concentrations of ethanol. They were then embedded in Araldite resin. Ultrathin sections were cut using an ultratome and stained by uranyl acetate saturated in 70% ethanol and lead citrate. Ultrathin sections of mice hearts were performed in the Sophisticated Analytical Instrument Facility (SAIF), formerly known as Regional Sophisticated Instrumentation Centre (RSIC) at North-Eastern Hill University (NEHU), Shillong, Meghalaya, using a JEOL transmission electron microscope JEM-1200, Ex, Japan.

III. RESULTS

1. **Ultra Structural Effect of *Apium graveolens* Extracts and HFD in the Cardiomyocyte:** The transmission electron microscopy (TEM) evaluation of the cardiomyocytes in both the control and the 200 mg/kg treatment groups revealed no major ultrastructural changes (Figures: 1, 2, and 3). At the center of each cardiomyocyte, a normal myofibrillar structure may be found, characterized by striations, a branching appearance, and continuity with the myofibrils that are near it. These myofibrils only

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contain a single nucleus. The nuclei do not exhibit any particular properties, and the membranes of the nuclei have been seen to stay unaltered (Figure 2). In addition, TEM revealed that the intercalated disc has large interdigitated cell junctions, including gap junctions, fasciae adherents, and desmosomes. These are the locations where myofibrils attach. The mice's heart muscle included a significant number of mitochondria (Figure: 1). they are made up of structures that have an asymmetrical form and are dispersed throughout the myofibrils. Moreover, they frequently congregate around the nucleus or underneath the sarcolemma. There does not appear to be any particular localization concerning sarcomeres. In the HFD group, the mitochondrial ultrastructure (M) displayed clear signs of morphological abnormalities, including the destruction of the mitochondrial membrane, the disorganization of the cristae, which caused them to appear significantly deformed, and the loss of their cristae (Figure: 1). Degeneration can be seen in both the nuclear membrane and the nucleolus (Figure: 2). In addition, perinuclear edema could be seen in the TEM micrographs of the myocytes. There is a reduction in the number of myofibrils (Mf), which exhibit characteristics consistent with degeneration and sarcomere disarray (Z-line). The TEM micrographs showed a significant decrease in muscle fiber mass, which indicated a significant degeneration of muscle fibers. On the other hand, treatment of the HFD group at a dose of 200 mg/kg (HFD + 200 mg/kg) showed a normal arrangement of muscle fibers, a minor enlargement of myofibrillar structure with striations, and the majority of the mitochondria still stay intact (Figure:3). The TEM micrograph of the nucleus has an uneven form but maintains its integrity (Figure:2).

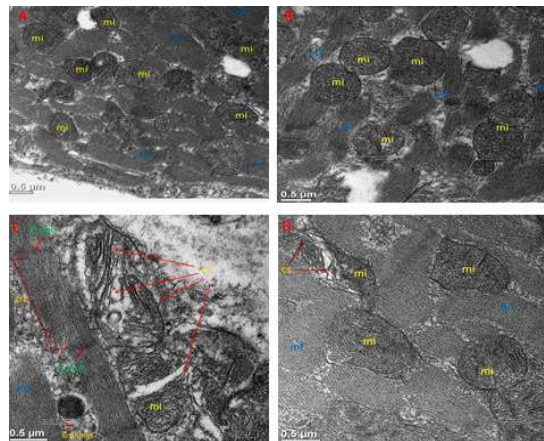


Figure 1: Representative electron micrographs depict ultrastructural characteristics of mitochondria in cardiomyocytes in experimental groups containing normal (a), 200mg/kg (b), HFD (c) HFD + 200 mg/kg of *Apium graveolens*(d). N: nucleus; Mi: mitochondria; Arrow: Z-line of myofibrils, Cs: Cistenea, Sr: Sarcomere.

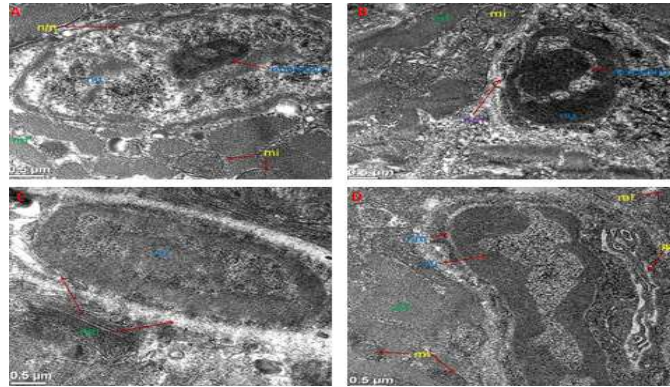


Figure 2: Representative electron micrographs depict ultrastructural characteristics of the Nucleus in cardiomyocytes in experimental groups containing normal (a), 200mg/kg (b), HFD (c) HFD + 200 mg/kg of *Apium graveolens*(d). N: nucleus; Mi: mitochondria; mf: myofibrils, ga: Golgi apparatus.

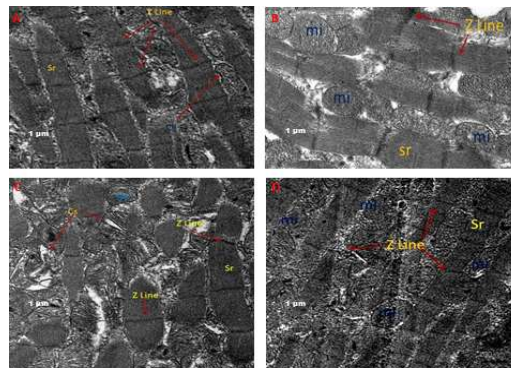


Figure 3: Representative electron micrographs depict ultrastructural characteristics of myofibrils in cardiomyocytes in experimental groups containing normal (a), 200mg/kg (b), HFD (c) HFD + 200 mg/kg of *Apium graveolens*(d). Mi: mitochondria; Arrow: Z-line of myofibrils, Cs: Cisteneae, Sr: Sarcomere.

IV. DISCUSSION

Evidence from our study suggests that treatment with a high-fat diet (HFD) for 12 weeks leads to cardiac dysfunction, including the disruption of myofibrils and cristae, as well as some measurable mitochondrial degradation and loss of sarcomere integrity. In addition, the mitochondria in the myocardium showed broad abnormalities, including morphological alterations such as reduced size, decreased density, and disruption of inner-membrane cristae, as well as functional damage, which manifested as a poorer efficiency of energy generation. These changes were accompanied by a disruption of the inner-membrane cristae. The transmission electron micrograph (TEM) of cardiomyocytes from HFD-treated mice also showed a considerable loss in muscle fiber mass, severe degeneration of muscle fibers, and breakdown of the nuclear membrane. This occurred in addition to an increase in oxidative stress in the cardiomyocytes and the breakdown of the cristae of the majority of the mitochondria. In the HFD-treated mice, damage may be seen in both the nuclear membrane

and the nucleolus. This demonstrates without a doubt that long-term feeding of mice on an HFD can result in cardiac damage. Despite decades of study, the mechanisms causing HFD-induced heart failure remain unclear. Several studies have found that oxidative stress, brought on by an HFD, is a key factor in the development of CVDs^{23,24}. Fatty acid accumulation is detrimental because it disrupts mitochondrial activity in cardiac and skeletal muscle cells. Mitochondrial dysfunction and structural impairment²⁵, decreased cardiac efficiency²⁶, and cardiomyopathy, in particular, due to lipid-induced apoptosis²⁷ are all linked to aberrant lipid metabolism. Cardiac tissue is vulnerable to oxidative injury due to its high oxidative capacity and its limited antioxidant enzyme composition²⁸. Over time, an HFD can cause cardiomyocytes to lose their structural integrity, which can disrupt cellular processes, make cells more vulnerable to stress, and ultimately lead to cell death, which may have long-lasting pathological consequences. The normal arrangement of muscle fibers (sarcomere), mitochondria, and nuclei after *Apium graveolens* extract (200 mg/kg b.w.) treatment, however, illustrates the extract's capacity to protect cardiomyocytes from the oxidative stress generated by HFD as shown in figures 1,2 and 3. The findings suggest that *Apium graveolens* may aid in the prevention of cardiac tissue from oxidative stress and its related diseases induced by a high-fat diet.

V. CONCLUSION

An ultrastructural examination concluded that HFD was the root cause of the damage that was done to the cardiomyocytes. Despite this, treatment with aqueous extracts of *Apium graveolens* has been shown to minimize damage to the heart, giving more evidence that the plant exhibits cardio-protective effects. In light of this, consumption of *Apium graveolens*, more commonly known as celery, may, as a result, assist in the prevention of heat-related illnesses.

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