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THE POTENTIAL OF MORINGA OLEIFERA ON MITOCHONDRIAL BIOGENESIS THROUGH INCREASES TOTAL OXPHOS UNITS EXPRESSION

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Abstract

Moringa oleifera grows in the tropical and subtropical country. Some benefit can get from moringa included to rehabilitation of malnutrition, and to increase quality of lactating mothers. However, effect *M. oleifera* on skeletal muscle especially to mitochondrial biogenesis remains unknown. This current experiment to determine the effect on moringa itself and combination with endurance exercise (eTR) on skeletal muscle. Sample are adults male Wistar rats which divided into exercise and non-exercise groups. The dose was 37 mg/ 200-gram body weight of extract of *moringa* leaf dissolved in water were consumed per-oral for 28 days and thirty minutes of treadmill/day for 24-day exercise were used to determine the effect of *M. oleifea* treatment. Western blotting (WB) was used to detect protein expression. These current results showed that treatment of extract of *M. oleifera* leaf mixed with endurance exercise enhance the protein OXPHOS sub-unit expression in the gastrocnemius (Gas) muscles. Conclusion this current result indicated that moringa oleifera blend with exercise potentially to increase mitochondrial biogenesis on skeletal muscle.

Keywords: *Moringa oleifera*, Mitochondrial biogenesis, Skeletal muscle.

1. Introduction

Human body have ability to respond to the physiological stressor included exercise. One of the kinds of response is increasing of mitochondria based on exercise need the cell organ to produce Adenosine Tri Phosphate (ATP) for exercise. On the other hand, mitochondria muscle has to succeeded to keep balance between un-healthy and healthy mitochondria to produce healthy muscle. This turnover prevents imbalance cell metabolism that can increase the prevalence of increasing of body weight, blood glucose, cardiovascular diseases and aging [1]. Several studies showed that such endurance exercise potentially increases metabolism in the skeletal muscle such as glucose transporter 4 (GLUT4) expression and also mitochondrial biogenesis. However, the mechanism of how mitochondria adaptation can be elucidated [2]. Mitochondrial biogenesis process produced enhance of numbers and quality of mitochondria because influenced of the activity especially exercise in order to produce more energy. Cell environment study suggest that to increase mitochondrial biogenesis need the master regulation which activated these processes optimally and effectively.

Based on the many previous study the Peroxisome proliferator-activated receptor-gamma co-activator 1 alpha (PGC-1α) is seemed look like to be the master regulator of mitochondrial biogenesis. Addition, PGC-1α will communication with nuclear respiratory factor 1 and nuclear respiratory factor 2 (NRF1 and 2) to activating mitochondrial transcription factor A (Tfam). Furthermore, Tfam have responsible duty to transcribe nuclear-encoded mitochondrial genes, to make ensure mitochondrial DNA (mtDNA) working through the process copied of RNA from DNA and produce and transfer protein. Previous study declared that acute endurance physical activity included sport, ability to regulated or enhance mitochondrial biogenesis via its ability to increase the PGC-1α mRNA and mitochondrial marker protein expression levels [3].

This result indicated that the regulator for metabolic adaptations when exercise potentially do by PGC-1α. ATP supply which needed by human cell body when their exercise is important situation in order to prevent exhaustion come to fast because depleted of ATP. On the other side if this condition cannot handle by mitochondrial to produce more energy on the next step the exercise must be terminated, and muscle cell will be death. In order to prevent this condition, exercise have to success to induction protein which have ability to controlling the transcriptional regulation of the Oxidative Phosphorylation (OXPHOS) system and the transcription, replication of mitochondrial DNA [4, 5].

Moringa oleifera, part of the Moringaceae family, is potential and effective in solving malnutrition problem. *M. oleifera* has variety of essential phytochemicals abundantly, especially in its leaves. One of the study showed that many beneficial effect of moringa because its high content on micro-nutrient and macro nutrient, for example high of protein, high of energy metabolic for macro-nutrient and high level of vitamins (A, B, C, and E), minerals like calcium, iron, selenium, zinc, and β-carotene and fats content and contain the 10 amino acids essential to human [6].

The previous study showed results that *M. oleifera* is beneficial for athletes to increase their performance. Previous study showed that moringa has ability to be an antioxidant and anti-fatigue for the swimming endurance athletes because its potentially effect to improve storing energy in the body and also its antioxidant

high level content have beneficial effect to decrease lactate acid level on the tissue [7].

Moringa oleifera effort to enhance carbohydrate metabolism can involve on the preventing and rebuilding integrity and physiological function from pancreatic β-cells in order to stimulated insulin activity to utilization of blood sugar and protecting increasing of blood sugar [8]. Furthermore, it has also been reported that the *M. oleifera* improves adipocyte functionality during adipogenesis and regulates the sirtuin 1 (SIRT-1) and PGC-1α pathway. Furthermore, this study explained that SIRT-1, UCP1 and PGC-1α needed by lipid metabolism especially to in thermogenesis modulating process [9].

Parallel with this study, other experiments also showed that complication on diabetic patients because of mitochondrial proton leak (UCP activity) which happen on the cell environment [10], not only diabetic but also in cardiovascular diseases [11]. These results suggested that *M. oleifera* involved in intracellular fashion through lot of mitochondrial metabolic pathways. However, the underlying mechanism of whether *M. oleifera* increasing mitochondria biogenesis on skeletal muscle still remains unknown. Based on that previous result, we interested to investigating the effect of M. oleifera on mitochondrial biogenesis protein marker on skeletal muscle included OXPHOS (Oxidation Phosphorylation) mitochondrial subunit.

2. Material and Methods

2.1. Animals

Twenty-four Wistar rat's male at 8 weeks of age were used for investigating the effect of *M. oleifera* on skeletal muscle. The rats were separated to four groups, namely control (without *M. oleifera*), Moringa (with *M. Oleifera*), eTr (endurance training without *M. oleifera*), and eTr + Moringa (endurance training with *M. oleifera*) groups. The rats were placed at rooms with a temperature around 22-24^{o}C, under a light-dark balance cycle, and given pellet and water ad libitum. The wistar rats were fed with the standard chow diet that provides 65.5% carbohydrate, 25% protein and 7% fat and around 5 % of micronutrient and mineral. Extract *M. oleifera* leaf was given orally with doses of 37 mg/200-gram of body weight rats. All procedures were followed the guidelines for our university animal laboratory guide and care protocol.

2.2. Treadmill training protocol

All rats were habituated to the environment for two weeks and continued with habituation on the treadmill with duration two weeks. For the training sessions, the treadmill was set to high intensity training because of that we used 30 m/minute. All training sessions were performed during the light cycle between 8 and 12 AM. Non-training rats served as the sedentary control (n=6). On the harvest muscle day, the rats were anesthetized use 5% inhaled isoflurane until one minute after the breathing stopped. Harvest time was done immediately after the last training sessions in each group.

2.3. Western blotting

The western blotting running based on our laboratory methods previously [12]. Briefly, we harvest muscle from the rats and lysed them in lysis buffers mixed with protease inhibitors. Protein sample were centrifuged and the combined with the sample buffers containing beta-mercaptoetanol and following by heat denaturation on temperature 95^0C for five minutes. Equal protein amounts of samples were undergoing into gel of Sodium Dodecyl Sulfate-polyacrylamide electrophoresis for Total OXPHOS subunit and loading control beta actin antibody primer for 60 minutes at 120V and then were transferred onto a nitrocellulose membrane (ATTO, Japan) for one hours at 200 mA at room temperature.

The membrane put in Tris-buffered saline (TBS) buffer pH 7.4 and added by Tween-20 and wait for 10 minute at room temperature in order to incubated membrane before applying of blocking buffer. Next step, the membrane was sitting on blocking buffer with concentration is 4% at room temperature for 1 hour. After 1 hour, the membrane was prepared to incubated with mouse monoclonal antibody to Total OXPHOS Rodent WB antibody Cocktail (#: MS604-G2750) and for loading control we use Mouse polyclonal antibody to β-actin (#: ab8316-GR120178-2) and antibodies incubated at 4°C for overnight and after that membrane rinsed by TBS-T for 3 times with duration 10 minutes every rinsed. On the next step, membrane have already to reacted with the second antibody and we use Anti-rabbit or antimouse IgG, for secondary antibody and incubation for 1 hours on the room temperature. In order to read a protein band expression, we were detected by Li-cor chemiluminescence (Biosciences, USA) with added firstly the ECL detection system (GE, USA) to enhance the signal. The signal level evaluated using Image J.

2.4. Statistical analysis

The data was showed as the mean ± standard deviation (SD) and analysed by One-way analysis of variance (ANOVA) in order to know effect of *M. Oleifera* on skeletal muscle. The post hoc Tukey-Kramer test was used to evaluated the significantly between control, only *M. oleifera*, endurance exercise (eTR), and endurance exercise with *M. oleifera with a* $p<0,05$.

3. Results and Discussion

3.1. *Moringa oleifera* increases food consumption intake but not increases body weight

This current result showed that food intake on all groups increased between the first week and after the fourth week (Fig. 1). Indeed, some studies showed that *M. oleifera* potentially increases food consumption of the animal because *M. oliefera* to animals is used to increase health, egg quality, performance abundantly [13]. On the other way, our result showed that even though *M. oleifera* increase food intake, it seems to not increase body weight on a group, only *moringa* and *moringa* combination with endurance exercise (Fig. 2). It is presumably can be assumed that *M. oleifera* has the ability to improve glucose transport. The previous study showed this evidence, which showed that *M. oleifera* decrease body weight, hepatic gluconeogenesis and increase insulin activity in animal [14].

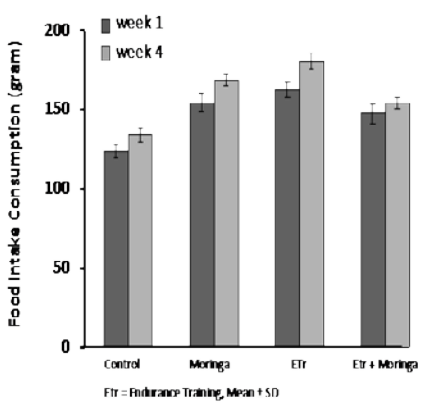

Fig. 1. The food intake of the rats after the first-week and fourth-week treatments.

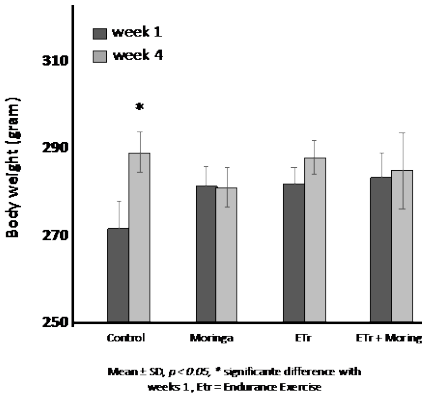

Fig. 2. The body weight of rats after the first-week and fourth-week treatments.

3.2. *Moringa* Oleifera increases mitochondrial complex subunits expression

Mitochondria is the cell organ which have responsibility to produced energy especially when exercise. Nutrient be the external source which consume by the human to get the energy have to involved on the metabolic pathway process. In order to oxidizing the nutrient, the enzyme oxidative phosphorylation (OXPHOS) or electron transport linked phosphorylation used by cell to release energy and continuing to produce ATP. These activities take place inside mitochondria, which produces energy, and these conditions are known as electron transport chain. The electron transport chain has four complexes which used to transfer of electrons in order to obtained energy. In this complex the pumping proton is happened from mitochondrial matrix into the mitochondrial membrane space because the electrochemical proton gradient situation on mitochondria cell. On the Krebs Cycle, electron carrier nicotinamide adenine dinucleotide (NADH) sending the electron and accepted by very large Complex I (NADH coenzyme Q reductase). Furthermore, the electron passes them onto a series of iron sulphur cluster and end up on a carrier molecule coenzyme Q (ubiquinone) / complex II. In this complex two hydrogen uptakes ions and from here, electron moving to complex III (cytochrome bc_1 complex). Complex II, get the electron from Flavin adenine dinucleotide (FADH2) molecules and in this section, protons do not across the membrane. Electron from complex III, finally passed to cytochrome c (Cyt c) and continuing flow to Complex IV (cytochrome c oxidase), in this complex oxygen molecular to water reduce because electron and ion hydrogen uses by Complex IV. Complex V which known as ATP Synthase now role be the ion channel which have ability to refluxing the proton to go back into mitochondrial matrix and this process will release the energy.

In order to investigated effect of *M. oleifera* on mitochondria biogenesis on skeletal muscle, this current result interested to examined the effect of *M. oleifera* on mitochondrial complex subunits expression. This result showed that *M. oleifera* itself increase complex I, and combination of *M.oleifera* and eTr showed that

moringa have adaptive effect of eTR to increases complex I (Fig. 3). Previous study shown the result that physical activity increases mitochondrial content and our recent result also in line with the previous study which showed that *M. oleifera* increased complex I activity [15].

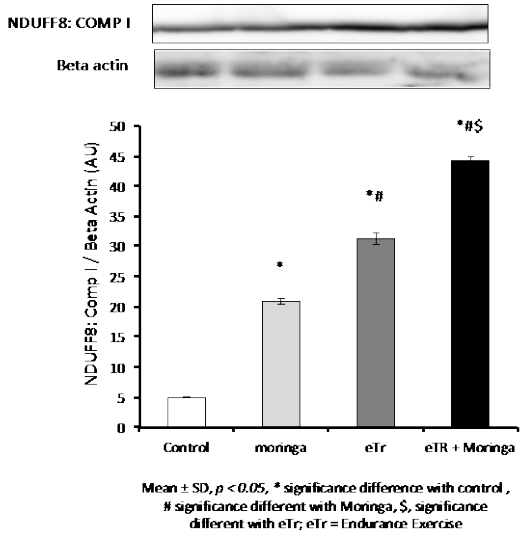

Fig. 3. *M.Oleifera* increases complex I total OXPHOS subunits expression.

This result showed that *M. oleifera* itself is able to significantly increase mitochondrial complex II protein expression of the experimental groups than the control group (Fig. 4). Furthermore, on the group eTR, there are no significant differences between eTR and combination eTR and *M. oleifera*. This result indicated that moringa itself has the ability to increases the complex II mitochondrial unit.

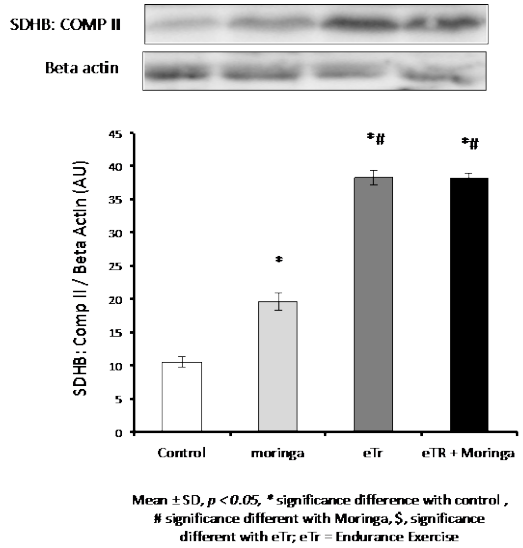

Fig. 4. *M.Oleifera* increases complex II total OXPHOS subunits expression.

Parallel with the result on complex I and II, our current result also showed that *M. oleifera* itself increase protein expression on complex III and V (Figs. 5 and 6). In addition, when combined with eTR, *M. oleifera* has a potential adaptive effect of eTR to increase complex III and V.

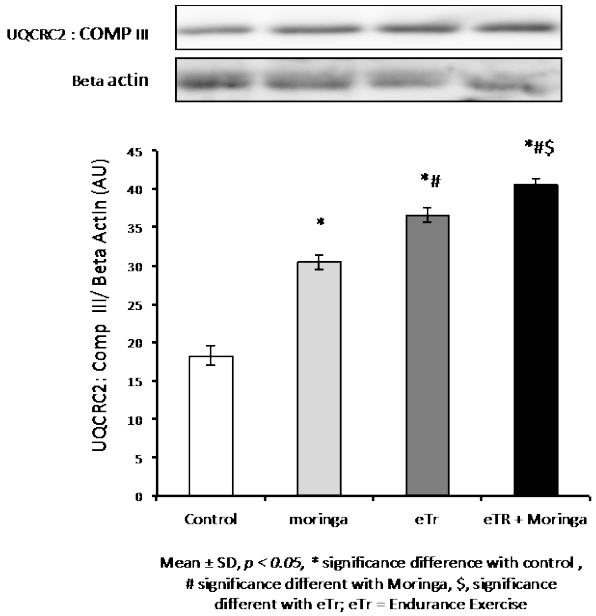

Fig. 5. *M.Oleifera* increases complex III total OXPHOS subunits expression.

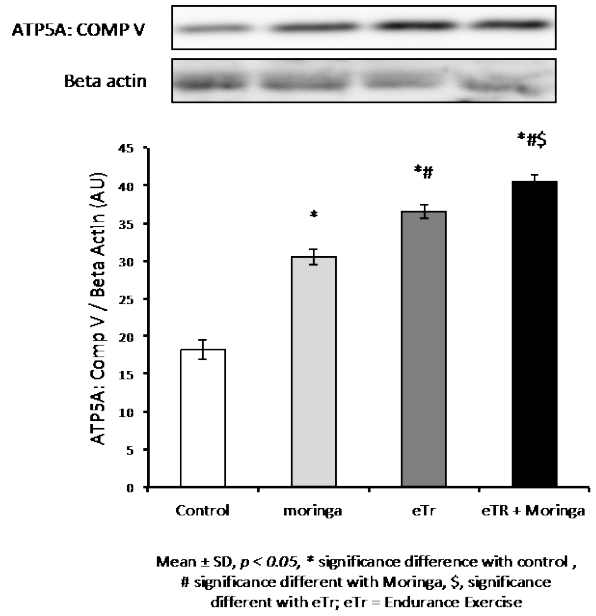

Fig. 6. *M.Oleifera* increases complex V total OXPHOS subunits expression.

4. Conclusions

This current study investigation has been made to determined effects of *M. oleifera* on skeletal muscle, especially on mitochondrial complex subunits protein expression. Furthermore, this current result revealed the determined effect of *M. oleifera* when combined with eTR (endurance exercise). The result indicates that *M. oleifera* potentially increases mitochondrial biogenesis based on our result that moringa increase mitochondrial subunit complex I, II, III and V protein expression. Furthermore, this current result showed that *M. oleifera* adaptive effect of eTR (endurance exercise) to increase mitochondrial complex subunit complex I, III and V.

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