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# EFFECT OF HIGH-DOSE *MORINDA CITRIFOLIA* (MC) LEAF ETHANOLIC EXTRACT ON SWIMMING ENDURANCE IN FEMALE SPRAGUE DAWLEY RATS

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## Abstract

*Morinda citrifolia* (MC) is a herb that is famous for its various medicinal properties. However, there has been little research on its effect on increased stamina and physical fatigue reduction. The purpose of this study was to determine the effect of high-dose oral intake of 50% ethanol MC leaf extract on exercise performance and physical fatigue in an animal model. Thirty female Sprague-Dawley rats were divided into five groups; Control (C); Sedentary (S); Exercise (EX); MC leaf extract 1000 mg/kg/day (MC); MC leaf extract 1000 mg/kg/day with Exercise (MC+EX). The endurance test showed a significant difference in endurance time between EX and MC groups, whereby intake of MC leaf extract without daily exercise lowered endurance level by 68.75%. These findings correlated with serum fatigue indicator analysis whereby significant increases in CK levels of MC group, and LDH levels in MC and MC+EX groups were noted when compared to other groups. Electron microscopy analysis on cardiac muscle tissue showed ultrastructural injuries in muscle fibers of the EX, MC and MC+EX groups whereas injuries to the gastrocnemius myofibres were not so prominent. Based on the study findings, it can be concluded that the consumption of 1000 mg/kg/day 50% ethanol MC extract disturbed internal physiology and resulted in injury to the muscle tissues thus lowering rats endurance capability. This shows that a high dose has unfavourable effects on an animal model while a lower dose may be more appropriate.

*Keywords*: *Morinda citrifolia*, exercise, endurance test, fatigue, swimming

## Abstrak

*Morinda citrifolia* (MC) ialah herba yang terkenal dengan pelbagai khasiat perubatan. Walau bagaimanapun, kurang kajian mengenai kesannya terhadap peningkatan stamina dan pengurangan keletihan fizikal. Tujuan kajian ini adalah untuk menentukan kesan pengambilan oral dos tinggi ekstrak 50% etanol daun MC ke atas prestasi senaman dan keletihan fizikal pada model haiwan. Tiga puluh ekor tikus Sprague-Dawley betina dibahagikan kepada lima kumpulan, iaitu kumpulan Kawalan (C); Sedentari (S); Senaman (EX); Ekstrak daun MC 1000 mg/kg/hari (MC); Senaman dan ekstrak daun MC 1000 mg/kg/hari (MC+EX). Keputusan ujian ketahanan menunjukkan perbezaan signifikan di antara kumpulan EX dan MC, di mana pengambilan ekstrak daun MC tanpa senaman menurunkan tahap ketahanan sebanyak 68.75%. Keputusan ini selaras dengan analisa penanda keletihan pada serum di mana terdapat peningkatan signifikan CK pada kumpulan MC, dan peningkatan signifikan LDH pada kumpulan MC dan MC+EX berbanding dengan kumpulan lain. Analisa mikroskop elektron pada tisu otot jantung menunjukkan kecederaan miofiber dalam kumpulan EX, MC dan MC+EX manakala tanda kecederaan pada otot gastroknemius tidak begitu ketara. Berdasarkan hasil kajian, dapat disimpulkan bahawa pengambilan ekstrak 50% etanol daun MC 1000 mg/kg/hari mengganggu fisiologi dalaman dan mengakibatkan kecederaan pada tisu otot justeru menurunkan ketahanan tikus. Ini menunjukkan bahawa dos yang tinggi mempunyai kesan buruk pada model haiwan sementara dos yang lebih rendah mungkin lebih bersesuaian.

*Kata kunci*: *Morinda citrifolia*, senaman, ujian ketahanan, keletihan, renang

## 1.0 INTRODUCTION

Low energy and fatigue negatively affects mental health and quality of life. Estimates of fatigue among the general public range from 2.72% to 75.7% depending on fatigue levels [1]. In general, women are more likely experience and to complain about fatigue compared to men [2], especially among those who are young and have high levels of education [3]. Fatigue is also the main symptom for several mental illnesses, including depression, anxiety and insomnia [4-6].

Fatigue is defined as difficulty to initiate and maintain voluntary activity accompanied by deterioration of physical activity performance [7]. The main cause of fatigue is difficult to determine, as there are many factors influencing fatigue. High-intensity physical activity reduces glycogen storage in the liver and skeletal muscle. It also increases the accumulation of metabolic products, such as lactic acid, inorganic phosphate and ammonia in the body [8]. High-intensity physical activity can also lead to the accumulation of reactive oxygen species (ROS), which causes oxidative stress in various organs in the body through lipid-induced peroxidation and mitochondrial damage [9]. Fatigue is often associated with the deterioration of autonomic function, neuroendocrine function, cognitive function and immune function, leading to an impairment that affects the control of immune-neuroendocrine interactions [10]. Thus, recovery from fatigue requires the removal of the metabolic products accumulated during strenuous physical activity. Hence, studies are focusing on natural products with high antioxidant capabilities to improve physical conditions, reduce fatigue and enhance stamina [11].

*Morinda citrifolia* (MC) or its local name "noni" or "mengkudu" is a shrub native to Asia and Polynesia. Its roots, fruits and leaves have been a part of traditional herbal medicine to treat common diseases and to maintain overall health. Recent natural product research has been aimed towards MC, amongst other herbs, due to the reason it is proved to have high therapeutic activities such as anti-cancer, anti-oxidant, anti-bacterial, antidyslipidemia and analgesic [12-16]. MC is also capable of eliminating free radicals, inhibiting low-density lipoprotein oxidation, controlling cholesterol, stimulating the immune system, controlling cell function and cleaning the blood [12, 17-19]. The MC leaf itself specifically, has been proven to have high antioxidant, anti-inflammatory and wound healing properties itself [20-22]. MC leaves are known for its high antioxidant activity, and it has been proven to be safe in acute, subacute, and subcritical oral toxicity tests on mice [23]. Preliminary studies have shown that a high dose of aqueous MC leaf extract is beneficial for the cardiovascular system as it lowers total cholesterol concentration and increases antioxidant activity within an ovariectomised atherosclerosis rat model (unpublished data). Therefore, using the same high dose of MC leaf extract and with regards to the lack of studies pertaining to the ergogenic properties of the MC leaf, we aim to study the ability of a high dose of MC leaf extract to increase stamina and reduce fatigue markers in an animal model.

## 2.0 METHODOLOGY

### 2.1 Plant Material and Extraction Method

MC leaves were obtained from the Institute of Bioscience, Universiti Putra Malaysia (Voucher number: SK 2877/15). 3.47kg of fresh MC leaves were dried at 40˚C for 3 days using a drying oven. 1.81kg of dried ground MC leaves underwent soxhlet extraction using 15L of 50% ethanol for 18 hours at 45-55˚C. The resulting yield was 406.5g (22.46%).

### 2.2 Study Design

A total of 30 female Sprague-Dawley rats (200-250 grams) were purchased from Universiti Kebangsaan Malaysia Animal House. Ethical approval from the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) was obtained prior to the study (UKMAEC No. FP/ANAT/2015/FAIZAH/29-JULY/697-AUG.-2015-AUG.-2016). One week of acclimatisation to the environment and diet was allowed before the experiment began. All animals were fed a standard rat chow diet and distilled water ad libitum and housed at room temperature (23 ± 1 °C) and 50%–60% humidity with a 12-h light/dark cycle (lights on from 06:00 to 18:00). The rats were equally divided into five groups (n = 6 per group); control (C); sedentary control (S); exercise training (EX); 1000 mg/kg/day MC leaf extract (MC); exercise training and 1000 mg/kg/day MC leaf extract (MC+EX). MC leaf extract was administered orally for 8 weeks. After the 8 weeks period, groups S, EX, MC and MC+EX were subjected to an exhaustive endurance test. Group C stands as the control group and was not subjected to the exhaustive endurance test. Groups S and EX acted as vehicle controls and received normal saline solution equivalent to individual body weight.

### 2.3 Swimming Exercise Training and Endurance Test

Exercise-training groups were made to swim for 60 min/day, five days/week for eight weeks [24]. All exercises were performed at the same time of day for each group and were continuously supervised. The endurance test involved forcing the rats to swim with attached weight loads corresponding to 10% of body weight to the rats tail. The endurance of each rat was recorded as the time from the beginning to exhaustion, which was determined by observing loss of coordinated movements and failure to return to the surface within 10 s [25]. Both exercise and

endurance test was performed in a cylindrical plastic container that is 70 cm in diameter and 120 cm in height, filled to a depth of 90 cm with water, and maintained at a temperature between 30°C and 33°C.

### 2.4 Determination of Biochemical Parameters

Blood glucose, creatine kinase (CK) and lactate dehyrgoenase (LDH) were analysed using commercial ELISA kits (Abcam), according to the manufacturer's instructions.

### 2.5 Histomorphometry Analysis

Skeletal muscle (gastrocnemius) and cardiac tissues were fixed overnight in 4% formaldehyde at room temperature. The muscle tissue samples were processed in paraffin wax and sectioned at 5 μm for hematoxylin and eosin (H&E) staining. Images were captured with Leica DMRXA2 microscope and VideoTest-Morphology 5.2 software (Digital Imaging Systems) was used for analysis.

### 2.6 Transmission Electron Microscopy (TEM)

Ultrathin sections of the heart and skeletal muscles were processed and prepared accordingly. The inspection was carried out using a transmission electron microscope (Philips HMG 400). The specimens were examined and micrographs were then taken for qualitative description.

### 2.7 Statistical Analysis

All data are expressed as the mean ± standard error of mean (SEM). Statistical differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey HSD post hoc test. Statistical significance was set at $p < 0.05$. All analyses were performed using with SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

## 3.0 RESULTS AND DISCUSSION

### 3.1 Effects of MC Leaf Extract on Swimming Endurance Test

The swimming endurance test showed a significant difference in swimming duration between groups MC and EX, while no significant differences in swimming duration were seen between groups S, EX and MC+EX (Figure 1). The average swimming duration for group EX was 32 minutes while the duration for group MC was 10 minutes. This showed that treatment of MC alone without exercise decreased endurance by 68.75% when compared to group EX, and indicated that group MC has less stamina and a lower endurance capacity when compared to group EX. The results also signalled that the supplementation of a high dose MC leaf extract without daily exercise reduces stamina and accelerates fatigue as groups EX and MC+EX did not show significant differences in the endurance test. The insignificant differences in swimming duration between groups S, EX and MC+EX may suggest that the 8-week swimming exercise regiment underwent by the female rats is not sufficient in building up stamina for the swimming endurance test. Matsumoto *et al*. (1996) has stated that rat models has several disadvantages when used in endurance studies whilst mice models would be a better option [26]. Nevertheless, the significant reduction in endurance capability as shown between groups EX and MC, raises alarms regarding the safety of high doses of MC leaf extract.

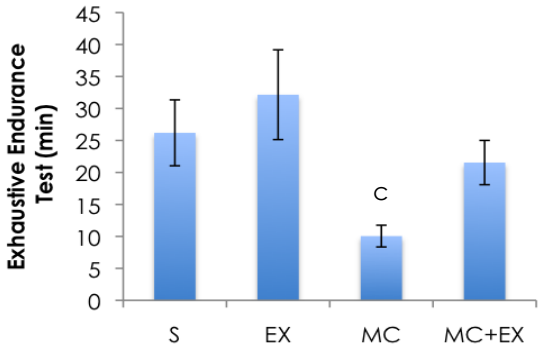


**Figure 1** Effect of MC leaf extract supplementation on swimming endurance test. P ≤ 0.05, c: vs EX

The findings of this study differed from the study of Mohamad Shalan *et al*. (2016) as they discovered that MC leaf water extract was able to increase swimming endurance capacity up to three times when compared to the control group. In their study, they looked into the effect of two different doses of MC leaf water extracts, 200 mg/kg/day and 400 mg/kg/day, on female mice over a period of 4 weeks. An endurance test was performed at the end of each week with a weight of 5% body weight attached on the mouse's tail. They found that the endurance capacity increased with every week of MC leaf water extract consumption, with the lower dose of 200 mg/kg/day having a better effect than 400 mg/kg/day [27].

### 3.2 Effects of MC Leaves Extract on Blood Glucose, Creatine Kinase (CK) and Lactate Dehydrogenase (LDH)

The glucose level of groups S, EX, MC and MC+EX were significantly higher in comparison to group C as a result of the strenuous endurance test, whilst serum LDH and CK levels of groups MC and MC+EX were significantly high when compared to groups C, S and EX (Figure 2). During the strenuous endurance swimming test, the rats body converts glycogen into glucose to meet the demand of the muscles [28, 29]. Thus there is an increase in serum glucose levels in the

rats subjected to the swimming endurance test. Biochemical variables such as glucose, LDH and CK are important markers for muscle fatigue after physical activity [28, 30, 31]. Increased activity of LDH and CK in serum after exercise is associated with changes in the integrity of cell membrane and muscle fibers damage. In this study, the fatigue indicators correlated with the result of the swimming endurance test. Group MC with the lowest endurance time has a significantly high level of CK and LDH. This indicated that the high dose of 1000 mg/kg/day 50% ethanol MC leaf extract induced tissue damage when consumed as a result of probable cellular toxicity [32].

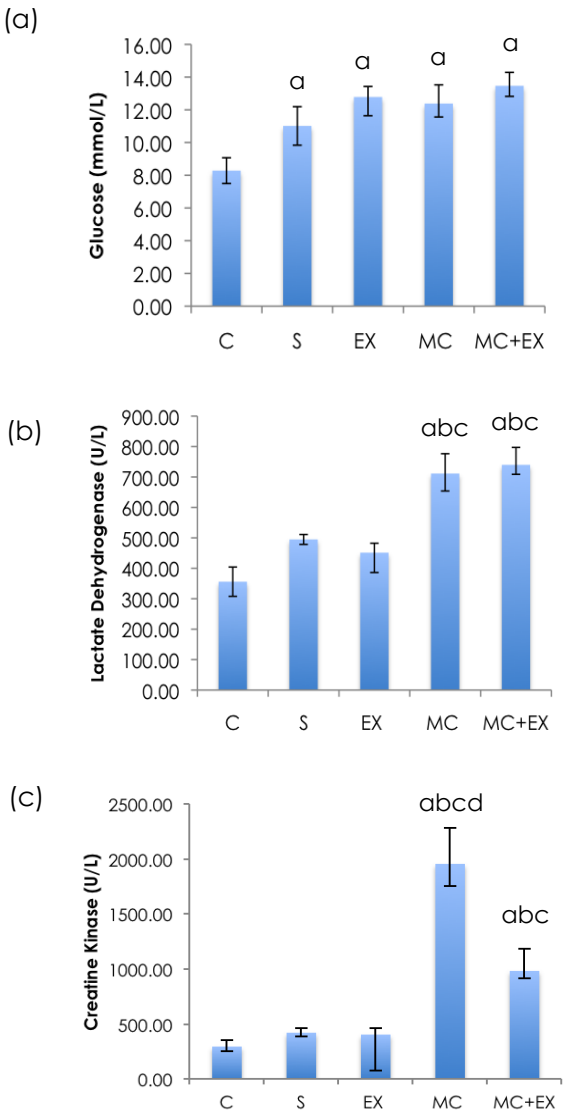


**Figure 2** Effect of MC leaf extract supplementation on (a) Blood Glucose (b) Lactate Dehydrogenase (c) Creatine Kinase. P ≤ 0.05, a: vs C, b: vs S, c: vs EX, d: vs MC+EX

### 3.3 Effects of MC Leaves Extract on Myofibre Histomorphometry and Ultrastructure

Histomorphometry analyses of the skeletal myofibres showed a significant increase in myofibre diameter in group EX and a significant increase in nucleus size in groups MC and MC+EX when compared to group C (Table 1 (a)).

The significant increase in skeletal myofibre diameter in group EX was the result of muscle hypertrophy due to swimming exercises performed on a daily basis. As swimming possess the features of strength and endurance exercises, it was able to build endurance due to repeated contractions of the gastrocnemius muscle and able to build strength as the gastrocnemius muscle receives external resistance from the surrounding water. Hence, muscle hypertrophy was seen in the group that performs daily exercise. This is in line with Paul *et al*. (2002) as they found that exercise leads to hypertrophy in the anterior gracilis, posterior gracilis and soleus muscles [33]. Myofibre hypertrophy, however, does not occur in group MC+EX. This may be due to cell damage indicated by the increased fatigue biomarkers. Groups MC and MC+EX instead exhibited an increase in nucleus size. This may have occurred due to cell division in the surrounding myofibres in response to the probable cellular damage in its surrounding [34].

**Table 1** (a) Histomorphometry of skeletal myofibre. (b) Histomorphometry of cardiomyofibre. P ≤ 0.05, * : vs C

(a)

| Group | Myofibre Diameter (μm) | Nucleus Size (μm²) | Number of Nucleus (per 10,000μm²) |
|---|---|---|---|
| C | 51.48 ± 0.55 | 8.30 ± 0.57 | 14.50 ± 1.88 |
| S | 52.17 ± 1.34 | 11.38 ± 0.50 | 14.33 ± 1.36 |
| EX | 58.32 ± 2.42* | 10.97 ± 0.58 | 12.5 ± 1.23 |
| MC | 55.86 ± 1.78 | 12.32 ± 1.34* | 9.83 ± 1.14 |
| MC+EX | 53.75 ± 0.72 | 14.54 ± 0.94* | 11.83 ± 2.50 |

(b)

| Group | Myofibre Diameter (μm) | Nucleus Size (μm²) | Number of Nucleus (per 10,000μm²) |
|---|---|---|---|
| C | 15.49 ± 0.62 | 27.56 ± 1.97 | 27.00 ± 3.10 |
| S | 16.89 ± 1.00 | 26.67 ± 2.38 | 34.00 ± 3.92 |
| EX | 17.22 ± 0.88 | 27.47 ± 4.47 | 30.50± 4.81 |
| MC | 18.29 ± 1.36 | 19.67 ± 1.27 | 30.67 ± 3.22 |
| MC+EX | 17.31 ± 0.30 | 29.06 ± 2.36 | 24.33 ± 2.14 |

Histological analyses of cardiomyofibre between all groups showed no significant difference in diameter, nucleus size and nucleus number (Table 1(b)). This shows that the exercise regiment subjected to the rats is incapable in inducing cardiac muscle hypertrophy. Several factors such as duration and frequency of the exercise regiment needs to be prolonged in order to obtain myocardial hypertrophy [35]. An increase in nucleus size and number is not seen as cardiac muscle cells lack proliferating satellite cells that respond to muscle damage as seen in skeletal muscle cells [36].

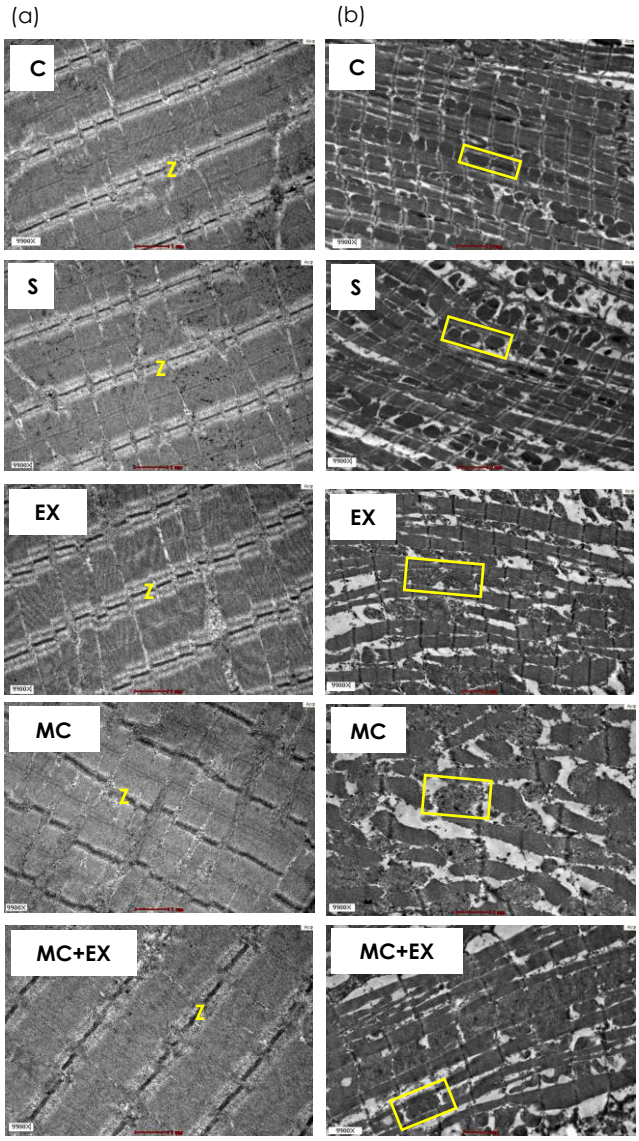


**Figure 3** (a) TEM images of skeletal myofibre (b) TEM images of cardiomyofibre (X9900) (Z-band (Z), Mitochondria (bounded by squares))

The TEM images of both cardiac and skeletal muscle exhibit ultrastructural damage, with the damage in cardiac myofibres being more prominent (Figure 3). Cardiac muscle TEM images of groups EX, MC and MC+EX showed characteristics of ultrastructural damage such as loss of normal structure, widening of the space between myofibrils and mitochondria swelling [37]. On the other hand, the damages at the skeletal myofibres were not that prominent and did not show signs of extreme Z-band disruption characterised by a wavy appearance running a zig-zag course and mild Z-band streaming [38].

Electron microscope scans show ultrastructural damage exhibited by cardiomyofibres to be more prominent than skeletal myofibres. Less prominent ultrastructural damage in the skeletal muscle is due to the presence of satellite cells that regenerate muscle fibres in response to muscle damage caused by intense exercise [39]. Cardiac muscle lack the presence of satellite cells thus exhibit prominent ultrastructural damage [36]. The electron microscope scans and the histomorphometry findings may present itself as having a noncorrelation as the ultrastructural damage seen may be too minute to give impact to the overall histomorphometry analysis.

### 3.4 General Discussion

Results from the swimming endurance test, serum fatigue indicators, histomorphometry and ultrastructural analysis suggested that the daily intake of a high dosage of 1000 mg/kg/day of 50% ethanolic MC leaf extract may have unfavourable properties leading to cellular damage. This however is in contradiction to a previous study that has claimed MC leaf extracts to be safe for oral consumption from concentrations ranging from 20 mg/kg to 2000 mg/kg in rodent models tested up to a period of 90 day [23]. The study tested MC leaves collected from different regions around the globe, namely French Polynesia and Cuba. Due to this, there is a possibility of diversity between the chemical compositions of the leaves, such as phytochemicals and antinutrients depending on the soil type of its place of origin. Marler *et al*. (2013) has documented the possibility of MC leaves being toxic and a health hazard as it is a member of the Rubiaceae, which holds the greatest number of aluminium accumulator species [40]. The study conducted by Mohamad Shalan *et al*. obtained leaves from the same source as the present study. They evaluated the toxicity of MC leaf and fruit aqueous extracts using 1-2 mg/ml of drinking water, which is equivalent to 100-200 mg/kg body weight, on a female mice model. They discovered that consumption of MC fruit aqueous extract at a high dose may cause weight loss, hepatotoxicity and mortality. On contrary, no unfavourable effects were seen by the consumption of both high and low doses of MC leaf aqueous extract. The same study also looked at the ergogenic affect of 200 mg/kg/day and 400 mg/kg/day aqueous MC leaf extract on female mice. They discovered that MC leaf aqueous extract, with the lower dose having a better effect, increased stamina and lessened fatigue by improving angiogenesis, mitochondrial biogenesis as well as antioxidant, anti-

inflammatory & stress responses. These findings however, does not disregard the possibility that a higher dose of 1000 mg/kg 50% ethanolic MC leaf extract, of having adverse effects thus negating the ergogenic properties displayed by its lower dose equivalent. Another probable factor to the unfavourable properties seen is that the 50% ethanolic extraction method may be the cause behind the myofiber damage. This suggestion however could be ignored as other studies using a 50% ethanolic extraction deem the method to be safe and suitable for oral administration [41, 42].

In summary, this study discovered that: (1) 50% ethanolic MC leaf extract supplementation reduced endurance exercise capacity without exercise training; (2) 50% ethanolic MC leaf extract supplementation increased accumulation of byproducts such as blood LDH and CK in both exercised and non-exercised groups; and (3) 50% ethanolic MC leaf extract supplementation induced prominent ultrastructural damage in cardiac muscle fibres. Due to this, a 1000 mg/kg/day of 50% ethanolic MC leaf extract supplementation may have unfavourable affects towards the body.

## 4.0 CONCLUSION

We discovered that the high dose of 1000 mg/kg/day over an 8 week period did not increase stamina and improve endurance. On the contrary, the high dose MC extract appeared to induce muscle damage thus decrease endurance capability, lower stamina and increase fatigue. This is supported by the increase in fatigue indicators as well as the damage in the ultrastructure. Further work using different doses of MC leaf extract as well as different extraction solvent needs to be conducted to confirm the possibility of MC leaf toxicity and ergogenic properties. This study gives an example of why further studies and stricter regulation should be placed on health risks associated with herbal medicine.

## Acknowledgement

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