EFFECTS OF *MORINDA CITRIFOLIA* LEAF ETHANOLIC EXTRACT ON EXERCISE ENDURANCE IN FEMALE SPRAGUE DAWLEY RATS

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ABSTRACT

Morinda citrifolia (MC) leaf is a well-known medicinal herb, however, there are insufficient studies regarding its effect on exercise performance and physical fatigue. This study showed the daily consumption of 1000mg/kg/day 50% ethanolic MC leaf extract reduced endurance capability and increases fatigue in a weight loaded swimming animal model. A total of 30 adult female Sprague-Dawley rats were equally divided into five groups; 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). Results revealed a significant difference in endurance time between groups EX and MC, whereby intake of MC leaf extract without daily exercise lowers endurance level by 68.75%. The endurance test findings correlated with serum fatigue indicator analysis whereby significant increases in CK levels of group MC, and LDH levels in groups MC and MC+EX were found when compared to other groups. In addition, ultrastructure analysis shows signs of cardiac tissue damage such as Z-band smearing and mitochondria bloating. In conclusion, the consumption of 1000mg/kg/day 50% ethanolic MC leaf extract appears to cause tissue damage. This suggests a probable toxic characteristic of the MC leaf that has yet to be explored. A lower dose may be more suitable for therapeutic purposes.

Keywords: *Morinda citrifolia*; exercise; endurance test; fatigue; swimming.

1. INTRODUCTION

Morinda citrifolia (MC) 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 [1]. The MC leaf itself specifically, has been proven to have high antioxidant, anti-inflammatory and wound healing properties itself [2]. However studies regarding its ergogenic properties are still insufficient.

Shalan et. al. claimed that 200mg/kg/day of aqueous MC leaf extract over 4 weeks could enhance performance by improving angiogenesis, mitochondrial biogenesis, antioxidant, anti-inflammatory and stress responses [3]. This research, however, attempts to study whether the claimed ergogenic properties of MC leaf extract still holds for a high dose 50% ethanolic extract of the MC leaf.

2. MATERIALS AND METHODS

2.1. Plant material, 50% ethanolic extraction

Morinda citrifolia (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 grounded MC leaf 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 acclimation 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. Groups S and EX acted as vehicle controls and received normal saline solution equivalent to individual body weight. After the 8 weeks period, all treatment groups were subjected to an exhaustive endurance test.

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. 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. 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 Blood Biochemical Variables

Blood creatine kinase, lactate dehydrogenase and glucose were analysed using commercial ELISA kits (Cayman Chemical Company), according to the manufacturer's instructions.

2.5. 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.

2.6. 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. RESULTS AND DISCUSSION

3.1. Effects of MC leaves extract on endurance test, blood glucose, creatine kinase (CK) and lactate dehydrogenase (LDH).

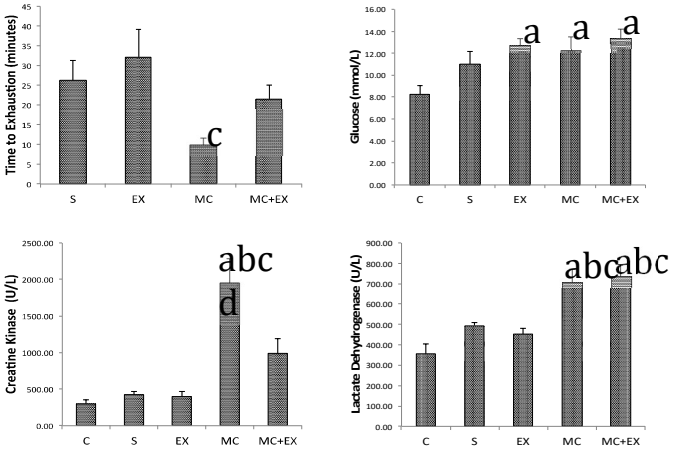

Figure 1. (a) Exhaustive Endurance Test (b) Blood Glucose (c) Blood Lactate Dehydrogenase (d) Blood Creatine Kinase. P≤0.05, a: vs C, b: vs S, c: vs EX, d: vs MC+EX.

A significant difference in time to exhaustion can be seen between the Exercise and MC groups. Treatment of MC alone without exercise decreases endurance by 68.75% when compared to group EX. There are no significant differences between groups S, EX and MC+EX. This indicates that 1000mg/kg/day 50% ethanolic MC leaf extracts decreases stamina and increases fatigue in contradiction to recent claims of the plants ergogenic properties. The fatigue indicators correlate with the endurance test [4]. Group MC with the lowest endurance time has a significantly high level of CK and LDH. This indicated that 1000mg/kg/day 50% ethanol MC leaf extract induced tissue damage when consumed as a result of probable cellular toxicity caused by the high dose of ethanol based MC leaf extract [5]. The glucose level of groups EX, MC and MC+EX were significantly higher in comparison to group C as a result of the strenuous endurance test. Regarding the main cause of toxicity, it can be argued that the main cause could be either the high dose of MC leaf extract or the 50% ethanol used for the extraction. This however is unlikely based on other studies that claim 50% ethanolic extractions are safe for consumption [6].

3.2. Effects of MC leaves extract on myofibre ultrastructure

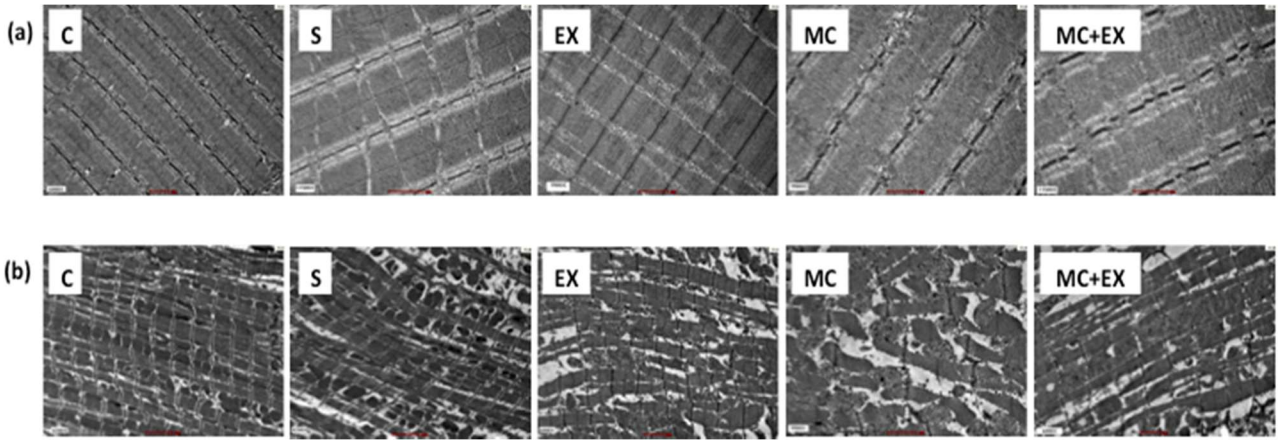

Figure 2. (a) TEM images of skeletal muscle (b) TEM images of cardiac muscle. (X9000)

The TEM images of both cardiac and skeletal muscle exhibit ultrastructural damage, with the damage in cardiac myofibres being more prominent. Cardiac muscle TEM images of groups

EX, MC and MC+EX show characteristics of ultrastructural damage such as loss of normal structure, myofibrillar disruption and mitochondria swelling [7]. On the other hand, the damages at the skeletal myofibres are not that prominent. However there are signs of Z-band disruption signaled by a wavy appearance running a zig-zag course and mild Z-band streaming [8]. Less prominent ultrastructural damage in the skeletal muscle is due to the presence of satellite cells that regenerate muscle fibres as a result of muscle degeneration caused by intense exercise. Cardiac muscle lack the presence of satellite cells thus exhibits clear and prominent ultrastructural damage.

CONCLUSION

We discovered that 1000mg/kg/day over an 8 week period does not increase stamina and improve endurance. On the contrary, the MC extract appears 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.

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