Malaysian Journal of Sport Science and Recreation
Vol. 18. No. 2. 244-254, 2022.
DOI: https://doi.org/10.24191/mjssr.v18i2.19576

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Received: 13 April, 2022 **Accepted: 22 June, 2022** **Published: 15 Sept, 2022**

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ABSTRACT

Ficus deltoidea (FD) is herbal plant that been used traditionally in Malaysia to treat various illness such as cold, regain energy in post-partum women and improve libido. Scientific studies also proved that FD possess medicinal properties such as anti-inflammatory, anti-nociceptive, anti-diabetic and wound healing properties. Currently, FD has been claimed to possess energy booster effect and a commercialized energy drink has been formulated using FD, but there was no scientific study on the effect of FD on physical performance. The purpose of this study was to evaluate the effect of FD aqueous extract on swimming time, muscle fiber size and blood testosterone level. Male Wistar rats (n=24) were used in this in vivo model of study. They were divided equally into 4 groups according to FD treatment dosage (0, 50, 200 and 800 mg/kg). FD treatments were given via oral gavage for 6 weeks. Physical fitness of rats treated with FD were evaluated using weight-loaded force swimming test (WLFST) while muscle fiber cross section area was measured to determine muscle fiber size. Data from WLFST suggested that FD supplementation did not improve swimming time in treated rats. FD was found to effectively induce muscle hypertrophy by increasing muscle fiber cross section area. In this study, FD was shown to exhibit ergogenic effect on rats by increasing lean muscle mass, without affecting testosterone level and swimming time.

***Keywords**: ficus deltoidea, Mas Cotek, muscle hypertrophy, physical performance*

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INTRODUCTION

FD is a native plant in South East Asia from the family of Moraceae. In Malaysia, the plant was commonly known as Mas Cotek, Serapat Angin and Telinga Beruk. There are few variants of FD available, but there are two most common variants that are widely used, namely Ficus deltoidea var. angustifolia (male plant) and Ficus deltoidea var. deltoidea (female plant) (Mohammad, Wei, & Bakar, 2012).

Almost all part of this plant been utilised in traditional medicine. The fruit is chewed to relieved headache, toothache and cold. The root and leaves was grinded and been applied externally to treat wound and soreness. It also been reported that the whole plant decoction had been used as herbal drink to strengthen up uterus after childbirth in women. It was also believed that this drink help in increase blood circulation, regain energy and improve sex desire (Sulaiman et al., 2008).

FD is one of the herbs that been claimed to had medicinal properties such as anti-nociceptive, anti-inflammatory and anti-oxidant properties (Abdullah, Hussain, Ismail, & Ali, 2009; Sulaiman et al., 2008). FD also been tested for wound healing properties. It was reported that FD extract had accelerate wound healing in rats (Abdulla, Ahmed, Abu-Luhoom, & Muhanid, 2010). Anti-pyretic properties of FD had been study in animal model. It was found that aqueous extract of FD had temporary anti-pyretic effect (Mohd Khir, 2010).

Aqueous and ethanolic extraction of FD also been studied for infertility effect in diabetic rats model. It had been demonstrated both extraction method can normalized testosterone level in diabetic rats (Nurdiana et al., 2017; Samsulrizal, Awang, Mohd Luqman Hakim Mohd, Idzham, & Zarin, 2011).

Aqueous extract of FD also been studied for anti-ulcer properties. It was show that certain dose of FD can reduce the size of stomach ulcer in rats (Fatimah, Mahmood, Hapipah, Suzita, & Salmah, 2009). Stomach ulcer recovery process requires the reconstitution of epithelial and connective tissue. These processes involve cellular proliferation, migration and differentiation (Beckert, Class, Farrahi, & Coerper, 2004). Cell proliferation and differentiation are characters that also possess by anabolic process in the body. This might be an insight that FD may possess anabolic effect that can promote tissue growth and speed up tissue injury recovery.

Based on previous studies, it is useful to study the effect of FD as androgenic and anabolic enhancer. Thus, this study was aimed to evaluate the effect of Ficus deltoidea aqueous extract on swimming time, muscle fiber size and blood testosterone level.

MATERIALS AND METHODS

Ficus deltoidea aqueous extract preparation

FD aqueous extract was prepared by Tropical Bioessence Sdn. Bhd. (683554 V). *Ficus deltoidea var. Terenganuensis* (Mas Cotek) was used to prepare the extract. Dried leaf of FD was washed twice and rinsed once with clean water. Plant decoction was prepared by boiling

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the plant in reverse osmosis water (RO), which acts as solvent, for 3 hours. The temperature was set at 100°C. The decoction was then filtered and concentrated by using Single Effect Concentrator until the volume of the extract reduce to 20% of the initial volume. Maltodextrin was added as the carrier and then the extract was dried using spray drier. Maltodextrin will also act as thickener and preservative to increase shelf life and maintain the stability of the extract. The dried extract powder was then kept in chiller for storage.

Animal

Male *Wistar* rats (24 rats) at 6 weeks old were used in this study. Rats were supplied by Takrif Bistari Enterprise. They were kept at Animal Experimental Laboratory, Faculty of Medicine and Health Sciences, UPM. The rats were caged in plastic cage with wood shaving as the bedding. Each cage only accommodates two rats. Cages were cleaned twice a week. The rats were fed with commercial pellet and water *ad libitum*. The temperature was maintained in range between 25°C to 29°C with 12-hour dark-light cycle. Animal handling and experiment was approved by Institutional Animal Care and Use Committee (UPM/IACUC/AUP-R031/2015).

Before the actual experiment begins, rats were test for their swimming ability. Only rats that can swim for at least 15 minutes, without load were included in this study. Rats were divided into 4 groups according to the doses of *FD* treatment (0, 50, 200 and 800mg/kg).

FD treatment procedure

FD treatments were administered through oral gavage technique. FD dried extract powder was diluted with distilled water to each treatment concentration. Each rat received 2ml of their respective dose. For control group (0mg/kg), 2ml distilled water were given to the rats.

Weight loaded force swimming test

Weight loaded force swimming test was done to measure the endurance capacity of each rats. Rats were loaded with steel washer weight of 6% of their current body weight. Clear rectangular acrylic plastic pool (30x30x60cm) was used. Water was filled to 50cm, adequate depth to prevent the rats from touching the bottom of the pool with their tail during swimming. This will make sure that the rats will swim continuously until exhaustion. Failure to rise to surface for 10 seconds was considered as fails to continue swimming. The rats were then rescued, dried using towel and place into their cage. Swimming time was recorded as the indicator of endurance capacity.

Blood testosterone analysis

This hormone assay was done using enzyme link immunoassay (ELISA) kit from Elabscience (Catalog No: E-EL-0072). This ELISA kit uses competitive-ELISA as the method. 96 wells microtiter plate provided in this kit has been coated with Goat Anti-Rabbit IgG to make it solid-phase secondary antibody. Sample, HRP-labelled testosterone and anti-testosterone antibody were added to each well to form a coated secondary antibody-anti-testosterone antibody-HRP-labelled testosterone complex. The amounts of bound labelled testosterone are inversely proportional to the amount of the testosterone in the sample. After washing the plate, substrate solution was added to each well. Substrate turns to blue catalyst with horse radish peroxidase.

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The enzyme substrate reaction was terminated by the addition of sulfuric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of testosterone in the samples was then determined by comparing the optical density of the samples to the standard curve.

Gastrocnemius muscle fiber cross section area measurement

Gastrocnemius muscle bundle that been kept in 10% formalin was cut (cross section) and processed using automated tissue processor (LEICA TP 1020). The samples was then embedded in paraffin wax and cut into 6µm thick and stained with hematoxylin & eosin (H&E) stain. Images were viewed under light microscope and muscle fibers cross section area was measure (Olympus Image Analysis Software, Cell^F) as the indicator of muscle fiber size. Areas with at least 10 intact muscle fiber with clear boarder were randomly picked and all the intact muscle fiber was measured in those area. Total of 50 muscle fibers cross section with clear border between each fiber were measured in each sample at x400 magnification. Muscle fibers with clear boarder indicate that it had not been rupture and the area is still intact. Total 250 muscle fibers were measured in each treatment group.

RESULTS AND DISCUSSION

FD did not affect body weight increment and swimming time

One-way repeated measure ANNOVA was conducted to evaluate the changes in body in each group throughout the experimental period (6 weeks). Generally, there were significant increments in body weight in every group during every time of data collection (Figure 1).
Sigwalt (2011) stated that weight loss can be a sign of over training or depression in rats (Sigwalt et al., 2011). In this study, all treatments and control groups had increased and almost identical body weight. This suggests that both FD treatment did not induce over training or depression on rats.

Force swimming test is a method of measuring the physical ability of the animal to swim. This test was normally done to evaluate fitness, endurance, energy or fatigue in animals (Jung, Kim, & Han, 2004; Sachdeva, Kuhad, & Chopra, 2011; Toda, Hitoe, Takeda, & Shimoda, 2016). The difference between weight loaded swimming and free swimming is that, in weight loaded swim test the rats will actively swimming to prevent drowning. Without weight loaded to their body, animals such as rat can be floating around without actively kicking or paddling. This is because rats can be considered as natural swimmer and they can learn to float if they had been exposed to swimming for period of time.

Weight loaded force swimming test can be considered as maximal test where the rats were force to swim continuously until failure. The advantage of maximal test in animal is that, it can eliminate the learning effect (i.e.: rats learn to float without paddling). This will ensure that the swimming time recorded is purely the time of the rats swim actively.

Weight loaded FST was aimed to measure fatigue time of rats during exercise and can be a parameter for their endurance capacity. This test was considered as maximum test since the test only stop when the rats were completely failed to swim.

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One-way repeated measure ANNOVA was conducted to compare weight loaded FST swimming time during Week 2, Week 4 and Week6 in all groups of treatment. There was no significant difference in swimming time when compared between Week 2, Week 4 and Week 6 in all treatment groups (Table 1).

Table 1: Weight loaded force swimming test time (minute)

Groups	Week 2 (minute)	Week 4 (minute)	Week 6 (minute)
Control	12.06±3.65	12.39±2.33	7.93±3.09
50 mg/kg	8.43±0.94	8.53±1.94	8.68±2.07
200 mg/kg	7.06±1.2	6.35±1.77	7.98±2.13
800 mg/kg	13.49±0.92	10.93±1	10.22±2.06

Results are mean ± SEM. There was no significant difference in swimming time when compared between Week 2, Week 4 and Week 6 in all groups.

These finding show that ingesting FD aqueous extract did not cause increment in swimming capacity of rats.

FD supplementation was hypothesized to improve weight loaded force swimming time after 6 weeks of treatment. After 6 weeks of training and FD supplementation, weight loaded force swimming time was decreased compared to the swimming time from Week 2. Even though the result was not statistically significant in all groups, the pattern of decreasing swimming time occurs in all groups of treatments.

Reduce in swimming capacity might be due to few factors such as aging and increase in body weight. Increase in body weight mean that the rats had to paddle harder to keep floating. There was suggestion that body weight had effect in exercising time in rodents (Palmer & Davis, 1982). Since body weight of rats in all treatment groups were increased, it can be a factor in reducing swimming capacity.

FD did not significantly increase blood testosterone level

A one-way between groups ANOVA was conducted to see the differences in blood testosterone level between all four treatments groups. There was no statistical significant difference in blood testosterone level [F (3, 16) = 0.818, p=.503]. Even though the result was not statistically significant, it can be seen that treatment with FD cause increment in blood testosterone level in dose dependent manners (Figure 2).

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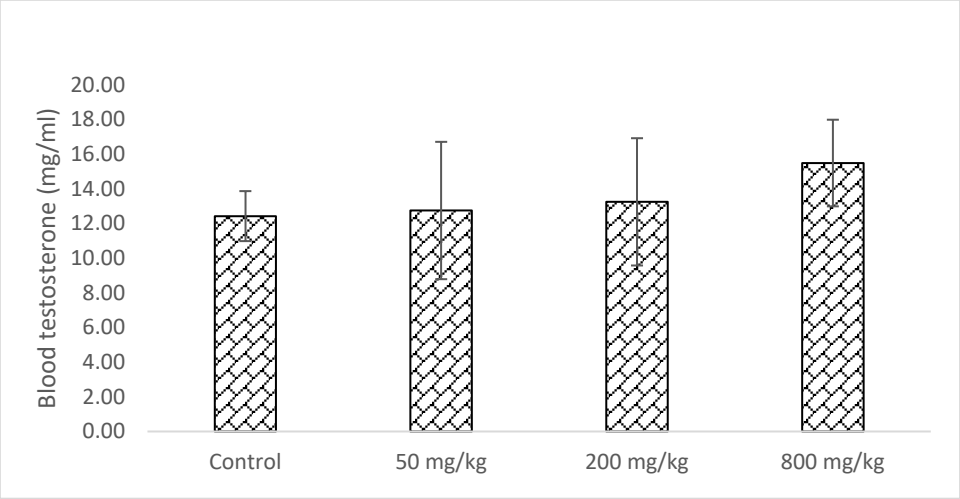

Figure 2: Blood testosterone level. There was no statistical differences between all groups. Results are mean ± SD

In this study, FD was hypothesized to increase serum testosterone level and there were previous studies that show that FD can alleviate testosterone level in diabetic rat's model. Even though blood testosterone was lower compared to normal control, other anabolic and androgenic properties such as body weight and sperm quality were also improved (Nurdiana et al., 2017; Samsulrizal et al., 2011).

Testosterone was known to act as androgenic (masculinizing)-anabolic (tissue building) hormone. Testosterone was able to both stimulate skeletal muscle protein synthesis and inhibit muscle breakdown by preventing inflammation (Urban, 2011). Anabolic effect of testosterone are varies among user. But, a lot of studies agree that testosterone can increase muscle mass. It was also found out that increase in muscle mass accompanied by increase in muscle strength, but not necessarily increase in contractile properties of skeletal muscle (Bhasin et al., 2004). Increase in testosterone level can be the sign of anabolic effect since this hormone involve in anabolic process. In this study, the change in testosterone level is small and insignificant. Despite there was no statistically significant different among groups, it can be said that swimming training and FD supplementation tend to elevate blood testosterone level.

FD increase skeletal muscle size

One-way ANNOVA test was done to check the effect of FD supplementation on muscle fiber cross sectional area. There was significant difference between groups of treatment in muscle fiber cross sectional area (Figure 3).

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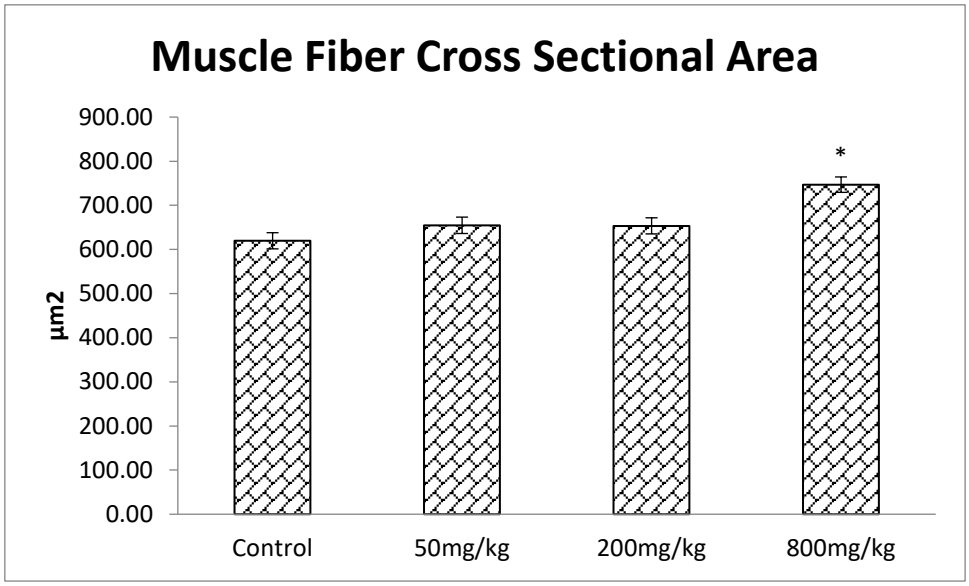

*Figure 3: Muscle fiber cross section area Results shown are mean ± SEM. (*p<0.05)*

From this result, it is clear that 800 mg/kg FD supplementation had significant effect in increasing muscle fibre cross section area or muscle size. Increase in muscle size was accompanied with dense nucleus (Figure 4 & 5).

At first, we hypothesized that muscle fiber size increase will be accompanied by increased in testosterone level, which is an anabolic hormone. Since there was no significant change in testosterone level, it was suggested that there are others factors that might influence the anabolic effect on muscle.

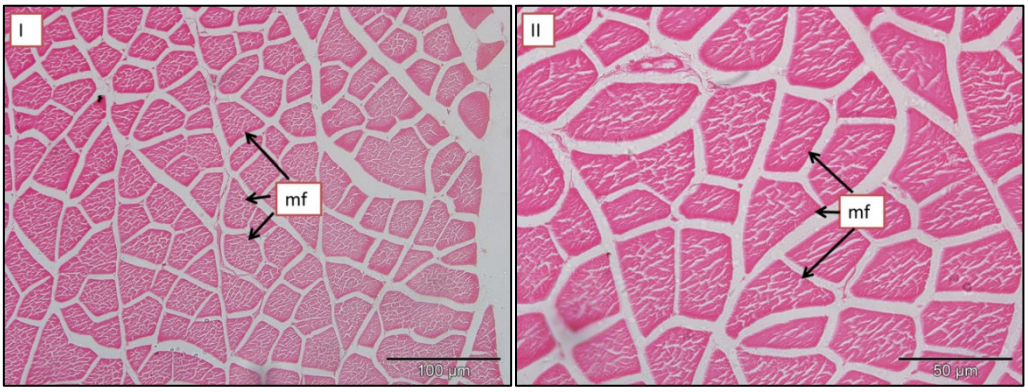

Figure 4: Representative pictures of muscle cross section from Control group at x200 (I) and x400 (II). (mf)=muscle fiber

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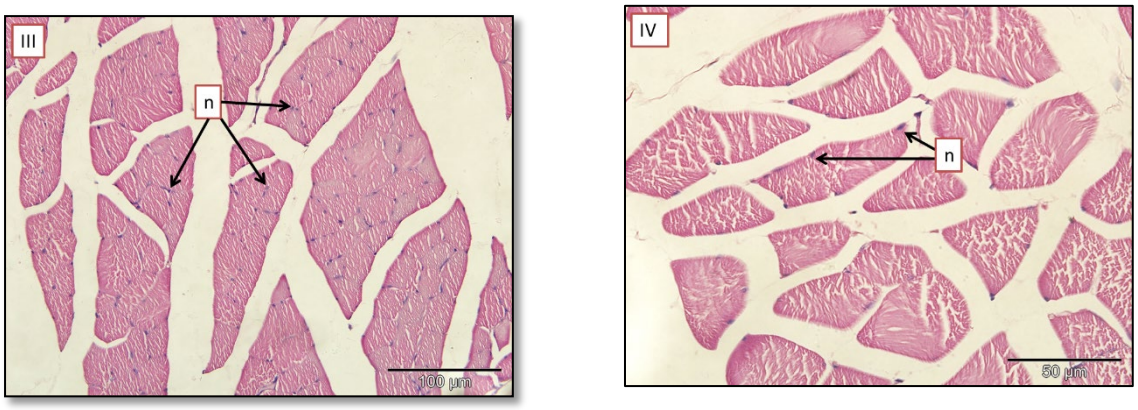

Figure 5: Representative pictures of muscle cross section from 800mg/kg group at x200 (III) and x400(IV). Dense nucleus (n) located peripherally on muscle fiber can be observe in both images. This effect is resembled to anabolic effect.

Testosterone prohormone properties is one of the suggested pathway since there was previous study that demonstrates FD increased testosterone level in diabetic model rats, which is their basal testosterone level is low compared to normal rats (Samsulrizal et al., 2011). Testosterone prohormone was known to be used to increase basal endogenous testosterone. Study by Leder on androstenedione, a testosterone prohormone show that the effect of oral supplementation of testosterone prohormone of serum testosterone level is dose dependent (Leder et al., 2000). Significant increase serum testosterone only can be seen if the dosage is high. A part from that, testosterone prohormone itself has weak properties as androgen-anabolic compound (Kraemer, Rubin, French, & McGuigan, 2002). This means that testosterone prohormones itself can upregulate anabolic process in the body without being turn into testosterone (Rasmussen, Volpi, Gore, & Wolfe, 2000).

Another suggested pathway is that FD might induce protein synthesis by activating hypertrophy intrinsic pathway without relying on testosterone and other anabolic hormone as mediator. Intrinsic pathway activation without relying on hormone can be seen in exercise-mediated muscle hypertrophy where membrane-derive molecule and tension-sensing pathway upregulate protein synthesis (West, Burd, Staples, & Phillips, 2010).

CONCLUSION

In conclusion, we found that FD induce muscle hypertrophy by increasing muscle fiber cross section area without elevating blood testosterone level and rats swimming time.

Authors contribution

Azhar Yaacob and Mohamad Taufik Hidayat Baharuldin contribute to designing study concept, data analysis and interpretation and revising the manuscript.

Azhar Yaacob involves in data collection and drafting the manuscript.

Mohamad Aris Mohd Moklas, Zulkhairi Amom and Mohamad Taufik Hidayat Baharuldin

contribute to study concept approval, data interpretation and given final approval.

Siti Zubaidah Nur Marthuan contribute to results interpretation and drafting the manuscript.

All authors give final approval and agree to be accountable for all aspects.

Acknowledgements

This study was funded by Universiti Putra Malaysia under research grant GP-IPS/2016/9509500

Conflict of interest

All authors declare that they have no conflict of interest. This study was funded by Universiti Putra Malaysia under research grant GP-IPS/2016/9509500

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