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Serum Testosterone Levels and Body Weight Gain of Male Rabbits Fed with *Morinda citrifolia* Fruit Juice

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ABSTRACT

Morinda citrifolia, popularly known as noni, has been reported to possess antidiabetic, antiseptic and antibiotic properties, as well as hypotensive and anticoagulant activities. *M. citrifolia* was also reported to possess immunomodulation characteristics, anticancer activity and may also be useful as an aphrodisiac. The effects of *Morinda citrifolia* fruit juice on serum testosterone concentration and body weight of twelve New Zealand White male rabbits were studied for 8 weeks. Three groups of rabbits (n = 3 each) were treated orally with dosages of 200 mg/kg, 400 mg/kg and 800 mg/kg of the fruit juice respectively. Another group of rabbits (n = 3) served as control. Body weights of rabbits were measured daily. Serum testosterone levels were determined every fifth day using enzyme immunoassay method. The results obtained showed that in all groups, serum testosterone level decreased as compared to the baseline values, but the changes were not statistically significant. Body weight gain was also not significantly different among all treatment groups. There was a negative association (r = - 0.365) between serum testosterone level and body weight of rabbits in the control group. However, the changes in serum testosterone level were not significantly correlated with the changes in body weight of rabbits in the treatment groups. The findings suggest that feeding *Morinda citirolia* juice to male rabbits for a short duration did not produce any significant changes to serum testosterone and body weight gain.

INTRODUCTION

Morinda citrifolia is a small tree of the family of Rubiaceae (Morton, 1992; Farine *et al*., 1996). It is popularly known as 'noni' in Hawaii and 'mengkudu' in Malaysia (Morton, 1992). The fruits of *M. citrifolia* are fleshy and ovoid, with a lumpy body (Morton, 1992; Barr *et al*., 1993). They have waxy, greenish-white skin (Morton, 1992). When fully ripe, they turn creamy-white and are edible, but with an unpleasant taste and 'cheesy' odour (Whistler, 1988; Morton, 1992). The flesh is juicy, bitter and yellowish with numerous red brown seeds (Morton, 1992). The ripe fruits contain

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fifty-one volatile compounds that include 20 acids, 7 alcohols, 11 esters, 2 ketones, 2 lactones and 9 other miscellaneous compounds (Farine *et al*., 1996). The major component is octanoic acid and the minor component is hexanoic acid (Barr *et al*., 1993; Farine *et al*., 1996). The metal content of the ripe fruits include sodium (204 mg/100g), potassium (2012 mg/100g) and calcium (23 mg/100g) (Barr *et al*., 1993). The fruits contain 52.3 % moisture and 24 to 158 mg of ascorbic acid per 100 g. Tests showed strong evidence of terpenes (Morton, 1992).

According to Heinicke (1985), the fruit is the most abundant and reliable source of proxeronine and proxeronase. Proxeronine is the precursor of the alkaloid xeronine. Xeronine is essential for proper cell function in the body and to sustain health. Xeronine is produced in the large intestine from proxeronine. Proxeronase is the enzyme necessary to complete the chemical reaction, which produces xeronine.

Ancient manuscripts handed down from generation to generation describe many uses of this plant. In Asian countries, *M. citrifolia* has been extensively used in folk medicine to treat diabetes, as an antiseptic, and as an antibiotic. It also has hypotensive and anticoagulant activities (Whistler, 1988; Morton, 1992; Farine *et al*., 1996). *M. citrifolia* was also reported to possess immunomodulation characteristics and anticancer activity. Numerous research has been carried out on these properties but no research has yet to prove that *M. citrifolia* can stimulate the endocrine system. It was claimed that *M. citrifolia* could support the male and female reproductive systems by solving hormonal problems. Solomon (2000) reported that approximately 10% of 10,000 male and female patients who consumed the juice of the fruit had enhanced enjoyment.

This study was undertaken to determine whether there is any increase in the serum testosterone concentration and body weight of male New Zealand White rabbits treated with three different dosages of *M. citrifolia* fruit juice; and to correlate the changes in serum testosterone level with the changes in body weight.

MATERIALS AND METHODS

M. citrifolia fruit juice

Ripe *M. citrifolia* fruits were collected from Shah Alam and allowed to soften and ripen fully. The fruits were weighed and cut into smaller pieces and the juice was extracted using TK-368 Takada Juice Extractor. The juice was sieved to obtain pure juice without fibres and seeds. Distilled water was added to the pure juice to make an end concentration of 1.0 g/ml. The juice was kept at 10°C in aliquots until used. Juice was prepared fresh every week.

Rabbits and variables measured

Twelve male New Zealand White rabbits (mean ± SEM, 2.40 ± 0.08 kg) were used in this study. The rabbits were housed in individual cages. They were fed 100 g of commercial food pellets per day and drinking water *ad libitium*. The rabbits were divided into one control group and 3 treatment groups with 3 rabbits per group.

The treatment groups received a dosage of 200, 400 and 800 mg/kg of the juice respectively via force-feeding using 1.0 ml syringes. A calculated dose of the juice according to the initial body weight of the rabbits was administered orally to each rabbit at 8 a.m. daily. The treatment lasted for a period of 8 weeks. Rabbits in the control group received drinking water *ad libitium*. Body weights of the rabbits were measured daily.

Blood samples were collected from the marginal ear vein of the rabbits at the beginning of the experiment and every 5

days. Blood collection was carried out using Venofix® 23G butterfly needle and 5 ml Terumo® syringes. Blood samples were placed into plain Vacutainer® silicone-coated tubes. Blood samples collected were allowed to clot at room temperature. The blood samples were centrifuged at 3000 g for 15 minutes to obtain serum. Serum samples were decanted into 1.5 ml Axygen™ Eppendorf tubes. The tubes were frozen immediately at –20°C and stored until required for analysis. Quantitative determination of serum testosterone level was carried out on thawed serum using testosterone enzyme immunoassay (EIA) test kit (Teco® Diagnostics, USA). An enzyme-linked immunosorbent assay (ELISA) reader was used to quantify the testosterone concentration

Statistical analyses

Data was presented as the means ± standard error of mean (SEM). Means of the changes in serum testosterone level and means of body weight gain of the rabbits were compared using One-Way Analysis of Variance (ANOVA). Pearson correlation test was used to measure the relationship between serum testosterone level and body weight of the rabbits. A p-value of less than 0.05 ($p<0.05$) was considered significant. Data were analysed using Statistical Package for Science Students (SPSS) 9.0 for Windows.

RESULTS

Serum testosterone concentration

The results obtained showed that there was a decrease in serum testosterone level compared to baseline values in all the groups (Table. 1). The reduction in testosterone level was highest in rabbits fed 400 mg/kg of *M. citrifolia* fruit juice (-2.31±0.46 ng/ml). However, the decrease in serum testosterone level was not significantly different between all groups. The changes in serum testosterone levels of rabbits throughout the study revealed a pulsatile pattern (Figure 1a and 1b). Fluctuations of values were more prominent in 400 mg/kg and 800 mg/kg as compared to 200 mg/kg.

Body weight gain profile

Body weight increased on the average of 0.50 kg from the initial level in all groups (Table 2). The increase in body weight was not significantly different between the four groups of treatment. Figure 2 shows the changes of body weight of rabbits throughout the study. Body weight of the rabbits increased gradually.

Relationship between testosterone concentration and body weight

Pearson correlation showed that there was a significant ($p<0.05$) association

Table 1. Changes in serum testosterone concentration of rabbits. Values are the mean ± SEM with n = 3 for each group.

Group	*Baseline (ng/ml)*	*Post-treatment (ng/ml)*	*Change from baseline (ng/ml)*	*% Decrease*
Control	21.33 ± 0.28	19.86 ± 0.40	- 1.47 ± 0.12	- 6.89
200 mg/kg	21.11 ± 0.41	20.59 ± 2.20	- 0.52 ± 1.79	- 2.46
400 mg/kg	19.93 ± 1.75	17.62 ± 2.21	- 2.31 ± 0.46	- 11.59
800 mg/kg	21.81 ± 0.43	20.29 ± 1.29	- 1.52 ± 0.86	- 6.97

Figure 1a. Profiles of serum testosterone concentration of rabbits measured every fifth day in groups of control and 200 mg/kg. Values are the mean ± SEM (n = 3 at each time point)

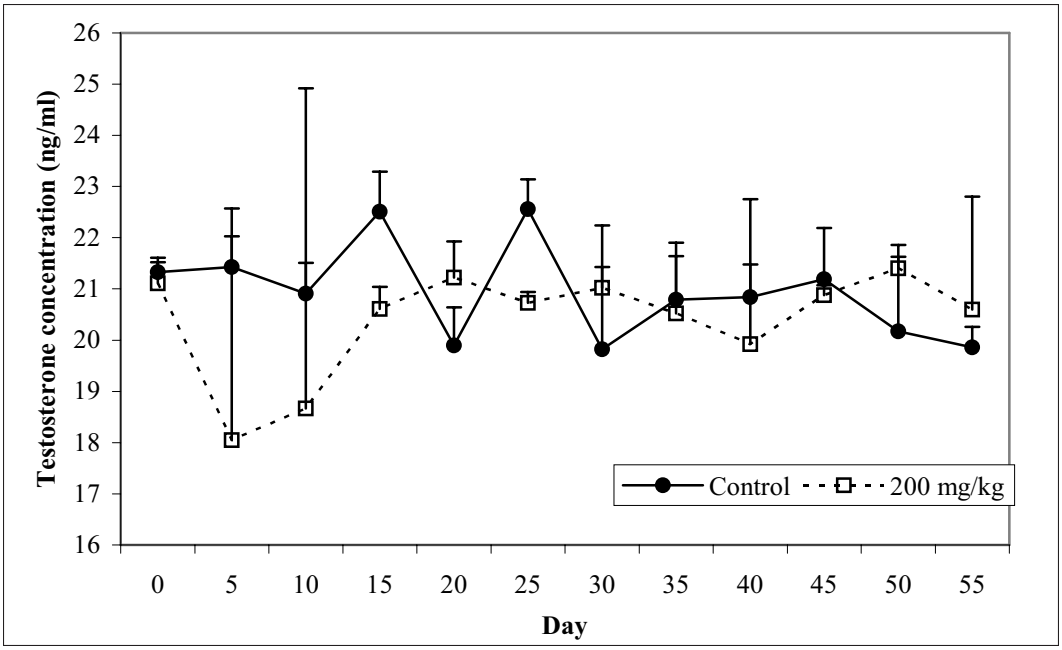


Figure 1b. Profiles of serum testosterone concentration of rabbits measured every fifth day in groups of 400 mg/kg and 800 mg/kg. Values are the mean ± SEM (n = 3 at each time point)

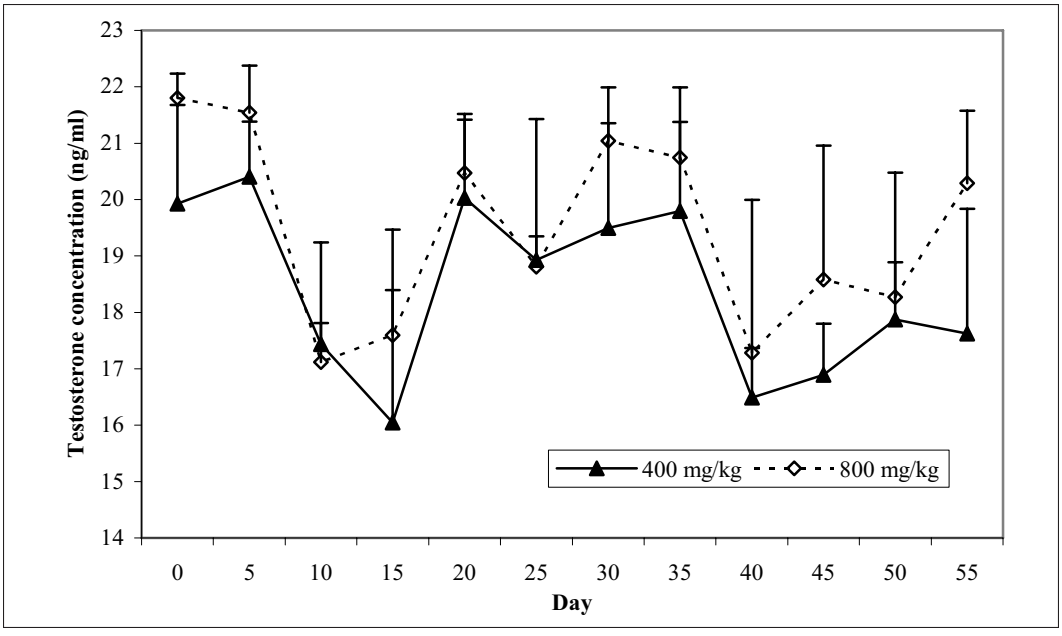
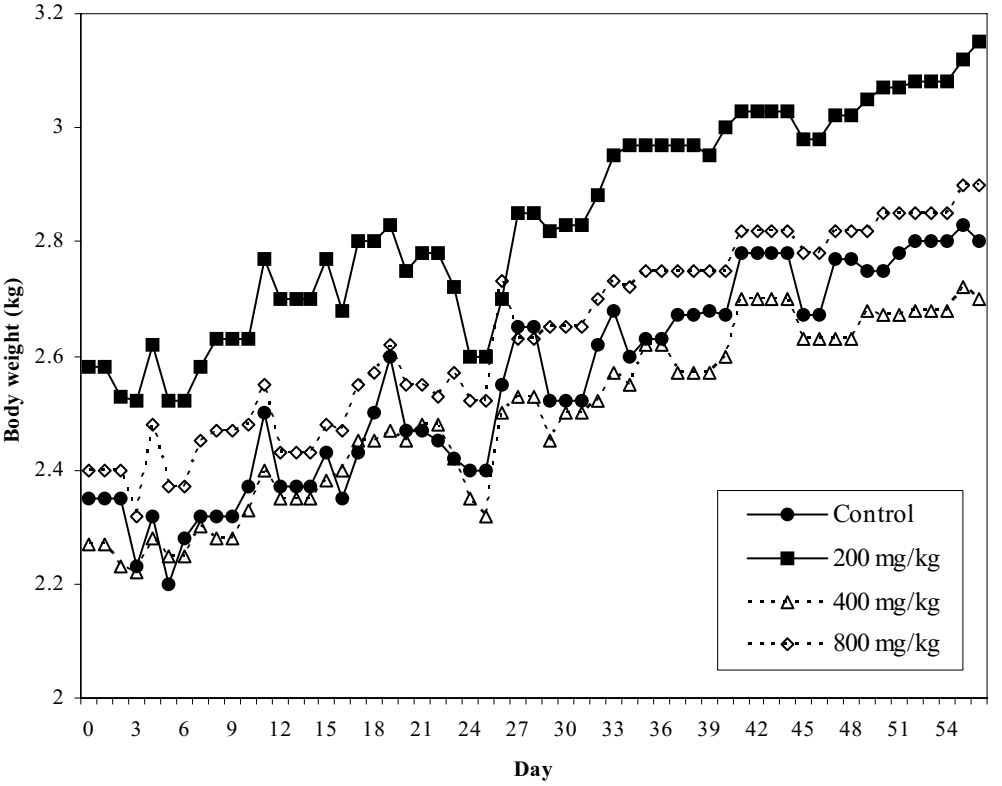


Table 2. Changes in body weight of rabbits. Values are the mean ± SEM with n = 3 for each group

Group	*Baseline (ng/ml)*	*Post-treatment (ng/ml)*	*Change from baseline (ng/ml)*	*% Increase*
Control	2.35 ± 0.23	2.83 ± 0.16	+ 0.50 ± 0.07	21.28
200 mg/kg	2.58 ± 0.17	3.12 ± 0.20	+ 0.54 ± 0.03	20.93
400 mg/kg	2.27 ± 0.14	2.72 ± 0.09	+ 0.45 ± 0.05	19.82
800 mg/kg	2.40 ± 0.05	2.90 ± 0.06	+ 0.50 ± 0.01	20.83

Figure 2. Profiles of body weight of rabbits measured daily throughout the study. Values are the mean ± SEM (n = 3 at each time point)



between serum testosterone concentration and body weight of rabbits in the control group. The correlation analysis indicated a negative relationship (r = - 0.365). However, the changes in serum testosterone concentration were not significantly ($p>0.05$) correlated with the changes in body weight in the other three treatment groups. Figures 3a-d are scattergrams showing the strength of relationship between these two variables in each group of rabbits.

Figure 3a. Correlation of serum testosterone level and body weight of control group

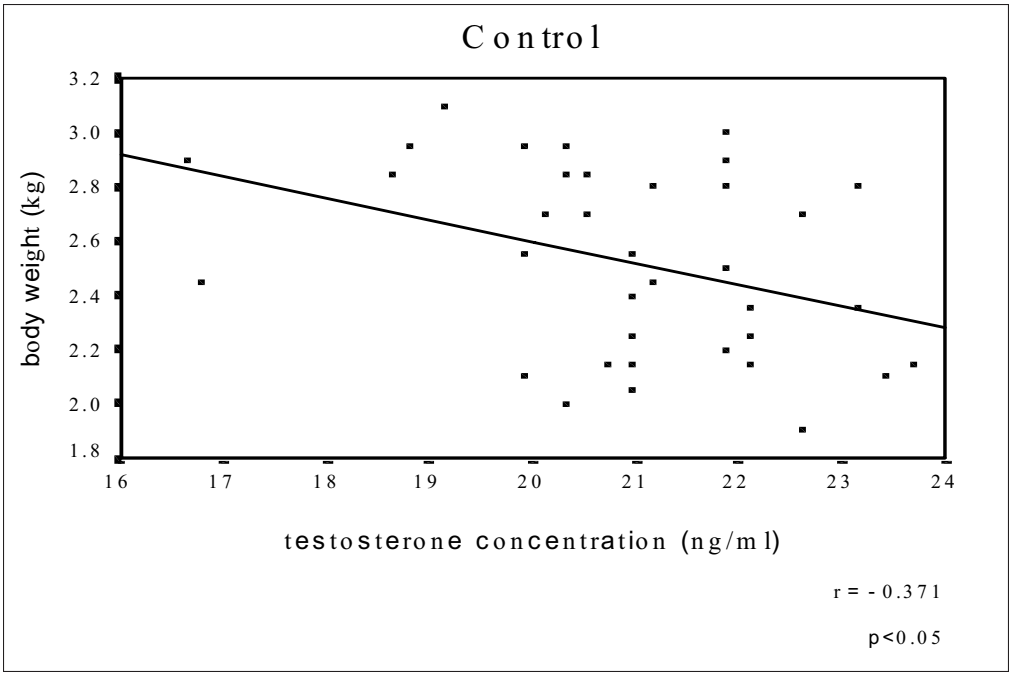


Figure 3b. Correlation of serum testosterone level and body weight of 200 mg/kg group

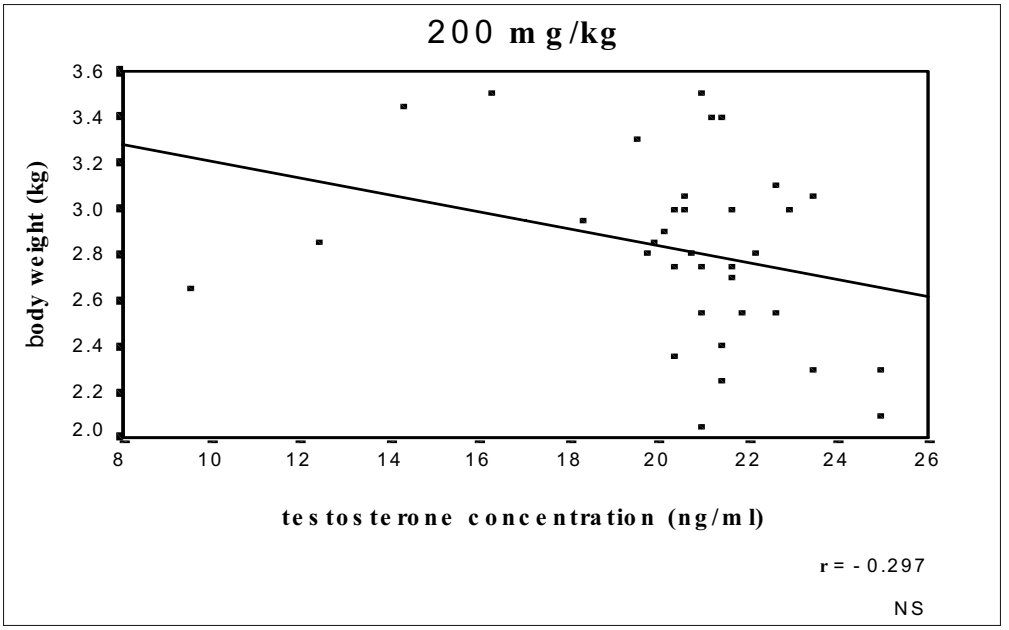


Figure 3c. Correlation of serum testosterone level and body weight 400 mg/kg group

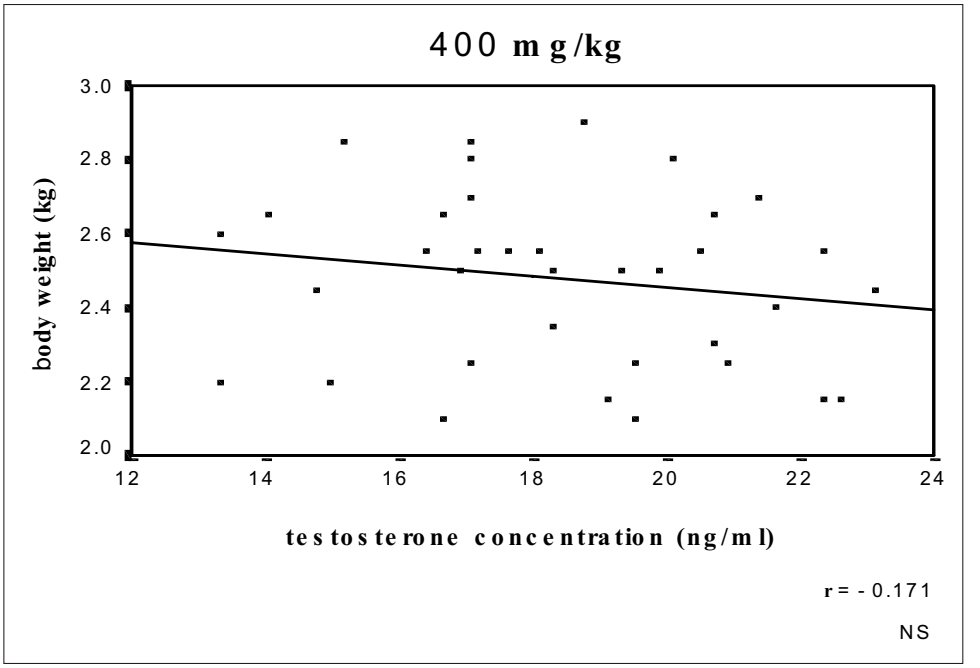
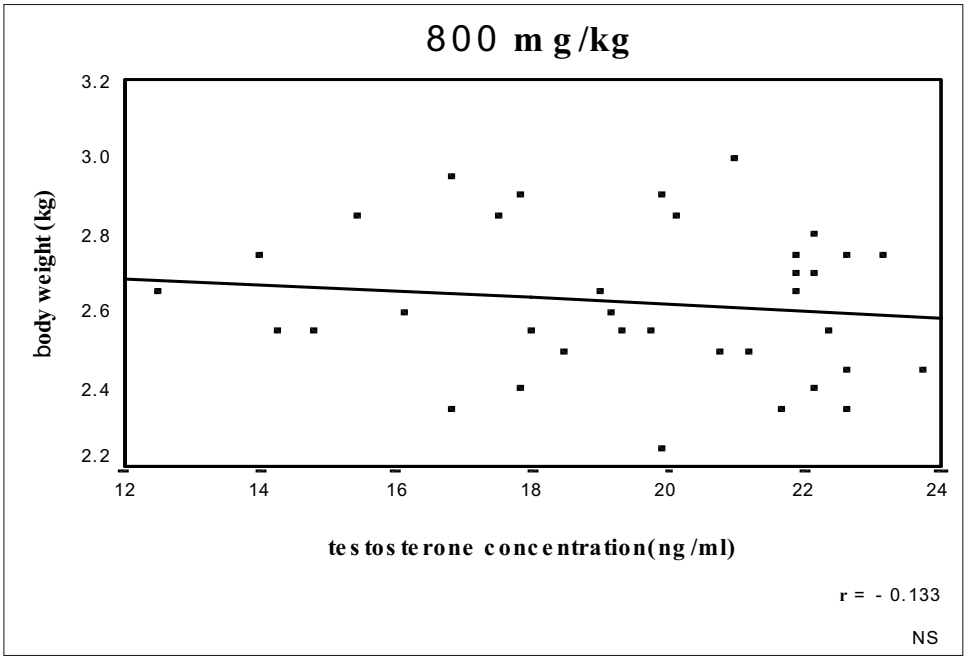


Figure 3d. Correlation of serum testosterone level and body weight of 800 mg/kg group



DISCUSSION

The results of the present study showed that repeated exposures to different dosages of *M. citrifolia* fruit juice did not produce significant changes in serum testosterone concentration and body weight of rabbits. Correlations between serum testosterone level and body weight of rabbits were not statistically significant.

Changes from baseline in serum testosterone concentration were not significant ($p>0.05$) in all groups. A possibility does exist that the dosage or duration of the *M. citrifolia* fruit juice administration might have been inadequate to produce significant effects in testosterone levels of rabbits in this study. The minimum dosage of *M. citrifolia* fruit juice used in this study was 200 mg/kg/day, which exceeded those consumed by humans, 130 mg/kg/day (Solomon, 2002). Therefore, it was expected that higher dosages (400 mg/kg and 800 mg/kg) would produce effects.

As shown in Figure 4, the serum testosterone levels were in a pulsatile rhythm. Serum testosterone levels of the 200 mg/kg group fell on day five but increased again until day 15 and remained quite constant throughout the study. This pattern shows the importance of homeostasis to prevent sudden severe changes within the body. Therefore, the decrease in testosterone level in the blood was detected by chemoreceptors in the hypothalamus. Hypothalamus increased the secretion of gonadotropin-releasing hormone (GnRH), which stimulates the release of luteinising hormone (LH) from the anterior pituitary. LH promotes the synthesis and release of testosterone to increase the level back to normal. (Marieb, 1998)

Meanwhile, the fluctuations of serum testosterone levels in 400 mg/kg and 800 mg/kg groups may be caused by the pattern of testosterone secretion. Previous studies that measure testosterone concentrations for 24 hours found a diurnal rhythm that peaked in the early morning and reached its nadir during the late evening (Mitamura, 1999, 2000). These studies were conducted on boys and girls, prepubertal and pubertal. Diurnal rhythm of testosterone secretion may also be true for rabbits. In another study by Winters *et al*. (1999), pulsatile pattern of testosterone release into spermatic vein plasma in human was revealed. The duration of the secretory events ranged from 15 to 90 minutes. Based on these findings, therefore, the level of testosterone during blood collection will definitely vary between each rabbit causing the fluctuating pattern.

There was no significant difference in body weight gain between all groups of rabbits. Since protein and caloric intake might influence the changes in body weight, daily feed intake of the rabbits were controlled at 100 g per day. This was necessary to make sure that body weight gain was not attributable to dietary considerations. It was not known whether the increase in body weight was due to increase in muscle mass, fat or lean body mass. Crist *et al.* (1983) reported that anabolic steroids did not substantially change body composition in nine volunteers experienced with progressive-resistance-weight training. There were no significant changes in lean body mass and percent fat in the study. This study suggested that the dosage and duration of testosterone cypionate administered were not adequate to produce significant improvements in the performance of the subjects.

Serum testosterone concentration was inversely correlated with body weight of rabbits in control group. There was no significant association between serum testosterone concentration and body weight of rabbits in the three treatment groups. Testosterone is a potent anabolic factor critical for the maintenance of lean body mass. Studies had shown that serum testosterone levels were positively correlated with body weight, lean body mass and muscle mass (Griggs *et al.*, 1989; Welle

et al., 1992; Grinspoon *et al.,* 1996; Wang *et al.,* 1996). Body weight and body mass index increased in sublingual testosterone replacement in hypogonadal men (Wang *et al*., 1996). The negative correlation of the control group may be due to additional factors that are not known.

In this study it can be concluded that repeated oral administration of 200 mg/kg, 400 mg/kg and 800 mg/kg of *M. citrifolia* fruit juice did not exhibit significant changes in serum testosterone level. Body weight gain was not significantly different between the three groups. Therefore, *M. citrifolia* fruit juice did not increase serum testosterone level and body weight of male New Zealand White rabbits. The results also showed that the changes in serum testosterone levels did not affect the body weight profile in any of the treatments. There was no significant association between the two variables in this study. Further studies with a bigger sample size with a minimum of six replicates should be used to determine whether a higher dosage of *M. citrifolia* fruit juice may produce an effect on testosterone level and body weight of rabbits.

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