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The effect of *Melaleuca cajuputi* methanolic leaves extract on body growth, puberty and sperm quality of juvenile male rats

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ABSTRACT

The aims of this study was to determine the effects of *Melaleuca cajuputi* methanolic leaves extract on body growth, the timing of puberty and sperm quality in juvenile rats. Twenty four Sprague Dawley male rats, four weeks old, were assigned to four different groups (n=6). The animals received standard maintenance diets supplemented with different levels of *M. cajuputi* methanolic leaves extract. First group served as a control and only received maintenance diets. Meanwhile another three groups supplemented with 50, 100 and 200 mg.kg^{-1} of *M. cajuputi* methanolic leaves extract, respectively, for six weeks. Bodyweight and the degree of preputial separation were monitored on weekly basis. At the end of the experiment, all animals were sacrificed and their epidydimis were subjected to sperm analysis. Over six weeks of experiment, the bodyweight increased significantly ($p<0.05$) over time for all groups but no differences ($p>0.05$) was observed between treatment. The number of male with fully preputial separation was significantly ($p<0.05$) higher in males supplemented with 200 mg.kg^{-1} of *M. cajuputi*. The sperm quality was increased with *M. cajuputi* supplementation. These results suggest that the *M. cajuputi* methanolic leaves extract improved sperm quality and reduced the age to achieve puberty in male.

KEYWORDS

Melaleuca cajuputi;
Puberty;
Sperm quality;
Preputial separation.

INTRODUCTION

Melaleuca cajuputi Powell is belongs to the family of Myrtaceae. This plant can be found indigenously across the hot and humid climate zone of Australia to the Asian mainland. It is able to populate coastal, subcoastal, riverbanks as well as the inlands. *M. cajuputi* grows as shrub or tree with single flexible trunk ranging from small tree to measure 25 metres in height. The leaves are alternately arranged, ovate to lanceolate and dark green to grey-green in colour. The flowers are produced in dense clusters along the stem and the fruit

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is a small capsule containing numerous minute seeds. The plant has been used traditionally for treatment of coughs, stomach cramps, burn and against influenza[1]. The oil from *M. cajuputi* is used in the Malay Archipelago for the treatment if internal disorders, intestinal problems and insecticide[2]. Pre-clinical data was shown *M. cajuputi* possess anti-inflammatory, anti-bacterial, anti-microbial, anti-dengue, anti-oxidant, anti-cancer and anti-convulsant activities[3-7]. It could also have a positive or negative effect on reproduction. However, much attention has not been given to the effect of this useful plant on male reproduction.

Previously, many papers have been published describing the association between plant materials and male reproduction. Some studies have reported that plant materials improve male's sexual function and other authors have shown that plant materials impair male's sexual function. Ajayi and co-workers[8] reported that *Titonia diversifolia* at a dose of 50 $mg.kg^{-1}$ increase the testosterone level, thereby improving spermatogenesis and other male reproductive profile. Meanwhile *Eurycoma longifolia* roots extract increases spermatogenesis by inhibiting the activity of phosphodiesterase and aromatase[9]. According to Hammami and co-workers[10], rats treated with *Allium sativum* showed an increased number of seminiferous tubules deprived of spermatozoa, apoptosis of testicular germ cells and a decrease of serum testosterone levels. Nwangwa[11] documented that *Xylopia aethiopica* affecting the sperm count, motility and viability in Wistar rats.

To date, there is a lack of data concerning the effect of *M. cajuputi* on male reproductive performance. The present study was designed to test the hypothesis that *M. cajuputi* supplementation could increase body growth and reproductive performance (sperm quality and the age at puberty) in juvenile male rats.

MATERIALS AND METHODS

Study area and animals ethics approval

The study was carried out between January 2013 and June 2013 at the Faculty of Applied Sciences Animals Facility, Universiti Teknologi MARA (UiTM). The procedures were carried out in accordance with the Malaysia legislation as assessed and approved by the Animal Ethic Committee of the UiTM.

Plants material and extraction

Melaleuca cajuputi leaves were collected in May-July 2012 from their natural habitat in Selangor, Malaysia. Plant material was authenticated by School of Environmental and Natural Resources Herbarium, Universiti Putra Malaysia. The plants were prepared by the method describes previously by several authors[12,13] with slight modification.

Fresh leaves were ground using a grinding mill and the powdered materials were extracted with methanol. Then the extracts were filtered through Whatman filter paper and the organic solvent was removed from the filtrate by rotary evaporator. The extract was reconstituted with distilled water prior to experimental assessment in different concentrations of the treatment.

Animals and experimental design

At the beginning of the trial, 24 juvenile's male rats were stratified by age and live body mass, before randomly allocated to four groups. All animals were placed in clean wired cages, with free access to food and water. Group 1 (n=6) received maintenance diet (commercial rat chow) and served as a control while another three groups were fed with maintenance diets supplemented with three different doses of *Melaleuca cajuputi* methanolic leaves extract. Group 2 (n=6) supplemented with 50 $mg.kg^{-1}$, group 3 (n=6) supplemented with 100 $mg.kg^{-1}$ and group 3 (n=6) supplemented with 200 $mg.kg^{-1}$ of *M. cajuputi* methanolic leaves extract for six weeks. *M. cajuputi* was supplemented by oral gavage.

Bodyweight and preputial separation measurement

Over six weeks of experiment, bodyweight and preputial separation was measured on weekly basis. Bodyweight was measured in rats by analytical balance (Mettler Toledo, CA). Meanwhile a scale of 1-5 was used to record the preputial separation, as described earlier by Stoker and co-workers[14] with slight modification. Score 1 indicated the prepuce covering the penis is not retracted, score 2 indicated the tips of glan penis had begun to project from the opening of the prepuce, score 3 indicated half of the glans penis was free

from preputial sheath, score 4 indicated that gland penis free from preputial sheath but not the filament and score 5 indicated fully preputial separation.

Sperm quality analysis

The rats were sacrificed by decapitation 24 hours after the last administration of the extract. Sperm was collected from the cauda epididymis into Toyoda-Yokoyama-Hosi (TYH) medium[15]. The sperm suspension was incubated in CO_2 incubator for 10 minutes (37 ^{0}C and 5% CO_2). Then the sperm suspension was analysed for sperm count by Makler Counting Chamber (Sefi-Medical Instruments, USA). Sperm morphology and sperm mortality were evaluated by eosin-nigrosin staining method[16]. A total of 300 sperm was counted on each slide under the light microscope at x400 magnification and the percentage of morphologically abnormal sperm (detached head and coiled tail) were recorded[17].

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for Windows. Results are expressed as the mean of six animals $\pm$ SE. The statistical significance ($p<0.05$) of differences between means was assessed by an analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Melaleuca cajuputi methanolic leaves extract increased sperm quality and reduced the age at puberty in male rats but no difference in live bodyweight was observed among the experimental groups.

The bodyweight was significantly ($p<0.05$) increased overtime for all groups but the changes of bodyweight was not different ($p>0.05$) between male given maintenance diet and supplemented with *M. cajuputi* (Figure 1). *M. cajuputi* methanolic leaves extract used in the present study seems unable to increase fat deposition, muscle and skeletal development. The stimulation of fat deposition, muscle and skeletal development is due to excretion of growth hormone[18]. The current study suggest that (i) the nutritional value in *M. cajuputi* leaves extract insufficient to promote body growth and (ii) the maximum dosage of *M. cajuputi*

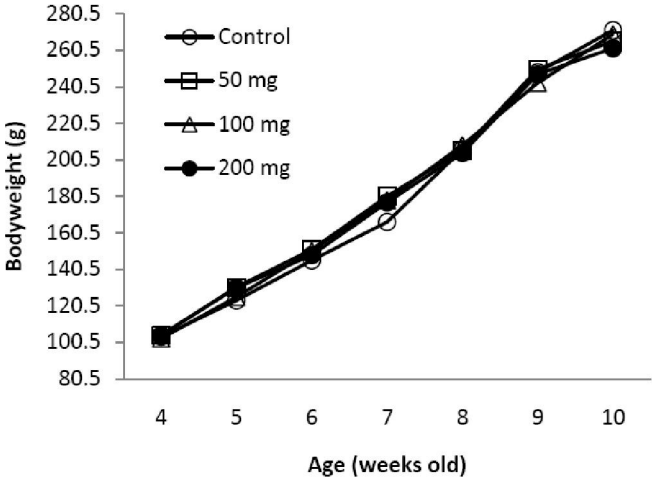

Figure 1 : Live bodyweight of juvenile male rats fed with different level of *M.cajuputi* methanolic extract (n=6 for each level) in daily diet over a period of 6 weeks.

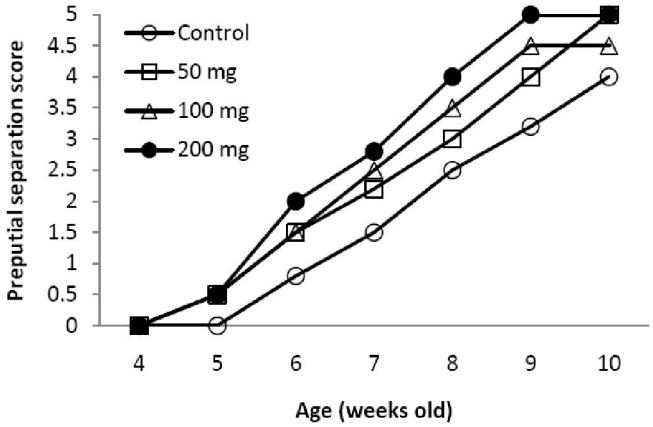

Figure 2 : Average preputial separation score of juvenile male rats fed with different level of *M.cajuputi* methanolic extract (n=6 for each level) in daily diet over a period of 6 weeks.

utilised in this study, 200 mg.kg^{-1} insufficient to increase growth hormone production by rat's hypothalamus.

The rate of preputial separation, leading to puberty was significantly ($p<0.05$) higher in males supplemented with *M. cajuputi* methanolic leaves extract compared to control (Figure 2). This finding suggests that *M. cajuputi* promoted the production of testosterone by rat's interstitial cells. The last decade many papers have been published describing the association between preputial separation, leading to puberty and testosterone production. Testosterone stimulated the separation of the foreskin from the penis, or fully preputial separation followed by puberty[14,19].

Sperm concentration increased significantly ($p<0.05$) meanwhile sperm abnormality reduced significantly ($p<0.05$) in males supplemented with *M.*

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cajuputi methanolic leaves extract compared to control (Figure 3 and Figure 4). The percentage of sperm mortality was not affected by treatments (Figure 5). We postulated that this finding associated with the capabilities of *M. cajuputi* leaves to promote the production of testosterone and spermatogenesis. The reduction in the number of abnormal sperm in males supplemented with *M. cajuputi* also maybe due to the potential of *M. cajuputi* in reducing the lipid peroxidation. Previous data by Turk and co-workers[20] revealed that antioxidant properties in plants material capable to reduce the number of abnormal sperm. Antioxidant properties also significantly boosted testosterone level, increased ejaculated volume, improved sperm motility and increased sperm total output[21].

In the current research we do not into account the histomorphometric of testis in experimental rats. However the fact that the sperm count increased in males

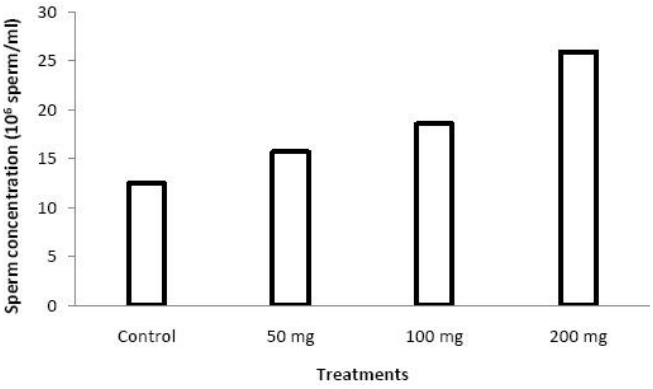

Figure 3 : Average sperm concentration (10^6/ml) of juvenile male rats fed with different level of *M.cajuputi* methanolic extract (n=6 for each level) in daily diet over a period of 6 weeks.

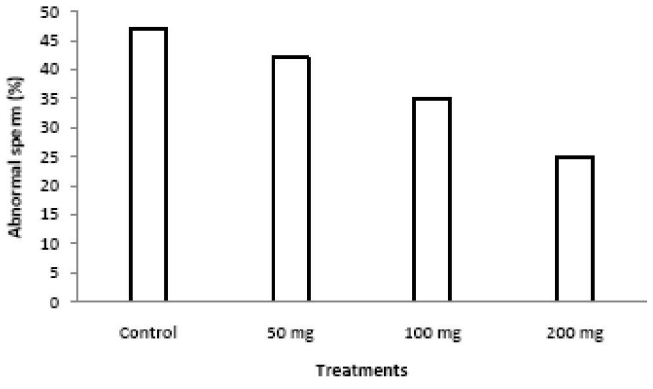

Figure 4 : Average abnormal sperm (%) of juvenile male rats fed with different level of *M.cajuputi* methanolic extract (n=6 for each level) in daily diet over a period of 6 weeks.

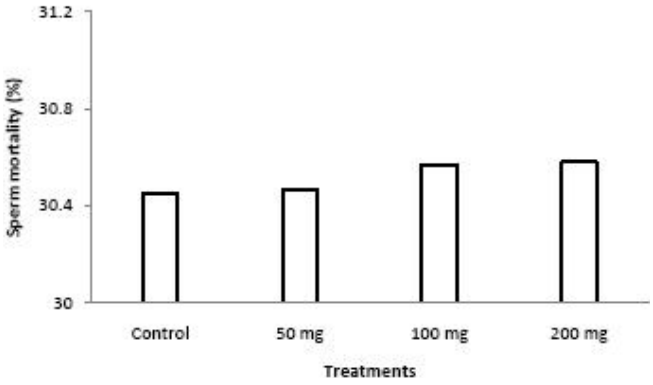

Figure 5 : Average sperm mortality (%) of juvenile male rats fed with different level of *M.cajuputi* methanolic extract (n=6 for each level) in daily diet over a period of 6 weeks.

supplemented with *M. cajuputi* over maintenance diet convinced us that the size of lumen and the rate of spermatogenesis maybe higher in supplemented group. For the future research, we recommended that (i) morphometric and histological observation should be conducted in control and *M. cajuputi* supplemented males, (ii) radio-immunoassay to determine the plasma concentration of growth hormone (GH), luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone and leptin.

In conclusion, the findings from the current study support the hypothesis that *M. cajuputi* methanolic leaves extract will improve sperm quality and reduce the age at puberty in male rats. However, the data also revealed that *M. cajuputi* supplementation does not have an evident effect on bodyweight. In light of that, further research is needed to reveal the nutritional values of *M. cajuputi* and to identify the mechanism of action by *M. cajuputi* in improving male reproduction.

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