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THE EFFECTS OF CURCULIGO LATIFOLIA DRY AND ETHANOLIC EXTRACTS ON SPERM QUALITY OF MICE MUS MUSCULUS

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ABSTRACT

Several Curculigo species have been reported to be widely used for the treatment of impotence and act as aphrodisiac. However, the effect of Curculigo latifolia Dryand on sperm quality, defined as the measurable parameters of the sperm sample which may determine its fertilising capacity, has not been completely explored. The objective of this study was to determine and compare the effects of the leaf and root extracts of Curculigo latifolia on the sperm quality of mice Mus musculus. Three groups of male mice were used in this study; the control group (Group 1) was forced-fed with saline solution, Group 2 with 500 mg/kg b wt of the root extract of C. latifolia and Group 3 with 500 mg/kg b wt of the leaf extract of the plant. The treatments were conducted for a duration of 14 days. The results of this study gave no significant difference in the weekly body weight and sperm morphology of the mice. Mice fed with the root extract showed a greater increase in sperm motility (75.33±2.60%) compared to Group 3 (74.00±5.00%) and Group 1 (64.33±2.73%). Meanwhile, mice fed with the leaf extract showed a higher sperm count (62.33±4.33 mill/ml) and sperm viability (28.00±4.04%) compared to Group 2 (58.67±4.10 mill/ml and 27.67±5.55%, respectively) and Group 1 (40.33±3.18 mill/ml and 23.33±2.96%, respectively). In conclusion, the leaf and root extracts of Curculigo latifolia appeared to have positive effects on sperm quality parameters which are linked to male fertility.

Keywords: *Curculigo latifolia Dryand; leaf and root extracts; sperm quality; Mus musculus; male fertility.*

1. INTRODUCTION

Medicinal plants possess a reservoir of active phytochemicals that can act as potential aphrodisiac molecules. There is a renewed interest in the search of traditional remedies from plants that can be used for the treatment of impotence and act as rejuvenator or aphrodisiac such as the genus *Curculigo* (Nie et al., 2013). *Curculigo orchioides* seemed to be effective in the treatment of erectile dysfunction and in the enhancement of the overall sexual performance in rats (Chauhan et al., 2007; Thakur et al., 2011). Phytochemical analysis of *C. orchioides* showed the presence of triterpenoids (curculigol), glycosides (curculignins A, B, C), curculigosaponins (curculigenins A, B, C, corchicoside A, curculigoside B) and alkaloids (yuccagenin, lycorine) (Garg et al., 1989; Rao et al., 1978; Kubo & Nakashimi, 1983; Mehata & Dubey, 1983; Mehata & Gawarikar, 1991).

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The demand for herbal drugs has increased in developed as well as developing countries because of their good aphrodisiac activity and safety. Normally and theoretically phenolic compounds such as flavonoids and phytoestrogens can be effective in fertility and the reproductive system (Khojasteh et al., 2016). Some phytochemicals have already been identified as potential aphrodisiac compounds such as 1,3,6,8-tetrahydroxy-2,5-dimethoxyxanthone and 1,6,8-trihydroxy-2,3,4,7-tetramethoxyxanthone isolated from the roots of *Securidaca longepedunculata* and may be used for large scale clinical trials in drug discovery programmes (Meyer et al., 2008).

C. latifolia Dryand or locally known as 'Lemba' or 'Pinang Puyuh' is a stemless herb that grows in Western Malaysia and its fruits have been used by the natives as sweetener. Curculin and neoculin from the fruit of *Curculigo latifolia* have been found to contain sweet-tasting proteins with a taste-modifying activity (Okubo et al., 2008). The plant has many beneficial effects including to ease joint pains, prevention of obesity and cardiovascular, anticandidal and cytotoxicity (brine shrimp assay), antidiabetic and hypolipidemic activities (Ishak et al., 2013; Lim et al., 2013).

Although the traditional use of *C. latifolia* as aphrodisiac was known, the phytochemical or scientific studies on *C. latifolia* in improving sperm quality defined as the measurable parameters of the sperm sample which may determine its fertilizing capacity, are limited (Suzuki et al., 2004). This study is beneficial in providing scientific information on male fertility for humans or livestock, which may help improve sperm quality demonstrated by sperm motility, sperm morphology and sperm count which are linked to male fertility. The plant may be an alternative natural treatment for the enhancement of reproductive performance.

2. MATERIALS AND METHODS

2.1 Plant Materials

Curculigo latifolia Dryand plants were collected at Sungai Petani, Kedah, Malaysia. The plant was identified by Dr Shamsul Khamis, the resident taxonomist of Universiti Putra Malaysia. The leaves and roots were washed and cut into small pieces and dried for 1 week at room temperature. The dried plant parts were separately ground into powder using a milling machine.

2.2 Preparation of Samples

The dried and powdered forms of leaves and roots of *Curculigo latifolia* Dryand were individually extracted with 80% EtOH at room temperature for 72 hours, dried in vacuum giving the crude ethanolic extracts (Napisah et al., 2004).

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2.3 Animal and Treatment

Male mice *Mus musculus* weighing 30-40 g with age of 8 weeks were used in this study. The mice were acclimatized to the laboratory conditions for a week and exposed to 12:12 hour light-dark cycle. A total of 15 mice were divided into 3 groups, comprising of 5 animals each. Group 1 served as control and received only saline solution. Group 2 was administered orally with 500 mg/kg b wt/day of the *C. latifolia* root extract and Group 3 was fed an amount of 500 mg/kg b wt/day of the *C. latifolia* leaf extract. The crude extracts were dissolved homogeneously in 2 ml/kg b wt of saline and administered using gavage once daily for 14 days (Saba et al., 2009).

2.4 Data and Collection

2.4.1 Body Weight

The body weights of the mice in each group were weighed using an electrical balance at weekly intervals and 24 hours before dissection.

2.4.2 Testicular Weight

After 14 days of treatment the animals were sacrificed by decapitation and an incision in the abdominal wall of each mouse was made. The cauda epididymides were carefully removed and both right and left testes were weighed.

2.4.3 Sperm Motility

The epididymides of each animal were placed in a drop of Phosphate Buffer Solution (PBS) and minced using forceps. Sperm were collected immediately and transferred into 200 µl PBS. The sperm suspension was loaded into the Makler Chamber and covered by a cover slip. Then it was visualized under a compound microscope immediately. The non-motile and motile sperm were calculated according to World Health Organisation criteria (NAFA, 2002). The procedure was repeated in 5 different areas and the percentage of sperm motility was determined.

2.4.4 Sperm Count

Sperm count determination was performed using the Makler chamber according to manufacturer's manual (Sefi Medical Instruments, USA) and World Health Organisation criteria (NAFA, 2002). The concentration of sperm (million/ml) for each mouse was calculated randomly from 5 different microscopic fields of the Makler chamber. The mean value obtained was multiplied by a factor of one million.

2.4.5 Sperm Morphology

The morphology of the sperm was evaluated by using Diff-Quik staining method (Rurangwa et al., 2004). The reagents for Diff-Quik staining are:

> Diff-Quik I: Stain solution I contains Eosin Y (1.22 g/l) in phosphate buffer with pH of 6.6 and sodium azide (<0.1%) as preservative.
>
> Diff-Quik II: Stain solution II contains Thiazine dye (1.1 g/l) in phosphate buffer also with pH of 6.6.

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Diff-Quik fix: Fixative solution contains fast green (0.002 g/l) in methanol.

Sperm suspension was smeared on the slide and dried. Diff-Quik solution was dispensed into a staining jar with lid and the slides were dipped into the fixative solution for 5 seconds. The slide was dipped in stain solutions I and II for 5 seconds each and allowed to drain after each dip. The slide was then rinsed with distilled water and dried before observing it under an inverted microscope. A minimum of 100 sperm cells was analyzed per data entry.

2.5 Statistical Analysis

Data were expressed as means ± standard error of the mean (SEM). The significant differences among the means of the treated groups were analyzed using One-way Analysis of Variance (ANOVA) against the control group. *P*-value lower than 0.05 ($p<0.05$) was considered as significantly difference.

3. RESULTS AND DISCUSSION

3.1 Body Weight and Testicular Weight

The effects of the *C. latifolia* Dryand root and leaf ethanolic extracts on body weight and sexual organ are shown in Table 1 and Figure 1 respectively. No significant effect was observed in the body weight and the testicular weight of the treated animals. After 14 days of treatment, the mice treated with the *C. latifolia* root extract (Group 2) showed the highest testicular weight (252.80±17.37 mg) compared to control (233.07±12.71 mg) and Group 3 (234.10±24.02 mg).

Table 1: Effect of the *C. latifolia* Dryand root and leaf ethanolic extracts on body weight in mice

Group	Body Weight (g)			
	Week 0	Week 1	Week 2	Week 3
Group 1	31.85 ±1.77	32.36 ±1.37	32.81 ±1.21	33.80 ±0.81
Group 2	33.97 ±1.04	33.55 ±1.57	32.86 ±1.28	34.25 ±1.15
Group 3	34.39 ±3.88	34.22 ±1.49	31.91 ±2.30	32.63 ±1.42

The values shown are means and their standard errors of the means (Means±SEM)

3.2 Sperm Motility

Figure 2 summarizes the effects of the *C. latifolia* root and leaf extracts on sperm motility of the treated mice compared to the control group. Sperm motility, the bioindicator of sperm quality is commonly used and easily analyzed (Rurangwa et al, 2004). This refers to the ability of the sperm to swim properly in order to reach and fertilize an egg.

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Generally, the percentages of motile sperm were higher compared to that of non motile sperm in all of the three groups. In addition, the percentages of sperm motility was significantly higher in both of the treated groups which were 75.33±2.60% and 74.00±5.00%, respectively, in Group 2 and Group 3 compared to 64.33±2.73% in the control group. It was also found that the non motile sperm in the control (Group 1) was highest (35.67±2.73%) followed by Group 3 (26.00±5.00%) and Group 2 (24.67±2.60%).

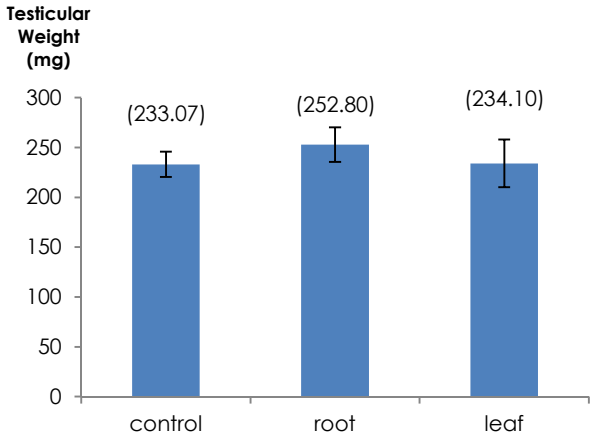

Figure 1: Effects of the *C. latifolia* Dryand root and leaf ethanolic extracts on testicular weight in mice. The values shown are mean values

3.3 Sperm Count

The results showed both groups that were treated with the root and leaf extracts of *C. latifolia* had significantly higher sperm count compared to the control with the highest value exhibited by leaf extract-treated group. The respective sperm count produced were 62.33±4.33 mill/ml (Group 3), 58.67±4.10 mill/ml (Group 2) and 40.33±3.18 mill/ml (Group 1), as shown in Figure 3.

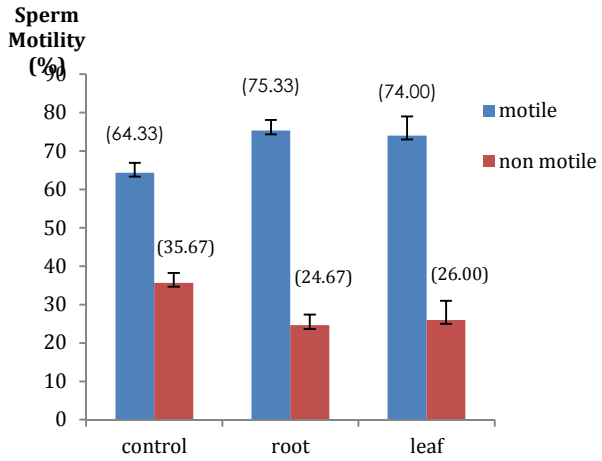

Figure 2: The percentages of motile and non motile sperm in different groups of mice. The values shown are mean values

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3.4 Sperm Morphology

Sperm morphology measures the percentage of normal and abnormal sperm. Sperm heads with a hook-like shape and straight tails were considered as normal sperm while other descriptions are considered as abnormal. Overall, there was no significant difference in the percentages of sperm morphology among the groups. The results showed that the percentage of normal sperm was higher compared to abnormal morphology in all groups. However, as shown in Figure 4, the control (Group 1) showed the highest percentage followed by the root extract-treated group (Group 2) and leaf extract-treated group (Group 3) with values of 89.00±3.06 %, 86.00±2.65 % and 83.67±2.03%, respectively. However, the differences were not statistically significant ($p>0.05$).

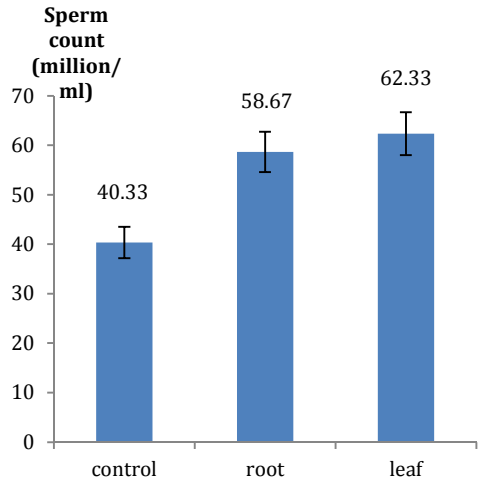

Figure 3: The concentration of sperm in control and treated groups. The values shown are mean values

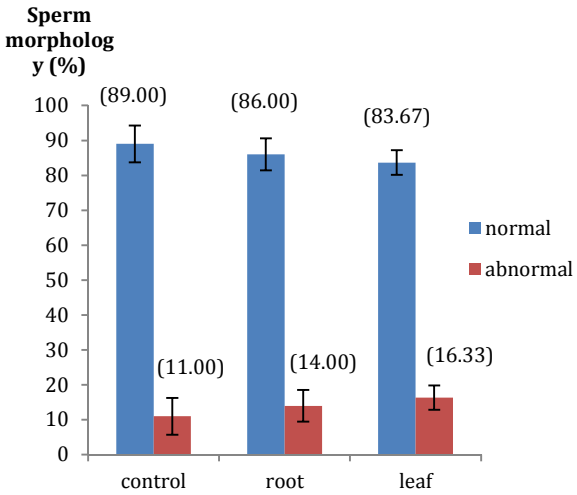

Figure 4: The percentage of normal and abnormal sperm in control and the treated groups. The values are the means

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4. CONCLUSION

The present investigation confirmed the root and leaf extracts of *C. latifolia* Dryand demonstrated positive effects on the sperm quality of mice especially sperm motility and sperm count which are linked to male fertility. Plants of the genus *Curculigo* are known to mainly contain phenols and phenolic glycosides, lignans and lignan glycosides, triterpenes and triterpenoid glycosides; acting together, may be responsible for the polyvalent activities of *Curculigo* including sperm quality. Therefore, a phytochemical study of these extracts needs to be done to identify the active compound(s) responsible for these effects. The results also seem to support the traditional use of *Curculigo latifolia* Dryand as aphrodisiac.

5. ACKNOWLEDGEMENT

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