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Comparative effects of *Leucaena leucocephala*, *Ficus deltoidea* and *Momordica charantia* on sperm quality and testosterone in Sprague Dawley rats

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ABSTRACT: Background: It is commonly known that plants have different effects on male fertility. **Aim:** The study was conducted to determine the effect of methanolic extracts of three medicinal plants (namely *L. leucocephala*, *F. deltoidea* and *M. charantia*) on male fertility. **Methodology:** Twenty four male rats were divided into four groups with six males each. Each group was supplemented with either distilled water (control group) or methanolic extract of *Leucaena leucocephala*, *Ficus deltoidea* or *Momordica charantia* at a concentration of 50 mg/kg bwt for 28 days. At the end of the feeding regime, blood was collected by cardiac puncture for testosterone estimation and all rats were sacrificed. Epididymis was collected from each rat and subjected to sperm quality analysis. **Results:** *L. leucocephala* showed no significant effect on male fertility, *F. deltoidea* significantly increased male fertility and *M. charantia* methanolic extract significantly decreased male fertility. **Conclusion:** It can be concluded that *M. charantia* more toxic towards male reproductive system compared to *L. leucocephala* and *F. deltoidea*.

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INTRODUCTIONS:

As noted in the scholar literature, plants have been used extensively to enhance or inhibit male fertility [1-3]. Various survey documented that rural society especially in under and developing countries use plants as an agents to improve fertility as well as pregnancy prevention. Current trends also shows that modern society is very interested in using plants as an alternative treatments. To complement this need, a detailed study is needed especially on the effects of plants taken on male fertility. The effect of the plant on male fertility whether

positive or negative effects still has an impact depending on the context, whether to promote or to suppress fertility. *Leucaena leucocephala* (Fig 1), *Ficus deltoidea* (Fig 2) and *Momordica charantia* (Fig 3) are some medicinal plants that are very popular among the Malay community in Malaysia. These plants are widely used by Malay's traditional healers to treat a variety of diseases and health issues including infertility. *L. leucocephala* or 'petai belalang' in Malay is a medium size tree belong to the family of *Fabaceae*. Previous authors reported that, this tree is very effective in treating stomachache, worm infections, diabetes mellitus and also demonstrated abortifacient effect [4-5]. Meanwhile *F. deltoidea* or 'mas cotek' in Malay is a large shrub belong to the family of *Moraceae*. There has been numerous studies showing that this plant has the potential to treat diabetes mellitus, toothache, infertility, and wound healing [6]. On the other hand, *M. charantia* or 'peria katak' in Malay is a perennial climbers belong to the family of *Cucurbitaceae*. Originally 'peria katak' was eaten as a salads and traditional vegetable but currently is increasingly popular as a supplement for alternative medicine. Scientific evidences shows that this plant has a potential as an antidiabetic, anticancer, antibacteria and antifertility among others [7]. The objective of this study was to compare the effects of these three plants on male fertility (sperm quality and blood testosterone levels).

Fig 1. *Leucaena leucocephala* leaves.

Fig 2. *Ficus deltoidea* fruits and leaves.

Fig 3. *Momordica charantia* fruits.

MATERIALS AND METHODS:

Chemicals and reagents:

All chemical used in this study were of analytical grade. Methanol and diethyl ether was procured from HmBG Chemicals (Germany). Testosterone ELISA Kit was supplied by Cayman Chemical Company (USA). Dulbecco's Modified Eagle Medium (DMEM) was obtained from Sigma-Aldrich (USA).

Animals and ethics clearance:

Sexually matured male rats (about 12 weeks old) weighing in between 180 to 220 g, procured from authorized breeder (Chenur Supplier Sdn Bhd, Malaysia) were used in the experiment. Rats were maintained under standard conditions (with 12 h of light and 12 h of dark cycle) and were fed with standard rodents pellets (Gold Coin Feed Mills Sdn Bhd, Malaysia) with free access to water. All experimental procedures were conducted under supervision of institutional Research Ethics Committee (UiTM Care 170/2017).

Plants materials and methanolic extractions:

M. charantia fruits were procured from local market, *F. deltoidea* fruits were harvested from local garden and *L. leucocephala* leaves were collected from their natural habitat. The specimens for all plants were identified by the botanist of the Herbarium, Universiti Pendidikan Sultan Idris (specimen WAA-1453, WAA-1454 and WAA-1455). Methanolic extract was prepared as previously described [8]. Part of the plants were washed with tap water and air dried at the room temperature. Then subsequently blended into coarse powder (Pensonic PB-3205DJ, Pensonic Holdings Bhd, Malaysia). The coarse powder was macerated in absolute methanol for three days at the room temperature before being filtered using Whatman filter paper number 1. The residue then was re-extracted twice following the same procedure. Meanwhile the filtrate was evaporated using

rotary evaporator to remove the entire methanol (Buchi Rotavapor R-210, Switzerland) and the concentrated crude extract was then kept in refrigerator at 4 ºC until use.

Sperm quality and testosterone assay:

Twenty four male Sprague Dawley rats were divided into four groups with six animals each. First group was supplemented with 2 ml/kg bwt distilled water and served as a control group. Second group was supplemented with 50 mg/kg bwt *L. leucocephala* methanolic extract (LLME), third group with 50 mg/kg bwt *F. deltoidea* methanolic extract (FDME) and group four with 50 mg/kg bwt *M. charantia* methanolic extract (MCME). All groups were treated for 28 days (four weeks). On the day of 29th, blood was collected by cardiac puncture under anaesthetic effect and then all rats were sacrificed by overdose of diethyl ether. The collected blood was centrifuged at 3000 rpm and 4 ºC for 15 min to harvest the plasma for testosterone estimation using Testosterone ELISA Kit (Cayman Chemical Company, USA) according to the manufacturer's instructions. Meanwhile epididymis was collected for evaluation of sperm quality. Each epididymis was minced in Dulbecco's Modified Eagle Medium (DMEM) using a small scissors. Then the sperm suspension was incubated in CO_2 incubator (37 ºC, 5 % CO_2) for 30 min. After that, a small aliquot of sperm suspension was transferred onto Makler Counting Chamber (Sefi Medical Instruments Ltd, USA) and observed under light microscope (Olympus CX-21, Japan) for sperm count and sperm motility evaluations. To determine the percentage of sperm with normal morphology, sperm was smeared, stained with Giemsa staining and observed under light microscope as previously described [9].

Statistical analysis:

All data were presented as means ± SEM and analyzed by ANOVA. P-value lower than 0.05 was considered as significantly difference [10].

RESULTS AND DISCUSSION:

Sperm quality and testosterone secretion did not differ significantly ($p>0.05$) among male rats supplemented with 50 mg/kg bwt of *L. leucocephala* methanolic extract (LLME) compared to male rats of control group (Table 1). There was no evidence that feeding LLME was detrimental to sperm quality and testosterone secretion, contradictory to the study by previous authors. Burawat and co-workers [11] documented that *L. leucocephala* significantly decrease sperm quality and sex hormones secretion in male rats. It has been proven that *L. leucocephala* crude extract contains a compound known as a mimosine that could affect male fertility [12]. The discrepancy in between the results of previous and recent study may be due to differences in experimental design. The current study used a relatively low concentration of LLME (50 mg/kg bwt) for a relatively short experimental period (28 days). Meanwhile study by previous authors [11-12] used higher dosage (either 1500 mg/kg bwt of crude extract or 60 mg/kg bwt of purified mimosine) for longer period of time (60 days). The current data suggest that the effect of LLME on the male reproductive system depends on the dosage and duration of exposure.

Observation on male rats supplemented with 50 mg/kg bwt of *F. deltoidea* methanolic extract (FDME) showed a significant ($p<0.05$) improvement in sperm quality and testosterone hormone production compared to control group and LLME supplemented rats (Table 1). It has been previously reported that *F. deltoidea* is rich in antioxidant constituents [6]. Therefore it is plausible to deduce that *F. deltoidea* antioxidant constituents boosted the testicular functions as reflected in the increased sperm quality and testosterone secretion. In addition, antioxidants works by eliminating reactive oxygen species, thus enables more quality sperm and testosterone to be produced in male rats [13] as observed in rats supplemented with FDME.

Meanwhile, male rats supplemented with 50 mg/kg bwt of *M. charantia* methanolic extract (MCME) showed a significant ($p<0.05$) decrement in sperm quality and testosterone secretion compared to other groups (Table 1). Observation by Naseem and co-workers [14] shows cholesterol accumulation in steroidogenic cells of rats treated with *M. charantia* alcoholic extract and is not synthesized into testosterone. The same barrier is also expected to be involved in the current experiment resulting in decrement of testosterone secretion, which in turn resulted in a suppression of sperm production (spermatogenesis). The adverse effects of MCME on fertility are not only reported in males but also in females, thus couple wishing to procreate should consume *M. charantia* with precaution [15].

CONCLUSION:

The popularity and commercialization of medicinal plants as supplements as well as alternative treatments should be in accordance with professional advice. This is

Table 1. Sperm quality and testosterone estimation in male Sprague Dawley rats treated with methanolic extract of *L. leucocephala* (LLME), *F. deltoidea* (FDME) and *M. charantia* (MCME).

Groups	Sperm count ($x10^6$)	Sperm motility (%)	Normal sperm (%)	Testosterone (ng/ml)
A - control (2 ml/kg dH_2O)	32.5±5.01[a]	58.4±3.95[a]	78.3±1.04[a]	1.07±0.29[a]
B (50 mg/kg LLME)	31.2±2.59[a]	61.6±2.49[a]	75.9±2.24[a]	0.94±0.13[a]
C (50 mg/kg FDME)	38.1±2.48[b]	79.3±2.27[b]	87.8±1.53[b]	1.51±0.36[b]
D (50 mg/kg MCME)	18.8±2.11[c]	32.5±5.12[c]	47.6±3.12[c]	0.63±0.18[c]

Values are presented as means ± SEM. Different superscripts within the same column shows significant difference at $p<0.05$.

due to some medicinal plants that are effective in treating certain diseases also have an adverse effects on the fertility of both male and female. The conclusion from this study indicated that LLME does not have any adverse effect on male fertility if taken at low doses and for shorter intervals. Meanwhile, FDME intake can improve male fertility. However, MCME should be used cautiously as it can cause infertility in male. In summary, it can be concluded that MCME more toxic to the male reproductive system compared to LLME and FDME.

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