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COMPARATIVE EFFECTS OF *Cosmos caudatus*, *Piper sarmentosum* AND *Premna cordifolia* ETHANOLIC EXTRACTS ON MICE (*Mus musculus*) SPERM PARAMETERS

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ABSTRACT

This study was carried out to compare the effects of ethanolic extract of *Cosmos caudatus* (CCEE), *Piper sarmentosum* (PSEE) and *Premna cordifolia* (PCEE) on sperm parameters of male mice, *Mus musculus*. Twenty four sexually matured male mice were used in this study. The mice were grouped into 4 groups of 6 mice each. The first group was given 0.9% saline solution and served as a control whereas groups 2-4 were daily administered with 500 mg/kg body weight of CCEE, PSEE and PCEE respectively, orally for 30 days daily. The body weight was recorded prior to, and after the treatments. At the end of the experimental period, the mice were sacrificed by cervical dislocation. The testis and epidydimis were collected and weighed. Then, the sperm suspension was prepared and subjected to sperm analysis (sperm motility, count and morphology). Bodyweight and reproductive organ weight (testis and epididymis) were not significantly different between control and mice treated with CCEE, PSEE and PCEE. Sperm count was increased in all groups compared to control but not significantly different. Sperm motility of mice treated with PSEE significantly ($p<0.05$) increased compared to control. Meanwhile sperm motility was not affected by CCEE and PCEE. PCEE significantly ($p<0.05$) decreased the percentage of normal sperm morphology compared to control. CCEE and PSEE also reduced the percentage of normal sperm morphology but the values were not significantly different. This study proves that all of the three plants affect the sperm quality, thus further study is suggested.

Key words: *Cosmos caudatus, Piper sarmentosum, Premna cordifolia, Mus musculus,* sperm parameters

INTRODUCTION

Traditionally, plants are widely used in curing reproductive system problems (Chauhan, 2014). In Malaysia, through folklore, some local plants are known to possess aphrodisiac properties such as *Eurycoma longifolia* or locally known as Tongkat Ali. Through research, it is known that Tongkat Ali able to increase testosterone serum level and it is known that testosterone plays a major role in spermatogenesis (Zanoli *et al*., 2009). For the past years, increased interest in herbs plant has contribute to research conducted in proving its efficacy in treating reproductive system problems. Other than posing androgenic activity, herbal plants also known to produce antioxidant activities, improving the reproductive system performance. For example, a study conducted by Awoniyi *et al* (2011) showed fermented rooibos and green tea able to protect testicular tissue against oxidative damage, reduced free radicals effectively and decreased defective spermatogenic cells.

In the process of mammalian reproduction, proficient spermatozoa production by testis plays a significant role as the haploid genome carrier to the oocyte (Kumari, 2011). The important indicators of human male fertility are the motility, viability, morphology and total number of spermatozoa. Other indicators are total fluid volume secreted by accessory glands and the composition of seminal fluid (Amidu, 2012).

The three plants chosen for this study are popular in Malaysia eaten as salad. Therefore, it is compelling to learn about its effect on reproductive system specifically on sperm parameters. *Cosmos caudatus* or locally known as *Ulam Raja* in Malaysia is freshly consumed or blanched in a boiling water. Its aroma and taste are able to enhance appetite (Mediani *et al*., 2014). The *Cosmos caudatus* plant can grow up to 30-250 cm in height. The leaves are compound, crossed opposite, pinnate,

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have pointed tips, flat edge, 15-25 cm in length and green in colour (Sulistyorini, 2010). The plant has been used traditionally to treat hypertension, enhancing blood circulation and supporting the firmness of bones (Mohamed, 2012). *C. caudatus* is also rich in antioxidants such as proanthocyanidins, chlorogenic acids, quecertin and its derivatives besides its anti-ageing properties (Shui *et al*., 2005).

Piper sarmentosum or known as *Kaduk* in Malaysia is a betel-leaf like plant that grows compatibly on damp soil in secondary forest (Rahman, 2010). Traditionally, the leaves were ingested for smoother digestion and treating fever while the roots have capability to treat coughing, toothache and fungal dermatitis on feet (Koh *et al*., 2009). The plant is rich in flavonoid, phenylpropanoid (ascaricin, α-ascarone), phenolic compounds (xanthophylls, tannins), calcium, iron, vitamin B1, B2, C, E and β-sitosterol (Navickiene, 2000).

Premna cordifolia or locally known as *Bebuas* in Malaysia is an edible plant to be taken raw or as one of the ingredients in cooking. It can be used to reduce fever and asthma. Meanwhile, the intake of young leaves by lactating mother can help in stimulating milk production (Mat Ali, 2008). Previous study showed that, the antioxidant activity of vegetable extracts has been correlated to their content of phenolic due to their property of scavenging free radicals and *P. cordifolia* recorded a total of 16.37 µg/mg phenolic content (Nazri *et al.*, 2011). *P. cordifolia* is a plant that is not well studied. There is neither literature on toxicity nor had its effect on the mammalian reproductive system been recorded.

Each plant used in this study consists of antioxidant properties that may help in protecting spermatozoa from excessive ROS (Amran *et al*., 2010). Previous study revealed imbalance of reactive oxygen species (ROS) concentrations and antioxidant scavenging systems lead to seminal oxidative stress leading to peroxidative damage of the sperm plasma membrane and impaired DNA (Agarwal, 2004). Thus, the aim of this study was to asses and compare sperm parameters of mice treated with *Cosmos caudatus*, *Piper sarmentosum* and *Premna cordifolia* extracts.

MATERIALS AND METHODS

Ethanolic Extracts Preparation

The young leaves used in this study were harvested in December 2014 from Kampung Melayu, Perak. The leaves were washed under running water, undergo cryogenic drying using liquid nitrogen and ground into powder using electrical grinder. Then, the powder form of the young leaves was left inside the freeze dryer (CHRIST Alpha 1-4 LDplus, Germany) for a week and later soaked in 80% ethanol for 48 hours. The crude extract was then evaporated to dryness using rotary evaporator (BUCHI Rotavapor R-210m, Switzerland) and concentrated using freeze dry. The crude extract was dissolved in 0.9% saline solution in order to obtain the desired extract concentration (500 mg/kg bwt).

Experimental Animals

A total of 24 sexually matured male mice (ICR strain, outbred) 8-10 weeks old and weighing 20-40 g were used in this study. Animals were housed in polypropylene cages with stainless steel grills at the animal holding room, Faculty of Applied Sciences, UiTM. Upon receiving the mice from the supplier (Chenur Supplier Sdn. Bhd.), the mice were acclimatized for a minimum period of 5 days in an air conditioned room (22 ± 3ºC) under a 12 h light: 12 h dark cycle. Throughout the duration of the experiment, all mice were given standard rodent pellets and water *ad libitum.* The study has been presented and approved by the UiTM Research Committee on the Ethical Use of Animals (UiTM Care: 111/2015).

Experimental Procedure

The mice were divided into 4 groups with 6 mice per group. Group 1 received 0.3 mL 0.9% saline solution (SS) and served as control. Groups 2 to 4 were treated with 500 mg/kg bodyweight of *Cosmos caudatus* ethanolic extracts (CCEE), *Piper sarmentosum* ethanolic extracts (PCEE) and *Premna cordifolia* ethanolic extracts (PSEE) respectively. The extracts were administered approximately 8 to 9 am daily for the duration of 30 days by oral gavage (Chenur Supplier Sdn. Bhd.).

Body and Organ Weight

All male mice were weighed two times in this study, at the beginning and at the end of the experimental period. On day 31, the mice were sacrificed by cervical dislocation. The cauda epididymis and testes were dissected out and weighed with analytical balance (FX-500i, A&D Company, Limited). Then, relative organ weight was calculated according to the formula by Perveen *et al* (2012):

$$\text{Relative organ weight (g)} = \frac{\text{Mean weight of organ (g)}}{\text{Mean final body weight (g)}} \times 100$$

Sperm Parameters Analysis

The epididymis was transferred into a 500 µl pre-warmed Dulbecco's Modified Eagle Medium

(DMEM) (Sigma-Aldrich Co.) and minced to produce sperm suspension. Sperm was allowed to swim-up for at least 15 minutes prior to sperm analysis. The prepared sperm suspension was maintained at 37ºC by placing in a controlled temperature water bath (WNB 45, Memmert, GmbH Co. KG) and quickly proceeds for sperm assessment.

Sperm motility

Sperm motility was assessed by pipetting 30 µl of the sperm suspension and placing it onto a clean glass slide. The motility of the sperm was observed and analysed. The sperms were classified into motile and non-motile sperm (WHO, 2010).

Sperm morphology

Sperm morphology evaluation was conducted by placing a drop of sperm suspension on a clean slide and stained using the eosin-nigrosin staining kit (FertiPro, Belgium) in accordance to manufacturer's manual. The well mixed mixture (sperm suspension and eosin-nigrosin staining) was smeared and air dried. Next, the slide was observed under the microscope for any defect on the sperm's tail, neck and midpiece or head and the sperms were categorised as normal and abnormal sperm. The data was expressed as a percentage of morphologically normal sperm. At least 200 spermatozoa were counted for each triplicate.

Sperm count

The sperm count was carried out by using improved Neubauer cell counting chamber (Marienfeld, Germany). One important precaution step was to heat the sperm suspension at 50º-60ºC prior to aspiration (50 µL) to the chamber. The chamber was placed in humid condition for 10 to 15 minutes prior to observation under the microscope to let the rendered sperm settle. Then the sperm was calculated using WHO Laboratory Manual 2010 as a guideline.

Statistical Analysis

The data was expressed as mean ± standard error of mean (SEM) and analysed by One-way Analysis of Variance (ANOVA) and post-hoc test of SPSS Software Version 20. A probability of $p<0.05$ was chosen as the criterion of statistical significance.

RESULTS AND DISCUSSION

Data were analysed and tabulated in Table 1. No significant difference recorded on the mean of increased body weight. CCEE treated mice showed the highest increment in body weight which was by 9 g. The result obtained was aligned with previous study that showed mice treated with 500 mg/kg CCEE demonstrated the highest body weight increased by the end of the experiment (Booh *et al*., 2015). All three treated mouse groups showed increased body weight indicating the extract does not impede the appetite.

PCEE treated mice showed the highest absolute testis weight (0.122 ± 0.003 g) whilst for cauda epididymis, the entire study group showed similar means of weight (0.02 g) (Table 2). As for CCEE treated mice, the highest mean relative testis weight (0.341 ± 0.029 g) was recorded and highest relative cauda epididymis weight was recorded by PCEE treated mice (0.078 ± 0.006 g) (Table 3). If there was a significant difference in organ weight increase, it

Table 1. Body weight of male mice treated with 0.9% saline solution (SS) and 500 mg/kg ethanolic extract of *Cosmos caudatus* (CCEE) *Piper sarmentosum* (PSEE) and *Premna cordifolia* (PCEE)

Groups	Increase Mean Body Weight (g)
SS	6.80 ± 0.58[a]
CCEE	9.00 ± 0.41[a]
PSEE	6.33 ± 0.88[a]
PCEE	8.80 ± 0.66[a]

Values with similar superscript in the same column showed no significant difference.

Table 2. Testis and epididymis absolute weight of male mice treated with 0.9% SS and 500 mg/kg ethanolic extract of *Cosmos caudatus* (CCEE), *Piper sarmentosum* (PSEE) and *Premna cordifolia* (PCEE)

Groups	Organ Weight (g)	
	Testis (g)	Epididymis (g)
SS	0.110 ± 0.004[a]	0.020 ± 0.000[a]
CCEE	0.120 ± 0.010[a]	0.020 ± 0.004[a]
PSEE	0.103 ± 0.003[a]	0.020 ± 0.000[a]
PCEE	0.122 ± 0.003[a]	0.020 ± 0.004[a]

All data values shows no significant difference ($p>0.05$).

Table 3. Testis and epididymis relative weight of male mice treated with 0.9% SS and 500 mg/kg ethanolic extract of *Cosmos caudatus* (CCEE), *Piper sarmentosum* (PSEE) and *Premna cordifolia* (PCEE)

Groups	Relative Organ Weight (g)	
	Testis (g)	Epididymis (g)
SS Control	0.338 ± 0.013[a]	0.062 ± 0.000[a]
CCEE	0.341 ± 0.029[a]	0.058 ± 0.013[a]
PSEE	0.288 ± 0.012[a]	0.052 ± 0.004[a]
PCEE	0.332 ± 0.006[a]	0.078 ± 0.006[a]

All data values shows no significant difference ($p>0.05$).

Table 4. Sperm parameter of male mice treated with 0.9% SS and 500 mg/kg ethanolic extract of *Cosmos caudatus* (CCEE), *Piper sarmentosum* (PSEE) and *Premna cordifolia* (PCEE)

Treatment Groups	Sperm count ($\times 10^6$ mil/mL)	Sperm motility (%)	Normal sperm morphology (%)
SS	24.40 ± 1.86^{a}	16.60 ± 4.26^{b}	92.78 ± 2.51^{a}
CCEE	38.69 ± 7.82^{a}	$33.87 \pm 7.51^{a,b}$	86.87 ± 3.36^{a}
PSEE	30.17 ± 5.89^{a}	47.17 ± 5.93^{a}	$82.67 \pm 2.17^{a,b}$
PCEE	39.40 ± 3.76^{a}	$39.00 \pm 5.90^{a,b}$	70.80 ± 3.76^{b}

Values with same super script in same column show no significant difference ($p>0.05$).

is an indicator of inflammation due to infiltration of inflammatory cells and presence of swollen cells. Testicular weights are considered valuable in toxicity studies because the changes in the testis weight reflect changes in the seminiferous tubules or presences of interstitial edema causing increase in organ weight (Michael *et al*., 2007). From the result obtained, there is no significant increase in organ weight in treated groups when compared to the control group. Thus, it indicates that the extracts used were not harmful towards the tested organ.

Analysed sperm parameter data (Table 4) showed that PCEE treated mice were recorded as the group with the highest sperm count (39.40 million/mL) while the lowest sperm count was by SS treated mice (24.40 million/mL). However, there were no significant differences among all of the groups. To date, there is a lack of data concerning the effect of *P. cordifolia* on sperm count. Previous study revealed that *P. cordifolia* is high in antioxidant activity which may facilitate in suppressing oxidative stress that lead to high sperm count compared to other groups (Mat Ali, 2008). Oxidative stress in testis capable of disrupting the steroidogenic capacity of Leydig cells which play an important role in spermatogenesis (Hales *et al*., 2005). Testes rely heavily on small molecular weight antioxidant factors for protection against oxidative damage including ions and a wide variety of free radical scavengers (Aitken & Roman, 2008).

PSEE treated mice showed highest percentage of motile sperm (47.17%) whereas the lowest percentage of motile sperm was by SS treated (16.60%). There was significant difference recorded between SS and PSEE ($p<0.05$) PSEE treated mice showed highest percentage of motile sperm (47.17%) whereas the lowest percentage of motile sperm was by SS treated (16.60%). There was significant difference recorded between SS and PSEE ($p<0.05$). *P. sarmentosum* is rich in antioxidants and vitamin E which is a major chain breaking antioxidant in the sperm plasma membranes (Bolle *et al.,* 2002). The phospholipids of the sperm's mitochondria are susceptible to lipid peroxidation. Antioxidants available in the *P. sarmentosum* suppressed the lipid peroxidation thus preventing from rendering the sperm immotile (Suleiman *et al*., 1996). Past studies disclosed a significant improvement in sperm motility after the used of antioxidants such as selenium and vitamin E (Moslemi & Tavanbakhsh, 2011).

In the study, the sperms were classified into two groups; normal or abnormal. Abnormal sperms were distinguished by the characteristics of hookless head, knobbed-hook head, banana-shaped head and coiled tail. SS treated mice recorded the highest percentage of normal sperm morphology (92.78%). Interestingly, CCEE treated mice followed closely behind with 86.87% which in parallel with previous study (Booh, 2015). PCEE treated mice showed significantly lower ($p<0.05$) normal sperm morphology ($70.80 \pm 3.76\%$) as compared to SS and CCEE treated mice. This showed that *P. cordifolia* may play a role in defect sperm morphology.

CONCLUSION

In conclusion, all plants extracts (CCEE, PSEE and PCEE) used in this study demonstrated increased body weight of all male mice groups. The extracts do not have the ability in hindering appetite. Hindered appetite will prompt limit to none food intake by the mouse, thus leading to important nutrients loss causes adverse effects on body form, function (hormone synthesis and spermatogenesis) and many more (Ahmed *et al*., 2010). PSEE and CCEE show potential in enhancing sperm parameter and further study need to be done. PCEE show significantly decreased in percentage of normal sperm morphology in comparison to control group and CCEE treated mice. More studies on PCEE need to be conducted in order to recognize its role in lowering the normal sperm morphology. Further phytochemical and pharmacological are currently under-going to investigate all studied plants mode of action.

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