

Short Communication

THE EFFECTS OF *COSMOS CAUDATUS* AND *PIPER SARMENTOSUM* AQUEOUS EXTRACTS ON MALE MICE FERTILITY

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ABSTRACT

**Objective:** This study was conducted to determine the sperm quality and fertility of male mice supplemented with *Cosmos caudatus* and *Piper sarmentosum* aqueous extracts.

**Methods:** The male mice were treated with either 0.2 ml of distilled water, 500 mg/kg bwt of *Cosmos caudatus* or *Piper sarmentosum* aqueous extract for the duration of four weeks. The body weight was checked on a weekly basis. Treated male mice then were mated with proven fertile females and the numbers of implantation sites were counted. At the end of the experimental duration, treated males were sacrificed by cervical dislocation and subjected to sperm analysis.

**Results:** Our data revealed that *Cosmos caudatus* and *Piper sarmentosum* increased the sperm quality and fertility in male mice. In addition, we found that the body weight was not affected by *Cosmos caudatus* or *Piper sarmentosum* aqueous extract.

**Conclusion:** In conclusion, *Cosmos caudatus* and *Piper sarmentosum* produced a remarkable increase in male mice reproductive performance.

**Keywords:** *Cosmos caudatus*, *Piper sarmentosum*, Sperm quality, *in-vivo* fertilisation

The use of plants or plant-based products to stimulate sexual desire and to increase reproductive performance is almost as old as the human race itself [1]. A number of herbal drugs have been validated for their effect on fertility and sexual behaviour. Ajayi and co-worker demonstrated that *Titonia diversifolia* promote spermatogenesis and testosterone secretion in male Wistar rats [2]. According to another report, *Abelmoschus manihot* improves sperm count, erectile index and sexual desire in Swiss albino mice [3]. Recently, a few authors reviewed Indian medicinal plants with aphrodisiac potential [4].

*Cosmos caudatus*, which is locally known as *Ulam raja* in Malaysia, is a herb of the family Compositae [5]. It is edible plant with purple or white ray florets, grows up to about 1 to 8 feet tall, hairless or sparsely hairy and leaves are finely dissected, 10 to 20 cm long [6]. In Malaysia, *Cosmos caudatus* is traditionally utilised for improving blood circulation, anti-aging agent, to promote fresh breath and to treat infections associated with pathogenic microorganisms [6-8]. Previous study has shown that this plant contains phenolic compounds that contribute to the antioxidant and anticarcinogenic properties of the plants [9]. Meanwhile, in male mice reproductive system, there are no negative effects imposed by *Cosmos caudatus* ethanolic extract [10].

*Piper sarmentosum* is commonly known as *kaduk* in Malaysia. This plant is herbaceous, creeping or erect. Ugusman and co-workers documented that *Piper sarmentosum* contains high antioxidant compounds such as vitamin E, carotenoids, xanthophylls, tannins and phenolics [11]. Previous studies reported that *Piper sarmentosum* has antibacterial [12], hypoglycaemic [13] and anti-malarial [14] properties. Traditionally, in a rural area of Kelantan State of Malaysia, a decoction of the leaves is drunk to treat malarial fever [15]. Meanwhile, the crushed leaves are mixed with water and used for bathing to treat kidney stones and difficulty in urination [16].

As far as our literature survey could ascertain, no attempts have been made to investigate the effect of *Cosmos caudatus* and *Piper sarmentosum* aqueous extracts on the male reproductive system. Hence, the present study was designed to compare the sperm quality and reproductive performance of male mice treated with *Cosmos caudatus* and *Piper sarmentosum* aqueous extracts. We hypothesized

that, *Cosmos caudatus* and *Piper sarmentosum* aqueous extract will increase sperm quality and fertility in male mice.

Mature leaves of *Cosmos caudatus* were collected from Selangor State, Malaysia. Meanwhile, mature leaves of *Piper sarmentosum* were harvested from the rural area of Kelantan State, Malaysia. Taxonomic identification was conducted by a botanist from Herbarium, Universiti Kebangsaan Malaysia. A voucher specimen DD-03/2014-Raja and DD-04/2014-Kaduk were deposited in Biology Laboratory, Universiti Teknologi MARA. The aqueous extract was prepared as previously described [17]. About 250 g of dried plant materials were boiled in 4000 ml of water until the volume of water is reduced to half. Then, the mixture was filtered and centrifuged at 3000 rpm for 15 min. The supernatant was freeze-dried to obtain a powder form of aqueous extract and was kept at 4 °C for later use.

All animal works in this study were conducted in accordance to Research Ethics Committee of the Faculty of Applied Sciences, Universiti Teknologi MARA. Eighteen adults and healthy male mice (25-35 g) were obtained and maintained in the Faculty of Applied Sciences Animal Facility. Animals were maintained under standard conditions with 12-h light/dark cycles and room temperature of 22 °C. The food in the form of dry chow pellets and water was available *ad libitum*. Eighteen proven fertile male mice were randomly divided into three groups. The first group (n=6) served as a control and orally treated with 0.2 ml distilled water. The second group (n=6) was supplemented with 500 mg/kg of body weight (bwt) of *Cosmos caudatus* aqueous extract. The third group (n=6) was supplemented with 500 mg/kg of body weight (bwt) of *Piper sarmentosum* aqueous extract. All the animals were treated with the respective treatments daily for four weeks, and the body weight was recorded on a weekly basis.

At the end of the four weeks of the experimental period, male mice from all groups were mated with proven fertile female. All male mice were kept in individual cages with sexually receptive females for five days (male to female ratio of 1:2). Females were checked daily for post-coital plugs for determination of mating. The presence of a vaginal plug was defined as a day 1 of pregnancy. Females were euthanized on day 17 or 18 post-copulation, and the number of implantation sites or foetuses was counted [18].

All males were sacrificed by cervical dislocation. Epididymis were excised, thoroughly washed with physiological saline and blotted on filter paper. Shortly after, the cauda epididymis was minced in 0.5 ml saline, incubated at room temperature for 2 min and subjected to quality sperm analysis. Sperm concentration, sperm progressive motility and sperm morphology were determined as previously described [19].

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for Windows. Body weight data was subjected to repeated measures analysis of variance. Sperm quality and fertility data were analysed by a two-way ANOVA.

Our result showed that sperm quality was affected by *Cosmos caudatus* and *Piper sarmentosum* aqueous extract consumption (table 1). Sperm count was increased significantly ( $p < 0.05$ ) in mice supplemented with 500 mg/kg BWT of *Piper sarmentosum* aqueous extract. The sperm quality of mice supplemented with 500 mg/kg BWT of *Cosmos caudatus* also was increased compared to control, but the difference was not significant ( $p > 0.05$ ). Sperm motility and the number of sperm with normal morphology were increased significantly ( $p < 0.05$ ) in mice supplemented with *Cosmos caudatus* and *Piper sarmentosum* compared to control. The number of implantation sites in female copulated with *Cosmos caudatus* and *Piper sarmentosum* treated male was increased but not statistically significant ( $p > 0.05$ ) compared to control.

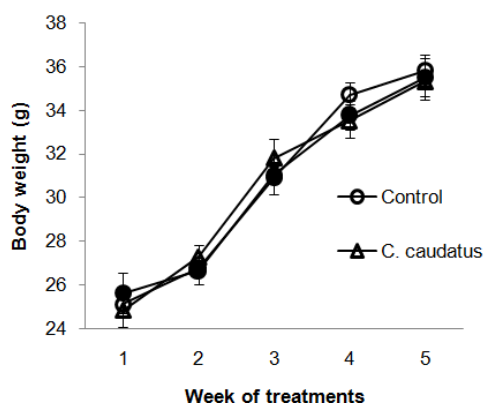
**Table 1: Sperm quality and fertility of mice supplemented with 0.2 ml of distilled water (control group), 500 mg/kg bwt of *Cosmos caudatus* aqueous extract and 500 mg/kg bwt of *Piper sarmentosum* aqueous extract**

Parameters	Control	<i>Cosmos caudatus</i>	<i>Piper sarmentosum</i>
Sperm count ( $10^6$ /ml)	48.67±3.6	51.81±2.4	65.33±2.7*
Sperm motility (%)	57.51±1.3	60.72±1.9*	62.83±1.4*
Sperm morphology (%)	72.45±1.9	76.86±1.3*	81.33±1.5*
Implantation sites per female (n)	9.29±0.3	11.17±0.5	11.45±0.7

Data are presented as mean±standard error mean (n=6). Asterisk (\*) indicated  $p < 0.05$  compared to control.

These results give support to the hypothesis that sperm quality and male fertility can be positively affected by *Cosmos caudatus* and *Piper sarmentosum*. It is probably necessary to mention that spermatogenesis and sperm quality was promoted partly by antioxidant properties. A number of *in-vivo* studies have proved that the spermatogenesis, sperm quality and fertility can be positively affected by antioxidant intake [20]. Medicinal and nutritional studies showed that *Cosmos caudatus* [9] and *Piper sarmentosum* [21] demonstrated strong antioxidant activities.

In addition, body weight was increased significantly ( $p < 0.05$ ) over time but there were no significant different ( $p > 0.05$ ) between the treatments (fig. 1). The data suggested that 500 mg/kg of body weight (BWT) of *Cosmos caudatus* and *Piper sarmentosum* aqueous extracts did not alter feeding behaviour or the nutrient metabolism compared to control.



**Fig. 1: Body weight of mice supplemented with 0.2 ml distilled water (control), 500 mg/kg bwt of *Cosmos caudatus* and 500 mg/kg bwt of *Piper sarmentosum* throughout the experimental period. Data are presented as mean±standard error mean (n=6)**

Similarly, another author reported that there were no adverse effects on the body weight of animals given *Cosmos caudatus* extract (100, 500 and 1000 mg/kg BWT) for 21 d [22]. Our data on *Piper sarmentosum* also in agreement with other researchers who reported that daily consumption of Piperaceae species gives no effect on body weight [23]. Overall, these suggest that supplementation of *Cosmos caudatus* and *Piper sarmentosum* may be beneficial in body weight management.

In conclusion, the results presented suggest that sperm quality and fertility in male mice positively affected by *Cosmos caudatus* and *Piper sarmentosum* aqueous extract consumption. Further pharmacological and phytochemical studies are currently in progress to investigate *Cosmos caudatus* and *Piper sarmentosum* mode of actions.

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#### CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper. All authors were involved in the writing, revision and final approval of the paper.

#### REFERENCES

1. Chauhan NS, Sharma V, Dixit VK, Thakur M. A review on plants used for the improvement of sexual performance and virility. *BioMed Res Int* 2014. doi.org/10.1155/2014/868062. [Epub 2014 Aug 18]
2. Ajayi AF, Jegede AI. Reproductive profile of male wistar rats treated with graded doses of aqueous extract of *Titonia diversifolia* leaf. *World J Life Sci Med Res* 2012;2:48-53.
3. Rewatkar KK, Shahzad N, Ahmed A, Khan MI, Ganesh N. A landmark approach to the aphrodisiac property of *Abelmoschus manihot* (L.). *Int J Phytomed* 2010;2:312-9.
4. Singh R, Ali A, Jeyabalan G, Semwal A. Current status of Indian medicinal plants with aphrodisiac potential. *J Acute Disease* 2013;2:13-21.
5. Mediani A, Abas F, Khatib A, Tan CP. *Cosmos caudatus* as a potential source of polyphenolic compounds: optimisation of oven drying conditions and characterisation of its functional properties. *Molecules* 2013;18:10452-64.
6. Rasdi NHM, Samah OA, Sule A, Ahmed QU. Antimicrobial studies of *Cosmos caudatus* Kunth. (Compositae). *J Med Plants Res* 2010;4:669-73.
7. Bodeker G. Health and beauty from the rainforest: Malaysian traditions of ramuan. Kuala Lumpur: Didier Millet; 2009.
8. Hassan WE. Healing herbs of Malaysia. Kuala Lumpur: Federal Land Development Agency; 2006.

9. Mohamed N, Khee SGS, Shuid AN, Muhammad N, Suhaimi F, Othman F, *et al.* The effects of *Cosmos caudatus* on structural bone histomorphometry in ovariectomized rats. J Evidence-Based Complementary Altern Med 2012. doi.org/10.1155/2012/817814. [Article in Press]
10. Booh MJ, Hashim M, Ismail NH, Daud D, Samsulrizal N, Yahya MFZR. Effects of *Cosmos caudatus* on sperm quality of mice, *Mus musculus*. Malays Appl Biol 2015;44:89-93.
11. Ugusman A, Zakaria Z, Chua K. Flavanoids of *Piper sarmentosum* and its cytoprotective effects against oxidative stress. EXCLI J 2012;11:705-14.
12. Atiax E, Ahmad F, Sirat HM, Arbain D. Antibacterial activity and cytotoxicity screening of Sumatran kaduk (*Piper sarmentosum* Roxb). Iran J Pharmacol Ther 2011;10:1-5.
13. Peungvicha P, Thirawarapan SS, Temsirikkul R, Wanabe H, Kumar-Prasain J, Kadota S. Hypoglycemic effect of water extract of *Piper sarmentosum* leaves in rats. J Ethnopharm 1998;60:27-32.
14. Najib NA, Rehman N, Furuta T, Kojima S, Takane K, Ali MM. Antimalarial activity of extracts of Malaysian medicinal plants. J Ethnopharmacol 1999;64:249-54.
15. Ong HC, Nordiana M. Malay ethno-medico botany in Machang, Kelantan, Malaysia. Fitoterapia 1999;70:502-13.
16. Ong HC, Norzalina J. Malay herbal medicine in Gemenceh, Negeri Sembilan, Malaysia. Fitoterapia 1999;70:10-4.
17. Parhizkar S, Che-Zainudin CZB, Dollah MA. Effect of *Phaleria macrocarpa* on sexual function of rats. Avicenna J Phytomed 2013;3:371-7.
18. Cammack KM, Antoniou E, Hearne L, Lamberson WR. Testicular gene expression in male mice divergent for fertility after heat stress. Theriogenology 2009;71:651-61.
19. NAFA manual on basic semen analysis. NAFA and ESHRE-SIGA; 2002.
20. Agarwal A, Sekhon LH. The role of antioxidant therapy in the treatment of male infertility. Human Fertility 2010;13:217-25.
21. Hafizah A, Zaiton Z, Zulkhairi A, Mohd-Ilham A, Nor-Anita M, Zaleha A. *Piper sarmentosum* as an antioxidant on oxidative stress in human umbilical vein endothelial cells induced by hydrogen peroxide. J Zhejiang Univ Sci 2010;11:357-65.
22. Radman HM, Yusof K, Saad QHM, Wan-Ngah WZ, Abdullah A. The effect of Ulam Raja (*Cosmos caudatus*) on drug metabolizing enzymes, lipid peroxidation and antioxidant status in mice liver. Int J PharmTech Res 2014;6:1213-25.
23. Mbongue FGY, Kamtchouing P, Essame OJL, Yewah PM, Dimo T, Lontsi D. Effect of the aqueous extract of dry fruits of *Piper guineense* on the reproductive function of adult male rats. Indian J Pharmacol 2005;37:30-2.