

EFFECTS OF *Cosmos caudatus* ON SPERM QUALITY OF MICE, *Mus musculus*

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ABSTRACT

This study was designed to analyse the effects of *Cosmos caudatus* on sperm quality of mice, *Mus musculus*. The daily intake of *Cosmos caudatus* was evaluated in determining its effects on male fertility. Four different concentrations of the crude ethanolic extracts which were 50 mg/kg, 125 mg/kg, 500 mg/kg and 1000 mg/kg, respectively were administered for 28 consecutive days. Sperm analyses includes sperm count, total sperm motility, sperm viability and sperm morphology were carried out using standard procedures outlined by WHO 2010. G3 group gives the highest mean percentage for change in body weight (9.60 gm), total sperm motility (56%) and normal sperm morphology count (62.8 sperm) as compared to the rest the group treatments. For the sperm count and sperm viability, G4 group showed the highest data; 56.5 million sperm/ml and 59.53%, respectively. The lowest data of the change in body weight and normal sperm morphology count of mice from G1 group which are 6.43 gm and 58.6 sperm, correspondingly. As for the sperm count, total sperm motility and sperm viability; the lowest data shown by G2 group; 39.4 million sperm/ml, 48% and 43.98%, respectively. The sperm morphology of all the treated male mice was normal except a few detected with abnormalities. The findings showed no negative effect of *Cosmos caudatus* on sperm quality of mice.

Key words: *Cosmos caudatus*, sperm quality, total sperm motility, sperm viability, sperm count

INTRODUCTION

Cosmos caudatus or locally known as “ulam raja” was listed in traditional Malay cuisine as salad, where it always eaten raw during meals (Ong, 2004) and is believed to boost blood circulation; and promote fresh breath (Burkill, 1966; Ismail, 2000). Enriched with anti-oxidant and anti-aging properties, *Cosmos caudatus* is an annual plant which bears either purple, pink or white ray florets and usually grows to 2 m in height (Nor Hafipah *et al.*, 2010). Previous study has revealed that this plant had high content of anti-oxidant. The study done by Wong *et al.* (2006) on the antioxidant activities of selected plants showed that *Cosmos caudatus* had the highest ferric ions reducing activity in the ferric reducing antioxidant potential assay. The metabolites present in this herbal plant include phenolic acid and flavonoids which are responsible for exhibiting strong antioxidant activity, not only to protect against lipid

peroxidation but also to scavenge free radicals (Ahmed *et al.*, 2012; Faridah *et al.*, 2006). This anti-oxidant property of *Cosmos caudatus* is really crucial to the reproductive system, especially in the testes. Hence, the aim in this study was to assess and compare the sperm quality of mice treated with different concentrations of *Cosmos caudatus* ethanolic extract.

MATERIALS AND METHODS

Ethanolic extract preparation

The leaves of the *Cosmos caudatus* were subjected to cryogenic grinding using liquid nitrogen before proceeding to ethanolic extraction. The crude *Cosmos caudatus* ethanolic extract was diluted in 0.9% saline (0.9 g NaCl per 100 ml distilled water) to the respective experimental concentrations prior to being administered to male mice.

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Experimental animals

Sexually matured male mice (7-8 weeks old) weighing 22 g – 32 g were used in the experiment. Mice were fed with standard mouse pellets (Gold Coin Feed Mills (M) Sdn. Bhd) and water given *ad libitum*. Mice were maintained under standard conditions of humidity, temperature with 12 hours of light and, 12 hours of dark cycle. The test mice were allowed to acclimatize for one week prior to the experiment.

Experimental procedure

Mice were divided into five groups including one group of control consisting of five mice per group. The treatment was administered for twenty-eight days by force feeding. The first group was fed with saline and kept as control. The rest were given the ethanolic extract of *Cosmos caudatus*, daily. The mice were divided into five experimental groups: Control group (normal diet + saline); G1 (normal diet + 50 mg/kg *C. caudatus* extract); G2 (normal diet + 125 mg/kg *C. caudatus* extract); G3 (normal diet + 500 mg/kg *C. caudatus* extract) and G4 (normal diet + 1000 mg/kg *C. caudatus* extract).

Collection of sperm and sperm preparations

Mice were sacrificed following a 12 hours fasting using the method of cervical dislocation and the ventral side was swabbed with alcohol before dissection. The sperm were collected from the cauda epididymides distally from the testes of mature male mice. The cauda was minced and transferred into 400 µl of a pre-warmed Dulbecco's Modified Eagle Medium (DMEM).

Sperm analysis

Sperm motility, sperm viability, sperm morphology and sperm count were carried out using standard procedures outlined by World Health Organization (WHO) 2010. Briefly, 30 µl of mice sperm suspension delivered onto clean glass slide and covered with coverslip. This preparation of sperm was examined using light compound microscope. The sperm viability and morphology were stained with VitalScreen™ eosin-nigrosin kit staining (FertiPro, Belgium). The sperm count analysis was carried out using MAKLER Chamber Count.

Statistical analysis

Descriptive analysis of ANOVA and post hoc test were conducted using SPSS software, version 20.0. A difference was considered significant at $p < 0.05$. All data were expressed as mean \pm standard error of the mean (SEM).

RESULTS AND DISCUSSION

The change in body weight of mice and all the sperm analysis showed no significant difference ($p > 0.05$) between four concentrations of 50 mg/kg, 125 mg/kg, 500 mg/kg and 1000 mg/kg with the control treatment group. The mean and standard error of change in body weight of mice and sperm analysis is shown in Table 1 and 2. G3 group gives the highest mean percentage for change in body weight (9.60 gm), total motility (56% progressive motility) and normal sperm morphology count (62.8 sperm)

Table 1. Change in body weight of mice and Sperm analysis of mouse spermatozoa

Treatment Groups	Change in body weight (gm)	Sperm count (x 10 sperm/ml)	Total motility (%)	Viability (%)
Control (Normal diet + 0.9% saline)	7.00 \pm 0.89	52.20 \pm 5.45	60.00 \pm 6.33	52.46 \pm 6.67
G1 (Normal diet + 50 mg/kg <i>C. caudatus</i> extract)	6.43 \pm 1.83	43.80 \pm 3.48	52.00 \pm 12.00	56.90 \pm 7.43
G2 (Normal diet + 125 mg/kg <i>C. caudatus</i> extract)	8.60 \pm 1.63	39.40 \pm 12.09	48.00 \pm 13.57	43.98 \pm 4.10
G3 (Normal diet + 500 mg/kg <i>C. caudatus</i> extract)	9.60 \pm 0.92	51.40 \pm 4.93	56.00 \pm 7.48	49.72 \pm 8.10
G4 (Normal diet + 1000 mg/kg <i>C. caudatus</i> extract)	7.80 \pm 1.38	56.50 \pm 5.75	55.00 \pm 0.50	59.53 \pm 6.09

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

Table 2. Normal sperm morphology count for all treatment groups of mice

Treatment Groups	Control (Normal diet + 0.9% saline)	G1 (Normal diet + 50 mg/kg <i>C. caudatus</i> extract)	G2 (Normal diet + 125 mg/kg <i>C. caudatus</i> extract)	G3 (Normal diet + 500 mg/kg <i>C. caudatus</i> extract)	G4 (Normal diet + 1000 mg/kg <i>C. caudatus</i> extract)
Normal sperm morphology	66.60 ± 5.44	58.6 ± 14.02	61.6 ± 10.01	62.8 ± 13.06	60.00 ± 11.29

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

as compared to the rest the treatment groups. For the sperm count and sperm viability, G4 group showed the highest data; 56.5 million sperm/ml and 59.53%, respectively. The lowest data of the change in body weight and normal sperm morphology count of mice from G1 group which are 6.43 gm and 58.6 sperm, correspondingly. As for the sperm count, total sperm motility and sperm viability; the lowest data shown by G2 group; 39.4 million sperm/ml, 48% progressive and 43.98%, respectively. The overall extracted sperm from all treatment groups were normal (out of 1964 sperm, 1488 sperm were normal; 75.8%) as compared to the abnormal sperm (24.2%). The abnormalities of sperm detected include looped and missing tail, acephalic and defects in head which were shown at Fig. 1. The fact that *Cosmos caudatus* is a medicinal herbal plant that contains potent biological compounds (Ahmed *et al.*, 2012) that enable it to exhibit strong anti-oxidant activity determined that there are no detectable negative effects of *Cosmos caudatus* ethanolic extract on mouse sperm quality and the results have shown so. However, the difference in concentrations administered onto much higher concentrations of *Cosmos caudatus* ethanolic extracts (500 and 1000 mg/kg) results in better sperm quality as compared to lower concentrations (50 and 125 mg/kg) of the ethanolic extract which in comparisons of change in body weight; 500 mg/kg extract compromise better result, sperm count, sperm total motility, sperm viability and normal sperm morphology count; G3 group shows the same pattern outcomes as the Control group compared to the rest of the treatment groups, as elucidated in the Fig. 2, Fig. 3 and Fig. 4. To date, there are no data regarding the effects of different concentrations of *C. caudatus* on sperm quality. However, one study showed at 500 mg/kg concentration of *C. caudatus*, it is able to repair bone damage due to the removal of ovaries of rat model (Norazlina *et al.*, 2013). The absence of significant difference of all statistical analysis that

were conducted indicates that this plant aids in maintaining better quality of sperm but at 50 and 125 mg/kg concentrations, they are not sufficient and the data obtained were lower than the standard normal value (WHO, 2010).

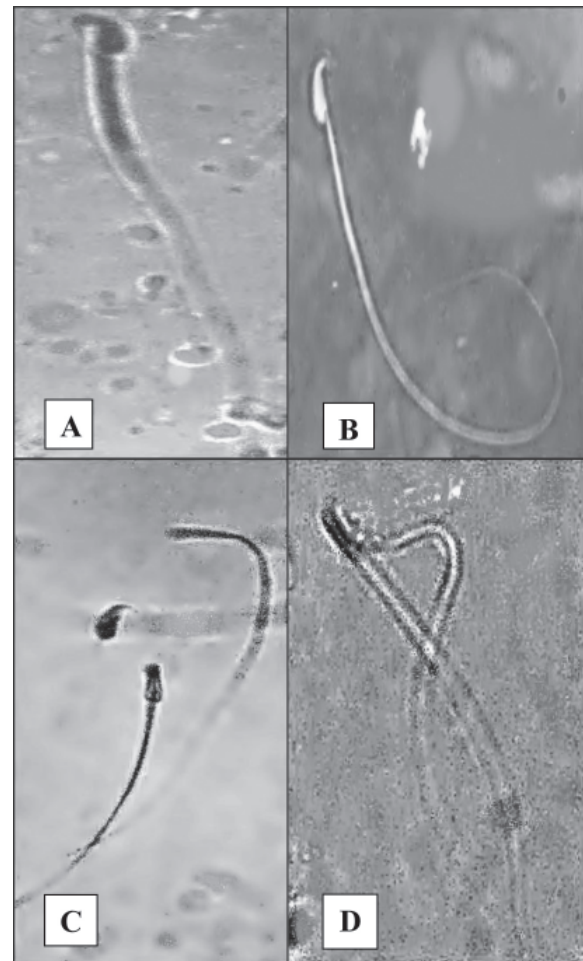


Fig. 1. Abnormalities detected in male mice sperm treated with *Cosmos caudatus* extracts. A: Normal sperm; B: Looped tailed sperm; C: Abnormal head, headless and acephalic sperm; D: Abnormal head and looped tail sperm.

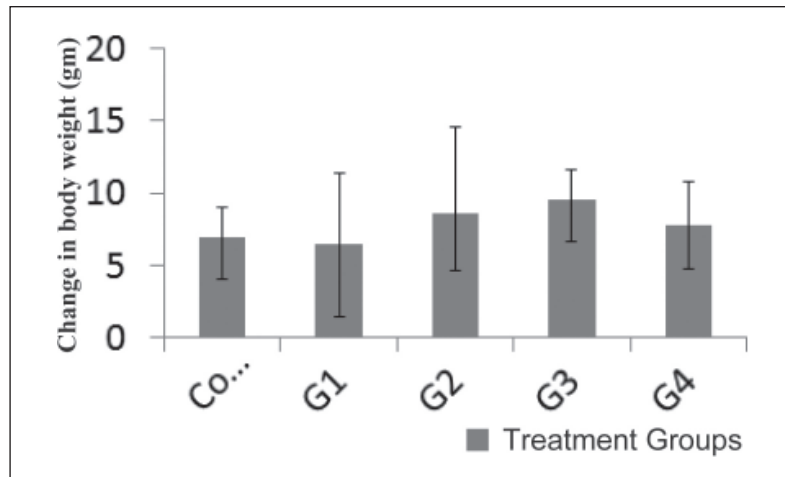


Fig. 2. Bar charts showed the comparisons between changes in body weight, gm of male mice from different treatment groups.

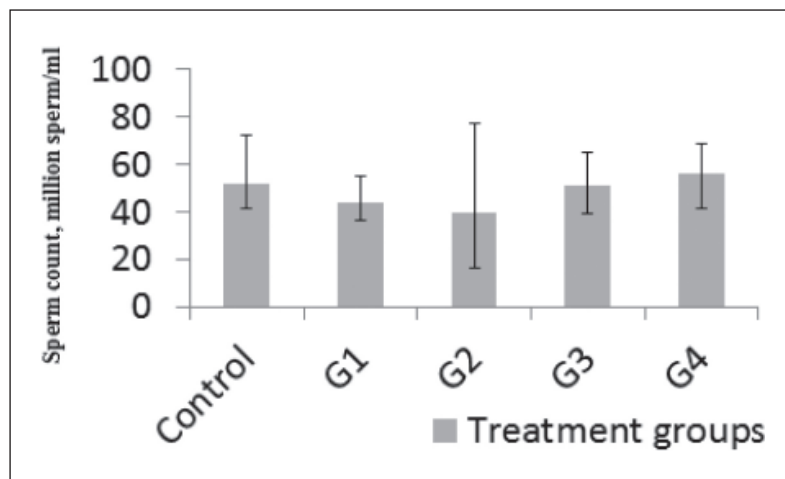


Fig. 3. Bar charts showed the comparisons between sperm count of male mice from different treatment groups.

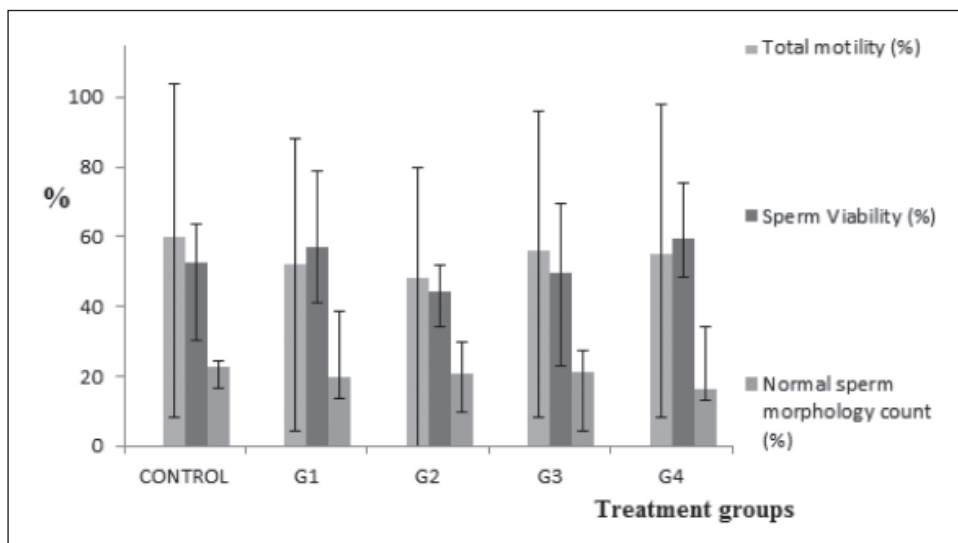


Fig. 4. Bar charts showed the comparisons between percentage in total motility, sperm viability and normal sperm morphology count with the different treatment groups of mice treated with *Cosmos caudatus* ethanolic extract.

CONCLUSION

Overall, there are no negative effects on mice sperm quality imposed by *Cosmos caudatus* ethanolic extracts. At the higher concentrations, the sperm quality is better maintained as compared to the lower concentrations.

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