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## REPRODUCTIVE TOXICITY OF PROCESSED SEEDS OF HORSE EYE BEAN (*MUCUNA URENSL.*) IN MALE RATS

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### ABSTRACT

**Purpose:** To investigate the effect of processed horse eye bean (*Mucuna urens* L.), on weight of testes and epididymes, conception rate, sperm count, sperm viability and sperm head abnormality.

**Methods:** Seventy-two mature albino rats (24 males and 48 females) of 12 weeks old with similar body weights were assigned to four groups of 6 male rats each and treated with processed horse eye bean at 0, 100, 200 and 300 mg/kg body weight respectively daily for 8 weeks. The male rats were made to sire the untreated female rats in the ratio of 1:2 for fertility test. The male rats were then sacrificed and the testes and epididymes were dissected out and weighed. The epididymes were processed for epididymal sperm count, viability and sperm head abnormality test.

**Results:** Rats treated with processed horse eye bean showed no significant ( $P>0.05$ ) effect on the weight of testes and epididymes, but treatment reduced the epididymal sperm count and sperm viability in dose-dependent manner when compared to the control. While it significantly ( $P<0.05$ ) increased sperm head abnormality. The conception rates were significantly ( $P<0.05$ ) reduced in a dose-dependent manner and directly proportional to sperm viability and sperm count; and inversely proportional to sperm head abnormality in all the treatment groups.

**Conclusions:** Processed horse eye bean still has some toxic effect on reproductive endpoints as well as conception rate. Hence, its indiscriminate use as soup thickener could result in reduction of spermatogenic activities and ultimately resulting in infertility, reproductive toxicity and dysfunctions.

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### INTRODUCTION

There have been reports that the consumption of horse eye bean (*Mucuna urens* L.) affects the consistency of semen and motility of sperm cells in the Eastern and South-Eastern parts of Nigeria where it is used as a soup thickener and frequently consumed in soup and stew (Udoh and Ekpenyong, 2001). Taylor (2004), reported toxicity and birth defects in experimental animals treated with horse eye bean. Udoh and Ekpenyong (2001) also reported the degeneration of sperm in testicular tubules, collapse of the villi in prostate gland and reduction of secretion in the prostate gland and seminal vesicles of male guinea-pigs treated with seeds of horse eye bean.

Horse eye bean (*Mucuna urens*) is often cracked and removed from the seed coats soaked for a period, and then boiled in water (Osei-Bonsu *et al.*, 1995; Elitta and Carsky, 2003), roasted or fermented to remove most of the toxic substances, which have been implicated in poisoning (Osei-Bonsu *et al.*, 1995). Many food scientists have reached a consensus that horse eye bean does not seem to pose a danger to humans, if proper cooking takes place prior to eating (Ravindran and Ravindran, 1988; Infante *et al.*, 1990; Osei-Bonsu *et al.*, 1995; Siddhuraju *et al.*, 1996).

In view of the insufficient information on effect of processed horse eye bean (*Mucuna urens*), this study set out to further explore these effects of processed horse eye bean on reproductive endpoints, reproductive toxicity and fertility of albino rats as mammalian model using short-term *in vivo* assays.

### MATERIALS AND METHODS

#### Plant material

Mature dry seeds of horse eye bean (*Mucuna urens* L.) were cracked and soaked in water overnight with various water changes, after which the seed coats were removed and the endosperm was boiled in fresh water for 30-40 minutes according to (Osei-Bonsu *et al.*, 1995; Elitta and Carsky, 2003). The water was discarded and the endosperm chopped into tiny pieces and sun-dried for two days, then pulverized into the processed horse eye bean for the study.

#### Animals

Seventy-two mature albino rats (24 males and 48 females) of 12 weeks old were obtained from the Animal House of Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria for this study. The rats were divided into four groups with five rats per group and

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housed in conventional wire mesh cages under standard laboratory conditions (temperature 25-30°C, 12 hours light and 12 hours darkness cycle). They were allowed free access to water and commercial feed throughout the period of the experiment. Generally, the study was conducted in accordance with the recommendation from the declarations of Helsinki on guiding principles in care and use of animals.

**Experimental design and procedure**

Four experimental groups of five male albino rats each with similar body weights were constituted in a Completely Randomized Design (CRD). The male rats were fed with the processed horse eye bean at 0 (control), 100, 200 and 300 mg/kg body weight respectively daily for 8 weeks. The processed horse eye bean was mixed with about 10-30% of the daily feed consumption and given in the morning, to ensure the consumption of the daily treatment dose; before the remaining feed was given later in the afternoon (Ekaluo *et al.*, 2009).

At the end of the treatment period, the treated and the control males were made to sire the untreated female rats for the fertility test. The male rats were then sacrificed under chloroform anaesthesia. The testes and epididymes were dissected out and weighed using Scout Pro SPU 601 electronic weighing balance. The epididymes were processed for epididymal sperm count, viability and sperm head abnormality.

**Fertility test**

Male rats from the treated and the control groups were introduced to untreated parous females in the ratio of 1 male: 2 females for a period of about four days. Thereafter, conception rate of the female rats was calculated according to Ikpeme *et al.* (2007).

**Table 1** Effect of processed horse eye bean on weight of the testes and epididymes, epididymal sperm count, sperm viability and sperm head abnormality in male rats.

Parameters	Horse eye bean (mg/kg BW)			
	Control (0)	100	200	300
Testes (g)	1.12 <sup>a</sup> ± 0.02	1.12 <sup>a</sup> ± 0.02	1.10 <sup>a</sup> ± 0.05	1.15 <sup>a</sup> ± 0.03
Epididymes (g)	0.32 <sup>a</sup> ± 0.07	0.33 <sup>a</sup> ± 0.05	0.34 <sup>a</sup> ± 0.05	0.33 <sup>a</sup> ± 0.07
Sperm Viability (%)	94.25 <sup>d</sup> ± 2.02	80.42 <sup>c</sup> ± 3.36	77.83 <sup>b</sup> ± 1.49	73.08 <sup>a</sup> ± 2.60
Sperm Count (x 10 <sup>6</sup> /ml)	6.86 <sup>d</sup> ± 0.05	6.20 <sup>c</sup> ± 0.03	5.40 <sup>b</sup> ± 0.06	4.40 <sup>a</sup> ± 0.03
Sperm head abnormality (%)	1.57 <sup>a</sup> ± 0.55	12.58 <sup>b</sup> ± 2.07	19.67 <sup>c</sup> ± 2.38	27.92 <sup>d</sup> ± 0.08

<sup>abcd</sup> [Values across the table with similar superscript are not significantly different at 5% based on ANOVA]

**Sperm count**

The epididymal sperm samples were obtained by macerating known weights of cauda epididymes in physiological saline in the ratio of 1:10 weight by volume. After vigorous pipetting to release the sperm cells. The suspension was filtered using an 80µm stainless mesh. Epididymal sperm count was obtained by cytometry using the improved Neubauer cytometer and was expressed as million/ml of suspension (Ekaluo *et al.*, 2008).

**Sperm viability**

The sperm viability test was determined using “Eosin-Nigrosin one-step staining technique” (Björndahl *et al.*, 2003). A portion of the sperm suspension was mixed with equal volume of Eosin-Nigrosin stain and five (5) air-dried smears were prepared on glass slides for each sample. The slides

were examined for percentage viability. Normal live sperm cells excluded the stain and appeared whitish, whereas dead sperm cells took up stain and appeared pinkish. Percentage viability was calculated based on the number of live sperm cells out of the total number of sperm cells observed.

**Sperm head abnormality test**

A portion of the sperm suspension was mixed with 1% eosin Y solution (10:1) for 30 minutes and air-dried smears were prepared on glass slides for the sperm head abnormality test. The slides were examined for percentage sperm head abnormalities in every 200 spermatozoa observed on each slide and five air-dried smears were prepared on glass slides for each sample. The percentage of sperm head abnormality was calculated according to Ekaluo *et al.* (2009).

**Statistical Analysis**

Data from conception rate, weight of testes and epididymes, and seminal analyses were subjected to the analyses of variance (ANOVA) while differences in means were separated using least significant difference (LSD) according to Obi (2002).

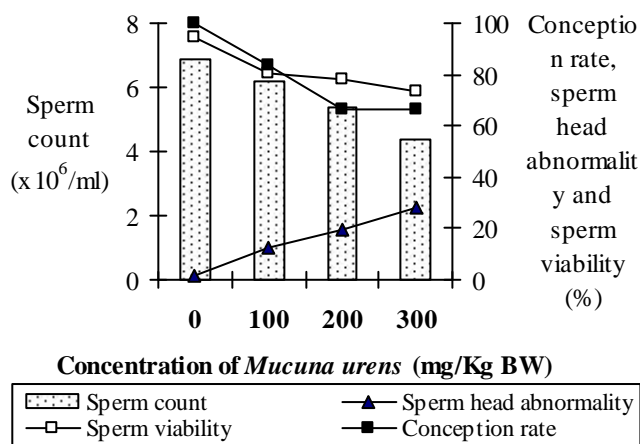
**RESULTS**

General observations showed that all the rats in the study looked healthy and there was a general increase in body weights of all rats in both treatment and control groups during the treatment period. The increases in body weights of the rats indicated that processed horse eye bean had no adverse effect on growth and body weight of the rats.

Table 1 shows that there was no significant (P>0.05) effect of processed horse eye bean on the weight of testes and epididymes even at highest level of the treatment.

The processed horse eye bean significantly (P<0.05) reduced epididymal sperm count and sperm viability in dose-dependent manner when compared to the control. While it also significantly (P<0.05) increased sperm head abnormality in a dose-dependent manner.

The conception rates of the untreated female rats that were sired by treated males were significantly (P<0.05) reduced in a dose-dependent manner when compared to the control. The observed relationship between epididymal sperm count, sperm viability, sperm head abnormality and conception rate is shown in Fig. 1. The conception rates were directly proportional to sperm viability and sperm count; and inversely proportional to sperm head abnormality in all the groups.



**Fig. 1** Relationship between sperm count, conception rate, sperm viability and sperm head abnormality in rats fed processed horse eye bean (*Mucuna urens*)

### Discussion

The processed horse eye bean did not have significant effect on weight of testes and epididymes which was contrary to the report of Udoh and Ekpenyong (2001), which showed degeneration of sperm in testicular tubules and reduction of secretion in the prostate gland and seminal vesicles of male guinea-pigs treated with unprocessed seeds of horse eye bean. The observed reduction in negative effects can be attributed to processing of the horse eye bean (Osei-Bonsu *et al.*, 1995; Elitta and Carsky, 2003).

The effect of processed horse eye bean on sperm count, sperm viability and sperm head abnormality; and their relationships agreed with the reports of Odeigah (1997) and Ekaluo *et al.* (2005). The effect indicates that the processed horse eye bean is still toxic to the mammalian model which is contrary to the views of Ravindran and Ravindran (1988), Infante *et al.* (1990), Osei-Bonsu *et al.* (1995) and Siddhuraju *et al.* (1996).

The reductions in conception rate, sperm viability, sperm count and increase in sperm head abnormality agrees with World Health Organization report (WHO, 1992) that, sperm head abnormality correlates more closely with fertilization rather than sperm count and sperm viability. Distortions in the fertility of male mammals are directly correlated to the distortions in spermatogenesis (Sharpe and Skakkebaek, 1993; Glover and Assinder, 2006). Reduction in spermatogenic activities has also been reported to result in infertility, reproductive dysfunction and toxicity (Greenspan and Stawler, 1997; Gelain *et al.*, 2005).

Processed horse eye bean still has some toxic effect on reproductive endpoints such as sperm count, sperm viability and sperm head abnormality, as well as conception rate. Hence the utilization of processed horse eye bean (*Mucuna urens*) as soup thickener should be done with caution since reduction in spermatogenic activities could result in infertility, reproductive toxicity and dysfunctions.

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