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INDUCED MUTATION EFFECT OF PROCESSED HORSE EYE BEAN (*MUCUNA URENS* L.) ON ALBINO RATS

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ABSTRACT

Purpose: To investigate the effect of processed horse eye bean on induced mutations in albino rats.

Methods: Forty-eight albino rats (24 males and 24 females) of 12 weeks old were used as the mammalian model for this study. The male rats were assigned to four groups and administered with processed horse eye bean at 0, 100, 200 and 300 mg/kg BW daily for 8 weeks. At the end of the treatment period the untreated female rats were sired by treated males in the ratio of 1:1. The males were then sacrificed and the epididymes were dissected out and processed for sperm count, sperm head abnormality test (SHAT) and induced mutation indices (IMI). The females were sacrificed and assessed for induced lethal mutation indices (ILMI).

Results: There were significant ($P < 0.05$) and dose-dependent decreases in sperm counts while sperm head abnormality, induced mutation indices (IMI) and induced lethal mutation indices (ILMI) increased significantly ($P < 0.05$) when compared with control and in dose-dependent trend.

Conclusions: The results show that processed horse eye bean had induced mutation effects on mammalian model

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INTRODUCTION

Horse eye bean (*Mucuna urens* L.) is usually found in home gardens of the Efiks, Ibibios and the Igbos of South-Eastern and Eastern parts of Nigeria, West Africa where it is particularly used as a soup thickener (Achinewhu, 1984).

Before eating mucuna beans, they are 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 (Duke, 1981; Osei-Bonsu *et al.*, 1995). Many food scientists have reached a consensus that mucuna beans 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).

According to de la Vega *et al.* (1981), Duke (1981) and Olaboro (1993), mature seed pods of horse eye bean are regarded as less toxic than green pods and, along with leaves, have been boiled and eaten as vegetables. Such foods have also been reported to be rich sources of potassium, magnesium, calcium, iron, proteins and amino acids. The seeds of horse eye bean contain 5-hydroxytryptamine, mucunine, mucunadine proteins, carbohydrates, tannins, and phytates, as well as essential and non-essential amino acids (Udoh and Ekpenyong, 2001).

Taylor (2004) reported that, powdered mucuna beans have been formulated into tablets or capsules and for the following properties: androgenic, anabolic, aphrodisiac, immunomodulator, analgesic, nervine, anti-inflammatory, anti-Parkinson's, febrifuge, antivenin, hypoglycemic, hypocholesterolemic and neuroasthemic. Traditionally it has also been used for abortions, cancers, menstrual disorders, blood cleansing, central nervous system and uterine stimulation.

Udoh and Ekpenyong (2001), 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 (*Mucuna urens*).

Induced lethal mutation is reported to be responsible for dysfunction of the gamete which is lethal to fertilized egg or developing embryo (Ehling, 1977; 1978). The frequency of induced lethal mutation is directly proportional to sperm head abnormality and inversely proportional to the sperm count (Odeigah, 1997; Ikpeme *et al.*, 2007; Ekaluo *et al.*, 2009).

In view of the insufficient information on the induced mutation and sperm head abnormality effects of processed horse eye bean (*Mucuna urens*), this study set out to further explore these effects of processed horse eye bean on albino rats as mammalian model using short-term *in vivo* mutagenicity assay.

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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) in an attempt to destroy any toxins that may be present. The water was discarded and the endosperm was chopped into tiny pieces and sun-dried for two days, then pulverized into the processed horse eye bean for the study.

Animals

Forty-eight healthy and sexually mature albino rats (24 males and 24 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 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 (Carbone, 2004).

Experimental procedure

Four experimental groups of six 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, 100, 200 and 300 mg/kg BW respectively daily for 8 weeks. The processed horse eye bean was mixed with 5g of commercial feed (30-40% of the daily feed consumption) and given to the male rats in the morning, to ensure the consumption of the daily treatment dose; before the remaining was given later in the afternoon (Ekaluo *et al.*, 2009). The male rats in the control group received only the commercial feed.

At the end of the treatment period the untreated female rats were sired by treated males in the ratio of 1:1. The male rats were then sacrificed under chloroform anaesthesia and the epididymes were dissected out and processed for epididymal sperm count and sperm head abnormality test (SHAT). The females were thereafter sacrificed and assessed for induced lethal mutation index.

Table 1 Effect of processed horse eye bean (*Mucuna urens*) on epididymal sperm count and sperm head abnormality in rats.

Parameter	Processed horse eye bean (mg/kg BW)			
	0	100	200	300
Sperm count (x 10 ⁶ /ml)	6.86 ^d ± 0.05	6.20 ^c ± 0.03	5.40 ^b ± 0.06	4.40 ^a ± 0.03
Sperm head abnormality (%)	1.57 ^a ± 0.55	12.58 ^b ± 2.07	19.67 ^c ± 2.38	27.92 ^d ± 0.08

^{abcd} [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. Epididymal sperm count was obtained

by cytometry using the improved Neubauer cytometer and was expressed as million/ml of suspension (Ekaluo *et al.*, 2005).

Sperm head abnormality test (SHAT)

The sperm suspensions were 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 and induced mutation index were calculated from the sperm head abnormality according to Ekaluo *et al.* (2009).

$$\text{Induced mutation index (IMI)} = \frac{\text{Sperm head abnormality (treated - control)}}{\text{Sperm head abnormality (control)}}$$

Induced lethal mutation assay

Two weeks after exposure to males, the female rats were sacrificed under chloroform anaesthesia and scored for total implants (embryos) which comprised of live embryos and early foetal deaths. Induced lethal mutation indices (ILMI) were calculated according to the procedures adapted from Odeigah (1997) and Ikpeme *et al.* (2007) for induced (dominant) lethal mutation index.

$$\text{Induced lethal mutation index (ILMI)} = 1 - \frac{\text{Live implants (treated)}}{\text{Live implants (control)}}$$

Statistical analysis

Differences between the mean sperm count, sperm head abnormality (sperm morphology), induced lethal mutation indices (ILMI) and induced mutation indices (IMI) of the control and treatment groups were compared for significance using the Analysis of Variance (ANOVA) test.

RESULTS

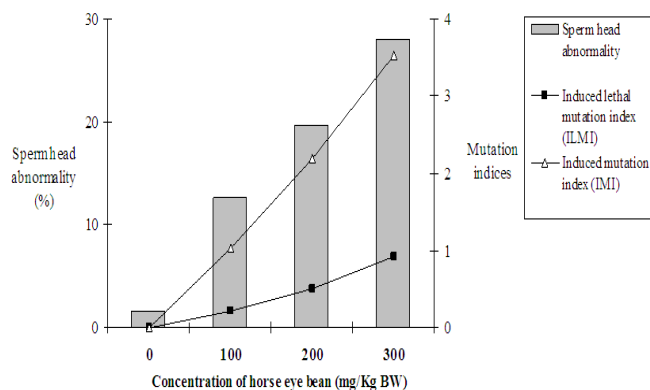
Sperm count and sperm head abnormality

The processed horse eye bean (*Mucuna urens*) had significant (P<0.05) and dose-dependent effect on the epididymal sperm count of the treated rats. The sperm count was significantly reduced from 6.86 x 10⁶/ml in control to 4.40 X 10⁶/ml in 300mg/Kg BW. It also had significant (P<0.05) and dose-dependent effect on the sperm head abnormality, with increasing percentage of sperm head abnormality from 1.57 % in control to 12.58, 19.67 and 27.92 % respectively for 100, 200 and 300 mg/Kg BW as shown in Table 1.

Induced mutation indices

Processed horse eye bean (*Mucuna urens*) increased significantly (P< 0.05) the induced mutation indices (IMI) of the treated groups in a dose-dependent fashion when

compared with the controls. A similar trend was also observed for induced lethal mutation indices (ILMI) for female rats sired by treated males. Fig. 1 showed positive relationship between induced mutation indices and percentage of sperm head abnormality. There was an increase in average dead embryo per female cumulating in an increase in induced lethal



mutation indices (ILMI) as shown in Table 2.

Fig. 1 Relationship between sperm head abnormality and induced mutation indices

Table 2 Induced lethal mutation indices (ILMI) for female rats sired by males treated with processed horse eye bean (*Mucuna urens*).

Daily treatment (mg /Kg BW)	Number of Females	Implants per female	Average live embryo per female	Average dead embryo per female	ILMI
0 (Control)	6	6.33 ± 0.47	6.00 ± 0.54	0.00	0.00
100	6	5.67 ± 0.40	4.67 ± 0.42	1.00 ± 0.33	0.22
200	6	4.83 ± 0.31	3.00 ± 0.42	1.83 ± 0.16	0.50
300	6	3.67 ± 0.20	0.50 ± 0.00	3.17 ± 0.31	0.92

Induced mutation indices

Processed horse eye bean (*Mucuna urens*) increased significantly ($P < 0.05$) the induced mutation indices (IMI) of the treated groups in a dose-dependent manner when compared with the control. A similar trend was also observed for induced lethal mutation indices (ILMI) for female rats sired by treated males. There was also a positive relationship between induced mutations indices and percentage of sperm head abnormality (Figure 1). There was an increase in average dead embryo per female cumulating in an increase in induced lethal mutation indices ILMI (Table 2).

DISCUSSION

Processing of the seeds of horse eye bean (*Mucuna urens*) was an attempt to destroy the toxins (Osei-Bonsu *et al.*, 1995; Elitta and Carsky, 2003); and make it safe for human consumption. It has not completely been achieved since the processed horse eye bean still produce in the treated rats some of the effects earlier reported by Udoh and Ekpenyong (2001), in male guinea-pigs treated with unprocessed seeds of horse eye bean; though in milder forms. Boiling did not completely eliminate the toxins as earlier suggested by Ravindran and

Ravindran (1988), Infante *et al.*(1990), Osei-Bonsu *et al.*(1995) and Siddhuraju *et al.*(1996).

The reduction in sperm quantity and quality shows the potency of even processed horse eye bean (*Mucuna urens*) in disrupting spermatogenic processes. The observed increase in percentage of sperm head abnormality and reduction in epididymal sperm count may have resulted from the alteration in the epididymal environment as earlier reported by Nwanjo *et al.* (2007), and according to Ekaluo *et al.* (2009), increases in the percentage of sperm head abnormality is an indication of the increase in the rate of induced mutations on the sperm cells at the level of spermatogenesis. The dose-dependent increases observed for induced lethal mutation indices agrees with earlier reports of Odeigah (1997) and Ikpeme *et al.* (2007) for induced (dominant) lethal mutation index. However, caution should be exercised in the usage of the term “dominant”; unless it is proven to be either “dominant” or “recessive” lethal mutation.

Increase in the percentage of sperm head abnormality and induced mutation indices which showed dose-dependent seemingly support the report of Taylor (2004) that horse eye bean had toxicity and birth defects in experimental animals. The results reveal that the processing method could not completely eliminate the toxins thus the processed horse eye bean still had induced mutation effects on the mammalian

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