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Ox-Eye Beans (*Mucuna urens* L): A Novel Indigenous Contraceptive

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ABSTRACT: *Mucuna urens* L, commonly called ox-eye beans, a well known and well consumed climbing herb in the Southern and South-Eastern States of Nigeria was tested for its contraceptive effects in male albino rats. The age-long claim by the locales that consume this herb, that it reduces fertility in the male, led to this investigation. A total of twenty-four rats, twelve males and twelve females were used for this study. Male albino rats (Wister strain) were divided into four groups viz: group I, group II, group III and group IV and treated with four - 0 mg/kg (control), 70mg/kg, 140mg/kg and 210mg/kg - different dosages of ethanol extract of the aerial parts of *M. urens* respectively for 14 consecutive days. After the treatment period, female rats were mated to the treated males at a ratio of 1:1. Fourteen (14) days after mating the treated male rats to untreated females, the female rats were sacrificed for the Induced dominant Lethal Mutation Assay (IDLMA). Results showed a significant difference ($P<0.05$) in the dominant lethal effect between the treated rats and the control. Mean life implants were 8.67±0.57, 7.00±1.00, 4.33±3.70 and 0.00±0.00 for treatment groups I, II, III and IV while the mean dead implants were 0.00±0.00, 2.00±0.57, 4.00±2.64 and 7.67±0.57 respectively. The induced dominant lethal indices calculated, were 0.0, 0.18, 0.50 and 1.00 for groups I, II, III and IV, respectively. Photographs of the *corpora leutea*, were obtained using a digital camera (DCR-HC48E, KODAK). The results have shown that the consumption of the ethanol seed extracts of *Mucuna urens* L could induce dominant lethal mutations in spermatocytes and early spermatids in the male, showing the mutagenic effect of the seeds of the plant *M. urens* and its contraceptive effects on the male reproductive system.

Keywords: Ox-eye beans, Albino rats, Dominant Lethal Mutations, Male contraceptives.

Introduction

Mucuna urens (L), is commonly found in many home gardens in the South-eastern parts of Nigeria where it is highly consumed as a soup thickener. It is a climbing herb that belongs to the sub-family Papilionacae and family, Fabaceae. The fruits are usually legume pods, with superior ovary containing mostly, three to four seeds. When fruits are mature, they split through dorsal sutures. The seeds are black when dry with hard seed coats and the seeds are usually without endorsperm. A seeming consensus exists among the food scientists familiar with *Mucuna*, as well as with the recent developers of *Mucuna* recipes, that the intoxication associated with eating *Mucuna* seed is, attributed to the high L-Dopa content of the raw seeds.

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It is, known across several South Eastern states as "Ibaba" and known as "Yerepe" in the Yoruba speaking parts of Nigeria. Oxe-eye beans is usually sold in the local markets during its harvest season, which is in the month of January. In our locality, Oxe-eye beans is prepared in various ways. It is used in stew (soup) preparations, where it is briefly dry-roasted (5-10 minutes) in a pot on fire until the seed becomes spotty brown. The roasted seeds are then pounded, sieved (to separate the seed coats) and added to a soup consisting of greens, vegetables, oil and sometimes meat. The soup is then cooked for at least 30 minutes. It is estimated that a cup containing about 20 seeds can feed approximately 10 people. It is consumed in the same way as the seed of egusi (melon), an extremely popular ingredient in Nigerian cooking. Sometimes, the Oxe-eye beans seeds and egusi are mixed to prepare the soup (Elitta and Carsky, 2003). Horse eye bean and devil bean are all common English names for *Mucuna urens*. One of its Sanskrit names, *atmagupta*, ("having hidden properties"), seemingly denotes its importance as a medicinal plant while another, *kapikachchhu* ("monkey's itch"), refers to the unpleasant characteristic of its many accessions (Armstrong, 1998).

Mucuna has repeatedly impressed farmers and researchers due to its high biomass production, weed suppression, and consequent beneficial impacts on main crop yields. Active components Oxe-eye beans include polyphenols or tannins, which can bind with proteins, lowering digestibility (Siddhuraju and Becker, 2003). In *Mucuna* however, most of the tannins are seemingly located in the seed coat, which is typically discarded in food preparation, rather than in the endosperm (Ravindran and Ravindran, 1988; Mary-Josephine and Janardhanan, 1992). Phytic acid, a component of all plant seeds, can reduce bioavailability of certain minerals and reduces the digestibility of proteins. Several researchers have quantified phytic acid levels in *Mucuna* seeds (Ravindran and Ravindran, 1988; Siddhuraju and Becker, 2003; Laurena *et al*., 1994).

Cyanogenic glycosides liberate hydrogen cyanide; a well-documented toxin upon hydrolysis but, fairly low to zero levels of hydrogen cyanide have been found in *Mucuna* (Ravindran and Ravindran, 1988; Laurena *et al*., 1994; Siddhuraju and Becker, 2003). Trypsin inhibitor activity and L-amylase inhibitor activities have been detected in *Mucuna* (Del Carmen *et al*., 1999; Siddhuraju and Becker, 2003). *Mucuna* had the third highest level of oligosaccharides of seven legumes evaluated in the Philippines (Farnsworth, 1994). Contraception is the use of hormones, devices or surgery to prevent conception. Most of the existing contraceptives today are targeted at the women folk. Apart from condoms, withdrawal method and vasectomy, effective male contraceptives are still under investigation (Wikipaedia, 2009). Mutagenesis could be harnessed as a tool for developing a safe and readily reversible male contraceptive, thus, the investigation of the mutagenic effects of the herb, *Mucuna urens* (L) on the sperm cells of male albino rats in order to establish its contraceptive effects.

Among the various procedures proposed for use in assessing the mutagenic potential of drugs, the dominant-lethal assay stands currently as one of the few tests for measuring mutagenic effects on germ cells (Ray *et al*., 2008). Several dominant lethal indices can be employed when examining dominant lethals in mammals; these include pre-implantation losses estimated based on *corpora lutea* counts, dead implants per female, dead implants/total implants, females with one or more post-implantation losses, and females with two or more post-implantation losses (Chellman *et al.*, 1986). In this study, the pre-implantation losses, number of life and dead implants per female and Induced dominant lethal mutation indices will be assessed.

The objective of this research study was to investigate the age-long belief by the locals (in areas where it is consumed) that, Ox-eye beans affects fertility in men.

Materials and Methods

Twenty-four (24) albino rats (12 males and 12 females) were used as mammalian models for this study. The rats, weighing between 100g and 150g were housed in groups of three each in rubber cages and kept under optimum laboratory conditions (ambient temperature of 25±3ºC, relative humidity, 50-55%, 12:12 dark: light cycle) and given food (commercial poultry growers mash from the Top Feeds LTD., Calabar) and water *ad libitum.*

Preparation of ethanol plant extract:

The plant extract was prepared following standard procedures. The dry seeds of Oxe-eye beans (fig. 1) were bought from a local market (Watt market) in Calabar South Local Government Area of Cross River State and identified by the Curator in the Department of Biological Sciences, Cross River University of Technology, Calabar. The seeds were washed under a flowing tap, chopped into pieces with a clean knife, air-dried for 3 days and then

pulverized using a blender (Laprivia 3000 Model, China). The powdered sample (fig. 2) was then extracted in a Soxhlet apparatus using Ethanol as the extracting solvent. 100g of the powdered sample was wrapped in filter paper (Whatmann's No. 40) and placed in a thimble in the main chamber of the Soxhlet apparatus. The soxhlet extractor was placed on a flask containing 400ml 80% ethanol and then equipped with a condenser. The ethanol was heated to reflux and the vapor travelled up a distillation arm to the chamber housing the thimble containing Oxe – eye beans powder. As the Soxhlet chamber filled up, the solvent automatically emptied into the distillation flask through a siphon. This cycle was allowed to repeat for 72hours at 80°C. The ethanol extract was evaporated using a hot air oven (STUARC scientific, England) for 24hrs at 50°C. One gramme (1g) of the paste extract was also dissolved in 10ml Corn oil (vehicle) to make up 100mg/ml concentration which was the stock solution. This was kept refrigerated at 4°C for later use (Siddhuraju, and Becker, 2003).

Fig. 1: Intact dry seeds of *M. urens*

Fig. 2: Pulverized *M. urens* seeds

Administration of extract

After an acclimatization period of two weeks, administration of the Oxe - eye beans extracts commenced and continued every day for 14 days. Three concentrations (doses) of the plant extract were fed to the male rats while the female rats were left untreated. These doses were 70mg/kg BW (gp.I), 140mg/kg BW (gp.II) and 210mg/kg BW(gp. III). The control animals (gp.I) were not fed any extract (0mg/kg BW). The doses were computed based on the initial body weights of the experimental animals. The experimental animals received the extract before their normal chow every day. Administration was done by feeding with oral gavage tube.

Mating

At the end of the extract administration, the male rats were mated with untreated virgin females for three days at a ratio of 1: 1 to examine the Dominant lethal parameters.
Mating was confirmed by examination of the vaginal plug. The rats were then separated again and the female rats observed closely for 14 days at the end of which period, they were sacrificed by cervical dislocation and thereafter, dissected. The uterine contents were examined to observe the number of implants and life and dead embryos in the *corpora leutea* for determining pre-implantation losses. Pictures were obtained using a digital camera (DCR-HC48E). Evaluation of Dominant lethal mutation was analyzed using the formula (Odeigah, 1997):

$$1 - \frac{\text{mean life implant in each trt. Group}}{\text{mean life implant in control}}$$

Statistical analysis

Results were reported in accordance with the CFR P798-024 Rodent Dominant Lethal Mutation Assay guidelines (1978). The Completely Randomized Design (CRD), with a one-way ANOVA, was used for the analysis of all data collected. Dunnett t-test was used to compare the means. Means were significantly different at $P<0.05$ level. All evaluations were done with the aid of the PASW (18) statistical package.

Results

The results of the Induced Dominant Lethal Mutation Assay of male rats fed with ethanol extract of seeds of Ox-eye beans (*M. urens)* for 14 days are presented in Table 1. Photos of the *corpora lutea*, Figure 3(plates A-D) of untreated female rats mated with treated males, at mid-gestation, have also been presented. The induced dominant lethal indices observed were 0.0, 0.18, 0.50 and 1.00 for groups I, II, III and IV respectively. Mean life implants in the *M. urens* treatment group were 8.67 ±0.57, 7.00 ± 1.00, 4.33 ± 3.70 and 0.00±0.00 for treatment groups I, II, III and IV respectively while the mean dead implants were 0.00±0.00, 2.00 ± 0.57, 4.00 ± 2.64 and 7.67 ± 0.57 for treatment groups I, II, III and IV respectively (Table 1). The Dunnett t-test showed a significant difference ($P<0.05$) in the dominant lethal effect of *M. urens* between the control and the treated rats. Pearson's correlation also gave a significant ($P<0.01$) correlation between the life and dead implants.

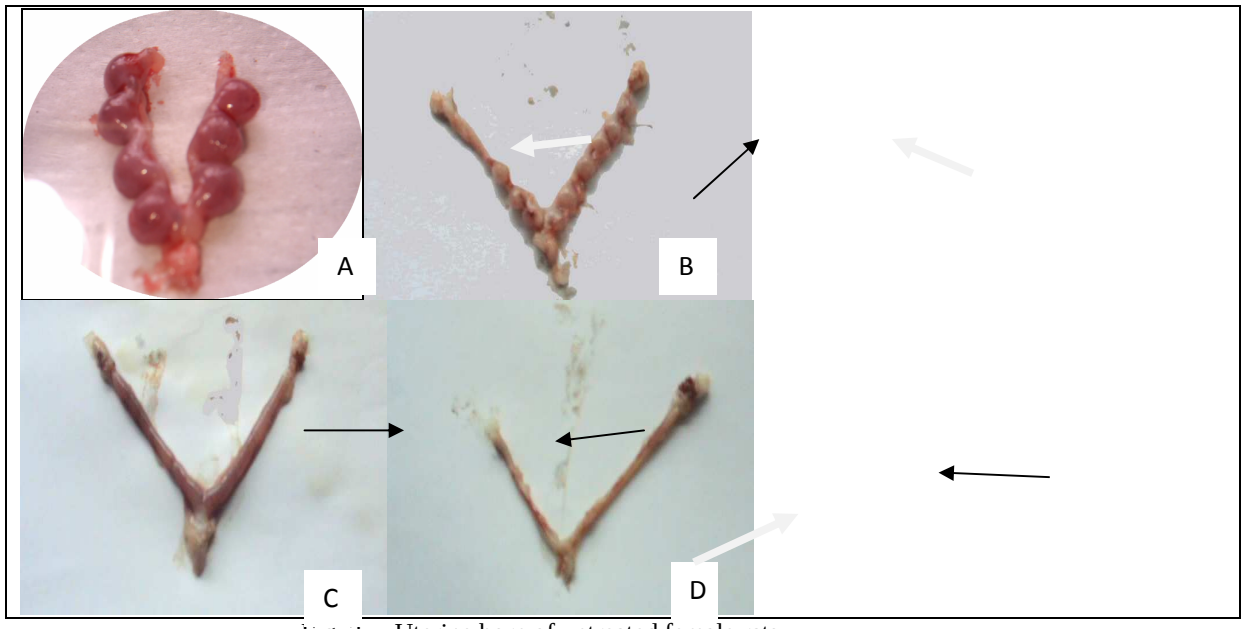

Fig. 3 Uterine horn of untreated female rats

Legend:

A – Uterine horn of female rat mated with control male rat showing healthy implantations (arrowed)

B – Uterine horn of female rat mated with male rat fed 70mg/kg of Ox-eye beans extract showing dead (black arrow) and life (white arrow) implants.

C – Uterine horn of female rat mated with male rat fed 140mg/kg of Ox-eye beans extract showing dead (black arrow) implants

D – Uterine horn of female rat mated with male rat fed 210mg/kg of Ox–eye beans extract showing few dead (black arrow) implants and no implantations(white arrow).

Table 1

Induced dominant lethal mutation (IDLM) in rats fed ethanol extracts of *M. urens*

Treatment	Daily treatment (mg/kg BW)	Number of females	Number of males	Induced dominant lethal index
	0	3	3	0.00
Oxe – eye beans				
(*M. urens*)	70	3	3	0.18

Table 2: Average live and dead embryos in uterine horns of female rats

Treatment	Treatment Dosage (mg/kg bw)	Average Live Embryo	Average Dead Embryo
Oxe-eye beans (*M. urens*)	0	8.67 ±0.57	0.00 ±0.00
	70	7.00 ±1.03	2.00 ± 0.57
	140	4.33 ± 3.70	4.00 ±2.64
	210	0.00 ± 0.00	7.67 ± 0.57

Discussion

Amongst the various procedures proposed for use in assessing the mutagenic potential of drugs, the dominant-lethal (D-L) assay stands currently as one of the few tests for measuring mutagenic effects on germ cells (Lachance and Reimann, 2003). In general, the animal treated is often a male because chemicals acting systemically on females may interfere with hormonal status, possibly interfering with the development of normal fetuses, or the chemical may act directly on the maturing oocyte, causing death other than by a dominant lethal mutation (Maxwell and Newell, 1973). Dominant lethal mutations are also characterized by the presence of chromosome bridges and fragments between dividing nuclei in the embryo (James and Smith, 1997). Thus, it can be concluded that most dominant lethality probably result from multiple chromosomal breaks in the germ cells (WHO, 1992).

The results from this study, showed a dose-dependent dominant lethal effect caused by ethanol extract of Ox–eye beans (*M. urens* L) administered to the rats (Table 1). The lethality of the mutation increased with increased dosage of the extract. Partington and Jackson (1963) had similar results when they treated male rats with alkane sulphoric esters. The induced dominant lethal mutations were highly significant ($P<0.05$). This alone is an indication that the test substance, the ox-eye beans extract had mutagenic effects in the experimental animals (Maxwell and Newell, 1973). The mutations observed as pre-implantation losses and dead implants could be attributed to damages in the sperm cells leading to inferior sperm quality as reported by Lister and McLean (1997).

It is believed that the spermatids and spermatocytes were arrested by components in the administered Oxe- eye bean seed extracts causing an inability of the germ cells to carry out their normal function of fertilization. The decrease in average live embryo with increase in dosage administered (Table 2), also shows the dose-dependent dominant lethal effect of *M. urens* in the rats. This indicates that the extracts had affected germinal tissues of the test species in accordance with reports of Ehling *et al*, (1978) and the CFR Rodent Dominant Lethal Mutation Assay guidelines, (2002). Results of mating between male rats treated with high doses of *M. urens* and the untreated females is an indication of the dysfunction of the gametes of treated males caused by the induced dominant lethals. According to Senod (1998) and Ray *et al.* (2008), this results could suggests that under the test conditions, the ethanol extract of the seeds of Oxe – eye beans (*M. urens* L) were genotoxic in the germplasm of the treated rats. This genotoxicity is also implicated as the cause of lethality in fertilized eggs or developing embryo (Sharpe, 1993). The sperm cells of the treated males must have been affected at some point in their formation resulting in the

observed pre-implantation losses in the uterine horn of female rats mated with male rats treated with the higher doses of the extract (Figure 3).

The mean live embryo observed in the *corpora lutea* also attests to the germinal lethality induced by the administered extracts as there was a dose-related decrease in number of life implants(Table 2). This agrees with findings of Chellman *et al*, (1986) and Ray *et al*, (2008). Since the *corpora lutea* is critical in hormone production at the early stages of conception, its reduction in number will also reduce hormone production and may, therefore be implicated in the inability of the conceptus to stay alive (WHO, 1992). At the highest dose (210 mg/kg BW), mostly dead implants were observed (Figure 3). This also strongly indicates pre-implantation losses due to the Ox-eye beans extract administered to the male. Thus, it can be surmised that at high doses, the ethanol extract of Oxe - eye beans (*Mucuna urens* L) inhibited implantation, hence conception, in untreated female albino rats.

Conclusion

This preliminary investigation allows the suggestion that the administered oxe – eye beans extract had altering effects on the germ cells in male rats. There is strong indication that the process of spermatogenesis in the treated male albino rats was disrupted in a dose-related manner by the administered ethanol extract. Reversibility studies are underway and it is believed, the results from these studies will go a long way to establish our own very palatable "Ibaba" as the first safe, available and affordable male contraceptive of our time. Studies on effective dosages are recommended for documentation and also, it is recommended that the antifertility components present in the ethanol extract be isolated and characterized using in-depth laboratory techniques like High Performance Liquid chromatography (HPLC) for the purpose of further *in vivo* tests in higher laboratory animals and also for subsequent human trials.

References

Armstrong, W.P. (1998). Unusual drift fruit from Costa Rica. *The Drifting Seed, 4* (2): 7-8.

Chellman, G. J., Bus, J. S. & Workings, P. K. (1986). Role of epididymal inflammation in the induction of dominant lethal mutations in Fischer 344 rat sperm by methyl chloride. *Protocol of the National Academy of Science, 83*: 8087-8091.

DelCarmen, J., Gernat, A. G., Myhrman, R. & Carew, L. B. (1999). Evaluation of raw and heated velvet beans (*Mucuna pruriens*) as feed ingredients for broilers. *Poultry Science, 78*: 866-872.

Ehling, U. H., Cumming, R. B. & Malling, H. B. (1978). Induction of dorminant lethal mutations by alkalyting agents in male mice. *Mutation Research, 5*: 417.

Elittä M. & Carsky R. J., (2003) Efforts to improve the potential of *Mucuna* as a food and feed crop: Background to the workshop. *Tropical and Subtropical Agroecosystems* 1: 47-55.

Farnsworth, N. R. (1994). Ethnopharmacology and drug development: Ethnobotany and the search for new drugs. *Wiley Chichester (Ciba Foundation Symposium), 185*: 42 – 59.

James, D. A. & Smith, D. M. (1982). Analysis of results from a collaborative study of the dominant lethal assay. *Mutation Research, 97*(4): 303-314.

Laurena, A., Rodriguez, F. M., Sabino, N. G., Zamora, A. F. & Mendoza, E. M. T. (1994). Amino acid composition, relative nutritive value and *in vitro* protein digestibility of several Phillippine indigenous legumes. *Plant Foods for Human Nutrition, 41*: 59-68.

Mary-Josephine, R. & Janardhanan, K. (1992). Studies on chemical composition and antinutritional factors in three germplasm seed materials of the tribal pulse, *Mucuna pruriens* (L.) DC. *Food Chemistry, 43*: 13-18.

Maxwell W. A. & Newell G. W. (1973) Considerations for evaluating chemical mutagenicity to germinal cells. *Environmental Health Perspective.* 6: 47–50.

Odeigah, P. G. C. (1997). Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. *Mutation Research, 389* (2-3): 141-148.

Oliver-Beaver, B. (1986). *Medicinal plant of tropical West Africa*. London: Cambridge University Press.

Partington, M. & Jackson, H. (1963). The induction of dominant lethal mutations in rats by alkane sulphoric esters. *Genetical Research, 4*: 333 – 345.

Ravindran, V. & Ravindran, G. (1998). Nutritional and anti-nutritional characteristics of *Mucuna* (*Mucuna utilis*) bean seeds. *Journal for Science of Food and Agriculture, 46*: 71-79.

Ray, pV. A., Holden, H. E., Salsburg, D. S., Ellis, J. H., Jr., Just, L. J. & Hyneck, M. L. (2008). Comparative studies of ethyl methanesulfonate-induced mutation with host-mediated, dominant lethal, and cytogenetic assays. Mutation Research, 15 (2): 99 – 102.

Senod, D. G. (1988). Effects of clomiphene citrate on anterior pituitary and male reproductive organs of bats (Rousettus leschenaulti). Proceedings of all India symposium on comp Endocrinology; Nagpur University.

Siddhuraju, P. & Becker, K. (2003). Comparative nutritional evaluation of differentially processed *Mucuna* seeds (*Mucuna pruriens* (L.) DC. var. *utilis* (Wall ex Wight; Baker ex Burck) on growth performance, feed utilization and body composition in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research, 34*: 487-500.

World Health Organization (WHO) (1992). *WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. Cambridge: Cambridge university Press.

Wikipaedia (2009). Sperm motility. Retrieved May 18, 2009 from http:/enwikipaedia .org