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Dominant lethal mutations in rats fed extracts of *Mucuna urens* (Linn.)

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Dominant lethal mutation assay was carried out on rats after being treated with graded doses of ethanol extract of the seeds of *Mucuna urens.* Male albino rats (Wistar strain) were caged in three groups labeled, groups II, III and IV and treated with three different dosages of the ethanol extract of the seeds of *M. urens*; 70, 140 and 210 mg/kg body weight (BW), respectively, for 14 days. The positive control animals (group I), were treated with distilled water for the entire period. At the end of the feeding period of two weeks, they were co-habited with virgin female albino rats at a ratio of 1:1 for 3 days. 14 days after mating, the females were sacrificed for the dominant lethal mutation assay. The results of the dominant lethal mutation assay showed that only female rats in group II had implants on the uterine horn, of all the treated groups. The rats in groups III and IV did not have any implants at all. Biological evaluations (pre-implantation losses) carried out showed 0, 76, 100 and 100% lethal mutations in groups I, II, III and IV, respectively. The statistical evaluations obtained showed $8.6^{b} \pm 0.47$, $6.6^{a} \pm 0.94$, 0 ± 0.0 and 0 ± 0.0 in groups I, II, III and IV, respectively. Photographs of the *corpora lutea* were obtained using a digital camera (DCR-HC48E, KODAK). The results obtained can be attributed to the induction of dominant lethal mutations in spermatocytes and early spermatids in the male Albino rats, showing the mutagenic effect of the seeds of the plant *M. urens* and its potential as a male contraceptive.

Key words: *Mucuna urens* seeds, dominant lethal mutations assay, mutagenesis, male Albino rats.

INTRODUCTION

The seeds of the herb, *Mucuna urens*, are commonly found in home gardens in the south eastern parts of Nigeria, West Africa, where the Efiks, Ibibios and Igbos use it as a major soup condiment for thickening. It is called “Ibaba” by the Efiks/Ibibios and “Ukpor” by the Igbos and is usually sold in the local markets during its harvest season which is in the month of January.

In Calabar and in its immediate localities, *Mucuna* seeds are prepared in various ways. The seeds are used in sws (soup) preparations, where they are briefly dry-roasted (5-10 min) 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. In that soup, they are allowed to cook for at least 30 min (Elittä and Carsky, 2003). It was estimated that

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a cup containing about 200 seeds feeds approximately 10 people. It is therefore used in the same way as the seeds of “egusi” (melon), an extremely popular ingredient in Nigerian cooking, and sometimes the seeds of *Mucuna* and “egusi” are mixed to prepare the soup (Ukachukwu and Obioha, 2000). Another recipe developed for preparing it involves cracking the seeds, two overnight soakings, various water changes, and boiling for at least 20 min (Elittä and Carsky, 2003).

Mucuna is typically planted near trees and climbs around the trees as support producing high amount of seed per plant. It is also planted by the fence or allowed to climb yam stakes together with yam. Both white- and black-seeded *Mucunas* (scientists in the region refer to these types typically as var. *cochinchinensis* and *urens*, respectively) are used but the white-seeded type is preferred (Sridhar and Bhat, 2007). In other localities where *M. urens* is found, it is known as velvet bean, *pica-pica*, bengal bean, *nescafé*, *ojo de venado*, *pois mascate, kara benguk*, *olhos de burro* (Esonu et al., 2001). 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. Horse eye bean, ox-eye bean and devil bean are all common English names for *Mucuna*. The seeds are also known as "sea beans," because they are commonly carried by rivers into the ocean (Armstrong, 1998).

Taxonomists classify *Mucuna* as a vascular seed plant belonging to the subclass Rosidae and order Fabales. 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 mainly related to the high L-Dopa content of the raw seeds. *M. urens* seed was chosen for this investigation because it is believed in our immediate localities, where it is consumed, that they lower the sperm integrity in men, thereby reducing erection and invariably, fertility. 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., 1974).

In general, the animal treated is a male because chemicals acting systemically on females may interfere with hormonal status, possibly interfering with the development of normal foetuses, or the chemical may act directly on the maturing oocyte, causing death other than by a dominant lethal mutation (Takagi et al., 2000). Several dominant lethal indices can be employed when examining dominant lethals in mammals; these include pre-implantation losses estimated on the basis of *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; www.setonresourcecenter.com, 2002).

The aim of this study, therefore, is to investigate the mutagenic effect(s) of the ethanol seed extracts of *M. urens* on the sperm integrity in male albino rats using the dominant lethal mutation assay (Plates I and II).

MATERIALS AND METHODS

The 24 albino rats (12 males and 12 females) used as mammalian models for this study were obtained from and housed in the animal house of the Pharmacology Department of the University of Calabar, Calabar. Preparation of the extract and sacrificing of treated and non-treated animals were carried out in the research laboratory of the same department.

Collection and preparation of seeds

The dry seeds of *M. urens* (Linn.) were bought from the local market (Watt market) and identified by the chief technologist in the Pharmacology Department of the University of Calabar, Calabar. The seeds were further air-dried for 3 days and then pulverized using a blender, Laprivia 3000, China.

Animal treatment

The rats, weighing between 100 and 150 g were housed in groups of three each in rubber cages and kept under optimum laboratory conditions (ambient temperature of 25±3°C; 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.*

Plant extraction

The plant extract was prepared by Soxhlet extraction following standard procedures. Ethanol was used as the extracting solvent since the powder was not soluble in water. The powdered sample (100 g) was wrapped in filter paper (Whatmann’s No. 40) and placed in the thimble in the main chamber of the Soxhlet apparatus. The Soxhlet extractor was placed on a flask containing 400 ml 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 of *M. urens* 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 72 h at 80°C. The ethanol extract was evaporated using a hot air oven (STUARC scientific, England) for 24 h at 50°C. 1 g of the paste extract was also dissolved in 10 ml Corn oil (vehicle) to make up 100 mg/ml concentration which was the stock solution. This was kept refrigerated at 4°C for later use.

Administration of extract

After an acclimatization period of two weeks, administration of the herb 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 70 mg/kg Body Weight (group I), 140 mg/kg Body Weight

Plate I. *Mucuna urens* seeds.

Plate II. Pulverized *Mucuna urens* seeds.

(group II) and 210 mg/kg Body Weight (group III) (Udoh and Ekpeyong, 2001). The control animals (group I) were not fed any extract (0 mg/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. At the end of the extract administration, the male rats were co-habited with untreated virgin females for three days at a ratio of 1: 1 to examine the dominant lethal parameters.

Experimental

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 lutea* for determining pre-implantation losses and pictures were obtained using a digital camera (DCR-

Table 1. Dominant lethal mutation assay of rats treated with ethanol extracts of *Mucuna urens.*

Treatment group (mg/kg BW)	No. of males	No. of pregnant females	No. of non-pregnant females	Biological evaluations
I	3	3	-	0
II	3	3	-	76
III	3	-	3	100
IV	3	-	3	100

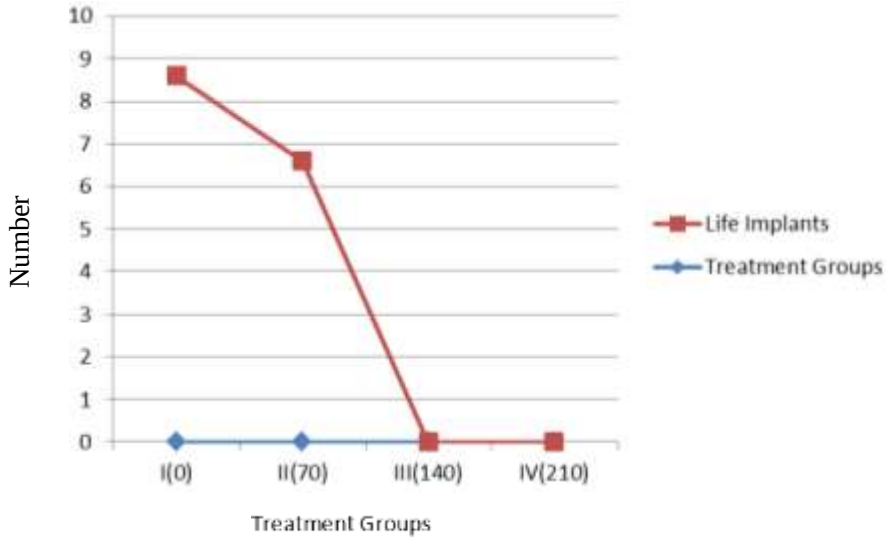

Figure 1. Life implants in the uterine horn of female albino rats.

HC48E, KODAK).

Data analyses

Biological evaluation of dominant lethal mutation was analyzed using the formula (http://www.setonresourcecenter.com, 2002):

1 - (Mean life implant in each treatment group / Mean life implant in control) × 100%

Results were reported in accordance with the CFR P798-024 Rodent Dominant Lethal Mutation Assay guidelines (1978). The statistical evaluation of the means and standard deviations of the observed implants were also carried out. All evaluations were done with the aid of the Genstat (7.2) statistical package.

RESULTS AND DISCUSSION

The results of the Dominant Lethal Mutation Assay of male rats fed with ethanol extract of seeds of *M. urens* for 14 days are presented in Table 1 and Figure 1. Photos of the *corpora lutea* (Plate IIIA to D) of untreated female rats mated with treated males, at mid-gestation, have also been presented. The biological evaluation of the mutations was observed as 0, 76, 100 and 100% lethal mutations in groups I, II, III and IV, respectively. This data which was obtained from the biological evaluations shows that the ethanol extract of the herb, *M. urens*, had lethal effects on the spermatocytes and early spermatids of the male albino rat (Udoh and Ekpeyong, 2001).

Pre-implantation losses were recorded in all the treatment groups, with rats in groups III and IV scoring no implants. On the other hand, the percentage implantation in the control rats was 100%. Means and standard deviations of life implants observed were $8.6^{b} \pm 0.47$, $6.6^{a} \pm 0.94$, 0 ± 0.0, and 0 ± 0.0 for the treatment groups I, II, III, and IV, respectively. These results are similar to those obtained by several independent workers on the dominant lethal effects of Acrylamide in male mouse germ cells. It was observed to induce dominant lethal mutations, heritable translocations and specific locus mutations in the mouse.

Shelby et al. (1986), Ehling and Neuhauser-Klaus (1992), Adler et al. (1994) Udoh and Ekpeyong (2001) showed the antifertility potentials of *M. urens* extract in

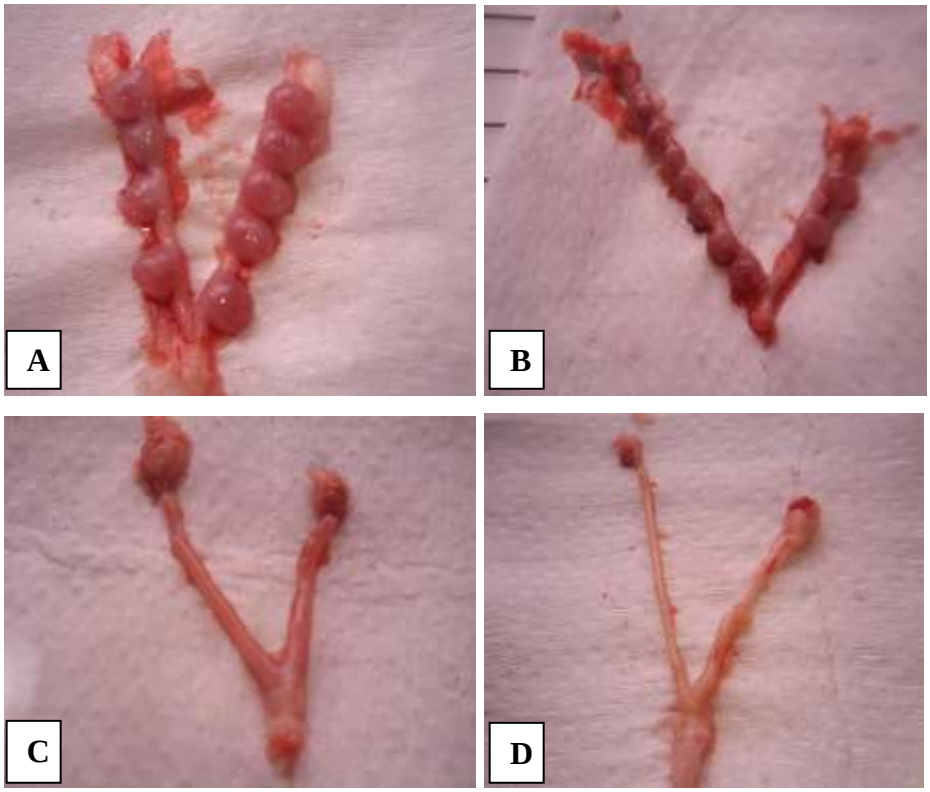

Plate III. A-Healthy implantation (group I); Plate B-Poor implantation (group II); Plate C- No implantation (group III); Plate D- No implantation (group IV).

male guinea pigs. From their findings, histological observations at high dose (140 mg/kg BW) showed complete degeneration of sperm in the testicular tubules. This will suggest that under the test conditions, the ethanol extract of the seeds of *M. urens* maybe genotoxic in the germplasm of the treated male rats (CFR, 2002).

Similar results were obtained in investigations on Neem plant (*Azadirachta indica*) when the Neem oil was shown to inhibit sperm motility *in vitro* (Riar et al., 1990; Upadhyay et al., 1990). This genotoxicity is also implicated as the cause of partial sterility in the lower doses and total sterility in the male rats at higher doses (Generoso et al., 2003). The *corpora lutea graviditas* counted also showed a dose-related decrease in number. 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, 1985).

Conclusion

The mutation observed in the treated rats at the different doses of treatment may be attributed to the effects of a component or some components of the seeds of *M. urens* acting either alone or in synergy. It is recommended that further studies on the effects of *M. urens* seed extracts in male animals with emphasis on mutagenesis be carried out. Characterization and further fragmentation to determine the active antifertility ingredient(s) is also suggested.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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