ISSN: 0975-833X

Available online at http://www.journalcra.com

SPECIAL ISSUE

International Journal of Current Research
Vol.3, Issue, 6, pp.323-327, June, 2011

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

MALE REPRODUCTIVE ENHANCING ACTIVITY OF *MUCUNA PRURIENS* LINN. SEED EXTRACT IN WNIN/GR-OB OBESE RATS – AN INFERTILE OBESE MUTANT RAT MODEL WITH PREDIABETES

Satish Kumar[a], Mir Yousuf Ali[a], P. Sailaja[b], S. Mahesh[b], M.V. Surekha[c], N.V. Giridharan[b] and N. Harishankar[b]

[a]Department of Ilmul-Advia (Pharmacology), Govt. Nizamia Tibbi College, Charminar, Hyderabad.
[b]National Centre for Laboratory Animal Sciences, National Institute of Nutrition (ICMR), Hyderabad.
[c]Department of Pathology, National Institute of Nutrition (ICMR), Hyderabad.

ARTICLE INFO

Article History:
Received 19th March, 2011
Received in revised form
13th April, 2011
Accepted 5th May, 2011
Published online 26nd June 2011

Key words:
Mucuna pruriens
Reproductive hormones,
Levator ani muscle,
Sperm count,
Spermatogenesis,
Gonaidal Index.

ABSTRACT

Aim of the study: *Mucuna pruriens* is often used in Unani medicine as an aphrodisiac and to improve male reproductive functions. Since systematic study on these aspects are few and far in between, we studied its androgenic effect on an established obese mutant rat model at our centre, which is obese and infertile.

Materials and Methods: 18 obese male rats with their corresponding 18 lean littermates were taken and divided in to three groups and were fed with the herbal seed extract at two different dose levels. Blood samples were collected from control and experimental rats to measure hormones like testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin. Levator ani (LA) muscle weight and complete semen analysis, gonadial index and testis histology were investigated.

Results: Testosterone, LH, FSH and prolactin hormone levels showed significant increase in experimental rats compared to controls. The LA muscle weight significantly increased and also sperm count and motility as well, in treated rats. The testis histology of experimental rats showed significant improvement in the architecture with densely packed spermatids in the seminiferous tubules.

Conclusions: *M. pruriens* ethanolic seed extract has definite positive effect on male reproductive functions in terms of hormone profile, organs weights, semen quality and quantity.

INTRODUCTION

Infertility is often a problem encountered under obese conditions, and this is well depicted by obese rodent models, where in a deligent selection of heterozygous carriers (+/-) only ensures continuous production of obese rats (Kasten, 1952). This is not restricted to animals alone and often humans equally affected. In women, obesity is frequently associated with menstrual disturbances with a high risk of androgenic ovulatory dysfunction (Robinson *et al*., 1993). Obese men relatively have low serum testosterone concentrations and they invariably respond to exogenous administration of testosterone, resulting in weight reduction (Martin *et al*., 1992). Among the obese models, only the A^y mice have been reported to have normal fertility and this was also restricted to young animals who were yet to become fully obese (Kasten, 1952). The testis of Ob/Ob, db/db and Ad/Ad obese mice was also found to be smaller than those of their corresponding lean littermates and often they remained undescended. Penis and scrotum are often poorly developed, but, despite reduced gonadal development, active spermatogenesis was observed in these obese species (Coleman & Hummel, 1970; Hellman *et al*., 1963; Hetherington and Ranson, 1942). To restore the infertility problems, compounds endocrinal in composition like thyroid cytomel, glucocorticoids and low doses of androgens and long acting testosterone esters are often tried (Charles, 1978). Apart from these hormones, traditional medicinal plants are increasingly used now a days to restore the infertility. It is often observed that, the leaf and seed extracts of some of these medicinal plants often more effective with fewer side effects (Jayanthi Abraham, 2011). *Mucuna pruriens* seeds often known as velvet beans is one among them and in Unani medicine it is extensively prescribed as an aphrodisiac (Suresh *et al*., 2009). Additionally they have anti Parkinson (Kasture *et al*., 2009), antidiabetic (Gupta *et al*., 2007) and antioxidant (Dhanasekharan *et al*., 2008) activities and also found to regulate steroidogenesis and improve semen quality in infertile men (Shukla *et al*., 2008). Though much work has been carried out on *M.pruriens* for its reproductive enhancing activity especially on males, systematic study on an obese animal model with infertility

Corresponding author: hsnemani2000@yahoo.com

is yet to be reported. In the present study we have studied the effect of *M.pruriens* seed extract on the male reproductive functions of an indigenously developed mutant obese rat (WNIN/GR-Ob) with infertility problem. WNIN/GR-Ob is an obese mutant rat with impaired glucose tolerance (IGT), established at our centre in 1997 (Giridharan *et al*., 1996; Harishankar *et al*., 2010). In this model we studied the effect of *M.pruriens* seed extract on testis and accessory glands weights, sperm count, sperm morphology and gonaidal index and LA muscle weight. The circulatory reproductive hormones like testosterone, FSH, LH and prolactin, in the control and *M. pruriens* seed extract treated obese and lean littermates. In this model we studied the effect of *M. pruriens* seed extract on testis and accessory glands weights, sperm count, sperm morphology, gonaidal index and LA muscle weight.

MATERIALS AND METHODS

Preparation of experimental drug

Experimental drug was prepared as per the modified method of Murugan and Uma Maheswar Reddy, 2009. *M. pruriens*, dried seeds were procured from Lala Dawasaz, Charminar, Hyderabad and the extraction was carried out using soxhlet apparatus. After extraction the ethanol was distilled off and the extract was concentrated on hot plate for evaporation and for further drying of the drug. Subsequently the drug was kept in clean container for feeding the experimental rats. For feeding the experimental rats, 24 gm of the drug was mixed thoroughly in 2 Kg powder of standard laboratory rat chow which had been developed and established at our centre. This rodent chow containing all macro and micronutrients prepared at our facilities (56% carbohydrates, 18.5% protein, 8% fat, 12% fiber, 55% of carbohydrate and recommended levels of minerals and vitamins). Handmade pellets were prepared from the mixture in the quantities of 0.5 gm and 1 gm, containing 6 mg and 12 mg of the drug respectively.

Animals and housing conditions

Eighteen WNIN/GR-Ob obese (-/-) male rats, aged 90 days, were taken for study along with eighteen lean (+/+) littermates. These animals were housed in standard polycarbonate cages (Techniplast, Italy) with top grill having facilities for holding pelleted feed and drinking water in polycarbonate bottles with stainless steel sipper tubes. The bottom of the cage was also steel grilled to facilitate free droppings of feces and urine and also to prevent coprophagy. Readily available autoclaved paddy husk was used as a bedding material in the cages. An ambient temperature of 22 ± 1° C with 14-16 air changes per hour, relative humidity of 50-60% and light/dark cycles of 12 hours duration were maintained in the animal rooms. Standard rodent chow prepared at our centre containing 20% protein, 6% fat, with recommended levels of vitamins and minerals, prepared and pelleted in our animal facilities was fed along with water *ad libitum*.

Drug treatment and end points

After three days of acclimatization with standard rat chow, 6 obese rats were fed with control diet (placebo group), 6 obese rats were fed with 0.5 gm hand made pellet containing 6 mg of the seed extract (normal dose group - ND), 6 obese rats were fed with 1.0gm handmade pellet containing 12 mg of the seed extract (high dose group - HD). Similar groups were made with lean littermates to serve as positive controls. The supplementation of seed extract continued for 45 days as per the schedule given by Suresh *et al* (2009). Animals belonging to the control and experimental groups were studied for growth and food intake studies till the end of the experiment. Daily food intake and weekly body weights were monitored in the control and experimental rats of both phenotypes. Blood was collected from supra orbital complex (Riley, 1960), for reproductive hormonal profile and subsequently the animals were euthanized by isoflurene inhalation to measure the reproductive indicators and organ weights, as well as for histology of testis.

Weight of gonads and accessory glands

The male reproductive accessory glands along with testis were separated from the viscera and the fat attached to these glands were removed and blotted on a filter paper. The weights were then taken on a Sartorius analytical balance (0.1 gm sensitivity) and kept in appropriate fixative solutions for further processing to study the histology.

Reproductive indicators

To measure the sperm count, sperm was collected from the cauda epididymis, and counted by using a haemocytometer in control and extract treated rats as per the method described by Raghunath *et al*., 1987. The intact sperm cells were only counted and the counts were expressed as 10^6 cells/ml of saline suspension.

Gonadial index

The weights of both testes in relation to the body weights were calculated for correlating it with the sperm production potential which indicates the gonadial index (Marks, 1990). The gonadial index was determined in control and extract treated obese and lean littermates as per the method described by Jayanthi Abraham (2011).

Levator Ani Muscle

LA muscle weight is often used as a bioassay for testosterone (Eisenberg and Gordon, 1950). The levator ani muscle is a horse shoe shaped muscle at the bottom of the large intestine surrounding the rectum. This muscle is under the control of steroid hormones and its weight is correlated with androgen concentration (Sala and Baldrati, 1953). The LA muscle is often expressed as wet weight. LA muscle was extirpated from control and extract treated obese and lean littermates as per, the modified technique of Rassaert *et al*., (1968).

Reproductive hormones

Hormones like testosterone, FSH, LH and prolactin in the control and ethanolic seed extract of *M.pruriens* treated obese and lean littermates measured by using kits supplied by Biosystem, Spain, based in the Elisa kit method.

Histology of testis

For this, animals belonging to control and treated group were fasted for 17 hours and sacrificed by isoflurene inhalation. Both testis were rapidly removed and fixed in 10% Bouwin' fixative and processed for histological studies. The transverse sections of the testis were stained with hematoxylin and eosin and observed under 10X eye piece graticule.

***Statistical analysis*:** Mean ± standard error were tested using one way ANOVA to check for any significant differences between the mean values of different groups. All the values given were mean ± standard error of mean (SEM). A probability level of less than 5% ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Food intake and body weight

Food intake significantly increased with increase in age and HD treated rats showed high food intake (29.58 ± 2.13 gm/per rat/per day) with high feed efficiency ratio. The initial trends in terms of the hyperphagia were seen in ND treated rats at 2nd week of extraction administration. However, subsequently there were no significant changes among obese control (24.46 ± 2.86 gm/per rat/per day) and ND (23.58 ± 2.9 gm/per rat/per day) treated rats. In the case of positive control lean rats, no significant changes were seen in terms of their food intake in ND and HD treated rats.

The body weights of control, ND and HD treated obese rats showed significant increase with increase in their age. Among the groups control obese rats (613.5 ± 7.89 gm) gained significantly higher body weights compared to ND (518.67 ± 10.94) and HD (606 ± 6.00) treated obese rats till the termination of the extract feeding ($P<0.01$). Among the treated obese groups HD rats gained higher body weights than ND rats ($P<0.01$). Similar results were obtained in lean littermates with no significant change in ND and HD treated groups ($P<0.01$). It was observed that within 15 days of seed extract treatment, the ND and HD treated obese rats had become more active and they resumed their grooming behaviour as well which was not seen in control obese rats. This overall improvement in their general wellbeing in terms of increased activity may be due to the increased utility of the metabolic fuel, as witnessed by the correction in glucose homeostasis and decrease in the body fat.

Reproductive parameters

Table 1 shows weights of the testis and accessory glands, sperm count and gonaidal index. Obese control showed significantly lower testis and accessory gland weights, and also low gonaidal index. Epdidydimal fat weight is high in the treated obese groups compared to obese control. Between ND and HD groups there was no difference in weights of testis and accessory glands. The LA muscle weight, sperm count also showed increase after completion of seed extract supplementation in lean as well as obese groups. It was observed earlier that, low testis with normal spermatogenesis and histology was seen in WNIN/GR-Ob obese rats compared to lean littermates (Harishankar thesis, 2001), which is in line with many other genetically obese rodents (Bray *et al*., 1976; Swerdloff *et al*., 1976; and Edmons and Withyachumnarnkul, 1980) as described above. From the results of this present study it becomes evident that the seeds of *M.pruriens* have a definite positive action on improving testis weight and sperm production (as indicated by sperm count) in treated obese rats. The testis weight was found to be less in control rats compared with ND and HD treated obese rats. The increase in the total weight of testis of ND and HD treated obese rats was 17.98% and 19.25% respectively. This also reflected in terms of the increase in sperm count as discussed below.

Table 1. Effect of ethanol extraction of *M. pruriens* seeds on reproductive accessory organ weigts in WNIN/GR-Ob obese and lean rats. (n=6).

Group	Testis (g)	Seminal vesicle (g)	Epididymus (g)	Epididymal fat (g)	LA Muscle (g)	Sperm count 10^6 cells/ml	Gonaidal Index
Obese control	2.6±0.17	0.65±0.28	0.6±0.24	9.73±2.28	0.18±0.2	109.83±8.59	0.44±0.16
Obese ND	3.17±0.12	0.87±0.27	0.7±0.1	10.05±3.67	0.22±0.11	148.75±5.14**	0.52±0.13
Obese HD	3.22±0.3	0.98±0.17	0.68±0.04	10.28±2.13	0.28±0.12	171.0±5.33**	0.617±0.06
Lean control	3.01±0.31	1.0±0.2	0.65±0.10	3.55±1.16	0.18±0.11	146.83±8.33	0.68±0.15
Lean ND	3.18±0.07	1.25±0.28	0.71±0.14	2.88±0.64	0.25±0.14	129.82±9.88	0.86±0.08
Lean HD	3.23±0.13	1.06±0.17	0.85±0.13	2.46±0.13	0.23±0.13	131.66±4.66	0.87±0.07

Values are mean ± S.E. * $P < 0.01$.

Table 2. Effect of ethanol extraction of *M.pruriens* seeds on reproductive hormone profile in WNIN/GR-Ob obese and lean rats. (n=6).

Reproductive Hormones	Obese group Control	Obese group Normal dose	Obese group High dose	Lean group Control	Lean group Normal dose	Lean group High dose
Testosterone (ng/ml)	0.39±0.10	0.45±0.09*	0.74±0.03*	4.16±0.91	24.16±2.06**	16.5±1.36**
LH (ng/ml)	0.42±0.11	3.85±0.33**	3.42±0.42**	0.94±0.14	9.12±2.05**	11.05±1.18**
FSH (ng/ml)	16.04±1.6	14.12±1.9**	27.98±2.4**	6.68±1.02	13.87±2.36**	8.69±1.07**
Prolactin (pg/ml)	5.34±.0.68	5.13±0.69	5.72±1.15	4.84±1.09	9.82±1.38*	6.86±1.10*

Values are mean ± S.E. * $P < 0.01$. ** $P < 0.001$,

The results of sperm count in response to the drug *M.pruriens* showed an increase with a 26.16% in ND and 35.77% in HD groups. The drug is found to have a profound effect at 45 days of 12 mg treatment (HD group). The increase in sperm count is found to be dose dependent and a significant increase is observed in HD treated group obese rats. This increase in sperm count may directly relate to androgenic action of the *M.pruriens* extract, as evidenced by the increased in LA muscle weight and testosterone levels in these animals and also the gonadotrpic levels. In fact, the weights of seminal vesicle and LA muscle were often taken as a measure of testosterone concentration and weight of LA muscle in particular is suggested as a bioassay for testosterone (Whitaker *et al*., 1983).

Hormones

The circulatory reproductive hormone levels were analyzed and given in Table.No.2. The obese control had low hormone levels compared to lean controls. But on comparison of seed extract,

the testosterone, LH, FSH and prolactin levels were significantly increased with the increase in the dose in both obese and lean rats compared to controls.

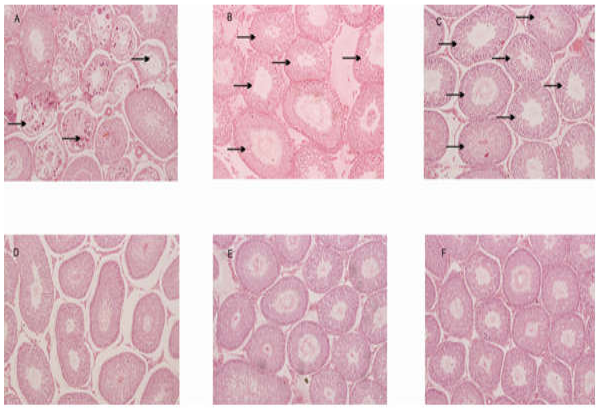

Fig. 1.

A. Microphotgraph of testis section of obese control group showing most of the semineferous tubules with maturation arrest at spermatogonia level and with giant cell formation. (H&E staining: 10X)

B. Microphotgraph of testis section of obese ND treated group showing few tubules with maturation arrest at spermatids stage and absence of maturation spermatozoa (black arrows) (H&E staining: 10X)

C. Microphotgraph of testis section of obese HD treated group showing an occasional semineferous tubules showing maturation arrest at spematid stage (black arrows) (H&E staining: 10X).

D, E, F. Microphotgraphs of testis section of lean control, ND and HD groups showing normal maturation of semineferous tubules (H&E staining: 10X).

The testosterone levels were significantly high in HD group followed by ND and control group (P<0.05) in that order. But, in the case of lean littermates ND treated rats had significantly higher testosterone than HD and control groups (*(P<0.001)*. LH and FSH levels were significantly increased in both ND and HD treated rats (P<0.001). The seed extract did not show any increase in the prolactin levels of obese rats belonging to control and treated groups. However, a significant increase in prolactin levels was seen in lean control and treated groups (*(P<0.001)*.

Generally the obese rodents have low reproductive indices in terms of reproductive hormones, organs weights, testis and accessory gland weights etc., Sinha *et al*., (1975) studied extensively on gonadotropin hormone (GH) and prolactin (PRL) in Ob/Ob mice and found that plasma levels of GH and PRL are reduced in obese mice compared to their lean littermates. Sex differences in levels of GH and PRL was observed in lean mice and were not so marked in obese mice. Previous studies in obese male Zucker rats at various age groups (4, 6 and 10 months) revealed inadequate sexual behaviour, occurring at an age of 4 months (Edmonds and Withyachumnarnukul., 1980). In these rats, the pituitary weight was found to be less and the concentration of LH and FSH were shown to be increased (Bray, 1977). The concentration of circulating gonadotropin was also abnormal and it did not show any response in fa/fa rats (Bray *et al*., 1973). However, in spite of limited reproductive capacity, a normal sperm production and motility were observed Zucker obese rats (Edmonds and Withyachumnarnkal, 1982). Earlier observations in these animals showed low levels of circulating testosterone and low weights of LA muscle (Sala and Baldratti, 1963). It is well evident that medicinal plants with potential reproductive enhancing activity will increase steroidiogenesis activity leading to increased testosterone concentration (Yakub, 2006). Similar mechanism might have been involved with respect to this herb as well as shown by the increase in circulatory levels of testosterone concentration at both ND and HD dose concentrations. LH and FSH hormones are produced by anterior pituitary of the brain and are necessary for maintaining testosterone levels in the body and an increase in the concentrations of bioavailable/free testosterone should cause increase in the concentration of LH and FSH (Yakub *et al*., 2007). Similarly, in the present study, we have observed that there is a significant in increase in the testosterone, LH and FSH concentrations in ND and HD treated groups. Prolactin hormone is developed by specialized pituitary cells called lactotrophs and increases the production of breast milk and suppresses the secretion of LH and FSH (Yakub, 2007). A high level of production often suppresses synthesis as well as testosterone production. In the present study there is a reduction in the prolactin hormone levels in ND and HD treated groups leading to increase in LH and FSH levels and there by increase in the testosterone as well. *M.pruriens* seed extract contains higher amounts of L-dopa (Levodopa), and it changes into dopamine after entering in to the blood stream. The increase in L-dopa, which is converted in to dopamine, is shown to be a powerful neurotransmitter leading to a strong aphrodisiac affect (Capochichi *et al*., 2002).

Testis histology

Grossly there were no changes in the morphology of testis in obese and lean littermates (Figure.1). Histologically most of the seminerous tubules with maturation arrest at spermatogonia level and with giant cell formation was seen in control obese rats (Photo micrograph - A). The testis of ND treated rats showed few tubules with maturation arrest at spermatid stage and absence of mature spermatozoa (Photo micrograph - B). Occasional seminiferous tubules showing maturation arrest at spermatid stage observed in HD treated obese rats (Photo micrograph - C). Normal maturation of semineferous tubules was seen in the lean littermates (Photo micrograph – D, E and F). The histology of the testis in lean animals showed normal histology as expected, while obese rats had a comparatively lesser packed male reproductive cells, which also reflected in low sperm count exhibited by them. On treatment significant increase in the number of primary spemtocytes, spematogonia, spermatids, sertoli cells and leydig cells were seen both in ND and HD seed extract treated groups compared to control. In conclusion, the data obtained in the present study, conclusively proves the male reproductive enhancing activity of *Mucuna pruriens* on WNIN/GR-Ob obese rats, a mutant obese rat with infertility problem. It is thus evident that this seed extract has definitely a potential to treat male infertility, especially associated with obesity and diabetes and can be suggested to treat male sexual disorders associated with obesity and diabetes.

Conflict of Interest: The authors declare that there are no conflicts of interests.

Acknowledgement: We are grateful to Indian Council of Medical Research (ICMR) for the financial support to carry out the studies. We thank Mr.N.Yadagiri, Lab.Asst for his excellent care and cooperation in the maintenance of WNIN/GR-Ob rat colony. We are thankful to Dr.Abdul Saleem, Asst.Prof, Dept. of Ilmul-Advia, (pharmacology), Govt. Nizamia Tibbi College, Charminar, Hyderabad, and

Dr. B. Sesikeran, Director, National Institute of Nutrition for keen interest in this work.

REFERENCES

Bray, GA, Saiduddin, S, York, DA and Swerdloff, RS. 1976. Effect of estradiol on uterine weight, thyroid function food intake, and pituitary weight of genetically obese (fatty-Zucker) and lean rats. *Proc. Soc. Exp. Biol. Med*. 153: 88-91.

Bray, GA, York, DA, Swerdloff, RS. 1973. Genetic obesity in rats. I. The effects of food restriction on body composition and hypothalamic function. *Metabolism*. 22 :435-442.

Bray, GA.1977. The Zucker-fatty rat: a review. *Fed. Proc*. 36:148-153.

Capo-chichi, LJA, Eillitta, M, Carsky, RJ, Gilber, RA. and Maasdorp, B.2003. Effect of genotype and environment on L-dopa concentration in Mucuna seeds. *Tropical and subtropical Agroecosystems*. 1: 319-328

Charles, WC, Julian, AG. 1978. Testosterone rebound therapy. *Fertil. Steril*. 29; 1-3

Coleman DL, Hummel KP. 1970. The effects of hypothalamics lesions in genetically diabetic mice. *Diabetologia*. 6:263-267.

Dhanasekharan, M., Tharakan, B., Manyam, BV., Antiparkinson drug. Mucuna pruriens shows antioxidant and metal chelating activity. *Phytother Res*. 2008. 22(1) 6-11.

Edmonds ES, Withyachumnarnkul B. 1980. Sexual behavior of the obese male Zucker rat. *Physiol Behav*. 24:1139-1141.

Edmonds, ES, Dallie, SK and Withyachymnarnkul, B**.** 1982**.** Reproductive system of the obese male zucker rat. Reproductive capacity, artificial insemination and plasma testosterone levels. *Biol. Reprod.* 27; 891- 897.

Eisenberg, E., and Gordon, GS. 1950. The Levator ani muscle of the rat as an index of myotrophic activity of steroidal hormones. *J. Pharmac. Exp*. There. 99: 38-41.

Giridharan NV, Harishankar N and Satyavani M. 1996. WNIN/Ob, A new rat model for the studies of obesity. *Scan.J.Lab.Ani.Sci*. 3: 131-138.

Gupta, R., Bajpai, KG, Johri, S, Saxena, A. 2007. An over view of Indian Novel traditional medicinal plants with anti-diabetic potentials. *Afr.J.Tradi.complement Altern Med.* 27: 1-17.

Harishankar, N, Vajreswari, A, and Giridharan , NV . 2011. WNIN/GR-Ob - An insulin-resistant obese rat model from inbred WNIN strain for studies on obesity associated with diabetes. (Manuscript accepted in *IJMR*).

Harishankar, N, Vajreswari, A, and Giridharan, NV. 2010. WNIN/GR-Ob - An insulin-resistant obese rat model from inbred WNIN strain for studies on obesity associated with diabetes. (Manuscript communicated to *IJMR*)

Harishankar, N. 2001. Establishment and characterization of an obese mutant strain developed from WNIN rat strain. Ph.D thesis. Osmania University . Hyderabad.

Hellman, B, Jacobsson L, Taeljedal, IB. 1963. Endocrine activity of the testis in obese-hyperglycaemic mice. *Acta Endocrinol* (Copenh). 44: 20-26.

Hemmes, R and Vaid, J. 1977. Reproductive inadequacy of genetically obese Zucker rat. Male response to female sex attractant pheromone. *Fed. Proc*. 36: 1165-1168.

Hetherington AW, Ranson AW. 1942. The relation of various biochemical lesions to adiposity in the rat. *J. Comp. Neurol*., 76:475-499.

Jayanthi Abraham. 2011. Effect of Mucuna pruriens seeds on Fertility of male Albino rats *Rattus norvegicus*. *J. Pharma. Res*., 4:233-236.

Kasten FH. Comparative Histological Studies of Endocrine Glands of Yellow (A[unknown]a) and Non-agouti (aa) Mice in Relation to the Problem of Hereditary Obesity. *Science* 1952; 115:647-649.

Kasture, S, Pontis, S, Pinna, A, Schintu, N, Spina, L, Longoni, R, N.Simola, Baller, M, Moreli, M. 2009. Assessment of symptomatic and newroprotective efficacy of Mucuna pruriens seed extracts in rodent model of Parkinsons's disease. *Neurotox. Res. Epub*. 15: 111-122.

Mårin, P, Holmäng, S, Jönsson, L, Sjöström, L, Kvist, H, Holm, G, Lindstedt, G, Björntorp, P. 1992. The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. *Int J Obes Relat Metab Disord*. 16:991-997.

Marks, HL 1990. Abdominal fat and testis weight in diveser genetic lives of Japanese Quail. *Poult. Sci.*.69: 1627-1633.

Murugan, M, Uma Maheshwara Reddy, C. 2009. Hypoglycemic and hypolipidemic activity of leaves of Mucuna pruriens in alloxan induced diabetic rats. *J Pharm Sci and Tech*.1: 69-73.

Raghunath,.M, Giridharan, NV**.** 1987. Effect of male anti fertility agent gossypol on pancreatic and intestinal proteases in the rat. *Nutr. Rep. Int*. 36: 756-761.

Rassaert, CL, Wolff, A and McDougall,EA. 1968. A new technique for extirpation of the levator ani muscle in the rat. *J. Endocrinol.,* 42, 601-602

Riley, V. 1960. Adaptation of orbital bleeding technique to rapid serial blood studies. *Proc.Soc.Exp.Biol.Med*.. 109, 751-754.

Robinson, S, Kiddy, D, Gelding,SV, Willis, D, Nithynanthan, R, Bush, A, Johnston, DG, Frank, S. 1993. The relationship of insulin insensitivity to menstrual pattern in women with hyperandrogenism and polycystic ovaries. *Clin. Endocrinol.* 39:368 -371.

Sala, G and Baldratti, G. 1963.A Long-Acting Anabolic Steroid: 4-Hydroxy- 19-nortestosterone- 17-cy clopentylpropionate. *Endocrinol*. 72: 494-495

Shukla, KK, Mahdi, AA, Ahmad, MK, Shankwar, SN, Rajender, SP, Jaiswar, SP. 2008. Mucuna pruriens improves male fertility by its action on the hypothalamus – pituitary gonadal axix. Epub. *Fertil and Steril* 92: 1934-1940.

Sinha YN, Salocks CB, Vanderlaan WP. 1975. Prolactin and growth hormone secretion in chemically induced and genetically obese mice. *Endocrinol*. 97:1386-1393.

Suresh, S, Prithiviraj, E, Prakash, S. 2009. Dose-and time-dependent effects of ethanolic extract of Mucuna pruriens Linn. seed on sexual behaviour of normal male rats. *J. Ethanopharmacol*. 122: 497-501.

Swerdloff RA, Batt RA and Bray GA. 1976. Reproductive hormonal function in the genetically obese (ob/ob) mouse. *Endocrinology*. 98, 1359-1364.

Whitaker, EM. Shaw, MA and Hervay. 1983. Plasma oestradiol-17 beta and testosterone concentrations as possible causes of the infertility of congenitally obese Zucker rats. *J. Endocrinol*. 99: 485-490.

Yakubu, MT, Akanji, MA and Oladiji, AT. 2007. Male sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. *Pharmacognosy Rev*.1: 49-56.

Yakubu, MT, Akanji, MA and Oladiji, AT.2005. Aphrodisiac potentials of aqueous extract of Fadogia agrestis (Schweinf.Ex. Hiern) stem in male albino rats *Asia..J Androl.*7:399-404.

Zucker LM and Zucker TF 1961 Fatty, a new mutation in the rat. *J. Hered.* 32: 275-78.

****