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Evaluation of Varying Concentrations of *Mucuna Pruriens* Seed Powder on Testosterone, Glutathione and Catalase Levels of Male Albino Wistar Rats

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Abstract
The antioxidant and aphrodisiac properties of Mucuna pruriens seed powder extract have been documented in the literature. This study evaluated the effect of varying concentrations of Mucuna pruriens seed powder on testosterone, antioxidant and semen parameters of male albino rats. Thirty rats were grouped into four; controls, and three other groups each respectively administered 0.4, 0.8 and 1.2mL extracts of Mucuna pruriens seed powder. The analytes were evaluated using standard methods. Statistical significance was set at $p<0.05$. Serum and seminal catalase was significantly higher ($p=0.031$, $p=0.003$) in the group 3 rats than the groups 1 and 2 rats. Seminal testosterone was significantly higher ($p= 0.004$) in group 3 rats than other groups. Groups 2 and 3 rats had the greatest improvement as observed in their sperm counts and catalase activity. Owing to the results recorded in this study, oral administration of Mucuna pruriens seed powder extract can improve testicular antioxidant and sperm count in combating infertility.

Keywords: *Mucuna pruriens extract,* catalase, testosterone, sperm count

INTRODUCTION
Mucuna pruriens also known as velvet beans in English, *Okotiekpo* in Efik/Ibibio tribe in southern Nigeria, is an annual climbing tropical legume of the family FABACEAE and genus *Mucuna* with up to 100 species (Ezeagu *et al.,* 2003). It is an annual herbaceous, vigorous climbing vine that grows 3-6 cm in height. It is indigenous to the tropical regions in Africa and Asia (Majekoduimi *et al.,* 2011). The leaves are mostly free and have trifoliate white or dark purple flowers hung on long clusters, pods are sigmoid and the seeds are ovoid having 4-6 seeds per pod (Buckles, 1995). *Mucuna pruriens* is a rich source of protein supplement of food and feed for humans and livestock (Siddhuraju *et al.,* 2001). Some ethnic groups in Nigeria use the pods and leaves as edible vegetable (Adebowale *et al*., 2005), while the seeds are used as thickener for soup and as vegetable oil (Ukachukwu *et al*., 2002).

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Medicinal plants being an effective source of both traditional and modern medicines are genuinely useful for primary health care (Sofowora *et al.,* 2013). World Health Organization (2009) has advocated traditional medicine as safe remedies for ailments of both microbial and non-microbial origin. Male reproductive dysfunction, poor sexual performances and loss of libido are common public health disorders as well as a major health challenge among humans in many parts of the world (Akinola *et al.,* 2010) and this has been a major concern over the years. Management of this reproductive dysfunction in many developing countries is expensive and may not be accessible and available to the poor. This has compelled humans in developing countries such as Nigeria to source for medicinal plants that are cheap, readily available and effective. One of such plants used by the herbal practitioners to remediate ailments in Nigeria is *Mucuna pruriens*, widely known as velvet beans (Ashidi, 2019).

The plant has been screened for its phytochemical constituents and reported to be rich in alkaloids, tannins, saponins, flavonoids and cardiac glycosides (Agbafor and Nwachukwu, 2011). The plant's seeds have been reported to improve fertility in male animals and humans due to its active compound L-3, dihydroxyphenylalanine (L-DOPA) (Mutwedu *et al.,* 2019). However, the exact mechanism of its action is unknown. But possibly, it may be as a result of its antioxidant adaptogenic and general nutritional properties (Nagashyana *et al.,* 2000). Infertility has been linked to several emotional, physical and socio-cultural problems with different etiologies (Greil *et al*., 2010). Male infertility accounts for about 40% of all cases and it is known that some conditions such as cryptorchidism, hypogonadism and genetic factors can cause infertility (Alahmar, 2017). However, no underlying cause can be identified for primary or secondary infertility in approximately 25% of couples which is termed idiopathic infertility. One of the mechanisms proposed for idiopathic male infertility is oxidative stress (OS) (Alahmar, 2019). Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the scavenging capacity of available antioxidants resulting in redox paradox. Spermatozoa are highly susceptible to the deleterious effects of ROS due to the large amounts of unsaturated fatty acids found in their cell membranes (Schuppe *et al*., 2008).

Mucuna pruriens seed powder is effective in combating the stress mediated compromise in spermatogenesis by maintaining the antioxidant level (Siddhuruji *et al.,* 2000). In addition, antioxidant, anti-inflammatory, neuroprotective, antidiabetic, antiepileptic, antibacterial, antiparasitic, antistress, depressant, antineoplastic, learning and memory enhancing properties of the plants have been reported (Uma and Gurumoorthi, 2013; Obogwu *et al.,* 2014). Catalase and glutathione (GSH) peroxidase form the main antioxidant system in semen and are present in both the intracellular and extracellular space (Ahangarpour *et al*., 2013). A study found that the levels of antioxidants in seminal plasma from infertile men were significantly lower than in fertile controls and the levels of ROS produced by spermatozoa were negatively correlated with sperm quality (Oliveira *et al*., 2015). The seeds of *mucuna pruriens* have been reported to be useful in the treatment of spermatorrhoea, leucorrhea, aphrodisiac and improvement of fertility status among humans around the world. Although several studies have validated the medicinal potential of *Mucuna pruriens* and its role in male fertility, only few have considered its role on oxidative stress markers in male infertility (Greco *et al*., 2005). The research was conducted to study the pro-male fertility and aphrodisiac properties of *Mucuna pruriens* seeds by evaluating the testosterone, glutathione and catalase levels of healthy male albino wistar rats.

MATERIALS AND METHODS

Collection of plant material and preparation

The dry seeds of *Mucuna pruriens* were obtained from of tropical plant farm, Abuja, Nigeria. It was properly de-hulled and identified at the Botany department, University of Calabar, Nigeria. The raw de-hulled seeds were air dried and ground into powder with a Binatone electric blender and later screened for its phytochemical and proximate compositions. The flowers, pods, leaves and beans of Velvet beans are shown in Plate 1.

P

Seeds. (Source: Fern, 2014)

Experimental animal

Thirty-two (32) healthy adult male albino wistar rats weighing between 108 and 150g were purchased from the animal house unit of the Department of Physiology, University of Calabar, Nigeria. The rats were divided into four groups and housed in wooden cages with steel net as its cover. The rats were randomly assigned to the cages and all housed under similar conditions of management and husbandry including regular feeding and washing of drinking troughs. The rats were allowed to acclimatize for 14 days at 30 ± 2 ºC maintaining a 12-hour night and dark cycle prior to the commencement of treatment. Throughout the study, all the rats were fed with standard rat chow pellets and clean drinking water. The rats in the test groups were administered the *Mucuna pruriens* seed powder extract by oral method dosages at 0.4, 0.8 and 1.2mL/body weight respectively. Proper feeding with rat chow and clean drinking water was continuous throughout the duration of the experiment. The ethical protocols guiding the use of animals for experiment were followed. Ethical approval was obtained from the Animal Research Ethics Committee of the Faculty of Basic Medical Sciences, College of Medical Sciences, University of Calabar, Nigeria.

Experimental design

The rats were grouped according to individual body weight into four experimental groups (groups 1, 2, 3 and 4) with eight (8) rats per group.

Group 1: Rats were fed with standard laboratory rat chow (Control).

Group II: Rats were fed with rat chow and received oral administration of 0.4mL extract of *Mucuna pruriens* seed powder

Group III: Rats were fed with rat chow and received oral administration of 0.8mL extract of *Mucuna pruriens* seed powder
Group IV: Rats were fed with rat chow and received oral administration of 1.2mL extract of *Mucuna pruriens* seed powder.
The quantity of feed given was measured and readjusted every 7 days in accordance to the body weight increase. However, clean drinking water was supplied to all the rats freely and the whole experimental exercise lasted for 4 weeks.

Sample collection and preparation
After 4 weeks of oral administration of raw and dried *Mucuna pruriens* seed powder, the rats were sacrificed and blood samples were collected via cardiac puncture after anaesthesia with intramuscular injection of ketamine (AuroMedics Pharma LLC, Eat Windsor, USA) at a dose of 100 mg/kg body weight. The blood samples were put in plain sample tubes and allowed to clot and retract at room temperature, and then centrifuged at 3000 rpm for 10 minutes. The sera obtained was later stored at -20 ^{O}C until time of analysis.

Semen analysis
The epididymis of the rats were surgically removed while the cauda epididymis of both sides were separated from testes and placed in a small Petri containing one mL of Dulbecco medium pre-warmed to 37 ^{O}C, The cauda epididymis was cut into several parts by a sharp microsurgical scissor to release the sperm into solution (Seed *et al*., 1996). The semen sample was analyzed for sperm morphology, motility and count.

Determination of biochemical analytes
Serum and seminal testosterone were analyzed with AccuBind Enzyme-linked Immunosorbent assay (ELISA) kits from Monobind Inc, USA). Rat Catalase (CAT) ELISA Kit from Elabscience Biotechnology Inc, USA was used to assay catalase. Glutathione was determined using the modified Ellman's method (Ellman, 1959).

Phytochemical screening and proximate analysis
The seeds were screened for its alkoloids, anthraquinone, glycosides, cardiac glycosides, flavonoids, saponins, phenols and tannins contents using the standard protocols described by Harborne (1973); Trease and Evans (1989); Sofowora (1993).

Statistical analysis
Computer software SPSS version 21.0 was used for analysis of data. Comparison of variables was done using analysis of variance (ANOVA) and Fisher's least significant difference (LSD) post-hoc test. Pearson's correlation was employed for correlation analysis. The probability value $p< 0.05$ was considered statistical significant.

RESULTS

The screening results of the phytochemical compositions of *Mucuna pruriens* seeds powder showed that it was abundant in saponin, anthraquinone, flavonoid and tannins. It also contained some amount of alkaloids, cardiac glycoside and phylobatanins (Table 1). Table 2 shows the initial, final and body weight change of the different test groups. There was a significantly lower body weight change in group 1 when compared to the controls ($p< 0.05$). Table 3 shows the analysis of sperm function parameters (pH, motile, non-motile, viable, non-viable, rapid progressive motility (RPM), sluggish progressive motility (SPM), forward progressive motility (FPM), residual cytoplasm and sperm count) in control and test groups. There was no significant variation in mean values of pH, motile, non-motile, viable, non-viable, RPM, SPM, FPM, residual cytoplasm among the group ($p> 0.05$). However, there was

a significant variation in the sperm count ($p< 0.05$) among the groups. Serum and seminal catalase were significantly higher ($p= .031$, $p= .003$) in group 3 rats than in groups 1 and 2. Seminal testosterone was significantly higher ($p= .004$) in group 3 rats than in other groups (Table 4). Glutathione levels however did not differ significantly among the groups. Groups 2 and 3 rats had the greatest improvement as observed in their sperm counts and catalase activity. Table 5 shows a significant positive correlation ($r = .898$, $p = 0.015$) between sperm count and serum testosterone in the control group.

Table 1: Phytochemical compositions of *Mucuna pruriens* seeds powder

Secondary metabolites	Results
Alkaloid	++
Anthraquinone	+++
Cardiac glycoside	+
Saponin	+++
Tannins	+++
Phylobatanins	+
Flavonoid	+++
Steroids	-
Phenols	-

key: - Absent, + Trace, ++ moderate, +++ Abundant (Pearson, 1976)

Table 2: Initial, final and body weight change of the different groups

Group	Control n=8	Test 1 n=8	Test 2 n=8	Test 3 n=8	F-ratio	p-value
Initial body weight (g)	115.33±2.30	112.83±2.29	116.00±2.45	121.00±7.48	0.724	0.551
Final body weight (g)	147.67±5.52	121.00±9.64	133.50±13.67	143.40±10.49	1.502	0.255
Body weight change (g)	32.33±6.12	6.00±12.50[a]	18.50±10.88	22.40±6.62	1.609	0.229

Values are presented as mean ±SEM
Post hoc
a= significantly different from control

Table 3: Analysis of sperm function parameters in control and test groups

Parameter	Control n=8	Test 1 n=8	Test 2 n=8	Test 3 n=8	F-ratio	P -value
pH	6.82±0.07	6.83±0.15	6.83±0.12	6.96±0.07	0.496	0.690
Motile (%)	22.5±6.02	33.75±11.43	47.5±10.31	32±8.60	1.393	0.284
Non-motile (%)	77.5±6.02	66.2511.43	52.5±10.31	68±8.60	1.393	0.284
Viable (%)	40.83±8.00	50±9.79	65±10.61	53±8.75	1.200	0.344
Non-viable (%)	59.17±8.00	50±9.79	35±10.61	47±8.75	1.200	0.344
RPM	8.33±4.59	20±8.16	30±7.91[a]	17±5.39	2.090	0.145
SPM	4.17±1.54	6.25±3.75	6.25±1.25	4±1.87	0.330	0.804
FPM	5±0.00	2.5±1.44	7.5±1.44	6±2.45	1.579	0.236
Residual	5.83±0.83	5±0.00	3.75±1.25	5±0.00	1.316	0.306
Sperm count (x 10^6 cells/mL)	3.87±0.79	23.53±5.26[a]	41.63±3.31[a, b]	39.46±7.91[a, b]	13.941	0.000*

Values are presented as mean ±SEM, * = significant at $p< 0.05$
Post hoc
a= significantly different from control
b = significantly different from test 1.
RPM= Rapid sperm movement
SPM= slow sperm movement
FPM= fast sperm movement

Table 4: Testosterone, glutathione peroxidase and catalase concentrations in control and test groups

Parameter	Sample	Control n=8	Test 1 n=8	Test 2 n=8	Test 3 n=8	F – ratio	P -value
Testosterone (ng/mL)	Serum	0.09±0.03	0.38±0.26	0.07±0.05	1.27±0.97	1.477	0.267
	Semen	1.04±0.42	0.64±0.11	0.56±0.09	3.14±0.63[a, b, c]	10.032	0.004*
GPx (IU/L)	Serum	35.32±1.52	40.02±8.34	47.26±13.31	39.83±3.61	0.520	0.675
	Semen	37.78±1.56	42.19±0.62	58.53±7.34	49.94±14.64	1.228	0.361
CAT (IU/L)	Serum	43.69±15.68	9.59±3.17	24.23±6.95	81.80±20.39[a,b]	4.308	0.031*
	Semen	76.13±30.73	140.47±10.47[a]	131.83±6.07[a]	18.77±0.73[a,b,c]	11.670	0.003*

Values are presented as mean ±SEM, * = significant at $p < 0.05$

Post hoc

a= significantly different from control

b = significantly different from test 1.

c = significantly different from test 1.

Table 5: Correlation of sperm count against testosterone and antioxidant parameters

		Testosterone (ng/mL)		GPx (IU/L)		Catalase (IU/L)	
		Serum	Semen	Serum	Semen	Serum	Semen
Control	r	0.898	0.054	0.140	0.244	0.806	-0.250
	p	0.015*	0.966	0.792	0.843	0.404	0.839
	n	8	8	8	8	8	8
Test 1	r	-0.835	-0.210	-0.136	0.119	-0.365	-0.696
	p	0.371	0.865	0.864	0.924	0.762	0.510
	n	8	8	8	8	8	8
Test 2	r	0.819	0.551	-0.076	-0.600	-0.117	0.442
	p	0.181	0.629	0.924	0.591	0.883	0.708
	n	8	8	8	8	8	8
Test 3	r	-0.388	-0.317	-0.842	0.692	-0.320	-0.886
	p	0.612	0.795	0.073	0.513	0.600	0.307
	n	8	8	8	8	8	8

* = correlation is significant

r = correlation coefficient

p = probability level

n = number of samples

DISCUSSION

Mucuna pruriens supplementation in infertile men has been associated with increased sperm count and motility (Shukla *et al*., 2009) indicative of its potential positive effect on male fertility (Gupta, *et al*., 2011). Moreover, the observed decline in the levels of serum testosterone with increasing levels of *M. pruriens* seed powder administration was in contrast to previous studies in men and rats. The increased serum testosterone levels in the treated groups compared to the controls could probably be due to mechanism of action of levodopa content of the seeds, in which increase in serum dopamine antagonizes prolactin's suppressive effect on libido and testosterone (Shukla *et al*., 2009). Increased testosterone was observed in infertile men without any impairment in seminal parameters following 5 gm of *M. pruriens* extract over 3 months (Shukla *et al*., 2009). Seminal testosterone was also increased in the

experimental groups (group 3) compared to the other groups. This improved spermiogram observed might be due to the presence of L-DOPA in *M. pruriens* seeds which can serve as a precursor of neurotransmitter, dopamine acting as a nerviness tonic that prevents male sterility. The effectiveness of using Mucuna seed powder over synthetic L-DOPA has been established by clinical trials (Hussain and Manyam, 1997). Kumar *et al*. (1994) reported that *M. pruriens* is rich in L-DOPA, a precursor to the neurotransmitter dopamine, a marker of sexual desire and pleasure (Molloy *et al*., 2006), besides having several other alkaloids and flavonoids. Therefore, reduction in abnormality following administration of *Mucuna pruriens* seed powder could be linked to high L-DOPA content of this antioxidant-rich plant (Sato *et al*., 1996). The improvement in sperm indices following treatment with *M. pruriens* seed powder might probably be due to protective action of antioxidants in this plant against oxidative stress during spermatogenic process. *Mucuna pruriens* seeds contain many bioactive constituents including alkaloids, coumarins, flavonoids and alkylamines, which play important roles in increasing its antioxidant capacity (Misra and Wagner, 2007). Moreover, treatment with *Mucuna pruriens* has been reported to contribute to proper functioning of male genital system and facilitate sperm transport (Fait *et al*., 2001). The results of the present study showed that oral administration of *Mucuna pruriens* seed powder increased the levels of serum and seminal catalase thereby scavenging the excess free radicals before they interact with other proteins and thus consumption of *M. pruriens* seeds can protect the normal function of the catalase in reproductive activity. This finding is consistent with other studies on effect of herbal extract of *M. pruriens* on Parkinsonian mouse model (Yadav *et al*., 2013; Prakash *et al*., 2013). Contrary to our study, previous researchers reported a significant increase in seminal plasma glutathione levels of streptozotocin induced diabetic rats treated with *M. pruriens.* Another study also reported *M. pruriens* as a known adaptogen and its alcohol extract reduces lipid peroxidation, maintains glutathione levels and superoxide dismutase (SOD) activity (Tripathi *et al*., 2001). Our study also observed a positive correlation between sperm count and testosterone levels in the control group. This is similar to the findings of earlier study by Tendayi *et al*. (2020) who reported a strong positive correlation between sperm count and testosterone though in humans. Testosterone is required for the processes that are critical for spermatogenesis including supporting meiosis and adhesion of elongated spermatids to sertoli cells and the release of sperm (Smith and Walker, 2014). Although the amount of nutrient intake was not measured in this study, the administration of *M. pruriens* seed powder resulted in improved sperm parameters. However, it has been reported that crude *M. pruriens* seed consumed in excessive quantity as food is poisonous to mammals (Vadivel and Pugalenthi, 2008). Therefore, the use of *Mucuna pruriens* in herbal medicine should be dosage regulated to avoid its toxicity.

CONCLUSION

The results from the present study suggest that oral administration of *Mucuna pruriens* seed powder extract may improve testicular antioxidant and sperm count which is useful in combating male infertility.

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Conflict of interest

The authors declare no conflict of interest.

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