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Full Length Research Paper

Effects of methanoic seed extracts of *Mucuna sloanei* on male sex hormones and sperm quality in rats

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This work was done to investigate the effects of methanoic seed extract of *Mucuna sloanei* on male sex hormones and semen quality on albino wistar rats. Thirty two male albino wistar rats weighing between 115 to 230 g were used, they were grouped into 4 of 8 rats each. Group i (control) received distilled water and normal rat chow. While test groups ii, iii, and iv received 100, 200 and 400 mg of the extract per kilogram body weight respectively for 30 days. Blood samples were collected from four animals per group and assayed for the following sex hormones: Lutuenizing hormone (LH), Prolactin (PLT), oestrogen, Progesterone and testosterone. Semen from the epididymis was analyzed for sperm count, sperm motility and morphology. Results showed statistically significant increase in the serum levels of testosterone and LH in test groups when compared with the control ($p < 0.05$). The result also showed a dose dependent increase in sperm count in the test groups when compared with the control ($p < 0.05$). In conclusion, methanoic seed extract of *m. sloanei* has significant positive effects on some male sex hormones and sperm count.

Key words*:* *Mucuna sloanei*, sex hormones, semen quality, albino wistar rats.

INTRODUCTION

Mucuna sloanei, commonly called “horse eye bean”, is an annual leguminous climber, with pods that are covered with hairs that irritate the skin when the fruit is mature and dry (Tuleun et al., 2008). *M. sloanei* has many local names in respect to different tribes and ethnic groups. In Nigeria, it is called ‘ukpo’ by the Ibos, ‘karasuu’ by the Hausas, ‘yerepe’ by the Yorubas (Nwosu, 2011,) and ‘ibabat’ by the Efiks (Obochi et al., 2007). The constituents of *M. sloanei* include crude proteins, carbohydrates, fat, crude fibers, moisture, ash, phosphorus, magnesium, calcium, sodium, iron, manganese, copper, tannins, glycosides, L-Dopa and zinc (Nwosu, 2011; Giami and Wachuku, 1997; Akpata and Miachi, 2001; Ijeh et al., 2004; Tuleun et al., 2008). The seeds are highly resistant to disease and pest and exhibit good nutritional qualities (Janardhanan and Vadiviel, 1994). *M. sloanei* has varied properties such as condiment or as garnishing for the main dish (Ukachukwu et al., 2002; Onweluzo and Eilitta, 2003); gelation properties (Nwosu, 2011); its medicinal properties include antidiabetic (Dhawan et al, 1980; Akhtar et al, 1990), antiparkinsonism (Hussain and Manyam, 1997; Molloy et al., 2006), anti-oxidant and anti-microbial (Rajeshwar et al., 2005a), aphrodisiac, anti-neoplastic, anti-epileptic (Sathiyanarayanan et al., 2007), enhances learning and memory (Poornachandra et al., 2005) and antihelminthic (Jalalpure, 2007). *M. pruriens*, a specie of *Mucuna* beans, has been shown to increase testosterone levels and sperm secretion (Amin et al., 1996; Muthu and Krishnamoorthy, 2011).

The World Health Organization (1993) defined infertility as no conception after at least twelve months of unprotected sexual intercourse. Infertility affects about 8 to 12% of the world’s population and in about half of cases men are either the single cause of it or contribute to the couple’s infertility (Cates et al.. 1985). According to

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the World Health Organization (1999), male factor contributes 40%, female factor 40%, both male and female 15% and unknown factors 5% of cases of infertility. Infertility is a major clinical problem, affecting people medically and psychologically (Raghuveer et al., 2010). It can also affect their economy, peace and harmony. Carlson et al (1992) reported that sperm counts are falling and that male fertility is in the decline. In Southeastern Nigeria, a positive male factor alone was found in 133 (42.4%) couples and female factor alone in 81 (25.8%) couples of the three hundred and fourteen couples evaluated for the cause of infertility (Ikechebelu et al., 2003). There is need to explore food items that may have beneficial effects on fertility as they may be cheaper, more available and accessible to the poor.

To the best of our knowledge, no work has been done on the effects of methanoic seed extracts of *m. sloanei* on sex hormones and sperm quality in albino rats, hence the essence of this work.

MATERIALS AND METHODS

Thirty male albino wistar rats weighing between 115 to 230 g, bought from the Animal House of University of Nigeria, Nsukka, were used for the research that lasted for 30 days. The rats were housed in a conducive environment, allowed two weeks to acclimatize and their health status closely monitored before and during the experiment. They were also fed with normal rat chow (Guinea Feeds PLC) and portable water *ad libitum*.

Preparation of extract

Pods of *M. sloanei* purchased from Orie Ugwu market, Umuna, Imo State, Nigeria, were cracked open, the seeds sliced into pieces, dried under ambient temperature and grinded using Laboratory Mill. 50 g of dry sample was macerated into 250 ml of methanol and poured into a mechanical shaker [Uniscope SM 101] for 24 h. Thereafter the mixture was sieved with a filter paper into a clean glass tube, the filtrate was concentrated using a rotary evaporator [Buchi, Switzerland] and was further concentrated to powder form using laboratory oven at 45℃.1g of the extract was dissolved in 20 ml of distilled water to give a concentration of 50 mg/ml of solution.

Toxicity test

The LD_{50} of the extract in mice was determined orally by Lorke (1983) method with slight modifications.

The thirty male albino wistar rats were grouped into four of 8 rats each. Group I (control) was fed with normal rat feed and water *ad libitum*. Test groups II, III, and IV were treated with 100, 200 and 400 mg per kilogram body weight of the extract respectively in addition to normal rat chow and water *ad libitum* for 30 days. The extract was administered orally and daily using syringes without needles between the hours of 8.00 and 9.00.

Sample collection and analysis

Blood samples

Blood samples were collected after 30 days by cardiac puncture after anaesthetising with chloroform. 2 ml of blood were collected from four rats in each group including the control group. The samples were carefully introduced into lithium containers free from anticoagulant and were properly labeled for example 1a, 1b, 1c and 1d. The blood samples were allowed to clot, retract and then centrifuged for 5minutes at a speed of 5000 revolutions per minute. The plasma was then collected, refrigerated at -20℃ and later assayed for the following sex hormones: Prolactin, oestradiol, progesterone, LH, FSH and testosterone using ELISA Hormone Test kits (Biotec Laboratories Ltd, UK).

Epididymal sperm count, motility and morphology

The rats were sacrificed at the end of four weeks by cervical dislocation. The testes from each rat were carefully exposed, excised and trimmed free of epididymis and adjourning fatty tissues. Caudal part of the epididymis was removed and placed in a beaker containing 1 ml physiological saline solution and allowed to stand for few minutes to allow spermatozoa swim out of the solution. The sperm count and motility were determined as described by Saalu et al. (2008). Briefly, few drops of semen were placed on a slide, two drops of warm 2.9% sodium citrate were added, slide covered with cover slip and examined under the microscope using ×40 objective for sperm motility. Sperm count was done under the microscope using the improved Neubauer's counting chamber (Haemocytometer). The sperm concentration was then calculated and recorded in million and expressed as (X) $\times 10^6$/ml, where X is the number of sperm in a 16-celled square. Light microscope at ×400 magnification was used to evaluate sperm morphology. Caudal sperm were collected from the original dilution for motility and diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). Five hundred sperm from the sample were scored for morphological abnormalities according to Atessahin et al. (2006). Briefly, in wet preparations using phase-contrast optics, spermatozoa were categorized. A sperm was considered abnormal morphologically, if it had one or more of the following features: rudimentary tail, round head, and detached head and was expressed as a percentage of morphologically normal sperm.

Statistical analysis

The results obtained from this study were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0 for Windows. Analysis of variance (ANOVA) was used to compare means, and values were considered significant at $P < 0.05$. Post Hoc multiple comparisons for differences between groups within groups were established using least significant difference (LSD). Results are presented as Mean ± S.E.M.

RESULTS

The oral LD_{50} of the extract in mice was calculated to be 3,872.98 mg/kg. The results showed statistically significant ($P < 0.05$) differences in the serum levels of testosterone and Luteinizing hormone in the test groups when compared with the control group. The increase is dose-dependent as increasing doses of the extract led to higher levels of the hormones. This is shown in Table 1. Table 2 shows that methanoic seed extract of *M. sloanei* has a significant dose dependent increase in sperm count in the test groups when compared with the control ($p < 0.05$). There was no significant ($P > 0.05$)

Table 1. Effects of methanoic seed extract of *Mucuna sloanei* on male sex hormones after 30 days.

Group	LH (ng/ml)	FSH (ng/ml)	Prolactin (ng/ml)	Oestradiol (ng/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
I	0.15 ± 0.50*	1.15 ± 0.35	0.75 ± 0.15	11.15 ± 5.05	1.35 ± 0.25	1.35±1.15*
II	0.35 ± 0.50*	1.35 ± 0.05	1.00 ± 0.20	15.75 ± 6.25	3.15 ± 2.35	3.25 ± 0.45*
III	0.45 ± 0.15*	1.60 ± 0.20	0.90 ± 0.20	15.20 ± 3. 20	1.80 ± 0.30	4.35 ± 1.55*
IV	0.55 ± 0.50*	1.15 ± 0.25	0.80 ± 0.20	14.40 ± 1.80	0.90 ± 0.70	5 .30 ± 0.00*

*The mean difference is significant at the 0.05 level.

Table 2. Effect of *Mucuna Slonei* seed extract on sperm count, motility and morphology after 30 days.

Group	Sperm count (ml)	Sperm motility (%)	Sperm morphology (%)
I	$28 \times 10^6 \pm 0.0$	80.0 ± 0.0	80.0 ± 0.0
II	$34.5 \times 10^6 \pm 2.5$	77.5 ± 2.5	77.5 ± 2.5
III	$43 \times 10^6 \pm 0.0$	80.0 ± 0.0	70.0 ± 0.0
IV	$377.5 \times 10^6 \pm 7.5$	82.5 ± 2.5	77.5 ± 7.5

*The mean difference is significant at the .05 level.

effect in sperm motility and sperm morphology.

DISCUSSION

This research was done to assess the possible effects of *M. sloanei* seed extracts on male sex hormones and semen quality using albino wistar rats over a period of 30 days. Toxicity test for the raw unprocessed seed extract of *M. sloanei* showed that at a dose of 3,872.98 mg/kg, it had a lethal effect on the experimental animals.

The results showed that after 30 days of oral administration of the extract, there were statistically significant increases in the serum levels of testosterone and LH in the test groups when compared with the control ($P < 0.05$). This is shown in Table 1. There were no significant differences in the serum levels of prolactin, oestrogen, progesterone and FSH in the test groups when compared with the control ($P > 0.05$). The *Mucuna* seed extract has dose dependent effects on the serum levels of the hormones. Extracts of *Mucuna pruriens* have been shown to increase serum and testicular testosterone levels (Amin et al., 1996; Muthu and Krishnamoorthy 2011). The increase in serum level of testosterone in the test groups may among reasons be due to the many constituents of the extract such as zinc and copper. Zinc can activate secretion and action of testosterone and can lead to increased efficiency of spermatogenic machinery and increased number of germ cells in the seminiferouse tubules (Pizent et al., 2003 and Abdella et al., 2011). Muthu and Krishnamoorthy (2011) observed that rats treated with extracts of *M. pruriens* have increased serum and testicular testosterone levels as well increase in the level of cholesterol in the testis and increased activities of alkaline phosphatase in the epidymis. They also noted increase in the weights of the testis, seminal vesicle and prostate. The increased weight and secretory activities of these androgen dependent organs may be due to increase in androgen biosynthesis, hence the increase in serum and testicular testosterone (Suresh et al., 2009). Again, cholesterol is the starting material for androgen biosynthesis (Carreau, 1996; Watcho et al., 2001).Thus increase in serum cholesterol level can lead to increase in testosterone level.

Luteinizing hormone is essential for the secretion of testosterone from the Leydig sells. It stimulates the Leydig cells to produce testosterone and the quantity of testosterone secreted is directly proportional to the amount of LH available (Sembulingam and Sembulingam, 2010). Leydig cells secrete testosterone by the stimulatory effects of LH (Udoh and Udoh, 2005b; Udoh et al., 2005a, Udoh et al., 2005c)

The results also showed that the seed extract of *M. sloanei* statistically increased the sperm count in the test groups when compared with the control ($P < 0.05$). This may be due to the observed increase in the serum levels of testosterone and LH caused by the plant extracts. Both hormones are very important for normal spermatogenesis. LH stimulates the Leydig cells to secrete testosterone and testosterone is responsible for the growth, maturation and transformation stages of spermatogenesis (Sembulingam and Sembulingam, 2010).

In conclusion, methanoic seed extract of *m. sloanei* has a beneficial effect on serum testosterone and LH and

improved sperm count in male albino wistar rats. It may therefore be considered in the management of infertility in males.

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