INTRODUCTION

Much work has been done over the years for restoring impaired fertility. Some compounds thus employed are mainly of endocrinal in composition such as thyroid cytomet, glucocorticoids, and low doses of androgens eg. mesterolone, Hmenopa, gonadotropin and clomiphene citrate and long acting testoster-
on one esters. Apart from hormones, metal ions and other substances, medicinal plants are also being employed for restoring impaired fertility. Herbal plant ingredients have found to be more efficient and with lesser side effects.

*Mucuna pruriens* has been found to have many beneficial properties some of which are antiparkinson activity, antidiabetic, antioxidant, metal chelating property, green manure and fodder which has been successfully intercropped with maize in different parts of the world. It was found to regulate steroidogenesis and improve semen quality in infertile men.

*Mucuna pruriens* which forms the basis of this research is found to be treated on the fertility aspects of male rats and to investigate the histological changes in the epididymis and testes after its administration. The concentration of the whole seed powder was adjusted to three doses 50, 100 and 200 mg with experimental duration of administration of the drug was 20, 40, 60 d. Complete semen analysis with basic determinants like sperm motility, sperm count, morphological character, gonadial index, histology of epididymis and testes were performed. The results revealed that there is a significant increase in sperm count and motility, decrease in the percentage of abnormal sperms. The histological studies were conducted for evaluation of enhanced effects on target organs testes and epididymis, which showed significant changes in the architecture of testes with densely packed spermatis in the seminiferous tubules in treated mice.

Key words: Gonadal index, histochemistry, sperm count, sperm motility.

MATERIALS AND METHODS

Experimental drug

*Mucuna pruriens* seeds were purchased and authenticated by Siddha Medical College, Arumbakkam, Chennai. One kilogram of seed was cleaned with double distilled water and dried. It was decorticated and the endocarp and was pulverized using Waring blender. The drug was passed through various sieves to ensure uniformity of the powder and was stored in clean containers for the study.

Experimental animals

*Rattus norvegicus* were purchased at the animal breeding centre of Tamil Nadu veterinary and Animal Sciences University (TANUVAS), Chennai. The animals chosen were of three months old weighing from 150 to 200 g. The animals were acclimatised to lab conditions for a period of seven to ten days and maintained in a clean environment free from pathogens. Clean water was provided ad libitum.

Dose and duration of *Mucuna pruriens*

The LD$_{50}$ of the seed powder extract was found to be 1000 mg/kg in albino rats. In another experiment the alkaloid extract of *M. pruriens* of one mg has found to bring a note worthy increase in the population of spermatozoa. Having this as the basis the concentration was adjusted to the whole seed powder and three doses were chosen 50, 100 and 200 mg. Duration of adminis-
tration of the drug was 20, 40 and 60d, with six animals in each concentra-
tion. Three control batches were maintained with ten animals in each batch. The drug was administered orally at the same period of each day.

Removing testis and epididymis for Semen analysis

The rats with known weight and maturity are chosen. They were sacrificed and the testis and epididymis being removed. The epididymis was kept suspended in tris diluent till the epididymis was opened to provide physi-

Dilution of the Semen

A drop of semen diluted to 0.5mL was placed on a watch glass to this a speck of eosin powder just to stain the sperm to a pale pink colour. The stained semen was drawn up to 0.5 reading in the red blood cell pipette. A filter paper was used to bring down the level to 0.5 marks. The tip of the pipette was removed and wiped dry and followed by 1% formal saline drawn up to marking 10% which results in a standard dilution ratio 1:200 formal saline is capable of killing and dispensing the cells evenly throughout the fluid. The proper mixing of the semen and diluents was ensured.

Gonadal index

The combined testis weight in relation to the body weight was being noted for correlating it with the sperm production potential. The cleaned testes was placed in tris diluent was placed and taken on a filter paper. This was weighed and the gross weight of both the testes was noted down and calculated.

Gonadal Index $=$Weight of both testes $\times$100

Body Weight

Motility and morphology of sperm

The motility was recorded in terms of forward progression called as weight is
Table 1. Analysis of variance for semen characters of Rattus norvegicus after treatment with Mucuna pruriens seeds

<table>
<thead>
<tr>
<th>Source</th>
<th>D.F</th>
<th>M.S.S. Sperm count</th>
<th>M.S.S. Motility</th>
<th>M.S.S. Abnormality</th>
<th>M.S.S. Gonadal index</th>
<th>M.S.S. body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between days</td>
<td>2</td>
<td><strong>575.45</strong></td>
<td><strong>104.71</strong></td>
<td><strong>30.02</strong></td>
<td>NS 0.04</td>
<td><strong>203.82</strong></td>
</tr>
<tr>
<td>Between treatment</td>
<td>3</td>
<td><strong>234.96</strong></td>
<td><strong>339.41</strong></td>
<td><strong>23.89</strong></td>
<td><strong>1.11</strong></td>
<td><strong>1549.74</strong></td>
</tr>
<tr>
<td>Error</td>
<td>78</td>
<td>13.48</td>
<td>8.3</td>
<td>6.54</td>
<td>0.18</td>
<td>43.53</td>
</tr>
</tbody>
</table>

N.S = Not Significant, M.S.S = Mean Sum of Squares, D.F = Degrees of Freedom

RESULTS AND DISCUSSION

From the results of this present investigation it becomes more evident that the seeds of Mucuna pruriens have a definite positive action on improving the sperm production in male rats. The epididymis of the rat was found to have the sperm reserve to the extent of 300.87X10^6 per epididymis which was found to coincide with the control values of the present study [13].

The results of sperm count in response to the drug Mucuna pruriens shows an increase with a value of 31.4 million/mL at 0.5 dilution, at 20d treatment in all the three dosage (Figure 1). The drug is found to have a profound effect at 60 d 200 mg treatment. The increase in sperm count is found to be dose dependent and a gradual significant increase is noticed at all the three doses and duration.

In connection with increased spermatogenesis and direct relation with L-dopa progressimilar work has been done by various scientists. This L-dopa is a precursor of dopamine and is able to cross the blood brain barrier. This is formed by L-tyrosine and is an intermediate in synthesis of catecholamine [14]. The presence of L-dopa in seed extract of M. pruriens has been reported to be at the range 0.64-1.52% by Modi et al. [13]. According to the results in (Figure 2) the effect of the drug M. pruriens on the body weight and gonadal index of male albino rats are found to be significant (P<0.01). The body weight is found to vary from 18.40 ±2.20 to 31.1 ±2.19 at 60 d and 35.11 ±2.01 at 200 mg dosage. Though a rise is seen at 20 d 31.72 ± 2.30 and 25.71 ± 1.75 at 50 mg there is a mild fluctuation in the weight at 40 d which could be due to seasonal changes. The testes weight is found to be significant in between doses when indexed with body weight. Correlation of sperm count, with that of testes
being suggested by many other scientists. This is attributed to the availability of testosterone and alkaloids to the gonads [8].

The data from the present study on the motility of sperm in response to the drug M. pruriens shows that all three dosage and duration recorded to be highly significant at (P<0.01). (Figure 3). The normal sperm motility of rat is 70% from the cauda epididymis [15]. The highest motility percent was noted at 60 d with 200 mg trial. The treatments between doses seem to have a prominent increase in overall mean percent of sperm movement. An increased motility propelling a rapid progressive movement is being influenced by the drug.

There are many deviations as from the normal sperm morphology. Head abnormalities are with a mark reduction size, double cells giant, amorphous, and conjoined sperm which may be pronounced in infertile sperm [9]. Results (Figure 4) reveal that the efficacy of Mucuna pruriens in bringing down the percentage of abnormality of sperm is pronounced from control value of 23.8%. There is a noticeable gradual decline in the abnormal sperm in relation to duration and dosage. Some of the abnormalities recorded where detached head, looped tail and crooked head (Figure 5). The decline of the mean percent of abnormal sperm might be due to the effect of alkaloids or L-dopa or other component of Mucuna pruriens in relation with the testes weight gain, increased sperm production and accelerated motility. Testosterone is correlated with sperm motility and luteinizing hormone to that of the sperm number [6].
A positive response was noted in the histological studies of epididymis and testes. A greater number of sperm cells were noticed in epididymis at the 60 d 200 mg (Figure 6 and 7) than in the sections viewed on 20 d treated animals (Figure 2 and 3). The testes sections showed a pronounced increase in the tubular diameter and spermatids with the increase in the dosage and duration of the drug as in 60 d, 200 mg. Similar trend was noticed in testes section exposed to 100 mg in all the three treatment period of 20 d, 40 d and 60 d (Figure 8-11) when compared to that of the control animals (Figure 5). In a study conducted by Jesse et al. [16], correlation of testes weight relative to body weight was recorded. From the results observed it becomes clearly evident that the testes weight is related with body weight, sperm cells produced and their motility and morphology are correlated (Table 1).

The potency to treat male infertility has been confirmed in the present study where encouraging results and observations have been noted. There is a marked increase in sperm concentration, motility and improved semen quality in response to treatment with Mucuna pruriens seed powder.

REFERENCES

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