CHAPTER V

RESULTS

AND

DISCUSSION
RESULTS AND DISCUSSION

The results of and inference drawn from the phytochemical and pharmacological investigations of the seeds of *Mucuna pruriens* Baker in this study are discussed here.

Of the four procedures attempted for extraction and isolation of alkaloids to obtain their maximal yield, procedure II yielded 0.02 per cent total alkaloids in the chloroformic extract affording four alkaloids. Procedure II was employed both to increase the number and yield of alkaloids. Procedure II yielded twice as much total alkaloids (0.04 per cent) as compared to the previous procedure. Six compounds were isolated in the ethanolic extract as total alkaloids, four of which were confirmed to be chloroform-soluble alkaloids (0.024%) and two, water-soluble alkaloids (0.016%). The increase in the yield was attributed to extraction effected in a Soxhlet apparatus (unlike 'refluxing' employed for alkaloidal extraction in the previous procedure) employing ethanol (95%) for alkaloidal extraction. Extraction in the Soxhlet apparatus allowed greater alkaloidal yield on account of repeat contact of redistilled solvent with the powdered seeds in the solvent cycles recurrently formed during extraction, causing the exhaustion of the drug of alkaloids. Procedure III was employed, with some modifications, to improve the yield further. It yielded 0.055 per cent total alkaloids containing six compounds, four of which as chloroform-soluble (0.035%) and two as water-soluble bases (0.020%).

The increase in CHCl₃-soluble bases was attributed to their
extraction at a specific pH 8 and concentration of alkaloidal extracts in an atmosphere of CO₂ to prevent oxidative reduction of the bases.

Procedure IV, developed by Ghosal, et al. (1971), was attempted with some modifications to attempt at increasing the yield further and verify their reported claim of 0.53 per cent alkaloids. Procedure IV yielded, 0.066 per cent total alkaloids, which was greater than in Procedure III, but the reported high yield (0.53 per cent) could not be achieved. For CHCl₃-soluble bases (0.035 per cent) the extraction at a modified pH of 9 assured greater yield of the bases than in Procedure III. For H₂O-soluble bases (0.031 per cent) separation of the bases by ammonium reineckate (in place of precipitating Dragendorff's reagent in Procedure III) assured greater yield than in the earlier procedure. Ion-exchange resins used to decompose ammonium reineckate-alkaloid complex was believed to have separated greater water soluble alkaloid than in Procedure III where Dragendorff's reagent-alkaloid complex decomposed by freshly precipitated silver oxide caused incomplete separation of the alkaloid, diminishing its yield.

The bases were separated in three steps. Their individual quantities and Rf values (TLC) are as follows:

First, as chloroform-soluble acetates (Compound A (0.007 g) (Rf 0.60) a viscous brown oily liquid and Compound B (0.008g) (Rf 0.92), a brown basic gum).

Second, as chloroform-soluble strong bases (Compound C (0.19)
(Rf 0.33), a white amorphous base, and Compound D (2.6g) (Rf 0.22), a brown basic gum.

Third, as water-soluble bases (Compound E (5.3g) (Rf 0.27) a colourless liquid base (studied as its chloride salt) and Compound F (0.006g) (Rf 0.36) a flaky white base).

Two TLC developers 1. n-BuOH - n-BuOH - AcOH - H₂O (85:40:15:20) for one-dimensional TLC and 2. n-BuOH - AcOH - H₂O (4:1:2) for two-dimensional TLC. For TLC in Procedures I to IV were chosen as they showed best resolution of six spots of alkaloids (among other developers) (Table 3), obtained in extraction and isolation procedures II, III and IV, using the marker Dragedorff's spray solution. Detection of alkaloids was carried out by alkaloidal precipitating reagents. Of the six alkaloids isolated from the ethanolic extract, A, B and F could not be identified and characterised on account of their exceedingly low yields. No crystalline derivatives of these compounds could be formed either. Two non-alkaloidal compounds were also isolated.

Compound G (12.0) was extracted from seed powder in aqueous acidic extract, and Compound H (9g) was extracted from petroleum ether extract using acetone and ethanol (for extraction of polar lipids fraction). The identification of individual compounds involved Co-TLC with their authentic specimens and their characterisation was done by melting point (m.p.) and mixed melting point (m.m.p.) determinations using Thiele's tube spectral analysis (the IR spectra of the isolated compounds were found to overlap the spectra of their authentic specimens, as
discussed below) and microanalysis (C-H-N estimations) which showed difference between the percentages 'found' of the elements of unknown compounds and the 'theoretical' elemental percentage of the authentic specimens to be negligible).

The compounds isolated from the seeds are discussed with their identification and characterisation as follows:

**Compound C:** (Rf 0.33) Co-TLC with authentic specimen of β-carboline; white amorphous base; m.p. 256-258°C; m.m.p. 257-258°C with β-carboline; picrate: m.p. 138-140°C, m.m.p. 138-139°C with picrate of β-carboline; IR (principal peaks: 1247 (A), 1450 (B) and 731 or 747 (C) showing overlapping of spectra of authentic β-carboline base; and C-H-N analysis: found: C (78.53%), H (4.79%) and N (16.58%); theoretical: C (78.49%), H (4.75%) and N (16.66%).

**Compound D:** (Rf 0.22) Co-TLC with authentic specimen of bufotenine base; buff coloured base with m.p. 175-177°C, m.m.p. 176°C with authentic specimen of bufotenine base; picrate: m.p. 103-104°C, m.m.p. 103-104°C with picrate of bufotenine; IR (principal peaks: 2950 (A), 2984 (B), 1470 (C) and 1482 (D) showing overlapping of spectra of the authentic bufotenine base; and C-H-N analysis: found: C (70.54%), H (7.88%), N (13.72%) and O (7.86%); theoretical: C (70.40%), H (7.89%), N (13.74%) and O (7.89%).
**Compound E**: (Rf 0.27), Co-TLC with authentic specimen of choline chloride; chloride salt prepared from hygroscopic base; crystalline substance, hygroscopic; m.p. 294-295°C (dec.), m.m.p. 294°C (dec.) with authentic choline chloride; IR (principal peaks: 1636 (A), 1477 (B), 953 (C) showing overlapping of spectra of authentic choline chloride; and C-H-N analysis (found: C (43.01%), H (10.11%), N (10.03%), O (11.46%) and Cl (25.39%); theoretical: C (42.90%) H (10.26%), N (10.26%), O (11.48%) and Cl (25.10%).

**Compound G**: (a white crystalline odourless powder; m.p. 280-282°C; m.m.p. 282°C with authentic L-dopa; IR (principal peaks: 1601 (A) and 1028 (B) showing overlapping of the spectra of authentic L-dopa) and C-H-N analysis (found: C (54.84%), H (5.63%), N (7.11%), and O (12.42%); theoretical C (54.82%), H (5.62%), N (7.10%) and O (12.46%).

**Compound H**: (a brownish yellow waxy substance; IR (principal peaks: 2940 (A), 1050 (B), 3400 (C), 1548 (D) and 2860 (E) showing overlapping of the spectra with authentic lecithin).

For pharmacological investigations in male albino rats, hydrochlorides of compounds C, D and G, and chloride of E were prepared. The solvent for all the four was chosen as water for injection (for intramuscular administration). Compound H was used in the same form and was dissolved in sterile arachis oil (for intramuscular administration).
The compounds were re-coded (and brought in a continuous sequence) for convenience of presentation in the observation tables as follows:

$S_1$ for $\xi$ (L-dopa), $S_2$ - $E$ (choline), $S_3$ - $D$ (bufotenine), $S_4$ - $C$ ($\beta$-carboline), and $S_5$ - $H$ (lecithin).

Code $S_6$ was given to the sample of seed powder (taken for study of overall seed activity); it was administered orally in the form of wheat flour pellets.

The compounds (test samples) were administered in the doses - $S_1$ (9.2 mg/kg), $S_2$ (200 mg/kg), $S_3$ (7.6 mg/kg), $S_4$ (5.6 mg/kg), $S_5$ (200 mg/kg), and $S_6$ (250 mg/kg) in rats in experiments 1, 2 and 3 after determining their $ALD_{50}$ by Horn's formula and table. One-fifth of $ALD_{50}$ of each sample was the administered dose in experiments 1, 2 and 3.

**In Experiment 1** on 'chromosomal studies' it was investigated whether the samples $S_1$ to $S_6$ produced any mutagenic changes in the germinal epithelial cell of rats. It was found that (Plates 1 to 8) no chromosomal morphological changes or any abnormalities were noted on completion of the last (30th) day of the experiment.

Normal chromosomes were observed, upon treatment with the samples $S_1$ to $S_6$, in the various stages of transformation of the germinal epithelial cells (into spermatozoa) - spermatogonia, primary and secondary spermatocytes, which were photographed at different magnifications.
The normal appearance of chromosomes strongly indicated absence of mutagenicity or toxicity of the samples S₁ to S₆ in the doses administered to the biological system.

In Experiment 2 (Table 6) concerning fertility studies on the immature castrated rats, the samples S₁, S₄ and S₆ were found to cause significant increase in weight of the accessory sex organs when compared with castrated Controls (Group III). The increase in weight of the reproductive organs was correlated with androgen-induced reversal of postcastrate retrogressive changes in epididymis, seminal vesicles and ventral prostate. It was strongly indicated that the samples S₁, S₄ and S₆ elicited androgenic response to effect these changes. Their activity was confirmed by comparing the changes with those effected by testosterone propionate, administered for androgen replacement therapy, in the immature castrated rats.

Samples S₂ and S₅ brought about insignificant changes in the weights of accessory sex organs, whereas the sample S₃ lowered their weight significantly, indicating contribution of S₃ to postcastrate retrogressive manifestations.

The modes of action of the samples S₁, S₃, S₄ and S₆ are discussed ahead, separately.

In Experiment 3A (Tables 7 and 8) the samples S₁, S₄ and S₆ were found to have effected significant increase in weights of the
reproductive organs - testes, seminal vesicles, prostate and epididymis (caput, corpus and cauda) in adult male rats both estrogen propionate-treated (to induce subfertility (Group III) rats and the normal Controls (Group IV). The samples exhibited reversal of estrogen-induced subfertility in Group III animals, which was attributed to the individual action of the samples S1, S4 and S6 caused via pituitary, effecting release of LH to result in testosterone secretion from Leydig's cells, thus counteracting the action of estradiol propionate (EP); EP acts via pituitary to cause sterility by inhibiting LH and gonadotropin release, indicating the role of the samples in reversing EP action at the pituitary level.

Samples S2 and S5 showed insignificant changes and sample S3 was found to have significantly lowered the weight of genital organs - in groups III and IV.

Samples S1, S4 and S6 exhibited reversal of anti-androgenic response (in Group III) elicited by estradiol propionate, whereas in Group IV they effected increase in weights of genital organs in normal rats, strongly suggesting androgenic activity. The modes of action of the samples S1 to S6 are discussed ahead.

In Experiment 3B, the histological examination of the genital organs in groups I to IV rats revealed that Group I (Controls) animals exhibited normal features of testes (with active spermatogenesis), caput and cauda epididymis (with normal tubular diameters) (Plates 13, 15 and 17).
Group II rats showed testes with spermatogenesis arrested at the spermatid stage, and reduced tubular diameters of caput and cauda epididymis. Cauda portion also showed thick intertubular stroma and desquamated germ cells (Plates 14, 16 and 18).

These retrogressive changes in the genital organ tissues were a result of the estradiol propionate (EP) - induced reversible sterility. EP is known to interfere with Leydig cells function and cause anti-androgenic changes - decline in growth of testes and accessory organs and changes in their histological features indicating subnormal or abnormal state of the organ, or induced sterility.

Group III rats receiving a combination of EP and a 'sample' in each subgroup (A₁ to F₁) showed for samples S₁, S₄ and S₆ active spermatogenesis, in the testes, with its all stages clear (Plates 14, 28 and 34), normal tubular diameter and germ cells and non-edematous stroma of caput and cauda epididymis (Plates 20, 21, 29, 30, 35 and 36).

These observations, identical to those in Group I controls, indicated that samples S₁, S₄ and S₆ caused reversal of the estradiol propionate-induced effects of sterility, to bring about normality.

Samples S₂, S₃, and S₅ showed no alteration of estradiol propionate-induced retrogressive changes. The testes showed spermatogenesis arrested at spermatid stage with atrophic Leydig cells in view (Plates 20, 25, and 31). The caput epididymis showed reduced tubular diameter and edematous stroma (Plates 23, 26 and 32). The cauda epididymis showed thick intertubular stroma and reduced tubular
diameter (Plates 24, 27 and 33).

In Experiment 3C on 'Sperm Count' (Table 9), Samples S₁, S₄, and S₆ produced significant increase in sperm population. Their activity strongly indicated the eliciting of androgenic response, evident from reversal of estradiol propionate (EP)-induced anti-spermatogenic activity (by virtue of its anti-androgenic action) in Group III rats. The samples effected significant increase in sperm count in Group IV (normal) rats. Samples S₂ and S₅ showed insignificant activity, whereas Sample S₃ brought about significant lowering of sperm count, suggesting contribution to EP-induced anti-spermatogenic action. The modes of action of S₁, S₃, S₄ and S₆ are discussed ahead.

In Experiment 3D on 'Sperm Motility' (Table 10) the samples S₁, S₂, S₄, S₅, and S₆ were found to bring about significant increase in per cent sperm motility in epididymis in Groups III and IV rats (Figs. A to N in when compared with Group I (Controls) and Group II (EP-treated) rats. The increase in per cent motility indicated reversal of estradiol propionate-induced decrease in sperm motility (Group II rats). Sample S₃ lowered per cent motility in Groups III and IV rats. The modes of action of the samples S₁ to S₆ are discussed ahead, separately.

In Experiment 3E, 'Fructose Estimation' (Table 11), the samples S₁, S₄, and S₆ were found to have significant rise in the fructose level in the coagulating glands of rats of Groups III and IV when they were compared with Groups I and II.
Their activity exhibited reversal of estradiol propionate(EP)-induced antiandrogenic action (Group II) that led to significant decline in fructose levels. The reversal of the sugar levels from the 'diminished' towards 'normal' suggested profertility activity. In Group IV, samples $S_1$, $S_4$ and $S_6$ brought about, individually, significant increase in fructose content in normal rats, indicating the effects to be the result of generation of androgenic response.

Samples $S_2$ and $S_5$ brought about insignificant activity, and $S_3$ lowered significantly the fructose content, thus contributing to estradiol propionate(EP)-induced depression in fructose level, a manifestation of subfertility.

In Experiment 3F, 'Estimation of Glycerolphosphocholine(GPC)' in epididymis of rats (Table 12), the samples $S_1$, $S_2$, $S_4$, $S_5$, and $S_6$ were found to exhibit highly significant increase in glycerolphosphocholine levels in Group IV animals and significant increase in Group III animals when compared with Groups I and II. The reversal of GPC content lowered in the animals by estradiol propionate(EP) treatment, exhibited a profertility change.

Sample $S_3$ brought about a significant decline in the GPC content and indicated contribution to the EP-induced depression of GPC content.

The modes of action of the samples $S_1$ to $S_6$ are discussed as follows with reference to their effect on the treated subjects in Experiments 2 and 3 (3A to 3F):
Modes of action: Samples $S_1$ to $S_6$ are discussed separately hereunder:

Sample $S_1$: Sample $S_1$ was chemically identified as L-dopamine, which is, physiologically, a neurotransmitter or a brain catecholamine, reported to play stimulatory role in male sexual behaviour. Strong hypersexual behaviour and hypergenitalism has been reported in a pre-pubertal boy and severe hypersexuality in a parkinson man.

In rat, L-dopa causes a selective accumulation of brain dopamine (DA) while serotonin levels are reduced. Stimulating effect of L-dopa has also been reported on copulatory behaviour of male rats with receptive females. In studying a 'test sample' for aphrodisical activity, it was found appropriate to use animals with experimentally lowered basal levels of sexuality.

A great deal of experimental evidence indicates that the sexual behaviour in male rats is stimulated by treatments that elevate brain dopamine concentration or decrease brain serotonin. In contrast, male sexual behaviour is suppressed by treatments that either block brain dopamine receptors or elevate brain serotonin levels. Consequently, a theory was proposed according to which male sexual behaviour is reciprocally controlled by a central dopaminergic stimulatory and serotoninergic inhibitory mechanism.\textsuperscript{3a, b}

$S_1$, which brought about increase in weight accessory organs in immature castrated rats (Experiment 2) and increase in weight
of testes and accessory organs in EP-treated and normal adult albino rats (Experiment 3), increase in sperm count and motility, and rise in fructose and GPC levels, is therefore strongly believed to have acted by elevating brain dopamine level.

In castrate rats up to 100 days of age testosterone supply from testes is disallowed, and so alternative androgen supplies are assured from adrenal X-zone. This zone atrophies when rats exceed this age.4

In rats in Experiment 2, the result of gonadectomy (castration)—i.e. of cutting off the testosterone supply, was strongly believed to have counteracted by S7 by causing pituitary stimulation to release ACTH for secretion of testosterone from adrenal X-zone.

In Experiment 3, it is strongly suggested that dopamine stimulated pituitary through hypotalamus to release LH which effected testosterone secretion from Leydig cells. The counteraction of EP-induced sterility at the pituitary level seemed most likely as dopamine influences CNS.

The changes effected by S7 indicated androgenic response that is typical of testosterone-induced activity in rats upon gonadectomy and hypophysectomy. This response is clearly exhibited in Experiment 2 after giving postcastrate testosterone replacement therapy.
Sample $S_2$:

Sample $S_2$, which was chemically identified as choline (used in the study as its salt—choline chloride) exhibited insignificant activity in Experiments 2, 3A, 3B, 3C and 3E. It increased only sperm count and GPC content significantly. Free choline is a precursor of acetylcholine which, it has been claimed, somewhat increases sperm motility. Free choline, itself, has been believed to exert some pharmacodynamic effects on the spermatozoa.\(^5\)

The role of choline in increasing glycerylphosphocholine levels is perhaps by causing increase in concentration of free choline in semen and accessory secretions, needed to play a role in metabolism of phospholipids, which may be preventing its precursor, glycerylphosphocholine from yielding free choline, resulting in accumulation of GPC.

Sample $S_3$:

Sample $S_3$, which was chemically identified as bufotenine, is also called pseudoserotonin and is reported to have serotonergic activity.\(^6\)

$S_3$, in Experiment 2, brought about significant decrease in weight of seminal vesicles, epididymis and ventral prostate. This activity is strongly indicated to be on account of rise in brain serotonin level by bufotenine which causes a reciprocal fall in brain dopamine level, leading to suppression of sexuality.
The decline in weight of accessory organs suggests drop in testosterone levels required from adrenal X-zone in castrates. The ACTH release from anterior pituitary through hypothalamus is believed to have been blocked (on account of its serotonergic activity) causing suppression of sexuality as a result of unavailability of testosterone from the adrenal X-zone.

S₂, in Experiment 3, brought about a significant fall in weight of testes and significant decline in sperm count and sperm motility, as also in fructose and glycylphosphocholine levels. The activity of S₂, as in Experiment 2, is strongly indicated to be a consequence of rise in serotonin levels which lead to diminished sexuality.

In estradiol propionate-induced reversible sterility is caused by its twin action of direct testosterone antagonism and blockage of gonadotropin and LH release from the anterior pituitary through hypothalamus, which in turn blocks testosterone release from the Leydig cells. S₂ was believed to have caused additive depression of sexuality with EP, which was confirmed on comparing Groups IV and II. Group IV rats showed significantly greater decline than Group II rats in weight of genital organs and drop in sperm count and motility, and fructose and GPC levels.

**Sample S₄:**

S₄, which was chemically identified as β-carboline, or norharman, is a known potent serotonin antagonist.⁷
In experiment 2, $S_4$ brought about significant increase in weight of genital organs, and rise in sperm count, motility, and fructose and GPC levels. This activity was strongly indicated to have been caused on account of direct antagonism of brain serotonin, effecting reciprocal rise in brain dopamine to result in hypersexuality. The hypersexuality is strongly indicated to have been affected by influencing anterior pituitary through hypothalamus to effect release of ACTH for secretion of testosterone from adrenal X-zone, an alternative source of testosterone which becomes important in castrates where supply of testicular testosterone is cut off.

The activity of $S_4$, in experiment 3, is proposed to have influenced anterior pituitary via hypothalamus (as a result of enhanced levels of brain dopamine) to effect release of LH that in turn caused secretion of testosterone from Leydig cells.

**Sample $S_5$:**

Sample $S_5$, which was chemically identified to be lecithin (synonym—phosphatidylcholine), was found to bring about insignificant changes in weights of secondary sex organs in Experiment 2, and in weights and histological features of genital organs, sperm count and fructose levels (Experiments 3A, 3B, 3C and 3E).

Sample $S_5$ exhibited significant activity of enhancing sperm motility and glycercylphosphocholine levels. The sperm motility enhancing activity was attributed to the supply of greater
quantities of free choline possibly as a result of metabolism of phosphatidylcholine in the epididymis. Free choline is known to exert a pharmacodynamic influence over spermatozoa. Rise in glycercylyphosphocholine (GPC) content in the epididymis is indicated to have been caused as a consequence of greater accumulation of free choline, which might have diminished the conversion of GPC (the precursor of 'choline') into 'free choline' that is essentially involved in phospholipid metabolism.

Sample S₆:

S₆, the seed powder, was found to exhibit significant activity of enhancing weight of accessory organs in castrated rats (Experiment 2), weight of genital organs, sperm count and motility, and fructose and glycercylyphosphocholine levels (Experiment 3).

All these effects strongly indicated the reversal of experimentally-induced sterility in gonadectomised (castrated) and chemically hypophysectomised rats by eliciting an androgenic response that is characteristic of the response generated in castrated rats receiving testosterone replacement therapy.

The androgenic response is strongly suggested to be generated by L-dopa which is present in a high percentage (upto 2 per cent) in the plant seeds and β-carboline, which, eventhough present in a much less quantity than L-dopa, exerted the profertility activity similar to L-dopa (S₇) on account of serotonin antagonism which only raises the level of 'brain dopamine'.³α
Unlike what has been reported by Ghosal et al that the aphrodisical activity of *Mucuna pruriens* Baker was attributed to serotonin and serotonin-like substance, Bufotenine (which is present in the seed), profertility activity was chiefly attributed to L-dopamine which has a significant role to play in causing hypersexuality unlike serotonin which diminishes sexuality when its concentration in the brain rises. The study finds the work of Ghosal et al concerning pharmacological study of aphrodisical property of the plant drug being attributed to serotonin, unconvincing and in just sharp contrast to the known activity of serotonin. L-dopamine has been found to be the chief causal factor of profertility changes, and is known to exert powerful aphrodisical action in man and other mammals, especially rats.

The contents of L-dopa has been found to occur in the seed sixty times more than bufotenine and β-carboline. It is suggested, therefore, the action of bufotenine (pseudoserotonin) which brought about contrasting activity to that exerted by L-dopa, was potentially masked or clouded by L-dopa.

Similarly, the action of β-carboline, which was found supportive to L-dopa activity, was most certainly potentially masked by the latter. In $S_6$ (the seed powder), the chief aphrodisical and profertility properties of the seeds are attributed primarily to L-dopa along with β-carboline. Seed powder, as the one-fifth of $ALD_{50}$ has proven to be very effective in reverting experimentally-induced sterility in rats.
As the seed powder is known for its use as a nervine tonic, the role of seeds in fertility, as studied in this work, now can be correlated with the psychosexual aspects of sex - its aphrodisical property in particular indicates the link of psychosexual sex with physiological sex. The seed powder, therefore, is deemed to be a conditioner of physiological, psychosexual and genital environments of sex drive and behaviour to accentuate fertility or reverse subfertility. 

Conclusively, *Mucuna pruriens* Baker seeds were found to have played a substantial role as a corrective in male subfertility arising from depressed androgenic levels. The seeds caused profertility changes which were typical of androgenic response generated in gonadectomised and hypophysectomised rats by androgen replacement therapy. 

The seeds which induced profertility changes manifesting significant increase in - weight of accessory sex organs, sperm count, sperm motility, fructose and glycercylphosphocho-line in secondary sex organs - strongly suggested that they have a much more elaborative role to play in male reproductive physiology than just as an aphrodisiac. 

The seeds will prove to be of considerable therapeutic value in subfertile males in whom malfunction of reproductive organs or lapses in sex physiology are associated with subdued secretion of testosterone, but, essentially, without any link with such damages as prostatic, adrenal or testicular
tumors, spermatic duct blockage, which require surgical
intervention, or those that indicate permanent impairment
of sexual function as in congenital sexual defects, or in states
of sterility and impotency which are diagnosed to indicate
severe fertility depression that can be treated by only
therapeutic doses of testosterone.

For effective treatment of male subfertility, however, the
minimum effective dose of seed powder remains to be studied.
MODE OF ACTION OF ACTIVE PRINCIPLES
OF THE SEEDS OF Ocimum gratissimum Baker
ON MALE FERTILITY IN RATS

TEST SAMPLES OF ACTIVE PRINCIPLES OF THE PLANT SEEDS

\[ S_1, S_2, S_3, S_4, S_5, S_6 \]

- **Experiment 2**
  - **Castrated immature rats**
    - (To note androgenic response)
  - **Normal rats**
    - (Untreated by estradiol propionate)
    - (Group IV)
  - **Functionally sterilized**
    - (By estradiol propionate)
    - (Rats to note influence on pituitary governed androgen secretion)
    - (Group III)

**Testosterone Propionate (TP)**
- (250 µg/rat/day)

**Group III**
- **Experiment 3**
  - **Increase in brain dopamine level**
  - **Increase in brain dopamine synthesis**
  - **Increase in brain dopamine level**

**HYPOTHALAMUS**

- **Stimulation of pituitary activity**
  - **Increase in testosterone level**
  - **Strong androgenic response apparent in increase in**

- **No influence on pituitary activity**
  - **No change in**

1. **Weights of**:  
   1. Seminal vesicles  
   2. Epididymis  
   3. Ventral prostate

2. **Levels of fructose (in coagulating gland)**
3. **Glycerylphosphocholine (GPC) in epididymis**
4. **Sperm count**
5. **Sperm motility**
REFERENCES:


